**Spawning, fertilizing, and dejellying eggs** 8/5/16

**Inducing Spawning:**

* general timing by days:
  + day 1 – feed oyster
  + day 2 or 3 - set up spawn; put animals on light box overnight
  + day 3 or 4 (after light box) – collect eggs, fertilize, dejelly
* general timing for spawning/fertilization/dejellying
  + set up the system so the light is on for 8 hours
  + about 1-2 hours after the light turns off, animals will start to spawn
  + after about another 1-2 hours, they should be finished (though it may take longer)
    - (fertilize 3-4 hrs after the light goes off)
  + plan to spend about an hour after you fertilize, to dejelly and wash the eggs
  + example:
    - set the timer to come on at 1 am, and go off at 9 am
    - animals will start spawning ~10-11 am, and continue for up to 2 hrs
      * so, maybe until 1 pm
    - fertilization and dejellying requires about an hour (→ 2 pm)
* 1-3 days after feeding oyster, place the dishes that you fed onto the light box. Remove the lids.
* set the timer to turn on and off at the desired time
  + make sure it is set to the **correct current time**
  + make sure the switch on the light box is **on**
  + make sure the sliding switch on the timer is set to **“timer”**
* cover the light box with the cardboard box lid lined with aluminum foil
* if you can, try to be there to make sure the light goes off at the correct time
  + this will tell you that you set it up properly
* materials:
  + animal bowl with ⅓X ASW, labelled “new females”
  + two small glass dishes. Label one “early eggs” and the other with the date
  + transfer pipettes
* when the light goes off, or when you arrive (if the light is already off)
  + move the dishes onto a lab bench, in the light (that is, not in the dark)
  + remove any egg masses and place them into the dish labelled “early eggs”
* whenever a packet of eggs is released, transfer it to a small glass dish
  + if you identify a female in a dish that is not labelled “female” or “♀”, transfer her to the dish labelled “new females”
* when you are ready (about 2-3 hours after the light goes off, or longer if you have time and the females are still spawning), add sperm water (from one of the cloudy dishes) to the eggs. Allow to fertilize for ~20 minutes.

**Dejellying eggs:**

* while the eggs are being fertilized, prepare cysteine
  + prepare 10 ml of 4% L-cysteine in 1/3x ASW
    - add 10 ml of 1/3x ASW to a 15 ml disposable tube
    - add 0.4 g L-cysteine to the tube
    - place on a rocker (or mix by hand) until the cysteine is dissolved
    - this solution is acidic!
    - to neutralize, add 300 μl of 10M NaOH
  + do not make cysteine too long before you need it, or it will go bad (and stink)
* 20-30 min after fertilization, transfer the egg masses to the tube of cysteine using a small transfer pipette. Carry over as little water as possible.
* after all the egg masses have been transferred, place on the rocker for 15 minutes
* remove from the rocker, and look to see whether all the jelly has been dissolved
  + if not, pipet the eggs up and down a few times to break up any remaining jelly (check to be sure no egg masses are stuck inside the pipette)
  + or, place back on the rocker for a few minutes, until the jelly has been dissolved
* remove from the rocker and place upright (in a test tube rack) to allow the embryo to settle (~5 min)
* remove most of the ASW, using a small transfer pipette. Place it in the waste container.
* for the first wash, use “sperm water” – cloudy water from a dish with males; you may use sperm water from several dishes
* allow the embryos to settle (~5 min) and remove the ASW.
* add fresh 1/3x ASW to the tube.
* repeat for a total of 3-4 washes
* after the washes, transfer the embryos to a glass dish, label with the date and time of fertilization, and place the dish in the incubator

**Clean up**

* any unused eggs go into the waste container (NOT down the sink)
* transfer females in the “new females” bowl in with the other females
* leave the bowls out overnight, to make sure the anemones are done.
* The next day, change the ASW, especially for dishes with males; female bowls may not require changing if the water is still clear.