In Situ Primary Productivity Protocol

A Description of Actual Procedures Used on the 1992 EqPac Survey and Time Series Cruises

Richard T. Barber

The conceptual basis for the anti-contamination procedures are, of course, the classic paper by Fitzwater, Knauer and Martin (1982), but other important comments on inhibition of phytoplankton are given in Chavez and Barber (1987), Price *et al.* (1986), Williams and Robertson (1989) and Marra and Heinemann (1987).

I. ^{14}C Solution

A. Anhydrous crystalline sodium carbonate is added to stock carbonate solution of 0.3 g carbonate in 1.0 liter of Nanopure water. The lot number of the NEN (New England Nuclear) $Na_2{}^{14}CO_3$ was 2653-074; the specific activity was 55.0 mCi/mmol. The designation number is NEC-088H.

B. The solution is made up and stored in Teflon containers that are cleaned as described below.

C. The ^{14}C solution is refrigerated, but allowed to come to room temperature before addition to the seawater.

D. The intended activity of the ^{14}C solution is 100 µCi/ml; however, in our procedure the activity added is measured for each profile, so variations in the initial activity are not a problem.

II. Cleaning

A. New bottles and labware are cleaned as follows: soak in a 2 % Micro solution for three days. Rinse three times with DI water, then soak overnight in DI water. Rinse again, then soak for two days in 0.5*N* HCl (Fisher trace metal grade). Rinse three times with Nanopure water.

B. After each use, the incubation bottles are soaked in a 10 % acid wash for 12 to 24 hours and then rinsed well (3 times) with Nanopure water.

C. Teflon stock bottles are cleaned by performing Micro and DI soaks as above. Then sequential three-day soakings are performed, filling first with 6 *N* HCl for three days, then with 2 *N* HNO_3 for three days, and then with the cleanest available 0.5 *N* HNO_3 for three days. Each filling should be followed with Nanopure rinses. Oven dry on a plastic tray at 65^{0} C.

D. Polyethylene or vinyl disposable gloves without talc are worn during Rosette handling and all other procedures.

III. Sampling

A. Eight light depths are sampled down to the 0.1 % I_0 light depth with custom made Go-Flo bottles on a ‘‘trace metal clean’’ General Oceanics rosette. The rosette was made by General Oceanics according to Moss Landing Marine Laboratory’s specification; it is called the EqPac ‘‘clean rosette.’’ The rosette was lowered on a Kevlar conducting hydroline with non-metal sheaves and a dedicated winch.

B. Samples were taken before dawn, usually at 3:00 to 4:00 am.

C. The 280 ml polycarbonate bottles are rinsed three times with sample water and filled. The water ‘‘fell’’ from the Go-Flo spout into the bottle without the use of a ‘‘filling tube’’. (McCarthy *et al* and Landry *et al.* always use a ‘‘filling tube’’ to reduce shear and turbulence that hurts microflagellates and ciliates. To avoid potential contamination, we do not use a ‘‘filling tube.’’)

D. Inoculation of 100 µl of ^{14}C solution is done in the radioactivity van with an Eppendorf disposable tip dispenser.

E. In addition to two bottles from each light depth a third bottle is taken from surface (‘‘100 % I_0 ’’) and the 8 % I_0 depth for the determination of time zero particulate ^{14}C counts. The 100 % and 8 % samples are inoculated and immediately filtered and treated identically to the incubated filters.

IV. Incubation

A. The two replicate bottles from each depth are placed in a nylon mesh bag and closed with polyethylene cable ties.

B. Each bag is attached to the polypropylene array line by ‘‘tuna’’ snaps that clip into rings that are spliced in the line at one-meter intervals.

C. The array line has a 50-lb lead weight at the bottom and two floats at the top.

D. A 20 m tag line connects the array line to the array spar buoy which has an aluminum radar reflector, 3M light reflector sheets, a reflective International Orange flag, a Novatech VHF transmitter and a Novatech xenon flasher. Batteries on the VHF and flasher are changed at each deployment.

E. The array is picked up after about 24 hours or about 4 am. The nylon bags are taken off and placed in a box as the array is recovered. The recovery takes about 20 to 30 minutes after the spar buoy is caught with grapnel hooks.

V. Filtration

A. Following retrieval of the nylon bags from the array line the bags and bottles are taken to the radioactivity van. One ml is taken from the 100 % and 8 % I_0 depth bottles and added to scintillation vials containing 1 ml of beta phenethylamine then 10 ml of Ecolume is added. The purpose of this procedure is to determine an added ^{14}C activity.

B. The samples are then filtered through Whatman GFF filters.

C. The filters are placed in scintillation vials and 0.5 ml of 0.5 *N* HCl added. The acidified filters are left for 24 hours in the hood.

D. 10 ml of Ecolume is then added and the vials capped and left for 24 hours.

VI. Counting and Calculations

A. The time zero, total activity and incubated samples are counted on the liquid scintillation counter with a wide window.

B. Carbon uptake for each light level is calculated as follows:

$$\text{Carbon} = ([DPM_{24} - DPM_0] \cdot 1.05 \cdot 24000)/DPM_{tot} \cdot \text{time})$$

DPM_{24} = CPM_{24}/efficiency of filters

DPM_0 = CPM_0/efficiency of filters

DPM_{tot} = (CPM_{tot}/efficiency tot) $\cdot$ bottle volume

1.05 = factor for preferential uptake of ^{12}C over ^{14}C

24000 = weight in mg/m^3 of the inorganic carbon in seawater

C. Carbon uptake for the water column down to the 1.0 % and 0.1 % light level is calculated using a trapezoidal integration. We note that each P.I. in JGOFS uses a different integration scheme. This is an area where some discussion might be useful.

Literature Cited

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