

C-MORE August 2007 cruise objectives and logistics

GENERAL CRUISE OBJECTIVES

The primary goal this year will be the characterization of the microbial assemblage and biogeochemical fluxes associated to summer increases in cyanobacterial biomass in the vicinity of Station ALOHA. This characterization will be compared to a sampling site where no biomass increase is detected. In addition, we will try to establish transects across a bloom region, or try to sample distinct areas where blooms are detected from remote sensing and SeaGliders, to assess the spatial heterogeneity of these blooms.

GENERAL CRUISE PLAN:

August 8th: Loading day

August 9th, 8:00 Departure from Snug.

1st scenario: If a bloom is remotely detected within 100km of Station ALOHA

August 9th to August 10th at 5AM: Transit to the bloom station

August 10th to August 13th in the morning: Sample and carry experiments within the bloom (considers the deployment of sediment traps for at least 72 hours on August 10th and carrying on deck incubation time series for 5 days [August 15th])

August 13th noon to August 14th evening: Series of stations to characterize the spatial heterogeneity of the bloom.

August 14th evening to August 15th 5AM: Transit toward Station ALOHA or a site within 100km of this site not displaying high accumulation of chlorophyll in surface waters.

August 15th to August 18th in the morning: Sample and carry experiments outside the bloom.

August 19th is left as a buffer and could be used to revisit the sampling site.

August 20th early morning - noon: start transit back to Honolulu.

2nd scenario: If blooms are not detected in the vicinity of Station ALOHA:

August 9th to August 10th at 5AM: Transit to 24N, 158W where increase sea surface chlorophyll concentration was observed on July 18 to 28. This location could change once we have developed the full MODIS chlorophyll statistics for the month of July for the study region.

We will use these statistics to assess the station that has had the largest change in chlorophyll concentration as well as the station that has not seen significant chlorophyll fluctuation within 100 to 200km radius north of Station ALOHA. These will represent our primary sampling sites, replacing the bloom and non bloom sites in the 1st scenario.

As in the first scenario, we will devote August 13th and August 14th to assess the spatial heterogeneity of the sampling region.

3rd scenario: A bloom develops during the cruise. We will modify the cruise plan accordingly in order to characterize the bloom evolution.

SPECIFIC OBJECTIVES PER RESEARCH TEAM:

MIT – DeLong/Chisholm

We are interested in tracking the bloom for microbial community expression analyses, if we catch it. If not, we can do the same for perturbation if that works. We also are seeing some really interesting gene expression at the chlorophyll max with respect to nitrification, and hope to do some shipboard incubations along these lines.

We will also work closely with Penny's group on this.

We are flexible and hope to give the expression analyses a real shakedown in the course of this cruise.

MIT- Boyle

Characterize the spatial trace-metal distribution within the bloom and non-bloom regions

UH- Rappé

We will analyze the structure of the microbial community throughout the course of this cruise using both community fingerprinting, cloning and sequencing, and fluorescence in situ hybridization techniques. We will concentrate these efforts on characterizing the 16S rRNA gene diversity in order to get a view of the microbial heterotrophic fraction of the ecosystem. We will also attempt some enrichment and isolation experiments in order to capture isolates for future laboratory experimentation.

UH-Bidigare

We will characterize

- 1) phytoplankton community structure through pigment analyses and
- 2) the distribution of cyanobacterial toxin BMAA.

Also, we will collaborate with Edwin Cruz Rivera in potential grazing experiments.

UCSC- Zehr

Our objectives are

- 1) Determine the distribution of major groups of diazotrophs and nitrate-utilizing cyanobacteria using flow cytometry and molecular techniques
- 2) Attempt cultivation of major groups of diazotrophs, including heterotrophs and diatoms
- 3) Examine the diel cycle of DNA compaction in unicellular diazotrophs
- 4) Perform N15 nanoSIMS experiments to demonstrate N fixation by uncultivated unicellular cyanobacteria, and demonstrate photosynthesis with C13
- 5) Collect metagenomic/metatranscriptomic samples for diazotrophic populations

WHOI-Repeta

Characterize the effect of blooms in high and low molecular weight dissolved organic matter

MBARI-Kolber

Test three on-line systems aimed at characterizing the spatial variability of photosynthetic activity in surface waters. 2 of the systems will remain in the KM.

