Maintenance of *Hyalella azteca* Cultures

1. SCOPE AND APPLICATION

1.1. This method is based on EPA 600-R-99-064 Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates 2nd Edition March 2000. It is used to maintain cultures of *Hyalella azteca* for use in acute standard reference toxicant tests and whole sediment toxicity tests.

2. SUMMARY OF METHOD

2.1. *Hyalella azteca* are maintained in two aquaria. The aquaria are cleaned at least monthly or more frequently if needed. Aquaria are flushed with well water weekly (valve fully open for 5-7 minutes).

2.2. Interferences

2.2.1. Contaminated water, air supply and food; over-population and failure to keep culture vessels adequately cleaned can be detrimental to the health of *H. azteca*. Culture activities are recorded in the *Feeding Log and Culture Record for Hyalella azteca and C. dubia* logbook.

3. EQUIPMENT AND SUPPLIES

3.1. 80 L *Hyalella azteca* aquarium, freshwater culture

3.2. 20 L *Hyalella azteca* aquarium, 10 ppth natural seawater (NSW) culture

3.2.1. Currently, only freshwater cultures are maintained. Saltwater cultures can be established as needed.

3.3. 5 x 5 inch terra cotta tiles with 1/8 inch silicone beads on the bottom of each tile, 18

3.4. Under-gravel filter plates (used to provide shade and shelter, not for filtration)

3.5. Fine mesh net

3.6. Razor-blade scraper

3.7. #25 sieve (710 µm mesh)

3.8. 5-gal bucket, rinsed with DI water

3.9. Blue scrub pad, for culture tanks only!

4. REAGENTS AND STANDARDS

4.1. Deionized Water

4.2. Well water or 10 ppth NSW

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5. CULTURE MAINTANANCE

5.1. ***Freshwater*:** Clean tanks once per week (Wednesday) unless tank conditions warrant cleaning more often (algal blooms, culture crash, cloudiness, etc.)

***Saltwater*:** Refer to these instructions ONLY when a saltwater culture is being maintained.

5.1.1. Gently remove the substrates and under-gravel filter plate and rinse off amphipods back into tank. Place substrates in a bucket of well water until the tank is cleaned.

5.1.2. If needed, clean the substrates. At the sink, scrub the substrates with a blue scrub pad and rinse well with DI water. Rinse the cleaned substrates with well water before returning them to the cleaned tank.

***Saltwater*:** Place substrates in a bucket of 10 ppth NSW or ASW until the tank is cleaned. Rinse the cleaned substrates with fresh culture water before returning them to the cleaned tank.

Some algal growth is OK and beneficial to the amphipods. Experience will teach you how much growth is too much.

5.1.3. Scrape the front glass with a razorblade to remove the algae. Frequently rinse the algae off the razorblade with well water. Leave the back, sides, and bottom unscraped.

5.1.4. Remove the airline and the heater (**unplug before removing!**) from the tank. Stir up the bottom debris so it becomes suspended in the water column. Avoiding any amphipods, net out the waste food and debris with a fine-meshed net.

5.1.4.1. The amphipods will normally drop back down to the bottom or swim to the sides of the tank after the debris is stirred up. With a little caution, the bulk of the waste should be able to be removed without netting many amphipods.

5.1.5. Once the waste has been removed, return the substrates, airline, and heater to the tank. Be careful not to crush any amphipods when returning the substrates.

5.1.5.1. Six tiles are stacked one on top of the other to provide concealment for the amphipods. The large tank has two stacks, the small tank and **saltwater** tank only have one.

6. MAINTAINING PROPER CULTURE DENSITIES

6.1. Amphipods may be held in quite dense cultures as long as the water quality and reproduction can be maintained at healthy levels.

Signs of overcrowding may include: Cloudy water, rapid waste buildup, abnormally high mortality rates, reduced reproductive rates, lack of algal growth on glass and substrates.

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6.2. If overcrowding is an issue, remove several hundred amphipods and rinse them through a #25 sieve back into the tank. The adults will be retained and the immature amphipods will go through. Repeat until the desired number of adult amphipods has been removed. Adults remaining on the screen are deposited into a Carolina dish for later disposal.

6.3. Euthanize the adults by placing them in a bucket of ice water.

7. FEEDING AND RECORD KEEPING

7.1. If the tank has just been cleaned, feed immediately following the cleaning rather than waiting until the next scheduled feeding.

7.2. If you plan on cleaning the tank in the morning, skip the morning feeding until after the tank is cleaned.

7.3. If the tank has not just been cleaned, feed the cultures according to their feeding schedule. Refer to SOP TA-02.00.

7.4. Record tank cleanings, feedings, mortalities, loss of air or water supply, and any removal of amphipods in the *Feeding Log and Culture Record for Hyalella azteca and C. dubia* logbook.

8. DATA ARCHIVAL

8.1. Use a black, waterproof pen for all bench sheets and logbooks.

8.2. Record preparation in the *Feeding Log and Culture Record for Hyalella azteca and C. dubia* logbook.

8.3. To correct raw data entries, place a single line through the incorrect or illegible entry. Write the corrected entry near the error, initial and date the correction. Do not overwrite entries.

8.3.1. The corrected entry is written as near its appropriate space as possible or else in the comment box on the appropriate bench sheet.

8.3.2. If the correction requires an explanation, write a comment and reference the error. Initial and date the explanation.

8.4. Use sequential alphabetical annotation to identify errors and corresponding comments (use A for first error and use A for corresponding explanatory comment; use B for second error, etc.).

9. SAFETY/HAZARDOUS WASTE MANAGEMENT

9.1. Proper laboratory protection, including lab hood or ventilation system, lab coat, gloves and safety glasses, is required when working with chemicals and unprocessed samples.

9.2. Refer to FDEP Laboratory Quality Manual for sample disposal

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9.3. Refer to the FDEP Laboratory Health and Safety Plan and Hazardous Waste Management

10. REFERENCES

10.1. EPA 600-R-99-064, *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates* 2nd Edition March 2000.

10.2. FDEP Laboratory Quality Manual
http://www.dep.state.fl.us/labs/library/lab_sops.htm

10.3. FDEP Laboratory Health and Safety Plan
https://www.fldepnet.org/content/dear/bureau-laboratories-safety-documents

10.4. DEP SOP TA-02.00, *Feeding Test-Organism Cultures*.

APPENDIX OF CHANGES

09/25/2008	Added Scope and Application, Summary of the Method, Interferences, References and Appendix of Changes
06/28/2013	Updated SOP format.
06/05/2014	Changed cleaning from weekly to monthly.
01/29/2015	Formatted for 508 compliance.
06/13/2016	Updated links.

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