

Possible Materials for Experimentation

Lesson 5 – Ocean Acidification: A Systems Approach to a Global Problem

Since students will design their own experiments and provide a list of materials to you, their list should be your main material list. However, we have prepared this list which contains materials commonly used during Lesson 5.

General Materials

- Beakers
- Pipets (disposable and/or reusable in a variety of sizes). Micropipettes are only required if you are using hemocytometers to count diatom cells.
 - http://www.carolina.com/lab-pipets/plastic-microchemistry-pipets/FAM_736982.pr?catId=10266&mCat=10216&sCat=10255&ssCat=&question=
- Al foil
- Dry ice, Alka-Seltzer, breath (source of CO₂), yeast, sugar, vinegar/baking soda
- Balance or scale

Materials students may want to use

- fish shake (fish emulsion)
- Infant iron drops (for 1mL trace minerals or vitamins per 1000mL seawater)
- Iron nail (non-galvanized)
- Chalk or Roloids (calcium carbonate)
- Copper Wire
- Magnesium sulfate (Epsom salts)

Shell Dissolution

- Crushed coral:
 - <http://www.carolina.com/tanks-and-accessories/coral-crushed-15-lb/163246.pr?catId=&mCat=&sCat=&ssCat=&question=coral>
- A gallon of sea shells:
 - <http://www.carolina.com/animal-habitat-accessories/sea-shell-assortment/261786.pr?catId=&mCat=&sCat=&ssCat=&question=shells>
- pH/CO₂ detection: paper, lime water, BTB (bromothymol blue) may be used if probeware is not available. Carolina has many options available for measuring pH.
- Biochamber: can be made from a large plastic container (cut/drill hole(s) on top). Use o-rings that can be purchased from Vernier for an extra good seal (though not required). Or purchase a biochamber complete with o-rings from Vernier.
 - <http://www.vernier.com/products/accessories/bc-2000/>
- Seawater: make using ESAW recipe or aquarium saltwater
 - <http://www.instantocean.com/>
 - or obtain natural seawater from a clean source and use a pressure cooker to sterilize. If not possible, multiple filtrations with a fine sieve can be sufficient. If

the water is fairly clean to begin with, filter once each day over 3 days and keep in the refrigerator. Use a fine mesh such as Use a fine mesh such as a [Nitex Bolting Cloth](https://shop.sciencefirst.com/wildco/517-nitex-bolting-cloth) (<https://shop.sciencefirst.com/wildco/517-nitex-bolting-cloth>) or some other fine mesh.

Diatom Experiments

- Diatoms (*Thalassiosira pseudonana*) are available from Carolina Biological:
 - <http://www.carolina.com/algae/thalassiosira-living/153110.pr?question=diatoms>
- Flasks: use glass ones and stopper with cotton. Or if you can obtain or already have 50mL vented tissue culture flasks, you can stack them to save space. They can be bought on ebay (least expensive). When using, be careful not to get the membrane in the cap wet.
- Seawater – made using ESAW recipe or aquarium saltwater as written above. If growing diatoms, you likely need to add nitrogen, phosphate, and silicate to your media to ensure they have the nutrients to grow. Sodium nitrate (NaNO_3), sodium phosphate ($\text{NaH}_2\text{PO}_4 \times \text{H}_2\text{O}$), and sodium silicate ($\text{Na}_2\text{SiO}_3 \times 9\text{H}_2\text{O}$) are good sources of these nutrients as indicated in the ESAW f/2 recipe.



Counting/Visualizing Diatoms

- Hemocytometer (aka Counting Chambers) – there are many types: glass (reusable), plastic (not reusable), pre-gridded slides, stick-on grids. Any of these can work.
 - o Standard Hemocytometers are ~\$200):
<http://labscientific.com/Cytology/Hemocytometer-with-Cover-Glass/>
 - o Plain Glass slides – there are many options for ordering. Click here for [one option](#). You can affix grids to the slide to help with counting. See our Diatom Techniques section in Lesson 5a for more information.
 - o Pre-gridded slides – again there are many options. Here are two sources:
<http://labscientific.com/Histology/Microscope-Slides/Grid-Slides/>
<http://www.flinnsci.com/store/scripts/prodView.asp?idproduct=18987>
 - o Stick-on grids for glass slides:
<http://www.flinnsci.com/store/scripts/prodView.asp?idproduct=19171>
 - o Plastic (disposable and non-reusable): Neubauer Improved C-chips (many sources for these can be found by searching “Neubauer C-chips Hemocytometer”).
 - o Spectrophotometry/spectroscopy may be used. Test your equipment first. Determining population growth using absorbance or transmittance may not be practical given the tendency for the silica of the diatom’s shell to scatter light and the variation in the amount of chlorophyll within the cells to give false readings.