Protocol for L4 sampling:Denise Cummings

Set up and labelling:

Set up filtration rig for SPM filtering (47mm frits) and glass filer holders. (When Jim gets the time he is going to lathe out new parts to make things a bit easier)

Set up glassware for DOC sampling (wearing gloves)

Label air bag for methane isotope collection and connect up pump

Set peristaltic pump for DNA extraction (on bench on way to engine room)

Label 3 slides for SPM

Label to small clean glass vials and lids for DOC

Label cryotubes for chlorophyll, 3 x 0m, 3 x 10m

Label 2 slides for CHN (3 filters into each)

Label alkalinity bottles 0m and 50m, silicone grease stopper

Label 4 brown cryovials, 2 at surface, 10m, 25m, 50m at L4

Label 4 white circular holders for PABs, 0m, 10m, 25m, 50m

Assess water requirements and decide on depths, write this in the CTD log book in the wheelhouse

At present it is first cast all surface water
Second cast 1,2=50m, 3,4=25m, 5-8=10m, 9-12=0m

Rough order of sample collection:

As soon as on site take airbag up to bow and fill sample approx 3/4 full.

Fill two of the carbuoys from large cool box with seawater from the hose, use these for SPMs, filter 3 x 4L

Fill another carbouy for DNA extraction, take this into alleyway before engine room with empty carbuoy and get the peristaltic pump going.

Get surface bucket of water and take temperature, record this and secchi depth.

Record zooplankton bottle numbers eg. 11/39 and 11/40

Record lugols and formalin bottle number eg. 9719

Once first cast is up (all surface) use the tubing and wearing gloves fill up Stephs 2 x 20L carbuoys (uses 4 CTD bottles)

1 CTD bottle for Rachel Beales VOC's (4 brown bottles) rinse and then overflow before adding stopper

1CTD bottle for DOC sample, rinse a few times and then fill.

1CTD bottle for my alkalinity, rinse first and then let overflow 3 times, if its not too rough then wearing safety glasses add 100ul mercuric chloride, if rough then wait until back at bench safely. Add stopper and invert.

1CTD bottle for Andy/Ian 3 x glass bottles for N2O and CH4 rinse first and then let overflow 3 times, if its not too rough then wearing safety glasses add 250ul mercuric chloride, if rough then wait until back at bench safely. Add stopper and invert, put on plastic clip to hold stopper on.

1 CTD bottle for nutrients, rinse 3 times and then fill.

1 CTD bottle for Glens flow cytometer samples. He has 12 clear plastic bottles labelled A1, A2, A3 = 0m, B1, B2, B3 = 10m, C1, C2, C3 = 25m, D1, D2, D3, = 50m Rinse bottle and then slowly fill with tube at bottom of bottle.

On the second depth cast take VOC's first, 2 bottles each at 10m, 25m and 50m

Take my alkalinity at 50m

Flow cytometer sample at 10m, 25, and 50m

CDOM bottles (brown glass in large coolbox) at 10m, 25m, 50m, rinse and fill.

Fill carbouys labelled L4, 10m, 25m, 50m

Fill my carbouy with tap with 10m water

Fill my small plastic bottle with surface CTD water

DOC: Filter onto ashed 47mm using the clean glassware, pour some of this into Duran bottle labelled filtered water to rinse it and then pour in the rest. Add 10ul of 50% HCl into DOC bottles and then pipette in 10ml into each. Clean all glassware with Mq and pack safely into the DOC coolbox. These samples are frozen upright to avoid contamination with plastic lid. These are put into freezer in the hold at end of trip. When there are enough samples they go to Alan Tappin at Plymouth University for analysis.

SPM's: Mix the water and filter 4 litres (or known amount) onto pre-weighed ashed 47mm filters, then rinse holder twice with Mq water(takes away salt deposits), take off holder and using finer Mq spray rinse into the centre of filter. Put filter into appropriately numbered slide and back at the lab these are dried in oven in seawater hall before weighing them and ashing and reweighing.

Set up filtration rig for 25mm filtration.

Pigments: Onto normal GFFs filter 1L , 2 x 0m, 1 x 10m, 1 x 25m, 1 x 50m, these are stored in small liquid nitrogen dewar on boat and put into large dewars back at the lab.

PAB's: Onto normal GFFs filter 1L , 1 x 0m, 1 x 10m, 1 x 25m, 1 x 50m, these are stored in small liquid nitrogen dewar on boat and put into large dewars back at the lab.

Fluorometric Chlorophyll: Onto normal GFF, 100ml x 3 at 0m, 100ml x 3 at 10m, put into liquid nitrogen and back at lab put into freezer in basement

CHN: Onto ashed GFF 200um meshed prescreened water, 6 x 250ml, put 3 into one slide and 3 into other, back at lab put into 60 C oven in basement

My alkalinity samples stay in their box on the boat until the box is full then I send them to NOC for analysis. (David Hydes, Sue Hartman).

Air Sample stays in box in my office until I have enough to send to Royal Holloway for analysis. (Dr Rebecca Fisher).

DNA: 2 x 5L are sampled and put into labelled bag and into liquid nitrogen, back at the lab they go into -80 C freezer

Fill lugols and formalin bottle with 10m water.

Salinity: First week of the month take samples for salinity. (Clear bottles under bench labelled P1-P30.) Unscrew cap and take out plastic insert, rinse and fill to the neck, mark in book the depth taken and corresponding bottle number. I will analyse these when I have 10 or more full bottles.

Whilst Victor is away: First thing before going to sea take vials with glutaraldehyde and ice packs from walk in freezer and take to boat. Add 2.95ml of each depth from the flow cytometer bottles (12 in all), keep cool and back at lab put samples in the fridge next to flow cytometer room114.