Brine Shrimp Preparation Protocol - Earley Lab

- 1. To determine which of the two brewers to process, look for the floating thermometer this signifies which brewer is ready.
- 2. Remove the thermometer and place in the other brewer. Unplug the overhead light. Remove the air stone and place in a bucket of water on the table (this prevents the other airlines from malfunctioning).
- 3. Use the baster to remove the eggs that are stuck on the sides of the brewer. Attach the clamp light to the side and angle it upwards to draw the shrimp to the top of the brewer (see figure below, photo on left).
- 4. Allow shrimp to settle for **15 MINUTES**. Unhatched eggs should sink to the bottom while shrimp and eggshells should float to the top.
- 5. Remove the bottom layer of sunken eggs from the brewer (see tan-colored stuff with blue box around it; figure below, photo on right) by opening the spigot. Resuspend this in 25 ppt water and place on the table near a light to allow any shrimp to swim to the top and unhatched eggs to sink to the bottom; below, this will be referred to as the "table bucket".





6. Attach the long clear tube to the bottom of the brewer and pass the remaining shrimp mixture through the plankton net into a 5 gallon bucket by opening the spigot. Do this until the brewer is completely empty. Allow all the 36 ppt water to drain through the plankton net into the 5 gallon bucket. *Place the 5 gallon bucket of 36 ppt water to the side and then arrange the plankton net so that you can hold it with one hand without the shrimp pouring out of the open ends (e.g., fold in a U-shape).

- 7. Using the green garden hose to the left of the brine shrimp table, fill the now empty brewer with a small amount of freshwater and drain out into a separate waste bucket to clear the spigot tubing of any egg residue.
- 8. Pour the shrimp (not the eggs) from the "table bucket" through a brine shrimp net. Dump the remaining unhatched eggs into the waste bucket.
- 9. Using the green garden hose, rinse the sides of the brewer and pass freshwater through the plankton net to rinse out the shrimp mixture (see step 6). Fill the brewer with freshwater to the top of the sticker on the brewer. Add the shrimp from your plankton net and from the brine shrimp net (step 8) into the brewer that has freshwater in it.
- 10. Allow shrimp to settle in the freshwater for 10 MINUTES. *Do not leave for longer otherwise shrimp may die (see photos below).





- 11. Remove the orange layer of shrimp from the brewer by opening the spigot and passing it through the plankton net into the waste bucket. Turn the spigot off once the shrimp layer approximately reaches the bottom of the brewer (shrimp layer shown by blue line in above right photo; it'll move down as you drain). Allow all the freshwater to drain out of the plankton net so all that remains is shrimp.
- 12. Resuspend the shrimp from the plankton net (step 11) in 25 ppt into one of the Nalgene containers. Use the blue hose attached to the 25 ppt saltwater reservoir to rinse the contents of the plankton net into the container and fill the container to the top.
- 13. Place a large air stone into both Nalgene containers and divide the shrimp from the one Nalgene container equally between the two. Dilute both containers with 25 ppt until desired amount is reached. *Currently it is 10.5 L total (5.25 L in each container).





- 14. Using the green garden hose and sponge on the brine shrimp table, thoroughly clean the brewer. Use the sponge to wipe down the entire brewer and to remove any algae/film build up. Open the spigot and rinse the brewer thoroughly with freshwater into your waste bucket.
- 15. Use the sponge to wipe down the airline and air stone to remove residue and egg debris and rinse these with freshwater too.
- 16. If it is MONDAY, TUESDAY or FRIDAY, pour the saved 36 ppt water (from step 6) into the brewer. Add more 36 ppt from the brine shrimp water reservoir (the trashcan to the left of the table) if needed to make the water line reach the top of the sticker. If it is WEDNESDAY, SATURDAY or SUNDAY use <u>NEW</u> 36 ppt from the reservoir to fill the brewer up to the sticker line (see bottom left photo below).
- 17. Place the air stone down into the bottom of the brewer so it fits snugly into the hole (see bottom right photo below). This will prevent eggs from settling at the bottom and not hatching.





- 18. Plug the overhead light back into the timer.
- 19. Grab the open can of brine shrimp eggs from the lab refrigerator and place 6 large scoops into the brewer. Return the can immediately to the fridge. DO NOT LEAVE CAN OPEN OR OUT FOR LONGER THAN NECESSARY!

