April 21, 2009

Sherr & Sherr method protocols for microzooplankton stocks and grazing/growth rates

Microzooplankton grazing rates on phytoplankton and sea ice algae: During the USCG Healy long spring cruise, March 28-May 6 2008, we completed 13 microzooplankton grazing experiments. Truncated dilution experiments were carried out following the protocol of Landry (1993) with triplicate 10 % whole water and whole water treatments. All carboys, bottles, and tubing used in setting up dilution assays were pre-soaked in 10% HCl and thoroughly rinsed with deionized water. Nitex gloves were worn during experimental set-up. Water for the dilution assays was collected in 30-liter Niskin bottles at a pre-determined depth, either the Chl-a maximum or a depth in the upper mixed layer corresponding to a depth sampled for phytoplankton production. Seawater was gently transferred into 50 liter carboys through silicon tubing. After collection of seawater, all other preparation steps were carried out in a temperature-controlled environmental chamber set at 0 to -1 ^{o}C under dim light (approximately 0.1 % of incident light). For dilutions, particle-free seawater was prepared by gravity filtration through a Pall 0.2 μm filter that was initially presoaked in 10% HCl and then thoroughly rinsed with deionized water. Five to seven liters of seawater were passed through the 0.2 μm filter before beginning collection of particle-free water for the dilutions. Experimental bottles were filled within two to three hours of sample collection.

Initial samples were taken from whole seawater samples for determination of chlorophyll-a concentration and for microscopic enumeration of microzooplankton abundance, biomass, and general taxonomic composition. Depending on the phytoplankton concentration, from 25 ml to 150 ml quadruplicate volumes were settled via vacuum filtration onto GFF filters in dim light. The filters were extracted in 6 ml of 90% acetone in 13 x 100 mm glass culture tubes at -20 ^{o}C for 18 to 24 hours. At the end of the extraction period, the filter was carefully removed from each tube, and the Chl-a concentration determined using a calibrated Turner Designs fluorometer. A solid chlorophyll standard was used to check for fluorometer drift at the beginning of each reading of Chl-a samples. 120 ml subsamples for determination of microzooplankton biomass and abundance were preserved with or 5% final concentration acid Lugol solution for inverted microscopy. Separate subsamples were preserved for inspection via epifluorescence microscopy with a three-step alkaline Lugol-sodium thiosulfate- 2% final concentration formalin fixation protocol (Sherr and Sherr 1993). Formalin-preserved samples were held at 2 ^{o}C for 12 to 24 hours to harden protist cells, and then settled onto 3.0 μm black membrane filters, stained with DAPI (5 μg ml^{-1} final concentration), and mounted onto glass slides which were stored at temperatures of -20 ^{o}C or lower until analysis.

Sampling during the first part of the cruise was under heavy ice conditions with low algal biomass in the water column; during the last 3 weeks open water with mass diatom blooms was encountered at some stations. We compared the rates of algal growth in whole water and in 10 % whole water diluted with particle-free filtered water over a 24 hour day-night cycle at light levels of 15% to 30% of surface incident light. We incubated separate sets of 10 % diluted water samples on the Ashjian/Campbell plankton wheel and also in our on-deck incubator when air temperatures were sufficiently warm to keep the incubator drain hoses from freezing. In four of the experiments, there were separate treatments with and without added ice algae. Growth rates

of algae were determined by change in chlorophyll-a (chl-a) concentrations from the initial to final times of the incubations.

In addition to the 1-day dilution assays, we did 5 longer term experiments lasting up to 12 days to determine growth rates of selected species or morphotypes of microzooplanktonic protists. Water was collected for 4 of these experiments at sites where diatoms were blooming, with initial chl-a values of 7 to 27 μg chl-a l^{-1}. Samples were held in 2-liter bottles in the dark in an environmental chamber set at 0 to -1 ^{o}C for two of the experiments, and for two experiments samples were incubated both at 0 to -1 ^{o}C and at 5 to 6 ^{o}C in a second environmental chamber. Samples were taken every 1 to 3 days for analysis of chl-a and microzooplankton abundance and biomass. One other growth experiment was done in the on-deck incubator at a light level of 30 % of incident. Water for this experiment was collected at Site NP-7 when initial chl-a concentrations were low, about 0.4 ug l^{-1}. Individual 2-liter bottles were sampled over a 12 day period. Protist growth rates were determined using the logistic growth equation based on change in biomass of ciliates and heterotrophic dinoflagellates > 40 μm and < 40 μm in size, and in two cases by change in abundance of recognizable protist species which were at sufficiently high numbers in the samples.

Microzooplankton abundance, biomass and general community and size composition was determined in whole seawater samples after samples collected at sea were returned to the laboratory. From 25 to 50 ml of Lugol-preserved samples were settled for a minimum of 24 hours and then the whole slide inspected. Inverted microscopy combined with a computer digitizing system was used to identify and measure individual microzooplankton and to convert linear dimensions to cell volumes using equations appropriate for individual cell shapes (Roff and Hopcroft, 1986). Samples on slides preserved for epifluorescence microscopy were inspected using an Olympus BX61 Epifluorescence Microscope with a multi-wavelength filter set to determine whether dinoflagellates counted in Lugol-preserved samples were heterotrophic or autotrophic; only heterotrophic dinoflagellate morphotypes were included in the microzooplankton data. All ciliate and dinoflagellate cells in each sample were counted, sized, and categorized into the general taxonomic groups of choreotrichous ciliates, oligotrichous ciliates, didinid ciliates, tintinnids, athecate dinoflagellates, and thecate dinoflagellates. From 80 to 460 protist cells were counted and sized in each sample inspected. Cell biomass for dinoflagellates was estimated using the non-diatom algorithm of Menden-Deuer and Lessard (2000) and for ciliates was estimated using both the Menden-Deuer and Lessard algorithm and the 0.19 pgC μm^{-3} value of Putt and Stoecker (1989). Ratios of heterotrophic dinoflagellate biomass as a fraction of dinoflagellate + ciliate biomass, and of > 40 μm sized microzooplankton biomass as a fraction of total microzooplankton biomass were calculated using the 0.19 pgC μm^{-3} value for ciliates, which is likely more accurate.

Images of ciliates, heterotrophic dinoflagellates, and other protists observed in the spring 2008 Lugol preserved samples are posted on the Sherr Lab webpage at:

http://bioloc.coas.oregonstate.edu/SherrLab/Microplankton%20images.html

References

Menden-Deuer, S., Lessard, E.J., 2000. Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. Limnology and Oceanography 45, 569–579.

Putt, M., Stoecker, D.K., 1989. An experimentally determined carbon: volume ratio for marine ‘‘oligotrichous’’ ciliates from estuarine and coastal waters. Limnology and Oceanography 34, 1097–1103

Roff, J.C., Hopcroft, R.R., 1986. High precision microcomputer based measuring system for ecological research. Canadian Journal of Fisheries and Aquatic Sciences 43, 2044–2048.