C-MORE Nutrient enrichment experiment – COURSE OUTLINE

Day 1: 3 hours

- Introductory lecture: key concepts in MO (45 min)
  - What is microbial oceanography?
  - Why is it important?
    - Food web
    - O_2 production
    - Climate
    - Nutrient recycling
  - Microbial diversity (4 major groups)
  - Abundance/size

- Initial sampling (4 hrs)
  - Discuss lab safety and proper lab technique
  - Set up lab
  - Make nutrient stock solutions in brown, labeled bottles and refrigerate
    - Add 4.462g $NaNO_3$ to 500ml deionized water to make 105 mM
    - Add 0.6118g K_2HPO_4 to 500 ml deionized water to make 7 mM
    - Add 9.87g Na_2SiF_6 to 500ml deionized water to make 10 mM

  - Collect 50L of seawater in five-10L carboys (only if single samples taken daily for ATP – for replicate analysis, need 100L of seawater to have 20L per treatment)

  - Spike the seawater samples with nutrient solutions to make 5 different treatments and **mix thoroughly**
    - Add 1.45 ml $NaNO_3$ to 10L seawater => 15µM NO_3 treatment
    - Add 1.45 ml K_2HPO_4 to 10L seawater => 1 µM PO_4 treatment
    - Add 1.5 ml Na_2SiF_6 to 10L seawater => 15 µM SiO_3 treatment
    - Add 1ml of $NaNO_3$ and 1ml of K_2HPO_4 to 7L seawater => 15:1 NO_3: PO_4 treatment
    - Leave 10L untreated seawater in one carboy for control

  - Aliquot treatments into labeled 1-L bottles (discard leftover water)
  - Filter T_0 samples in acetone (see experimental design)
  - Cover tubes in aluminum foil and place in cooler on ice
  - Transfer samples to -20^o C freezer when finished filtering
  - Place bottles in incubator on a 12:12 or 14:10 day:night cycle

Day 2: 3-4 hours

- Nutrient lecture (1 hr)
  - Nutrient dynamics
    - Light
      - Properties
      - Pigments

    - Nutrients – source and purpose
        - NO_3, PO_4, Fe and Si
    - Nutrient sources: upwelling, storm run-off, eddies
    - Growth curves
- Course structure and experiment
    - Nutrient enrichment experiment
        - Add different nutrients to test effect on growth
    - Biomass: how do you measure it?!
        - Chlorophyll
            - found in all photosynthetic cells, incl. dead ones
            - confounding with shift in chl:cell with depth
        - ATP – accurate indicator of live cell abundance, but incl. heterotrophic cells too

- Discuss assignment: formal lab report
- Hypothesis testing: outline research questions for lab reports
- Take T_0 reading on fluorometer
- Take samples for chl and ATP (45 min)

Day 3: 2 hours
Make visual observations about experiment
Take samples for chl a, ATP
Analyze samples from previous day (chl a)

Day 4: 2 hours
Make visual observations about experiment
Take samples for chl a, ATP
Analyze samples from previous day (chl a)

Day 5: 2 hours
Make visual observations about experiment
Take samples for chl a, ATP
Analyze samples from previous day (chl a)

Day 6: 5 hours
Measure chl AND ATP
Statistics lecture
    - Mean
    - Standard deviation
    - Confidence intervals
Compile and plot data in Excel (have them follow written instructions and then walk them through the process on the projector)
Visual and statistical analysis of data
Summarize experience/lessons learned
Evaluations