MICROSCOPY WORKSHOP FOR PHYTOPLANKTON AND
BACTERIOPLANKTON IDENTIFICATION
SEPT. 21-25, 2008
Hawai'i Institute of Marine Biology, Coconut Island, Hawai'i

Participants

The workshop is open to 12 US students (M.Sc., Ph.D.). Presently, the workshop has budgeted for costs to include 8 mainland US- and 4 Hawai'i-based students. Applications from students from under-represented groups are strongly encouraged.

Travel & Accommodations

Participants will stay at the Lanai suites at Hawai'i Institute of Marine Biology (HIMB) at Coconut, Island. These are shared occupancy rooms with 3-4 single beds and separate bathrooms. Two meals (breakfast and lunch) will be catered and dinner will be off-site at local businesses. Transportation, including airfare and travel to/from airport in Hawai'i will be provided by workshop.

Cost

The National Science Foundation funded Center for Microbial Oceanography: Research and Education (C-MORE) will provide full scholarships to the mainland participants for the course activities, including travel and accommodations (includes meals), however, we encourage students to apply for other sources of funding that C-MORE can supplement or match.

Preliminary Program

Sunday, Sept. 21, 2008

900 - 1700	Arrive, settle in to accommodations, set up computer accounts, sign up and scheduling for the free time on Friday.
1700	Welcome BBQ.

Monday, Sept. 22, 2008

900 – 10:15	**Lecture:** Overview of algal cell structure, algal classifications, and cell counting chambers (Sedgewick- Rafter, Palmer-Maloney, hemocytometer) by Foster and Carpenter.
10:30-11	2 Student Presentations (15 min./presentation)

References:

Phycology. 1992. Robert E. Lee. Cambridge University Press, New York, NY.
Algae. 2000. Linda E. Graham & Lee W. Wilcox. Prentice Hall Inc., Upper Saddle River, NJ.

1100 - 1145	**Cruise prep:** tour of facilities, safety regulations, and the gathering of supplies for Group I's small boat operation to collect phytoplankton samples and field measures.
1200 - 1300	**Lunch** (After lunch, individuals divide up into 2 six-person groups: Group I for field and Group II for lab)

1300 - 1500 **Group I:** small boat op to collect plankton samples with net, whole water, and take field measures of temperature, salinity, water clarity (secchi disk).

1500 - 1700 **Group I:** upon return, preserve and process samples for FISH (day 3), chlorophyll *a* (chl*a*) extraction, flow cytometry, and DAPI staining.

1300 - 1700 **Group II:** Microscopy introduction, sample preservation techniques, and cell counting lab. A general review of epifluorescent and light microscopes, slide and sample preparation, and counting chambers. Using green and blue excitation, students will begin to identify phycoerythrin and chl*a* containing cells, respectively. Introduction to cell identifications for major phytoplanktonic groups (diatoms, dinoflagellates, cyanobacteria, etc) and enumeration of DAPI stained cells.

2000 - 2100 **Group I:** filter samples preserved earlier in the afternoon for FISH.

Tuesday, Sept. 23, 2008

900 - 1015 **Lecture:** Review of fluorescently labeled *in situ* hybridization (FISH) method, TEM coupled with immuno-cytochemistry assays, and whole cell immunolocalization (Rappé and Foster).

10:30-11 2 student presentations

References

Giovannoni, S.J., M. Rappé. 2000. Evolution, diversity and molecular ecology of marine prokaryotes, p. 47-84. In: D.L. Kirchman (ed.), Microbial Ecology of the Oceans. John Wiley & Sons, Inc, New York, N.Y.

1100 - 1145 **Cruise prep:** gather supplies for group I's small boat operation to collect phytoplankton samples and field measures.

1200 - 1300 **Lunch** (After lunch, individuals divide up into 2 six-person groups again: Group I for lab and Group II for field)

1300 - 1700 Same as Monday, but Groups I and II switch activities.

2000 - 2100 **Group II:** filter samples preserved earlier in the afternoon for FISH.

Wednesday, Sept. 24, 2008

900 - 1100 Participants prepare bench space and review the FISH and immunolocalization protocols. Participants divide into four groups of 3 individuals each to begin hybridization of preserved samples for FISH.

1100 - 1200 **Lecture:** overview of bacterioplankton diversity, cell structure, abundance & distribution by Rappé.

References:

Lin, S., S. Henze, P. Lundgren, B. Bergman, and E.J. Carpenter. 1998. Whole-cell immunolocalization of nitrogenase in marine diazotrophic cyanobacteria, Trichodesmium spp. AEM. 64: 3052-3058.

Foster, R.A., B. Bergman, E.J. Carpenter. 2006. Unicellular cyanobionts in open ocean dinoflagellates, radiolarians, and tintinnids: ultrastructural characterization and immuno-localization of phycoerythrin and nitrogenase. J. Phycol. 42: 453-463.

1200 - 1300 **Lunch**
1300 - 1400 **Lecture (continued):** overview of bacterioplankton diversity, cell structure, abundance & distribution by Rappé.
1400-1500 4 student presentations
1400 - 1700 Participants again divide into four groups to finish processing preserved samples for FISH and whole cell immunolocalization of nitrogenase/phycoerythrin assays.

Thursday, Sept. 25, 2008
900 - 1045 **Lecture:** Overview of culture strategies, media recipes, and considerations when attempting isolations by Carpenter, Foster, Rappé, and Waterbury.
11-12 4 Participant presentations
1200 - 1300 **Lunch**
1300 - 1800 ALL DAY: 1-2 hr. comprehensive and scheduled time for each student on Epifluorescent microscope(s). Partners are recommended. Open review and repeat of staining techniques, and general open discussion and questions. PM-organized snorkel to reserve (voluntary).