WATER COLUMN TIME SERIES PROTOCOL
CHLOROPHYLL SAMPLE PROCESSING AND ANALYSIS
Moorea Coral Reef LTER

Sample Processing

GET TOGETHER:

- 47 mm GF/F filters Chl filters are not muffled
- 5, 47 mm in-line polycarbonate filter holders with connecting vacuum tubing and an in-line valve on each side of the filter holder.
- Forceps
- Large funnel that fits into the mouth of the 4-liter carboys.
- 1000 ml graduated cylinder
- vacuum pump
- 20 liter glass vacuum bottle connected to a 4 liter filtration flask as a trap
- Labeled foil squares, folded in half
- Water samples in 4, 10, and 20 liter carboys
- 5 clothes pins

SAMPLE FILTERING:

Sample Preparation: Filter in the "Flume lab" where lines are already present for hanging the bottles.

- Place filter in filter holder with tweezers
- Screw filter holder together with the bleeder valve facing upward toward the sample bottle and the gasket on top of filter (on the bleeder valve side of the filter).
- Place 1000 ml of sample in a 4-liter bottle and hang the bottle so that the spigot slopes downward. (To fill the bottle with new sample, **vigorously shake the appropriate 10-liter or 20-liter carboy**, pour 1000 ml into the graduated cylinder and empty the cylinder into an empty 4-liter bottle using a funnel). It is best to filter the Chlorophyll for each station and depth right after the POC samples for that station and depth have been filtered because the bottles will not need to be prerinsed since they already contain water from the appropriate station.
- Connect a filter to each 4-liter sample bottle. Turn vacuum pump on; vacuum should be around 7 mm Hg. Loosen the cap on the 4-liter carboys to allow air to enter. Open the valves to each filter. Tip each filter holder and twist the bleeder valve to allow the filter holder to fill with water. Use pliers if necessary. Be careful not to lose water through the bleeder valve.
- Clothes pin the appropriately labeled foil squares to the counter top under each bottle. Check the whole system for leaks.
- Pump only until filter dries, do not maintain vacuum on dry filters. Turn the valve between the holder and the pump to disengage the vacuum. Be sure to empty the filter flask occasionally. DON'T GET SEA WATER IN THE PUMP!!
- Using forceps carefully fold each filter in half and place it inside the appropriately labeled foil square. Crimp the corners of the foil to seal the filter inside.
- Freeze the samples at -80°C and bring back to Santa Barbara on dry ice or -80°C ice for analysis. Store in the freezer in Santa Barbara.
- Two replicates are done of each sample.

LOG EVERYTHING:

Record ALL information on log sheets. Make a note on the log sheet if any problems were encountered during filtration; i.e. a torn filter, leaking funnel - ANYTHING. Fill out entire log sheet

– it may take a little time now, but it's worth it in the long run! Check your labeling. Be sure not to mix the POC and Chlorophyll samples.

CLEAN EVERYTHING:

Rinse all sample bottles 3X with bottled water. NEVER USE UNFILTERED STATION WATER
Rinse all filters 3 times with bottled or Nanopure water.
If this is the last that the bottles will be used for some months, acid wash them and allow the acid to sit for several hours. Then rinse 3-4 times with filtered station water and 3 more times with bottled water.

<u>CHLOROPHYLL SAMPLE ANALYSIS</u>: Turner TD10-AU in Brzezinski Lab of MSI Analytical Lab

Sample preparation:

1. Remove the filters from the freezer and place each individually in labeled 15 ml falcon tube. Fold filter to ¼ with chlorophyll side out. Samples may be stored up to 3 months.
2. Add 10 ml of 90% Acetone to each tube, shake vigorously and store in the freezer in the dark for 24-48 hours, shaking a couple times more over that time.
3. Be sure to arrange use of the fluorometer with Janice Jones (893-7061) or the MSI Analytical Lab a few days ahead.

