Bigelow | National Center for Marine Algae and Microbiota

Phytoplankton Culturing Techniques Course

September 25th – 29th, 2018
https://ncma.bigelow.org/culture-course

Monday, Sept. 24th

Day	Time	Activity	Instructor
Mon.	All day	Students arrive	

Tuesday, Sept. 25th

Day	Time	Activity	Instructor
Tues.	7:30	Continental Breakfast Mezzanine	NCMA
Tues.	8:30	Welcome and Introductions: • **Safety Practices & Training** • Domestic issues (food breaks, bathrooms, accommodation, emergency drills). • Introductions; What do you want to get out of the course (survey summary) • Course outline/syllabus/manual • Internet access, course resources on website. • An introduction to Bigelow Laboratory & NCMA	**Sara Rauschenberg** Mike Lomas
Tues.	11:00	Tour of NCMA, Greenhouse, and seawater Facility	Mike Lomas
Tues.	12:00	Lunch on Mezzanine	EBBGS
Tues.	13:00	Introduction to lab/materials/supplies Microscope 101 - Lecture and demo on using microscopes and iOS device	Mike Lomas Bigelow IT

Tues.	13:45	**Laboratory Demonstration**
		Student Stations & prep for the week ahead.
		• About alcohol burners and other material at each student station
		• Make pipette mouth pieces
		  ○ Drawing micropipettes from Pasteur pipettes & capillaries
		• Sterile technique exercise.
		  ○ Pipetting into organic media
		  ○ Testing benches, work areas with agar plate
		  ○ Testing Pipettemen for utility and sterility
			Pete Countway

Day	Time	Activity	Instructor
Tues.	14:45	**Laboratory Demonstration** • Isolation techniques – picking cells (fresh sample and L1 for this exercise) – set up microtiter plates for isolation. • Carm's 'cell sucker' system • Microcapillary cell picking • Dilution-Extinction isolation • Set up plates for culturing • Discuss axenic vs. bacterized (xenotobiotic) cultures • Bacteria freeing, pros and cons	Carm Tomas & Pete Countway
Tues.	15:45	**Coffee Break**	

Tues.	16:00	**Lecture/Lab:**
		Principles of Flow Cytometry, Cell Sorting and discussion of different counting methods.
		• Monitoring Algal Growth
		  ○ Hemocytometer
		  ○ Fluorometer
		  ○ Flow Cytometry
		  ○ Growth curves
			Nicole Poulton

Tues.	18:00	Dinner on your own	

Wed, Sept. 26th LUNCH SPONSORED BY…

Day	Time	Activity	Instructor/Vendor
Wed.	7:30	Continental Breakfast on Mezzanine	NCMA
Wed.	8:15	Meet at Bigelow dock for ½ day cruise	R/V Snow Goose
Wed.	8:30	**Sampling Trip (Damariscotta river)** • FlowCam demo in the field • GoFlo (bottle) casts to profile and collect phytoplankton • Plankton tows to collect phytoplankton • Environmental measurements (temp/salinity) via CTD	Pete Countway Carm Tomas Harry Nelson & Frances (FIT) NCMA staff
Wed.	12:30	Lunch on the mezzanine	EBBGS
Wed.	13:30	**Laboratory Demonstration** • FlowCam Demo and analysis of sampling trip samples. • Picking cells from cruise collections for culture isolation and genetic identification	Harry Nelson (FIT) Carm Tomas Pete Countway
Wed.	15:00	Break	

Day	Time	Activity	Instructor
Wed.	15:30	**Laboratory Demonstration** • Continue with isolations • Identification of field isolates via microscopy • Lysis of single-cell isolates for later whole genomic amplification (WGA) & PCR • Whole genome amplification setup	Carm Tomas Pete Countway
Wed.	18:00	Dinner on your own	

Thurs., Sept. 27th

Day	Time	Activity	Instructor
Thurs.	7:30	Continental Breakfast on Mezzanine	NCMA
Thurs.	8:30	**Lecture: History of Seawater Media** Early Attempts of media making – • Erd schriber • The Provosoli & Guillard eras • Defined media – • Enriched sea water media Preparing sea water for media • Filtration – filter types and effective pore sizes • Sterilization – methods • Processing glassware • Matter of salinity • Routine Media for maintaining numerous cultures Formats for cultivation • Batch modes • Semi-continuous • Turbidostats • Chemostats	Carm Tomas
Thurs.	10:15	Break	
Thurs.	10:30 NCMA media room	Laboratory Demo – Making culture medium (1/2 of class)	Joan Blanchette
Thurs.	10:30 Ocean modular lab	Laboratory Demo – Impact of differential media enrichments – Microscopy (1/2 of class) • Cell picking/isolations from enrichments	Mark Hurd Pete Countway
Thurs.	12:00	Lunch on Mezzanine	EBBGS

