WATER COLUMN TIME SERIES PROTOCOL
Phytoplankton Primary Production – modified JGOFS ^{14}C method
Moorea Coral Reef LTER

Supplies: Almost all ^{14} C supplies are kept in a labeled gray box in the dry lab.
0.5N HCl
ß-Phenethylamine – prevents radio-labeled inorganic CO_2 from escaping to the atmosphere
Scintillation Cocktail – use ScintiSafe Econo 2 from Fisher – WATCH EXPIRATION DATE
Pipette tips, 25mm GF/F filters, glass beaker for cocktail, gloves, lab coat
Filtration rack, filtration bottles and trap for pump, tubing
Incubation bags of varying mesh, yellow floats, rubber bands, rope to go across incubation tank
Bubbler, acid, bubbling stone, and hoses for bubbling of filtrate

<u>Supplies to gather that are not in the box</u>:

Pipetters (1 each 10-100 µL, 100-1000 µL, 5 ml)
^{14}C working solution – approximately 80 µCi/ml in pH 9.5 H20 (in refrigerator)
Forceps, diapers, safety tray (in lower cupboard), vacuum pump, 3 buckets, dry waste box
250ml polycarbonate bottles for incubation - acid washed / rinsed (grey box above cupboard)
7 ml glass scintillation vials (in lower cupboard), labeled, in scintillation counter racks
Paper towels
Data sheet
Fill squeeze bottle from box with seawater (filtered if available)
Geiger Counter (in cupboard, must be returned to UCSB for calibration once per year)
*Geiger background is 50-60
Beckman LS 6500 Liquid Scintillation Counter

<u>Steps:</u>

1. Check with Biocode ahead to be sure it's ok to use their hood. Clean out the hood.
2. Turn on the Scintillation Counter (and printer) so it can warm up for a couple of days.
3. Label 7 ml scint vials for initial (rT) ^{14}C concentration (1A-T, 1B-T, 1D-T etc) and filters (1A, 1B, 1D etc). There will be 2 sets of vials for each sampling day (labeled 1-8 for OS and LTER1 sampling day, 1-9 for L1,F1,CB,CS sampling day)
4. Dispense 100 µL of phenethylamine into the rT samples in the hood. Cap and set aside. (This can be done day of sampling or day of processing. Do both 1T-8T and 1T-9T at same time).
5. **<u>Sampling</u>**:
   a. Collect samples (2 replicate light bottles, 1 dark bottle at each station and depth), rinsing each bottles 3 times before filling. Keep in cooler until returned to lab (within 3 hours)
   b. Inoculate by depth, not by number order (ie: 1's and 6's are both 1m samples). Add 100 µL ^{14}C working solution to each bottle (approximately 8 µCi per bottle)-put pipette in, discharge, lift while still holding down, put back in other side and pipette out. MIX and note time on data sheet **!!PIPETTE CAREFULLY!!**
   c. Place the bottles in the corresponding light bag in an outdoor tank with running seawater in full sunlight away from tank edges to avoid shading. Dark bottles go into dark cloth bags (rolled and closed with rubber bands) and are also placed in the outdoor tank in a mesh sampling bag weighted to sink them to the bottom (avoids heating). Incubate for 24hours. Note weather conditions throughout incubation. Check periodically that they are not touching.

Light levels for samples as follows:

Reef Samples (Lagoon 1, Fringe 1, Cooks Bay)

1 m- 100% light (turkey bags)
5 m - 58% light (bag of one layer grey screen)
10 m - 30% light (bag with 2 layers grey screen)
20 m- 15% light (bag with 3 layers grey screen)

Forereef (LTER 1) and Offshore

1 m- 100% (turkey bags)
5 m - 58% (bag of one layer grey screen)
10 m - 40% (1 layer grey, 2 layers nytex screen)
25 m – 24% (2 layers grey, 1 layer nytex)
75 m – 7% (4 layers of grey screens)

6. Processing:

After 24hours, remove the bottles from the tank and process as follows on the contamination tray in the hood. Lay diapers below everything, including floor, trays, and draped over railing.

Total radioactivity

1 – with a clean, new pipette tip, withdraw 100 µL from the sample bottle and add it to the scintillation vial containing the phenethylamine (rT) – draw and expel the volume twice to rinse tip before drawing the actual sample to go in the scintillation vial (use same tip for L and D bottles) – swirl the sample into the phenethylamine

2 – immediately add 5ml cocktail to the vial with a 5 ml pipette and cap.