OSU-Letelier/White

1. Characterization of the inherent and apparent optical properties inside and outside of cyanobacterial blooms. To this endeavor, we have an 'cyanocage' consisting of an ac-9, hyperspectral ac-s, seabird CTD, and phycoerythrin, CDOM and chl-a fluorometers.
2. Daily deployment of a PRR or HyperPro II for characterization of the ambient light field.
3. Collect samples for determination of the C/N/P ratio of particulate matter and the intracellular fractionation of particulate phosphorus.
4. Conduct surface net tows to concentrate the >64 um size-fraction of POM. These samples will be used to assess the size distribution of Trichodesmium colonies, their photosynthetic activity, as well as isolate Trichodesmium biomass for C/N/P analysis. surface waters as well as Determine the distribution of major groups of diazotrophs and nitrate utilizing cyanobacteria using molecular techniques
5. Organize the deployment of 24-hr free-floating gas arrays. We will conduct paired ¹³C and ¹⁵N incubations in 4-L PC bottles (light and dark) for measurements of whole-water N and C fixation. Water for these arrays as well as time-zero POM will be procured from the rosette in pre-dawn sampling.
6. Coordinate on-deck nutrient enrichment incubation experiments.

Logistic Requirements

MIT-Delong

Water budget

For each amendment experiment, which we'd like to conduct 2x at a non-bloom station and 1x and a bloom station we'll require:

220 liters from ~75m

15 liters from 500-700m (whichever depth mesocosm is using)

- Pre-dawn to allow for on deck handling w/o giving Prochlorococcus a substantial light shock
- We need >30 hrs between each experiment
- Prior to each amendment experiment we'll require one full cast (~250L) for concentration of HMWDOM unless continuous flow-thru is sufficient.

For each of 5 bloom transect ctd stations we'd like

12L at each of four depths (25m, 75m, 125m, 500m)

Flow thru sampling

~300L required from non-bloom region >24 hours prior to each amendment experiment.

8-16hrs (?) (bloom/non?) to collect large quantities of cell mass for lipid analysis

On-deck incubator space

For each of 3 ~30 hour experiments, we plan to incubate 8-20L carboys in the large on-deck flow-thru incubator. Each carboy has an approximate 25cmx25cm footprint.

Sample storage

For DNA samples we'll need ~ 2 ft² of freezer space (preferably -80)

For RNA samples we'll need a 20-30L dewar on-board for flash freezing and storage of samples in liquid N₂.

Upon return we'll need ~25L liquid N₂ for charging dry-shippers for transport.

Lab space

We'll need bench space, near a sink, to accommodate 3 scientists and associated pumps/filters. (5'x4' each)

MIT-Boyle

Water budget/wire time

will use the one-sampler ATE once on each station along with a five- sampler vane cast, just like we did on KM0703 except we will NOT want to get the wire angle forward ever (and should stop the cast immediately if they can't keep the line vertical or streaming aft).

Unless you have come up with some new system, I assume that you'll be using the same 3/8" line and capstan system that we used on KM0703.

Laboratory space?

Sample storage?

UH-Rappé

Water Budget:

Ideally sample 8 to 12 different depths per CTD cast in the upper 1000 m of the water column, twice a day (e.g. 10, 45, 75, 100, 125, 150, 175, 200, 400, 600, 800, and 1000 m). Of course, this can be modified to fit into a "consensus" sampling scheme, if one evolves. For the combined FISH and molecular work, we would like to get 5L per depth.

Lab space:

Essentially we need bench space surrounding (i.e. on both sides) a sink. We have had success with the first "L" shaped bench as you walk in to Lab 2, right in front of the door. If that would not work, then there are two spots in the back room of Lab 2 that would work as well; basically, the two bench spaces surrounding each of the two sinks in that back room.

UH-Bidigare

Water Budget:

- 1) 2L samples from core measurements for pigment HPLC analyses
- 2) Samples from net-tows for BMAA analyses

Lab Space:

Bench space for filtration units adjacent to space for Cruz-Rivera who will be looking at the characterization of grazer's community.

Sample storage?

UCSC

Water budget

- 1) Water: A minimum of 4L water collected from 6-8 depth between surface and 200 m for filtration DNA and flow cytometry per day or per major station.
- 2) Water: 3-4 times maximum during 10 day cruise, we will require about 50 L from one depth to set up the nanoSIMS N15 experiments. This 50 L could be from one cast (then requires incubator space for 24 2-L bottles), or 25 L from beginning of day, and 25 L from beginning of night period (incubator space for 12 2-L bottles needed per experiment).
- 3) Possible large volume surface pumped samples for metagenomics, depending on coordination with DeLong group, access to pumps, logistics, etc. Low priority since DeLong will be doing metagenomics.
- 4) Incubation space for 6-9 2-L bottles continuously (if possible), and up to 24 2-L bottles for the nanoSIMS experiment which will be performed 3 times total during the cruise.
- 5) Access to flow cytometer 4-6 hours per day to sort Group A unicellular cyanobacteria and sort, for subsequent on-board qPCR.

