BIOLOGICAL ASSAY PROTOCOL
2015-2016

Materials for Runs

- Collection bottles (with creek water) for every site
- Two Beakers of living *Ceriodaphnia dubia*
- Dixie paper cups
- Explorer Meter--pH/Conductivity/Temperature
- Control (Wasser) water (4 cups Arrowhead for every 1 cup Evian)
- Plastic covered container for Wasser Water
- Potassium chloride solution (10 g/L KCl)
- Distilled (DI) water (in dispenser)
- Graduated cylinders
- Disposable Pipettes
- Plastic syringes
- 33 labeled Replicate vials
- Replicate tray
- Light table
- Test tube brushes
- Beaker brush
- Alconox powder detergent
- Tank: with pump, light, heater
- Data sheets
- 400 micron and 100 micron sieves

DAY 1

1. Wash all necessary glassware (the replicate vials, the graduated cylinders, the syringes). To do so, fill a Dixie cup with a ¼ inch of Alconox. Fill it with tap water to create soapy water. Pour soapy water into all of the replicate vials, and gently scrub the pieces inside and out with a test tube brush. Carefully rinse each piece with tap water, and then with distilled water. After rinsing the replicate vials, place them upside down on a paper towel to allow them to drain. Repeat with the graduated cylinders and the empty beaker, except with placing the graduated cylinders on their sides instead of upside down at the end. Rinse all the pipettes and syringes with DI water.

2. If necessary, calibrate the two Explorer pH/Conductivity/Temperature Meters, which have both pH monitors and the conductivity testers. The Explorer kits include two controls each: one bottle of fluid at a pH of 4, and another at a pH of 7. To calibrate the pH meter, first make sure the meter is set to "pH." Then, after pouring distilled water on the tip of the meter, insert it into one of the controls, and adjust the pH dial on until the reading on the screen matches the pH of the control, or comes very close to it. Calibrate the meter using BOTH controls on BOTH machines and make sure

that the tip of the meter is rinsed for each and every test. The conductivity tester does not have to be calibrated, but when using it, make sure the Explorer is in the conductivity setting.

3. Create Wasser Water, which is used as the control. Pour four parts Arrowhead® water with one part Evian® water into the plastic covered container if there is not enough already. The Wasser Water can be stored and reused, as long as it is kept in the covered container.

4. Create Reference Toxicant (RT_1 and RT_2). For RT_1, measure out 100 mL of Wasser Water with the control syringe, and then add this to the plastic cup labeled "RT_1." Then, measure out 1.2 mL of 10 g/L KCL with the RT syringe and then add this to the RT_1 cup. To make RT_2, measure out 100 ml of Wasser Water in a graduated cylinder and then add this to the plastic cup labeled "RT_2." Then, measure out 5 mL of 10 g/L KCL and add to the plastic cup.
    a. NOTE: RTs and the Wasser water are controls used for QAQC (Quality Assurance Quality Control) which tests if our results are valid.

5. Fill each RT replicate with 10 mL of the appropriate RT using the syringe. Rinse the syringe with distilled water when you change RTs.

6. Collect creek water samples (the bottles labeled "pool") from the Chemistry Study. There should be six collection bottles: site A, site B, site C, site D, site E, and site F.

7. Pour water from each collection bottle into the appropriately labeled plastic cup. For example, water from site D will be poured into the plastic cup that says "site D." Fill each cup about ¾ of the way full with water (about 100 mL).

8. Test the pH and the conductivity of each cup, just as was done in steps 6 and 7. Rinse the instruments before and after testing each cup. Record the data on the data sheet.

9. Wet the 100 microns sieve with DI water. Filter the sample water through the sieve into another disposable plastic cup. Label the cup with the site.

10. Turn the light table on. Set the beaker of the *Ceriodaphnia dubia* on the light table.

11. Place the 400 micron sieve in to a 2oz Solo cup, and add a small amount of Wasser Water (ensure the sieve is wet). The cup should be on top of a deep tray. Pour the Cerio culture through the sieve. Always keep the sieve in the the cup (don't strand the Cerio in the drained sieve).

12. Pour a small amount of Wasser water through the sieve, this will wash out the Cerio that are smaller than 400 microns (do not suspend the material at the bottom of the culture jar). Save the culture liquid and pour back into the beaker, once cleaned with

Alconox and distilled (this can be one of the biweekly cleanings of the Cerio colonies).

13. Carefully, using a pipette, extract *Ceriodaphnia dubia* from the sieve, and squeeze out small droplets containing them into each of the replicate vials (3 vials per each site A-F, control vials, and RTs). Each replicate should have exactly five adult *Ceriodaphnia dubia* in it. Be careful not to agitate the vials, as this could move the drops of water and strand the Cerio on the dry surface. Record the number of *Ceriodaphnia dubia* in each replicate on the data sheet provided.

14. Each site has one control replicate. Each replicate vial will be filled with 10 mL of Wasser Water. Fill each replicate using the water from the "Control" cup using the syringe labeled for Wasser Water.

15. Each site will have three replicate vials filled with water from the site. For example, site D will have vials "D_1," "D_2," and "D_3." Use the syringe for the appropriate site to extract 10 mL of water from each site's collection bottle, and fill the replicates with the water (i.e. D_1, D_2, and D_3 will all have 10 mL of water). Rinse each syringe with distilled water after use.

