Feeding Test-Organism Cultures

1. SCOPE AND APPLICATION

1.1. These methods are based on EPA 821-R-02-012 "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms" and practical experience maintaining cultures in the laboratory. They are used for organisms cultured in the laboratory and organisms purchased from vendors.

2. SUMMARY OF METHOD

2.1. Unless otherwise noted, organisms are fed the appropriate quantity and type of food once daily Monday through Friday, as well as on weekends and holidays. At the discretion of the toxicity lab coordinator, maintenance of laboratory cultured organisms (*Ceriodaphnia dubia*) may be suspended for one calendar day, but not two consecutive calendar days during a weekend or holiday period.

3. EQUIPMENT AND SUPPLIES

3.1. 1 mL and 10 mL Volumetric pipettes

3.2. 2.5 mL Plastic transfer pipettes

3.3. 0.2 mL Pipettor (fixed)

3.4. YCT, prepared per SOP TA-01.01

3.5. *Pseudokirchneriella subcapitata*, prepared per SOP TA-01.04

3.6. *Artemia nauplii*, prepared per SOP TA-01.03 (less than 24-hour old nauplii are fed to test organisms)

NOTE: Toxicity Pipettes and Pipettors are calibrated per SOP TA-06.32

3.7. *Feeding Log and Culture Records for Ceriodaphnia dubia* logbook

3.8. *Artemia nauplii Culture and Feeding* logbook.

4. FEEDING METHODS

4.1. ***Ceriodaphnia dubia***

4.1.1. Mass Culture: Feed 2 mL of YCT and 2 mL of *Pseudokirchneriella subcapitata,* mixed, per liter of culture using a disposable transfer pipette. A new pipette is used for each feeding.

4.1.1.1. *C. dubia* mass cultures are kept in a minimum of two vessels (4 L beaker(s) and/or 8" diameter Carolina dish(es)), on tray B3 in room B246. The vessels are labeled "*Ceriodaphnia dubia*" "Mass Culture

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A, *B*, etc., with the date the culture was started (or renewed/changed weekly) and MHWCD batch #.

4.1.2. Chronic-test culture: Feed 0.2 mL of a mixture of 50% YCT and 50% *Pseudokirchneriella subcapitata* per 20 mL of culture medium daily during test duration (i.e. per 30-mL disposable plastic cup). Organisms are fed two hours prior to test initiation and renewal.

4.1.3. Acute-test culture: Feed 0.2 mL of a mixture of 50% YCT and 50% *Pseudokirchneriella subcapitata* per 20 mL of culture medium on day 0 and day 2 of the test (i.e. per 30-mL disposable plastic cup). Organisms are fed two hours prior to test initiation and renewal.

4.1.4. *C. dubia* chronic cultures are kept in Incubator #4 in room B246. Feed these cultures 0.2 mL of a mixture of 50% YCT and 50% *Pseudokirchneriella subcapitata* per 20 mL of culture medium daily (i.e. per 30-mL disposable plastic cup), including Saturday (see Section 2 - 2.1). When the C. dubia are transferred to a new tray, the food is added to the new organism cups prior to transferring the *C. dubia* into the new cups.

4.2. ***Cyprinella leedsi, Pimephales promelas, Menidia beryllina, Americamysis bahia***

4.2.1. Place purchased organisms in 4 L plastic Carolina® dishes (1 per culture).

4.2.2. Feed 1-3 mL *Artemia nauplii* per Carolina® dish, once per day.

4.2.2.1. *C. leedsi* and *P. promelas* should be > 1day old; *M. beryllina* should be > 5 days old and *A. bahia* should be 1 day old before they are fed artemia. Organisms are generally received at appropriate ages to be fed upon arrival.

4.2.2.2. Check the bottom of each dish for accumulation of *Artemia nauplii* and adjust the amount used accordingly. Remove and discard excess/dead artemia from the culture dishes (daily).

4.2.3. During testing, feed fish or mysids 0.2 mL of artemia 2 hours prior to renewal and after renewal. Artemia is prepared and counted per SOP TA-01.03.

5. DATA ARCHIVAL

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

5.2. Record feedings in the appropriate *Culture Records* logbook.

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

5.3.1. The corrected entry is written as near as possible to the error or in the comment box on the appropriate bench sheet.

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5.3.2. If the correction requires an explanation, write a comment and reference the error with a letter. Initial and date the explanation. Do not attempt to put all information (correction and comments) into a small space.

5.3.3. 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.).

6. SAFETY/HAZARDOUS WASTE MANAGEMENT

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

6.2. Refer to Laboratory Quality Manual for sample disposal.

6.3. Refer to the Laboratory Health and Safety Manual and Hazardous Waste Management.

6.4. Read MSDS information for reagent handling and disposal.

7. REFERENCES

7.1. EPA 821-R-02-012 Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms.

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

7.3. DEP SOP TA-01.03*, Artemia Nauplii* Culture.

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

7.5. DEP SOP TA-06.32, Calibration and Verification of Mechanical and Disposable Pipettes in the Bioassay-AGP Lab

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

7.7. Laboratory Health and Safety Manual and Hazardous Waste Management https://www.floridadep.net/resources/health-safety-manual

APPENDIX OF SIGNIFICANT CHANGES

01/22/2008 Added Scope and application, Summary of Method and Appendix of Changes. Deleted *D. pulex* chow from Materials. Added “4 mL of YCT and 4 mL of *Pseudokirchneriella subcapitata*” to *D. pulex* Step 1.

09/30/2013 Major revision. Removed feeding instructions for newly hatched fry and adults that were cultured in-house that are now being purchased.

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02/09/2015	Formatted for 508 compliance.
09/11/2015	Updated references.
10/07/2016	Updated references.
10/06/2017	*Hyallela azteca* section removed
08/29/2018	Updated Font. Revised the frequency for feeding organisms.
07/02/2019	Removed unused SOP from references
08/26/2020	Added test feeding instructions
07/09/2021	Updated for clarity, edited to reflect universal language, checked for grammar, updated SOP names, and updated reference link.
07/31/2023	Edited grammar and punctuation. Formated font to be Calibri. Updated link.

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