Primary Productivity Protocol

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CALCOFI PRIMARY PRODUCTIVITY PROTOCOL
(H:\word\instruct\ANALYTICAL\Prd_inst_2012SH.doc 2/2012)

PRE-CRUISE PREPARATIONS

1. Check well in advance of cruise to be sure there is enough isotope ready to be used. This amount may vary depending on specific activity desired. Also, check scintillation fluid and all other supplies on the supply list.
2. Be sure a copy of this protocol and a "Request for Isotope usage on SIO Vessels" (see sample forms section at the end of this Protocol) have been sent to the ship scheduling office. These items should be submitted three months in advance if possible. Submit these also for cruises NOAA vessel just as though it were an SIO vessel to extend licensing to the vessel.
3. Incubator tubes should be cleaned and calibrated prior to each cruise. After the Incubator tubes are calibrated, a new "desired depth" sheet must be prepared using the new calibration values. The new calibration values must also be entered into the PIC module of CODES for determining sampling depths at sea.
4. Polycarbonate bottles used for incubations should be cleaned with MICRO and acid rinsed prior to each cruise.
5. Rosette bottles used for Productivity sample collection must be MICRO cleaned and acid rinsed prior to each cruise.
6. Make a set of stick on labels scintiallation vials with sequential numbers for use on the cruise. Each CalCOFI productivity experiment uses 18 vials; typical cruises have 15 or 16 experiments.

ON SHIP PREPARATIONS

7. While setting up aboard ship make sure the Van drain hose is tightly connected. Check to be sure it does not leak and has unimpeded flow. The end of this hose should extend over the side and slightly below the sea surface. Make sure there is no chance of leakage from this line onto the ship deck. This hose should be clearly labeled as Van waste, and reserved for use only as a Van drain.
8. Check to be sure the drain valves beneath the sink are set initially so the sink will drain overboard. Be sure you understand how the valves are to be set for overboard draining of the sink.
9. Check to be sure there are seven liquid waste jug/vacuum traps empty and ready for use. One should be mounted at the left side of the radioactive filtration basin and connected to the vacuum pump system, ready to go for filtrations. Six should be in the waste containment tray on the floor below the counter.

DAILY AT SEA OPERATIONS

1. Early in the day (or late the previous day) figure out which station will be the productivity station and calculate Local Apparent Noon (LAN).
2. Prior to the CTD cast determine the Secchi depth. During the CTD down cast use the PIC module of the Codes program to integrate the productivity depths (or if necessary "Productivity Cast Worksheet" as set by the Secchi depth, into the hydrographic cast depths, as set by the hydrographic parameters observed on the down cast. If necessary add extra depths to the cast to preserve close spacing where needed for the hydrographic parameters. Be conservative, it is better to over sample than to leave gaps. Begin filling out the "Primary Productivity Data" sheet.
3. Ideally the CTD cast should be timed so the samples can be drawn, inoculated almost immediately, and placed in the incubators at LAN. Ideal timing is seldom possible. The CTD cast may be done early, with the CTD coming back on deck as much as 1.5 hours prior to LAN. If the cast is done early, the samples should be set aside in a cool dark place until they are inoculated. Make every effort possible to have the samples in the incubator by LAN +/- 15 minutes. In case of disasters and delays, samples may go into the incubator as late as LAN + 1 hour. If delays beyond LAN + 1 hour are encountered the experiment may be cancelled.
4. Productivity samples are drawn into the numbered set of 280 ml Polycarbonate bottles, two clear bottles and one dark bottle at each productivity sample depth. Productivity samples are normally drawn after all the other samples. If time is running short, they may be drawn immediately following the oxygen samples. While drawing samples, use the dark bottle sleeves and try as much as possible to shield the samples from light.
5. Samples are inoculated with 0.2 ml of C14 stock (approximately 5-20 micro Curies, depending on the precise concentration of the stock) using a repeating pipette fitted with a small diameter extension tip. The first squirt from the

Eppendorf pipette is not accurate, so it must be squirted back into the ampoule. Inoculations must be carried out in subdued light, using the "night lights" and the hood light in the Van. Prior to the inoculation procedure a drain plug will be placed in sink in the event of a spill to prevent isotope being carried downstream into the ocean to allow time for isotope to be treated with acid cleaning solution. All inoculations must be carried out in the containment basin area of the Van. Gloves must be worn for this operation. Absorbent paper need not be placed in the containment basin while adding the isotope because the basin is designed so it can be flushed directly into the sink. The leftover isotope must be flushed into the liquid waste jug/vacuum trap to be disposed of later along with the filtrates from the experiment. Pipette tips must be rinsed at least three times, with the rinses drained into the liquid waste jug/vacuum trap. Use one of the 1st filter funnel as a miniature sink for this rinsing. The pipette tips and ampoule parts should then be treated as radioactive trash, and should be put in the plastic "sharps" container provided. The inoculation must be totally finished and the incubation bottles tightly capped before removing the sample box from the containment basin. Do not open the sample bottles for any reason outside of the Isotope Van.