16. If you see Cerio floating on the surface, use one drop of water from the appropriate location (matching the labeling of the phial) from a distilled pipette to return the Cerio to the water column.

17. Perform QAQC by having a team member look over each replicate after you have finished pipetting, in order to confirm that each vial contains exactly 5 adult *Ceriodaphnia*.

18. Fill three phials designated as temperature phials with 10 mL of tap water, which Cerio will not be added to.

19. Place the replicates in the replicate tray. Place the replicates in the correctly labeled slot, so that all of the site A replicates will be in one column, and so on.

20. Place the tray in the tank. The tray should be submerged in just enough water so that the very top surface of the tray touches the water. **THE REPLICATES THEMSELVES SHOULD NOT BE SUBMERGED.**

21. Make sure the tank heater, light and pump are on, and cover the top of the tank with the lid.

DAY 2

1. Turn on the light table.

2. Remove the replicate tray CAREFULLY from the tank. Place or hold the replicates over the light table in order to count the number of living *Ceriodaphnia dubia*. Count the number of adults (ignore any young Cerio that were born during the test and any dead Cerio, which stay on the bottom of the vial and do not move when the vial is tapped), and record the total for each on each replicate on the data sheet. Make sure not to count non-*Ceriodaphnia* objects, molts left behind - though they are usually transparent, or *Ceriodaphnia* corpses. If you are having trouble distinguishing between these and live Cerio, swirl the replicate gently to see if they move on their own or not.

3. Measure temperature in the three designated temperature phials.

DAY 3

1. Repeat steps 1 and 2 from DAY 2 for sites A, B, C, D, E, and F.

2. Measure temperature in the three designated temperature phials.

3. Measure pH and conductivity (one for each replicate) using the probe. Record under final values.

4. Dump the contents of all replicate bottles in the sink before replacing the bottles in the replicate tray.

Note: Depending on which sites Chemistry is able to obtain water from, we may not perform all the steps listed under **Day 1** on one day. For example, last year we would prepare the vials for Sites A, B, and C, as well as the RTs and Controls on the first day, and the vials for Sites D, E, and F on the second, and record the data for the next two days for each accordingly.

FEEDING

1. Two people will sign up on Google Docs for each upcoming week to feed the *Ceriodaphnia* cultures during lunch or after school on each day of the week. Remember to feed the *Ceriodaphnia* if you have signed up for it! If it is a day with a run after school, the Cerio must be fed during lunch (they have to be fed 1-2 hours before the run).

2. Retrieve the YCT vials and the frozen Selenastrum from the refrigerator and bring it to the creek room. You will have to use the creek room key to unlock the door if no one is inside.

3. Lift the lid off the tank and carefully take both Cerio beakers out. Set them on the counter away from the edge.

4. Using a disposable pipet, transfer 5 mL of YCT into a 10 mL graduated cylinder. Both of these are located in a paper bag in the cabinet over the counter. Pour the YCT in the graduated cylinder into one of the beakers. Repeat with the other beaker, making sure not to pour the YCT into the same beaker twice.

5. Using the same pipette, squirt 4 mL of Selenastrum into the graduated cylinder, then pour that into the beaker. Do this for the second beaker as well. You may have to unfreeze the Selenastrum first by partially submerging its container into the water in the tank until some of it has entered liquid form.

6. Double the amount of YCT and Selenastrum given before a weekend. If you decide to clean the colony beakers on a Friday, make sure to feed after cleaning, not before.

7. Rinse both the graduated cylinder and the pipette in the sink and then distill with DI water, and return them to the bag.

8. Remembering to take the key with you, exit with the YCT vials and bag of Selenastrum, and return them to the refrigerator.

CLEANING

1. Depending on the size of the colonies, we may dump one of the beakers every week. To do this, simply take one of the beakers of Cerio outside and slowly pour the water onto the grass, making sure not to splash it everywhere.

2. Use tap water, Alconox, and a beaker brush to clean the beaker over a sink. Clean and rinse thoroughly, and make sure there are no soap bubbles left behind.

3. Fill the beaker back up to 700 mL or about halfway with Wasser Water.

4. Take the other beaker of *Ceriodaphnia* and slowly pour about half of it into the newly cleaned beaker.

5. Pour Wasser water into the uncleaned beaker until it is about ¾ full. Return both beakers to the tank and replace the lid.

6. We will clean alternate beakers every week. For example, if we dump and clean Beaker 1 on one week, the next week we will dump and clean Beaker 2, and so on.

7. If the colonies are not large enough for one to be dumped, pour the top 2/3 of the colony into a third beaker (or as much as looks clear). Pipette out juveniles from the bottom third of the beaker (making sure not to pipette any leftover food/waste) and place them the third beaker. Then dump out the original beaker and clean it with Alconox. Repeat, for moving the Cerio from the second beaker into the original beaker.

PREPARING YCT

1. See **Preparation of YCT for *Ceriodaphnia* Food** PDF. We will be scaling down the measurements by a factor of ten.

FURTHER READING (STILL MANDATORY)

1. See **A Simplified Acute Toxicity Testing Protocol With *Ceriodaphnia Dubia***, which gives extensive background information and explains what we do and why more thoroughly. *Do* read the whole packet, but it is not necessary to memorize all the information it presents. **However**, ideally by the second semester you should be able to perform the steps for each run, as well as feeding and cleaning without having to refer to this instructions packet, and know the general significance of each step.

If you have any more questions please feel free to email your managers!