**Brine Shrimp Bioassay for Plant Toxins**

1) Use an unnumbered vial and fill it with 5 mL of sea water. You will use this vial in step #4 with sea water as a “ruler” for filling all vials to a final volume of 5 mL.

2) Put 1-2 mL of sea water into all 20 of your numbered vials. You can do this using the squirt bottle and estimate the volume.

3) Add 10 live brine shrimp to all 20 of your numbered vials. Be sure to not include any dead ones.

4) Use the squirt bottle to make the final volume 5 mL using your unnumbered “ruler” vial to estimate 5 mL.

5) Leave the caps off and place the vials back into your box to keep them from spilling.

6) Friday, at least one person from each group needs to come to lab and count the live and dead/missing brine shrimp in all 20 vials. Record the number of live shrimp for each individual vial (don’t pool your data).

Counting shrimp works well if two people from each group work together. The lab will be available 8 am – 5 pm Friday but you may have to knock or ask the someone in the biology office to help you open the door. Record your numbers and save them for lab next week.

**Evolution of Antibiotic Resistance**

For the directed evolution lab, you want to pick colonies from your plate that are within the “zone of inhibition”. Pick two colonies from each plate and repeat the procedure from last week. You will want to resuspend your picked colonies in 50 µL of sterile water to facilitate spreading them onto the plates. You have 4 plates to work with this week.