Maintenance of *Daphnia pulex Cultures*

STEPS **COMMENTS**

Scope and Application

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 *Daphnia pulex* bioassay test organism cultures are maintained so as to provide suitable test organisms should the laboratory begin testing with *D. pulex*.

Summary of the Method

Cultured organisms are fed and transferred on a regular basis to prepared vessels containing fresh media. Health of the organisms is monitored and recorded in the *Culture Records for C. dubia, D. pulex and H. azteca* logbook.

Interferences

Improperly prepared culture vessels, culture media and food can be detrimental to the survival and reproduction of cultured *D. pulex*.

Materials

1. *Daphnia pulex* "Mass" culture

Daphnia pulex are not maintained as a source of test organisms so a "test species" culture record is not maintained at this time; however, culture feeding and maintenance is recorded in the "*Culture records for*…" laboratory notebook. The "Mass" culture is kept in a 4 L beaker on the culture tray labeled "B4". The *Daphnia pulex* "Reserve" culture is kept in an 8" (20.32 cm) Carolina® dish on Tray B4.

2. *Daphnia pulex* "Reserve" culture

3. Well water
4. Siphon tube
5. One 4-L beaker
6. Carolina dish, 8" (20.32 cm)
7. Wastewater bucket, 5-gal (18.93 L)
8. Clean large bore transfer pipette
9. *Culture Records for C. dubia, D. pulex and H. azteca* laboratory notebook.

Methods

Mass Cultures

1. Remove the "mass culture" from tray "B4" and place it on the bioassay work table. Siphon off approximately half of the water and *D. pulex* from the "current" culture into the waste bucket.

Clean once per week (Friday) unless culture conditions warrant cleaning more often (algae blooms, culture crash, cloudiness, etc.).

2. Pour the remaining culture water and *D. pulex* into the clean 4 L beaker.

3. Label the new, clean beaker with "*Daphnia pulex* Mass Culture", the "date", and your "initials".

STEPS	COMMENTS
4. Add well water to the new beaker to bring the volume to the 3-L mark. Return the new culture to tray "B4".	
5. Feed the culture per SOP TA-02.00.	
6. Dispose of the organisms in the waste bucket per Step 7 of the "Reserve Culture" method.	See page 3
7. Place the old culture beaker on the dirty glassware cart for cleaning.	
8. Record culture maintenance and feeding in the *Culture Records for C. dubia, D. pulex and H. azteca* laboratory notebook.	

Reserve Cultures

1. Fill the clean 8" (20.32 cm) Carolina dish 3/4 full with well water.	Clean once per week (Friday) unless culture conditions warrant cleaning more often (algae blooms, culture crash, cloudiness, etc.).
2. Remove the "reserve culture" from tray "B4" and place it on the bioassay work table. Using the transfer pipette, carefully transfer 50-75 of the *D. pulex* to the new, clean dish.	Transfer both large and small organisms (approximately half large and half small).
3. Label the new, clean dish with "*Daphnia pulex* Reserve Culture", the "date", and your "initials".	
4. Place the new "reserve culture" on tray "B4".	
5. Feed the culture per SOP TA-02.00.	
6. Pour the culture water and remaining daphnia in the "old" "reserve dish" into the waste bucket.	
7. Euthanize the organisms in the waste bucket by filling the bucket with ice and letting it sit for 20-30 minutes. Pour the iced water and euthanized organisms into the laboratory sink and flush the sink with tap water.	
8. Place the old culture dish on the dirty glassware cart for cleaning.	
9. Record culture maintenance and feeding in the *Culture Records for C. dubia, D. pulex and H. azteca* laboratory notebook.	

STEPS **COMMENTS**

References

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.
2. Feeding Test-Organism Cultures, DEP SOP TA-02.00
3. Biology Quality Manual, FDEP Bureau of Laboratories, March 2008

Appendix of Changes

9/25/2008 Added *Scope and Application, Summary of the Method, Interferences, References and Appendix of Changes*