NCOG Sampling Method

Parent Category: References (/references.html)
Category: Methods (/about-calcofi/methods.html)
Last Updated: 07 October 2015

NCOG Sampling For DNA and RNA Samples for NCOG Project (http://www.calcofi.org/field-work/bottle-sampling/ncog-project.html)

Sample **all** Prodo (~ 16 stations) and Cardinal Stations (10 stations)

Cardinal Stations = Line 90 (120.0, 90.0, 70.0, 53.0, 37.0); 82.47 (Santa Barbara Basin); Line 80 (55.0, 70.0, 80.0, 100.0)

Prodo and Cardinal Stations may overlap: Prodo stations = consistent time; Cardinal Stations = consistent stations

Sample Depths (typically 4 depths)

- Sample 10m **(DNA & RNA sample)** RNA filter 1 to 8 L mixed layer/surface
- Chl max depth **(DNA & RNA sample)** RNA filter 1 to 8 L
- 170 m **(RNA sample only)** RNA filter 6 to 8 L DIC are typically sampled here
- 515 m **(RNA sample only)** RNA filter 6 to 8 L DIC are typically sampled here
- 3500 m **(RNA sample only)** RNA filter 8 to 10 L *would really like this sample if available

Shallow Station Depth Sampling

- Bottom Bottle Depth **Less than 515m -** sample 10m, Chl max depth and 170m (3 depths)
- Bottom Bottle Depth **Less than 170m -** sample 10m, Chl max depth (2 depths)
- Bottom Bottle Depth **Less than 170m** and the Chl max is around 10m **-** sample 10m (1 depth)

Water Budget

If bottles available trip a duplicate bottle at 10m and Chl max depth

If LTER is getting a duplicate 10m, there may need to be 3 bottles tripped at this depth. (First bottle for CalCOFI, second bottle for LTER, and third bottle for NCOG RNA)

If 2 DIC samples need to be drawn from one of the NCOG depth, you may need an additional bottle tripped to have enough water for the NCOG sample.

DNA Sampling (10m and Chl max only)

***LTER and volunteers will take care of the labeling and filtering**

Sampled exactly like the POM samples using the same volumes (0.5, 1.04 or 2.2 Liter Bottle)

Volume depends on the amount of Chlorophyll in the water

*May filter smaller volume if water budget is tight or may not filter at all

*Sample from whatever bottle has water available from that depth

Brown sample bottles – Label D(nisken bottle number) eg D18 or D23

Use combusted GF/F, combusted foil and place the two samples into one cryovial

Label foil D(niskin bottle number)

Label Cryovial CC(Cruise) D(cast number), Station, Bottles and Volumes

Example CC1511OC D001

93.26.7

B 6, 9 .5,1.04L

Store in Liquid Nitrogen in the POM section and label Cryocanes D1, D2, ect…

RNA sampling (4 depths)

*Always keep the filter and samples in the dark, do not leave sample unfiltered for more time than absolutely necessary and record any time sample left unfiltered greater than 30 min. on log sheet.

*Make sure sample comes from the extra bottle

**Change out all 4 of the masterflex tubing in the pump head before cruise and approximately halfway through the cruise

-For high Chlorophyll depths (shallow/chl max) may need only ½ full bottle (less than 4 L) but if water is available draw 8 L and filter until desired color change on filter.

-For low Chlorophyll depth (170, 515, 3500m) try to get as much sample as available, ideally 8 L

Label color coded sample bottle - write the nisken bottle number on the color tape

Collect water, set up and **label sterivex filters**: Cruise, Station, Cast and Bottle

Place sterivex filter back into plastic if filters are labeled early

Example: CC1511OC

93.26.7

C 1 B 6

Organize samples and filtrate bottles

Rotate and secure tubing in pump head and close latches (allows for even wear on tubing)

Place one end of the tubing into the sample and the opposite end with the filter into the filtrate bottles

Start pump, make sure the pump is flowing in the correct direction and check for leak in the tubing or the sterivex filter

Record time in PST for start and end of filtration

*Stop filtering after 30 minutes or when some color is visible on the filter

Pump speed - 330 rpms

Detach sterivex filter from tubing and remove excess water from the filter

- use a 10 ml syringe 3 to 4 times to push any excess water
- flick the filter a few times to get out any excess water
- plug both ends with putty

Place the 10m and Chl max together in on foil pack and the 170m and 515m together in another foil pack

Label Foil: Cruise, Station, Cast and Bottle Numbers

Example: CC1511OC

93.26.7

C 1 B 6 & 9

Place samples in Liquid Nitrogen. Use center cryo-cane for flash freezing samples and then move samples to proper location in the dewar. Best to make foil pack a little large so when you place in cryo-cane the sample does not float out

Measure filtrate with a graduated cylinder and record volume of filtrate for each depth

Cleanup

Pump about 300ml of milli-Q water through the tubing, shake out any excess and rinse the outside of the tubing with the rest of the water in the bottle, wrap tubing up in a Ziploc bag to keep clean throughout the cruise.

Turn pump off and wipe off any excess salt water on pump head

Open latches and allow slack in tubing located in the pump head (tubing will last longer)

Post cruise

Scan data sheets and email to all points of contact; samples will remain in LTER dewar and LTER will coordinate pickup