3. When all 3 bottles have been sampled, SHAKE THE 3 VIALS VIGOROUSLY FOR AT LEAST 30sec.

4 – place the vials into the rack to be counted immediately (see counts procedure below).

BE SURE TO KEEP THE VIALS IN THE CORRECT ORDER LEFT TO RIGHT IN THE RACK WITH THE NUMBERS ON THE RACK FACING YOU

Filtration

*When filtering, keep an eye on the waste jar to prevent flooding the pump-AFTER 4 SETS IT WILL BE FULL!

1 - filter entire contents of each sample bottle on to a 25mm GF/F filter. Using a squirt bottle of filtered seawater (FSW), do 2 small rinses of the sample bottle to remove any trace ^{14}C from inside. When the filter is dry, rinse down sides of funnel with small amount of FSW, record time when first bottle is finished. Place the empty incubation bottle into a 5-gal bucket. Pump set no higher than 7 inches Hg on gage.

2 - Place each filter in a labeled glass 7ml scintillation vial. Fold, boat like, with edge touching bottom of vial-keep lids off until you have added HCl and cocktail. Inside the fume hood, pipette 0.250 mL 0.5N HCl onto each filter & let stand for several hours

3 – Then add 5 ml cocktail, cap and shake vigorously for at least 30seconds (or to 150 counts)

4 – When finished filtering, rinse filter rig with seawater or freshwater to clean out tubes

5 – count samples on the Beckman 6500 scintillation counter in the Gump lab using the **USER 2 program** (rack with User 2 card). Count rT's immediately; count filter samples several days later if possible (you can run both days/sites together later). Wipe each vial with a kimwipe before counting and make sure lids are tight

7. Clean-up

1. Fill a bucket half full with seawater outside in a good breeze, add 5 ml HCl, submerge all contaminated plasticware. Stand upwind. Let the bottles sit a few minutes, then rinse all plasticware with seawater and 3 rinses of bottled/nanopure water.

2. Contaminated filtrate- In the Hood, screw the lid with the 2 cm bore tubing onto the top of a filtering bottle and prop the end of the tubing into the outflow stack of the hood. Place the bubbling stone into the filter bottle through the hole near the top. Add 5 ml of HCl with a pipette (same blue pipette, new tip) and immediately seal the hole with the cork on the bubbling tube. Plug in the bubbler and bubble for <u>at least 1 hour</u> with the hood on. Dispose of <u>decontaminated</u> seawater down the drain.

3. Wipe down all surfaces with lightly acidified water, including the floor.

Wipe Tests- Wet GFC filters and rub each across about 1 square foot of wetted surface at 6-7 locations where the work occurred. Pay special attention to the floor and sink. Run the samples and keep a record of the results on the ^{14}C excel file on the server.

Counts:

Warm up the Beckman scintillation counter . (The ^{14}C standard is stored in the lower vegetable bin of the refrigerator in the LTER Lab if a recalibration is wanted. For the DPM program used, the manual does not recommend recalibration). For sample counting be sure the first rack of samples has the "User 2" card on it. Put all samples in racks and place the red HALT rack at end. Double check that all numbers are in order, and that cards on back of rack are inserted correctly so machine can read.

Press Main Menu
Review and Edit User Program
Select Alldredge.
Should look like this:
Count time: 20 min
Isotope: 14C
2 sig: 1
Bkg sub: 51
Factor: 1
Units: DPM (disintegrations per minute)
Turn on printer
Press Main Menu
Select "Automatic count"
Press Start
Be sure to carefully read the Scintillation manual so that you understand how this instrument works.
When printed sheet comes out, label with date and sites
Usually about 7100-8000 counts
Enter DPMs onto datasheet

Calculations:

DPM values are converted to daily productivity rates as follows:

Production (mg C/m^3/d)=((SDPM/V)*(W*0.25*10^{-3})/TDPM)*(1.05/T)

SDPM = DPMs in filtered sample
V = volume of filtered sample in liters
TDPM = total ^{14}C DPMs in 0.25ml aliquot

W =	DIC concentration in samples (~25000mg C/m^3)
$0.25*10^{-3}$ =	conversion of pipette volume to liters
1.05 =	correction for the lower uptake of ^{14}C compared to ^{12}C
T =	time in days

*Keep careful record of radioactive materials: concentration is higher than allowed, but amount is lower.

*Take back C14 samples every 2-3 trips…up to 2 flats on a trip