6. Set up the filtration area prior to end of incubation time. Load the filtration funnels with new 25mm Millipore type HA filters. Be sure to note the space left in the waste jug/vacuum trap. We need to fill these jugs ¾ fill line, avoid sucking filtrate into the secondary trap. Three days per jug, this requires careful attention on the part of the technician.
7. Samples should be removed from the incubator at Civil Twilight (CT) +/- 15 minutes. The samples should then be filtered immediately, using 0.45 micron type HA Millipore filters. Filtrations should be carried out under subdued light. Gloves must be worn while filtering and during cleanup following filtration. Do not open the sample bottles until they are over the containment basin. Pour samples into respective filter funnels, ~140 mls at a time. A 1ml subsample is required from the dark bottle to determine the exact activity added to each bottle. To this an equivalent 200ul of ethanolamine has been added to the Ecoscint to keep the 14C in solution. Assign a number series and note the appropriate number in the comments section.
8. Give both the sample bottles and the filter funnels two small rinses (about 20ml) with seawater drawn from a deep Niskin bottle to rinse down any phytoplankton and isotope clinging to the sides. Collect these rinses on the filter and in the liquid waste jug/vacuum trap. While the samples are filtering rinse out the empty productivity sample box with 10% HCl. The sample box rinse can go down the sink. After these rinses put the sample bottles in the sink for further rinsing.
9. Sample filters should then be removed and placed in a LSC vial. Add 0.5 ml of 10% HCl to each filter. Be sure the filter lies flat in the vial and is covered with acid. The vials are then allowed to sit uncapped overnight inside the fume hood. After the filters are removed, put the filter funnels in the sink for further rinsing.
10. Be sure the "Primary Productivity Data" sheet has been completely filled out.
11. Rinse all the Polycarbonate sample bottles, caps, and filter funnels at least three times with large volumes of seawater. No matter what, do not rinse with ships fresh water due to metals contamination. Make all items ready for the next days work. These rinses can be allowed to drain directly over the side. Finally, give each bottle a 10% HCL rinse. At the end of cruise, rinse HCl out of the bottles to prevent it from being put in the waste jug with the Micro rinse in Sverdrup hall.
12. The radioactive filtrate and rinse water in the waste jug/vacuum trap must be retained and returned to EH&S at the end of the cruise. Fill out the "Waste Collection Log" on a daily basis. Label waste accordingly. For logging purposes the "Liquid waste collected" at sea is estimated to be the total activity contained by the vial, minus 0.1 percent estimated to be taken up by the phytoplankton on the filters.
13. Next morning add 10 ml of Liquid Scintillation Cocktail (currently MP Biomedical Ecolume) to each vial and cap the vials firmly and shake them vigorously.

WEEKLY WIPES

1. Wipe test the van. Floor, bench and sink is sufficient for weekly tests. Diagram the wipes on a "Radioisotope Survey and Monitoring Form". Date the wipes accordingly. Required by Ships Operation/Radiation Safety.

END OF CRUISE

1. Rinse incubator tubes and manifolds with fresh water. Allow the tubes to air-dry and repack them in their bags. Disconnect and pack plumbing and manifolds for storage ashore.
2. While still at sea, thoroughly clean the Van. Wash the bench tops and floor, with a final wipe using dilute HCl. Rinse the filtration manifold with fresh water. Do these cleaning operations before the wipe tests.
3. Wipe test the van. Diagram the wipes on a "Radioisotope Survey and Monitoring Form".

Complete the "CalCOFI Isotope Balance Sheet" for the cruise and the "Shipboard Radioisotope Usage Form". The "Shipboard Radioisotope Usage Form" and "Radioisotope Survey and Monitoring Form" must be signed by the P.I. and Chief Scientist. The "usage" form and the "survey" form must be forwarded to the Ship Scheduling office, with copies to E.H. & S. Be sure also to keep copies of all these completed forms in the CalCOFI isotope record notebook.

4. Before the Van is removed from the ship, be sure the drain hose is removed and properly stowed, and that the drain is covered to prevent it from dripping.

HANDLING WASTE

1. Keep a careful watch on the level of filtrate in the liquid waste jug/vacuum trap. When it is filled to the full line, a new jug must be put in place. On a normal CalCOFI cruise it is convenient to switch the liquid waste jug/vacuum trap after three stations, rather than filling it to the maximum. A typical cruise will have about 15 productivity stations, using 5 jugs. Put the new jug in the filtration containment basin, and carefully move the trap top from the old jug to the new jug, taking care not to dribble fluid around. Cap the jug tightly with the cap from the new jug. Immediately fill out and attach a radioactive waste tag. Carefully lift the filled jug from the filtration containment basin and place it in the containment tray on the floor beneath the counter.

2. Prior to the cruise seven vacuum trap/waste, jugs should be prepared.

3. Do not remove the liquid waste jug/vacuum traps from the isotope van while the van is on the ship. Immediately following the cruise, contact EH&S to arrange for pick-up of the waste jugs. They will come to MARFAC to pick them up directly from the van.

GENERAL PRECAUTIONS

1. On board UNOLS vessels involved in natural abundance isotope work shoe covers must be worn, or a pair of shoes may be left in the Van to be worn only while working in the Van.

2. Items from inside the van such as mops, sponges, brooms, chairs, etc., must not be moved from the Van for use in other parts of the ship.

3. If a spill should occur, the liquids sponged up from the floor should be put into a liquid waste jug/vacuum trap using a large funnel. All mop-squeezings from any mid-cruise or end-of-cruise cleaning of the Van floor should also be put into a liquid waste jug/vacuum trap.

4. The fan mounted in the Van is designed to ventilate fumes out. Both acetone vapors and C14 labeled CO_2 are vented by the fan. It should be left on throughout the cruise.

5. All handling of open isotope containers should be done over the containment basin in the Van. This should be clear from the protocol above. There is no reason to have an open isotope container anywhere other than over the containment basin at any time during our procedures.

6. The Van must be clearly labeled for Isotope isolation. Be sure all scientific personnel are aware that access to the Van is restricted to authorized personnel only.