**Sea anemone care and feeding** 8/5/16

In a lab in which animals are used, it is everyone’s responsibility to care for animals and to check that they are healthy and well-maintained. Each student (or group) will take care of one “stack” of animal dishes. You are responsible for feeding your stack the appropriate food each week, changing medium as needed to keep animals clean, and for spawning the animals when it is your turn, to obtain embryos.

**General:**

* feed the animals brine shrimp 1-2x per week (once - twice per week)
* in a week in which the animals will be spawned, feed them oyster early in the week
  + do not feed them brine shrimp that week
  + clean the animals the day after an oyster feeding
    - you may need to transfer them to a clean bowl with fresh sea water
* prepare ⅓X artificial sea water (⅓X ASW) whenever a carboy is empty
  + CAUTION: do not tilt the carboy to get the last dregs of ASW; you will get solid, undissolved salt which is BAD for the anemones
* spawning and cleaning embryos takes a while; make sure you are prepared
* all glassware marked “E” (for embryos) should be hand rinsed with water only: NO detergent, NO soap, NO chemicals.

**Artificial sea water:**

* materials:
  + Instant Ocean (large tub with purple lid) - under the lab bench in UWBB 274
  + large weigh boat, stirrer - on the bench in UWBB 274
  + 2 liter beaker (marked “E”) – on the drying rack above the sink in 274
  + bubbler
  + 9 liter carboy
* open the tub of Instant Ocean
* using the beaker inside the tub, and the large weigh boat on the lab bench, measure out approximately 83.75 g
* to the large (2 liter) beaker marked “E”, add ~1800 mls RO water
* add the large stir bar to the beaker, place the beaker on the stir plate, and start the stirrer
* slowly add the salts to the stirring water
* allow to dissolve for ~30-60 minutes; the salts will not dissolve completely
* turn off the stirrer, transfer the beaker to a lab bench, insert a bubbler, and cover
  + cover with a large petri dish; this reduces splattering and evaporation
* allow to bubble overnight
  + bubbling overnight both oxygenates the ASW and allows the CO2 in the sea water to equilibrate with CO2 in the air, stabilizing the pH
* the next day, place the beaker on the stirrer to suspend undissolved salts
* pour into a clean, empty 9 liter carboy
* add RO water to the beaker, suspend salts; add this to the carboy. Repeat until the carboy is filled to the 7 liter mark; this is 1/3 x ASW.
* write the date on the tape on the outside of the carboy
* notes:
  + because of humidity, the salts in the tub can be difficult to break up. Use a clean screwdriver if necessary
  + not all the salts will dissolve on the stirrer, but we add them to the carboy anyway, as some will dissolve over time
  + ***CAUTION***: when we get close to the end of the 1/3x ASW in the carboy, be careful not to tip the carboy to get the last dregs of ASW. If you do that, some undissolved salts will come out. These adhere tightly to the anemones, and are bad for them. You will have to spend a lot of time picking salts off the anemones…

**Brine shrimp:**

* materials
  + clean separatory funnel (on ring stand in UWBB 270)
  + brine shrimp (*Artemia*) cysts (in 4° refrigerator in UWBB 273)
  + measuring spoons (by the sep funnels)
  + bubbler
* *make sure the stopcock is closed* on the sep funnel
* fill with ⅓x artificial sea water to the widest part of the funnel
* add ¼ teaspoon brine shrimp (*Artemia*) cysts to the funnel
* place a bubbler into the sep funnel (bubbler is a pipet connected to an air pump)
* after 10-15 minutes, swirl the sep funnel gently to rinse off any cysts stuck to the sides
* brine shrimp will hatch in ~36 hours
  + cysts are brown; the hatched larvae are orange
* after hatching, remove the bubbler, and shine a light from the side, toward the bottom of the sep funnel
  + brine shrimp are phototactic (they swim toward light)
* allow the brine shrimp to settle for ~10 min
* place a small beaker under the sep funnel, and remove the hatched (orange) brine shrimp
  + use a small beaker marked “E” from the drying rack in UWBB 274
  + it’s OK if you get some unhatched cysts, but you should get mostly hatched shrimp
  + add 1/3 x ASW to ~50 mls
* place the bubbler into the beaker (tip: lean the bubbler against something so it doesn’t fall)
* add a few drops of brine shrimp to each of your dishes
  + brine shrimp can be used for up to 48 hours after hatching
* clean up
  + rinse the sep funnel – with water only! - and place it back in its holder with the stopcock open to allow it to dry
  + you can also use the bottle brush by the sink in UWBB 274
  + once you are done with brine shrimp in the small beaker, this can be rinsed by hand (water only!)

**Oyster:**

* **IMPORTANT**: anemones should not be exposed to light for more than about an hour. You may wish to take dishes out 1 or 2 at a time to prevent too much light exposure
* materials
  + small, clean petri dish (a used petri dish is fine, if it is clean)
  + razor blade (used is OK, if clean)
  + forceps (two pairs)
  + oyster – from -20° freezer; see below
* remove your animals (see note above) from the incubator and remove the covers from the dishes
  + the anemones will “open up” after a few minutes in the light
* remove a dish of oyster from the -20° freezer
* quickly – while it is still frozen - slice off 3-4 thin slices of oyster (1-2 mm thick)
* transfer the slices to the clean petri dish
* using the razor blade and forceps, chop into small pieces (1-2 mm square)
  + it’s OK if some are not cut
* put the rest of the oyster back in the freezer
* feed the oyster chunks to the anemones, using the forceps
  + you may need to tear larger pieces in two with forceps
* after you have fed each dish, put it aside and let it sit for 5-10 minutes to give the anemones a chance to grab and ingest the food
* cover the dishes and place them back in the incubator
* the next day, remove any uneaten oyster
* change the ASW (transfer the anemones to a clean dish if necessary)
* put the anemones back into the incubator

**Oyster stock in -20**° **freezer**:

* Purchased at Whole Foods
* Opened with Oyster Knife / Gloves in Servetnick lab
* Stored one Oyster per petri dish, *wrapped in parafilm*, in -20° **freezer**
* Clearly label storage date of each oyster