Protocol: Rotifer Husbandry and Amictic Egg Harvest

To make 22ppt seawater:

Combine 800ml of seawater (Wet Lab) and 200 mL of RO water in a 1L media bottle. Filter this solution through a 0.22μm vacuum filter and transfer to a new, clean 1L media bottle.

To make *T. chuii* media:

Request 800ml of *T. chuii* from the hatchery and add 200ml of filtered seawater (above). Perform a cell count on the new media using the Coulter Counter.

1. Power on the Coulter Counter.
2. Make sure upper limit (Tu) is set to 20μm and the lower limit (Tl) is set to 6μm.
3. Press the "functions" bottom and set to "fill system" using the arrow keys.
4. Press start.
5. Follow the prompt by ensuring the beige filling tube is attached to diluent reservoir.
6. Place a clean sample cup containing 9ml of seawater at the sampling station.
7. Press start and wait for filling to finish. While waiting, sign in on the record sheet.
8. After the machine is filled, place a new sample cup with 9ml seawater on the sampling station (this is your blank).
9. Press set up and then hit the start key to begin the count. Record the Blank measurement.
10. Pour the blank down the drain, rinse with tap water, and refill with 9mL seawater and 1 mL of algae. Swish to mix and place on sampling station. Press start to count.
11. Repeat for a total of three times to get three algae counts.
12. After the three algae counts are obtained, press "functions" and use arrows to select "drain system."
13. Follow the prompt by switching the beige filling tube from diluent vessel to the waste container.
14. Place empty vial on sampling tray and press start to drain.
15. After draining has finished make sure the Coulter Counter gets turned off.

Standard Rotifer Husbandry:

B. plicatilis are maintained at 22ppt seawater supplemented with the single-celled green algae *Tetraselmis chuii*. Rotifers are passaged 1:4 every 3 days to maintain a robust, reproductive, and healthy population.

Passaging rotifers is easy:

1. Obtain a clean and sterile rotifer bottle (250 ml Nalgene beaker).
2. Add 75 ml of fresh *T. chuii* algae to the bottle.
3. Transfer 25ml of the previous passage to the new bottle.
4. Volumes can be adjusted up to 200 ml per bottle based on need (e.g., 150 ml algae and 50 ml rotifers)

Amictic Egg Harvest:

An amictic egg harvest is performed by sieving a culture of 2-day old rotifers and collecting them in 22ppt filtered seawater in approximately ¼ the original volume. The animals are vortexed to release eggs, and the culture is centrifuged to pellet amictic eggs.

The volume of culture can be adjusted based on the needed number of age-synchronized neonates. For example, 200ml of culture should be sieved and resuspended in 50ml of seawater; 400ml of culture should be sieved and resuspended in 100ml of seawater.

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1. Obtain two Pyrex dishes. Fill one approximately ¼ full and the other ¾ full with 22ppt filtered seawater.
2. Using a serological pipette, sieve 100 ml of rotifer culture through the filter into the ¼-full Pyrex dish, allowing the culture media and algae to flow through.
3. Transfer the sieve to the second ¾-full Pyrex dish. Using the serological pipette, pipette up until the animals are resuspended in ¼ of the original volume (25 ml). Transfer this to a 50 ml Falcon conical tube. ***NOTE:*** ***the goal is to have 50 ml conical tubes half-full.***
4. Repeat steps 3 and 4 above with the remaining rotifer culture, transferring to new 50 ml conical tubes.
5. Once rotifers are collected in ¼ volume 22ppt seawater, vortex on high for precisely 1 minute. ***NOTE:*** ***keeping conical tubes only half-full ensures that vortexing creates a shear force capable of stripping eggs from rotifers.***
6. Centrifuge the conical tubes at 300 x g for 5 minutes in a swinging bucket centrifuge.
7. Carefully remove the majority of the supernatant using vacuum aspiration, making sure to not disturb the pellet at the bottom of the tube.
8. Resuspend the pellet in 10ml of seawater and transfer to a 10cm petri dish.
9. Clear the culture of any live, swimming animals. This takes time.
10. After the plates are cleared, incubate the eggs at room temperature for 6 hours.