Maintenance of *Ceriodaphnia dubia* Mass and Chronic-Bioassay Cultures

1. SCOPE AND APPLICATION

1.1. This method is based on EPA-821-R-02-012 *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*, 5th Ed. October 2002 and the test organism culturing experience of laboratory personnel. It is used to ensure that *Ceriodaphnia dubia* (*C. dubia*) bioassay test organism cultures are maintained to provide suitable test organisms. Tests affected by this SOP are BSY-AS-FCD, BSY-AD-FCD, BSY-CS-FCD and BSY-CD-FCD.

2. SUMMARY OF METHOD

2.1. Culture vessels (cups and beakers) are prepared by soaking or washing. Cultured organisms are fed and transferred on a regular basis to prepared vessels containing fresh media. Reproduction, mortality, and health of the organisms is monitored and recorded in the *Feeding Log and Culture Record for C. dubia* logbook.

2.2. Interferences:

2.2.1. Improperly prepared culture vessels, culture media and food can be detrimental to the survival and reproduction of cultured *C. dubia*.

3. EQUIPMENT AND SUPPLIES

3.1. Mass Culture

3.1.1. *C. dubia* used for "Mass Culture" (The neonates from Monday counts)

3.1.2. 2 L beakers (2) and/or large, 20 cm, Carolina® dish

3.1.2.1. Other suitable culture containers may also be used if they meet the material guidelines found in EPA 821-R-01-012. Substitute the appropriate container name in place of "beaker" as needed.

3.1.3. Labeling tape and waterproof black marker

3.2. Chronic Cultures

3.2.1. *C. dubia* chronic culture 'A', *C. dubia* chronic culture 'B', *C. dubia* chronic culture 'C' and *C. dubia* mass cultures 'A' and 'B'. Additional cultures as needed.

3.2.2. 1 oz Plastic cups, 90

3.2.2.1. Preferably, presoak new cups with deionized (DI) water for at least 12 hours before use; else, rinse with DI water (spray) from the Toxicity lab DI hose.

3.2.3. 30 place Plexiglas trays, 3

3.2.3.1. Label one tray "*C. dubia* chronic culture A", one tray "*C. dubia* chronic culture B" and the other "*C. dubia* chronic culture C."

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3.2.3.1.1. Label along the front of the tray. The left rear corner contains cup #5. The right rear corner is the 30th slot and it contains the reserve cup, labeled "Res." The cups across the front of the tray from left to right are #1, #6, #11, #16, #21, and #26.

3.2.4. On Mondays, an additional 90 cups and 3 additional 30-place Plexiglass trays are labeled "Adults for Neonates Culture A", "Adults for Neonates Culture B", and "Adults for Neonates Culture C".

3.2.4.1. One 60-place tray can substitute for two of the 30-place trays. Label as if it were two 30-place trays. A piece of tape separates the tray into two 30-cup sections.

3.2.5. Graduated cylinders or Erlenmeyer flasks (250 mL or 500 ml are easiest to handle)

3.2.6. Dropper bulb (use with Pasteur pipet)

3.2.7. Pasteur pipet or large-bore plastic transfer pipet

3.2.8. Light table

4. REAGENTS AND STANDARDS

4.1. MHWCD (Moderately Hard Water for *C. dubia*)

4.2. YCT (Yeast, Cereal Leaves, Trout Chow) (food for *C. dubia*)

4.3. *Pseudokirchneriella subapitata (P. sub* formerly *Selenastrum capricornutum* -algae for *C. dubia*)

4.4. Deionized water

5. MASS CULTURE PREPARATION

5.1. Label two clean 2 L beakers "Mass Culture A" and "Mass Culture B". Write the current date on the label and the current MHWCD batch #.

5.2. After the chronic cultures have been transferred on Mondays, empty remaining *C. dubia* into the beakers labeled "Mass Culture."

5.2.1. Neonates from culture tray "C" can be split so that half are transferred to "Mass Culture A" and the other half to "Mass Culture B".

5.3. Add MHWCD to the new "Mass Culture" beakers to bring the volume to approximately 1.8 liters.

5.4. Take the old "Mass Culture" beakers to the sink and empty them. Rinse with water and place the dirty beakers on the "to be cleaned" glassware cart.

5.5. Place the new "Mass Culture" beakers on Culture rack B4

5.6. Feed cultures per SOP TA-02.00.

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5.7. Record feeding, cleaning, parameters and media replenishment in the *Feeding Log and Culture Records for Ceriodaphnia dubia* logbook.

5.7.1. Parameters for the "Mass Culture" beakers are typically done on Thursdays along with the DI water parameters but can be done any day of the week. DO, pH, temperature, and conductivity are taken and recorded.

6. CHRONIC BIOASSAY CULTURE PREPARATION

6.1. Empty the DI water from the cups soaking in the Plexiglas trays labeled *C. dubia* chronic culture 'A', 'B' and 'C.' On **Mondays**, empty the DI water from the cups soaking in the Plexiglas trays labeled "Adults for Neonates." If cups were not soaked the night before rinse cups with DI water three times.

