LBNL Sampling Protocol
R/V Brooks McCall
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Water Sampling

The CTD rosette on the Brooks McCall contains eleven 4L niskin bottles. Two niskin bottles were used to sample five different locations along the depth profile. The depths vary depending on the depth to sea floor and the presence and location of the plume determined by fluorometry readings. Once the CTD has collected samples and is on deck, each niskin bottle is subsampled for each research team. Our team is collecting nine liters of sample from three niskin bottles, one below the plume, at the plume and above the plume.

* With assistance of crew, we used a bucket to collect surface water. If the surface water sample was contaminated with oil, we collected a few hundred mL of water and filtered it and placed any large oil globules into microcentrifuge tubes.
Avoid locations that may be affected by ballast water

Samples are immediately taken to the laboratory workstation for processing

INT respiration test

- Add 2 ml sample to INT vial/solution (sterile 50mL screw cap test tube containing 1mL INT solution)
- Seal tube with cap, place in the dark, incubate 2 h (set timer)
- Add 300uL formaldehyde solution
- Store at 4°C. STOP here. Samples will be extracted back at LBNL

AODC counts

- Add formaldehyde 1 to 10 to sample (in a 15 ml Falcon tube: 1mL formaldehyde and 9mL sample).
- Store at 4°C until microscopy

Chemistry

- *Stable Isotopes/PLFA*
- Approximately 125 mL sample sterivex syringe filtered (0.2 um)
- Injected into an evacuated 125 mL serum vial with a 60 mL syringe, any head space pulled out with second syringe
- Once vial is full continue filling syringe until 700ml total of sample passes through sterivex filter. Store in whirl pack bag for PLFA analysis
- Store at 4°C

VOA analysis

- Fill VOA vial half way with sample, add 20 uL of half concentrated HCl. Then fill completely eliminating air bubbles once cap is screwed on.
- Stored at 4°C

Nutrient chemistry

- A 100 mL subsample saved in a 125 mL HDPE bottle for nutrient chemistry
- Stored at -20°C

Omics

Three subsamples simultaneously filtered on filtering manifold and Super 0.2 uM filters

- Attach 3 Nalgene water filters to the filtering manifold. Make sure to use vacuum grease.
- Check that tubing is secure and properly attached to the waste bottle. Also check nobs on individual filters and make sure pump is turned on (the clear plastic tubing is in case waste bottle overflows)
- Pour sample into filters (keep track of how many rounds)

- DNA filter (genomics/PLFA) (800mL)
- RNA filter (transcriptomics) (800mL)
- Proteomics filter (approx 1.1L)

- Use sterile disposable tweezers used to remove filters
- Place DNA filter into Whirl-Pak bag
- Place RNA filter in 15 mL Falcon tube. Add 13 mL RNA later using serological pipette
- Place Protein filter into Whirl-Pak bag
- Freeze immediately in freezer