Lab Space:

Bench space (not including FCM or microscope) for small qPCR instrument and laptop, and for peristaltic multi-head filtration pumping system (with 8 2-L sample bottles) with access to drain (sink)

Sample storage

Small amount of freezer space for assorted water samples.
Liquid nitrogen for 40 L dry shipper before and after cruise.

WHOI

Water Budget

Low molecular weight dissolved organic matter:

1) four vertical profiles at 6 depths: approximately 25, 50, 100, 300, 500, and 1000 m I need 20 L from each depth. Water will be filtered from the rosette into carboys and then processed in the lab.

2) surface (spatial) coverage: up to four additional stations in & out of the bloom. Water (20L) from near surface (5-100 m) collected via rosette.

High molecular weight dissolved organic matter:

This water is for Ed DeLong's incubation experiment:

I will plumb the system into the ship's clean seawater intake to concentrate organic matter nearly continuously during the cruise. I would like to take samples in and out of the bloom- but this depends on how close to the surface the bloom gets- e.g. changes in

Chl profile. Depending on the chlorophyll profile, I may want to take up to two full casts in the chlorophyll maximum of the bloom.

Trace metal DOM sampling:

I deploy a Teflon line from the deck and use a deck mounted, air driven pump to pull seawater up to fill Teflon-lined 55 gallon drums. The water is then pumped through a column. We did this on the April cruise. It can be set up on deck in a semi-protected area. I would like to take at least one sample each in and out of the bloom. The sampling time is about 90 min on station to pump the water in the barrels.

Lab space:

Low molecular weight DOM- the processing requires about 3-4 ft of bench in a wet-lab area. I will be processing lots of water, so it is best done in an area with easy access to the deck and where water can be splashed around without anyone getting too upset.

High molecular weight DOM: The system has a footprint of about 3x4 and is immovable once it is set up. It is 2 m high when set up, so I need that much vertical clearance. It also makes a fair amount of noise-I've run it in the laboratory area, but people are usually not happy with it there. It can be set up in a protected area on the deck- but needs to be near a clean seawater tap. I've pasted a not so good picture of the HMWDOM pump set up below.

TM DOM- this can be done on deck in a semi-protected area. The only caveat is that the tubing has to reach from the barrels to the pump and over the side, so the set up cannot be too far (more than 15-20 feet) from where the pump and hose are deployed.



HIGH MOLECULAR WEIGHT DOM SAMPING SYSTEM

Sample storage?

MBARI

Water Budget

Access to flow through water system

Lab space:

At least 6 linear feet of bench space in the proximity of the sea water flow through system.

OSU

Water Budget/wire time requirements:

- 1) Optics Cage : In practice, using the constant speed capstan on the KM, deployment time required to reach **~40-45 minutes** to deploy the cyanocage to 200m and recover the package. A hollow-core cord threaded through the A-frame was used to deploy this instrumentation.
- 2) The PRR can also be deployed via the capstan and will similarly require ~45 minutes to reach 200m and be recovered on deck. If the HyperPro is used, it will be deployed manually from the fantail deck.

- 3) Surface net tows can be conducted concurrent with CTD deployments, from the fantail under the supervision of a martech or another individual with communication to the bridge.
- 4) For each gas-array deployment (capacity = 6 depths, 4-4L bottles), we will collect water from pre-dawn arrays for time-zeros (PC/PN) and fill array bottles for duplicate measurements of light/dark bottles for each depth. (See Below Spreadsheet for planned isotope additions)
- 5) When water is available, we will collect **8-L samples** from all available depths for PP-fractionation, **4-L samples for PC/PN**. And 500-ml samples for PAM fluorometry.

Lab space:

- Bench space for setup of filtration rig, PAM fluorometer and compound microscope.
- Space in the staging bay space for storage of the cyanocage and data download.
- data download space for cyanocage
- Access to PRR computer in CTD control room

Sample storage:

Primarily in 20L liquid nitrogen Dewar.

OSU Collaborations:

We have discussed collaboration with the Zehr group in the cultivation of diatom-cyanobacterial symbioses. We have also discussed with numerous groups coordinated addition experiments where surface water samples will be spiked with water from below the euphotic zone (~75m) and below the DCM. Incubations will be monitored for changes in biomass and community structure.

OSU Appendix: Isotope Additions

1. We should require ~150 ml of the 400ml $^{15}\text{N}_2$ that we will have available. There will be no shortage of ^{13}C bicarbonate.
2. Per 4L bottle, we plan 2ml additions of $^{15}\text{N}_2$ and 0.5 ml mM ^{13}C -bicarbonate.