6.2. Use a cylinder or flask to pour 20 (± 2) mL of MHWCD into each of the cups from Step 6.1.

6.3. Place the trays containing the *C. dubia* chronic 'A', 'B' and 'C' bioassay cultures on the light table.

6.4. On **Mondays**, count the neonates and record their number in the *Culture Records for Ceriodaphnia dubia* logbook. Also, record any dead or missing adults. Using a Pasteur pipet with a dropper bulb or a plastic transfer pipet, transfer one neonate to the corresponding new culture cup (1-29). Transfer 10 neonates from the old reserve cup to the new one. Any dead or missing adults should be replaced using adults from the reserve cup. At the same time, transfer the adult from the old culture to the corresponding cup on the "Adults for Neonates for Tuesday" tray to produce <24-hour old neonates for use in Tuesday testing.

6.4.1. If a culture cup does not contain at least eight healthy neonates, a neonate is transferred from a cup containing eight or more healthy neonates. A note is made in the culture logbook if this occurs. More than one cup can be loaded from a cup containing more than eight healthy neonates.

6.4.2. While transferring, do not crush the *C. dubia* by capturing them against the side or bottom of the cup. When releasing the *C. dubia* into the new cup, release them under the surface of the liquid by gently squeezing the dropper bulb or transfer pipet.

6.4.3. The neonate transferred to the culture cups on Monday grows into the adult that is transferred on Wednesdays and Fridays, and that is replaced the following Monday.

6.4.4. If *C. dubia* tests are not being run, the adults that would be transferred to the "Adults for Neonates for Tuesday" tray should be transferred to a glass Carolina dish instead. Label the dish "Adults for Neonates", the date and time, and the MHWCD batch #.

6.5. On **Wednesdays** and **Fridays**, repeat Steps 6.1.-6.4. Transfer all adults in the old reserve cup to the new reserve cup.

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6.6. After all transfers are complete for the day, feed the newly transferred *C. dubia* (refer to SOP TA-02.00). Food can be added to the culture cups after the MHWCD is poured and prior to the transfers. Place the trays in Incubator #4 (maintained at ~25 ºC).

6.7. Empty remainder of young neonates and adults from the old culture cups into the culture room sink.

6.8. Rinse the inside of each empty cup with a strong stream of DI water, then fill the emptied cups with DI water and allow to soak until the next transfer.

6.8.1. If a cup has a film build-up that the DI water cannot dislodge, discard the cup and replace it with a new one.

6.9. Record daily feeding in the *Feeding Log and Culture Record for Hyalella azteca and C. dubia* logbook. (Feeding on Culture transfer days are also recorded in the *Culture Records for Ceriodaphnia dubia* logbook).

7. DATA ARCHIVAL

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

7.2. Record transfers in the *Culture Records for Ceriodaphnia dubia* logbook. Record culture maintenance and feedings in the *Feeding Log and Culture Records for Ceriodaphnia dubia* logbook.

7.3. 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.

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

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

7.4. 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.).

8. QUALITY CONTROL

8.1. Preferably, new cups are soaked with DI water for at least 12 hours before use; else, cups are rinsed with DI water (from the Toxicity lab DI water hose). The rinse is to remove any potential water-soluble contaminants from the inner surface of the cups.

8.2. To keep the cultures healthy, mass cultures are generally cleaned and replenished every Monday unless cleaning is needed sooner (the water develops a foul odor, excessive algae growth, the cultures crash, etc.).

8.3. Chronic-bioassay cultures are transferred every Monday, Wednesday, and Friday. Transfer days can be adjusted to account for holidays. Based on knowledge of when

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samples will be received, this ensures that the organisms will be an appropriate age for the test(s) being performed.

8.4. Culture media and food are prepared per their respective SOPs using sterile glassware and aseptic techniques to prevent contamination.

9. SAFETY/HAZARDOUS WASTE MANAGEMENT

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

9.2. Read MSDS information for reagent handling and disposal.

10. REFERENCES

10.1. EPA 821-R-02-012 *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*, 5th Ed. October 2002.

10.2. DEP SOP TA-03.01, *Preparation of 20% Dilute Mineral Water (DMW)*.

10.3. DEP SOP TA-03.15, *Preparation of Synthetic Moderately Hard Freshwater*.

10.4. DEP SOP TA-01.01, *YCT (Yeast, Cereal Leaves, Flake-Fish Food, or Trout Chow) Preparation*.

10.5. DEP SOP TA-01.04, *Pseudokirchneriella subcapitata* Culture for *Ceriodaphnia dubia* Food.

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

10.7. FDEP Laboratory Quality Manual https://floridadep.gov/dear/florida-dep-laboratory/content/dep-laboratory-quality-assurance-manual-and-sops

10.8. FDEP Laboratory Health and Safety Plan Health and Safety Plan

10.9. MSDS information sheets.

APPENDIX OF CHANGES

09/25/2008	Added Scope and Application, Summary of the Method, Interferences, References and Appendix of Changes.
06/27/2013	Reformatted SOP
06/05/2014	Updated procedure with ‘C’ culture included
01/29/2015	Formatted for 508 compliance
06/08/2016	Updated links
05/29/2018	Updated procedure with “Mass Culture” parameter information and “Adults for Neonates” Carolina dish information. Update logbook names.

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