In the Brzezinski or Analytical Lab:

Ensure that lights are turned off and shades drawn to avoid degrading the Chl

1. Put about 1.5" of room temperature water in the square white styrofoam cooler
   Place vials in cooler ~30 minutes prior to analysis to allow samples to reach room temperature
2. Centrifuge the falcon tubes for 5-8 minutes at maximum speed. Use the 3 outer spots (inner ones make the tubes tip), full speed. Then put them in the racks inside the cooler to let the temperature equilibrate (approx. 30 minutes). Keep the lid on the cooler to keep samples in the dark.
3. The fluorometer should be left on at all times, but if it has been turned off, let it warm up for 30 minutes prior to use

Avoid calibration screen and avoid pressing the * button!

Ent = Main Menu, HOME = Data Display
Check Fluorometer settings:
Pre-average delay (Ent, 1, 6, 3, 10 sec.) 10seconds
Averaging period (Ent, 1, 6, 3, 15 sec.) 15seconds
Blank subtract (Ent, 2, 1, 2, YES YES)
Autorange (Ent, 2, 4, 3, AUTO) YES

Run Solid Standard:

Record values for both L and H (rotate solid standard) on the Analytical Lab Log found in the fluorometer toolbox

BLANK:

4. If you forgot to prepare a filter blank during filtration, SHAME ON YOU! (preparing the blank during filtration is desirable because it ensures that the blank undergoes the same treatment as the sample) You can make one now by placing a filter into a scintillation vial and adding 10ml of 90% acetone, shake until filter becomes transparent and put it in the cooler with the samples.

5. Blank the fluorometer using a 90% acetone blank (without filter) – fill a borosilicate glass tube with 90% acetone and cover tightly with parafilm. Wipe down the tube with a kimwipe to remove all fingerprints and smudges, place the tube in sample chamber on fluorometer and cap.

Blank as follows:
from screen 2.0 (Calibration)
press <1> to access screen 2.1 (Blanking) then
press <1> to bring up 2.11 (Run Blank)
insert 90% acetone blank
press <0>when the reading is stable and the time count (TC) is stable at 8 seconds. (value should be 0).

The value of the blank has now been entered

6. Note the %value in the comments column on the log sheet. The fluorescence value of the blank can be read on screen <3.2> (Diagnostic Info) or HOME screen.

To obtain value for blank:
access screen 2.12 (Subtract Blank)and choose NOT to subtract blank
go to HOME screen and press <*> to start average
record value of blank in the Rb column
return to 2.12 and choose YES to subtract blank
Place acetone blank in correct orientation (so that you can return it to the fluorometer facing the same way) in the cooler while running the samples – the blank will be re-read at the end of sampling

FLUORESCENCE READINGS:

7. Decant a centrifuged sample from its falcon tube into a glass test tube being careful to leave any particulate matter in the vial. Wipe the tube with a kimwipe to ensure there are no fingerprints or smudges
   Fill vial about 3/4 full.
8. Return the fluorometer display to the HOME screen
9. Insert sample

if >999 , it won't read, go to 3.2
when <999, press <*> to start pre-average delay. The beep gives the average
if 3.2 doesn't allow reading, you need to dilute: 5ml sample, add 5ml 90% acetone
note dilution on log sheet – record volumes used for dilution

Wait until time constant (TC) is 8 seconds.
Janice tip: If reading is still fluctuating after reaching TC=8, the test tube is probably blurry. Take out the sample and kimwipe. If you wait for it to stabilize, sample can heat up and volatilize.

10. read fluorometer readout (Rb) and write scale (range) and reading in log
11. add 2 drops 10% HCl and invert to mix. Take Ra reading (approx. 0.5 of Rb), write scale and reading in log
12. Pour dregs into waste container; place the falcon tube in the trash and test tube in glass waste box.

Ra measures phaophytes and should be about ½ of the chlorophyll.

FINAL BLANK:

13. When all samples have been run, place the tube containing the acetone blank back into the fluorometer in its proper orientation and re-read the blank value as follows:

Access screen 2.12 and choose NOT to subtract blank
Go to HOME screen and press <*> to start sample averaging
Record value of blank in the Ra column
Return to 2.12 and choose YES to subtract blank

Log Book Example:

Sample #	**Range**	**Rb**	**Range**	**Ra (Acid)**
449	Med	88.0		52.6
450	Med	88.5		53.4
--- *Line breaks between 10's*	---	---		---
451, etc…				