Day	Time	Activity	Instructor
Thurs.	13:00 NCMA media room	Laboratory Demo – Making culture medium (other 1/2 of class)	Joan Blanchette
Thurs.	13:00 Ocean modular lab	Laboratory Demo – Impact of differential media enrichments – Microscopy (other 1/2 of class) • Cell picking/isolations from enrichments	Mark Hurd NCMA staff
Thurs.	14:30	Break	
Thurs. Thurs.	15:00 cont.	**Lecture/Lab: Taxonomy** How to use 'Identifying Marine Phytoplankton' Book and discussion on Morphological Taxonomy. • Microscopic analysis of NCMA strains from the book (see separate handout).	Carm Tomas NCMA staff
Thurs.	16:30	• PCR setup of WGAs with 18S rRNA gene primers	Pete Countway
Thurs.	18:00	Dinner on your own	
Thurs.	20:30	Circadian rhythms lecture and bioluminescence demonstration	Mike Lomas

Thurs.	15:00	**Lecture/Lab: Collecting & Counting Samples**
		• How to collect samples
		  ○ Grabs, net tows, & pumps
		  ○ Other methods
		• On site data needed
		  ○ Temperature & salinity
		  ○ Other variables
		• How to store samples until arriving to the laboratory
		• How to ship samples to offsite lab
		• To preserve or not preserve – that is the question!
		• Methods –
		  ○ Chambers –
		  • Sedwick Rafter Cells 1 ml
		  • Palmer Maloney 0.1 ml
		  • Haemocytometers
		• Settling methods
		  ○ Movable settling chambers
		  ○ Fixed chambers
		• Care and cleaning
			Carm Tomas
			
			(Link back to Wednesday sampling trip)

Friday, Sept. 28

Day	Time	Activity	Instructor
Friday	7:30	Continental Breakfast on Mezzanine	NCMA
Friday	8:30	**Lecture/Lab: Culturing continued** • Other methods – Antibiotic drop cleaning • Antibiotic wash methods • Agar plate techniques • Phototaxis • Gravity separation Culture accounting techniques • Assigning tentative names • Culture codes, keeping records • Sharing cultures – Purification Methods • Droop Method • Antibiotic plates • Antibiotic suite on bucket of seawater	Carm Tomas
Friday	10:00	Break	
Friday	10:30	**Laboratory Demonstration** • Transferring cultures • Working with agar plates, transferring colonies to growth medium. • Streaking out stocks on agar plates/slants	NCMA staff Pete Countway
Friday	12:00	Lunch on Mezzanine	
Friday	13:00	**Laboratory Demonstration** Cryopreservation (group A)	Mark Hurd NCMA staff
Friday	13:00	Laboratory Demonstration PCR isolation for ID (group B)	Pete Countway
Friday	14:30	Break	
Friday	15:00	**Laboratory Demonstration** Cryopreservation (group B)	Mark Hurd NCMA staff
Friday	15:00	Laboratory Demonstration PCR isolation for ID (group A)	Pete Countway
Friday	17:00	Happy hour – meet at Boothbay Craft Brewery to talk about algae and enjoy a tasty local beverage	Everyone
Friday	19:00	Dinner on your own	

Saturday, Sept. 29th

Day	Time	Activity	Instructor
Sat.	7:30	Continental Breakfast on Mezzanine	**NCMA**
Sat.	8:30	**Lecture/Data analysis** Phytoplankton Molecular Taxonomy & DNA-based identifications • BLAST sequences from single-cell isolates	Pete Countway
Sat.	9:45	**Break**	
Sat.	10:00	**Lecture - Mass Culture** **Laboratory Demonstration** • SeaCAP bag setup • Photobioreactors in greenhouse	Mike Preston Pete Countway
Sat.	12:00	Lunch on Mezzanine	
Sat.	13:00	**Laboratory Demonstration** Harvesting large volumes • Centrifuge • TFF • Spray drying	Mike Preston Pete Countway
Sat.	15:00	Break	
Sat.	15:30	**Course wrap up** • Finish mass culture demonstrations and related harvesting activities • Packing up algal isolates for shipment/transport • Final Q&A session	Mike Preston Pete Countway Carm Tomas Mike Lomas
Sat.	18:00	Lobster Bake at Bigelow Dorm Certificate of Completion presentations	**Everyone**