Artemia nauplii Culture

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. It is used to prepare food for bioassay test organisms.

2. SUMMARY OF METHOD

2.1. *Artemia* cysts are placed in a separatory funnel with artificial seawater (ASW). The cysts are aerated for ~24 hours. Aeration is stopped; the nauplii are allowed to settle and are then emptied into a light box for collection. Collected nauplii are aerated and used as organism food as needed. Culture information is recorded in the *Culture Records for Artemia* logbook.

3. EQUIPMENT AND SUPPLIES

3.1. 2 L Separatory funnel (1 L can be used)

3.1.1. Adjust number of funnels to suit laboratory needs. One funnel will hatch sufficient nauplii for 3-4 chronic definitive bioassays.

3.2. Funnel holder

3.3. *Artemia* cysts

3.3.1. Ensure that appropriate chemical analyses are performed for each lot (refer to USEPA 821-R-02-012) or that a suitability test is performed per TA-01.07.

3.3.2. See SOP TA-01.05 for the decapsulation procedure if necessary to increase yield.

3.4. Aeration device (tubing and a 1 mL pipet), connected to the FDEP compressed air outlet(s)

3.5. Light box

3.6. Siphon tube (50-60 cm length, ~ 4 mm diameter)

3.7. 1 L jars, 2

4. REAGENTS AND STANDARDS

4.1. Deionized Water

4.2. Artificial seawater (ASW), 15-20 ppth salinity prepared per SOP TA-03.02.

4.2.1. Natural seawater (NSW) diluted to the appropriate salinity can be used instead of ASW

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5. CULTURE PREPARATION

5.1. 24 hours before nauplii is needed for food, use the 1 L jar located on the ASW tank to pour 1600-1800 mL of ASW into the 2 L separatory funnel.

5.1.1. ALWAYS rinse the ASW collection jar with deionized water prior to placing it into the tank.

5.1.2. DO NOT ALLOW ARTEMIA CYSTS TO ENTER THE ASW TANK.

5.2. Place 1 heaping teaspoon of *Artemia* cysts into funnel.

5.2.1. If bacterial growth in test chambers fed with *Artemia* is an issue, refer to Step 7.2. in the QC section of this SOP.

5.3. Place funnel into funnel holder.

5.3.1. Funnel should be placed in a water bath or incubator but can be kept on the counter in B246 to maintain the water/cyst temperature at ~25 ºC.

5.4. Place the aeration device into the funnel and moderately aerate the cysts.

5.5. Record the “date,” “set-up time,” “*Artemia* lot number,” “date used,” and “time used” in the *Culture Records for Artemia* logbook.

5.5.1. “Date used” should be the day after the set-up date.

5.6. For morning feedings, remove aeration device and let *Artemia* settle for at least 5, but not more than 10, minutes.

5.6.1. Settling for more than 10 minutes may kill *Artemia* due to suffocation.

5.7. Add 1 L of ASW to the light side of the light box. (For Steps 5.7. through 5.14., refer to **Figure 1**.)

5.8. With the divider down, drain the settled *Artemia* from the funnels into the dark side of the light box.

5.9. Once the water has stopped swirling, turn on the light and **slowly** lift the divider until the ledge on the divider can rest on the lid of the dark side of the box.

5.9.1. The lid must be lifted slowly to prevent swirling the water and drawing *Artemia* cysts into the light side of the box.

5.10. Place a towel over the light side of the box and wait (~10 – 15 minutes) for the *Artemia* to swim to that side.

5.11. Empty the “48-hour” *Artemia* jar and rinse with DI water.

5.12. Pour contents of “24-hour” *Artemia* jar into the 48-hour jar and rinse the 24-hour jar with DI water.

5.13. Place the 48-hour jar on tray B-2, aerate, and add ASW to fill the jar approximately 2/3 full.

5.13.1. 48 hr old *Artemia* can be used to feed culture organisms, but not test organisms.

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5.14. Use the siphon tube to siphon out the concentrated *Artemia* from the light box into the 1 L jar labeled "*Artemia* 24- hours."

5.15. Feed the appropriate organisms.

5.15.1. Organisms that *Artemia* are fed to include: *C. leedsi, P. promelas, A. bahia,* and *M. beryllina*. See SOP TA-02.00.

5.15.2. If there appears to be a nutritional problem in organisms fed exclusively *Artemia nauplii*, refer to Step 7.3. in the QC section of this SOP.

5.16. Fill 24-hour jar containing *Artemia* approximately 2/3 full with ASW, aerate, and use for afternoon feeding. After feeding, return jar to tray B-2 and resume aeration.

5.17. Rinse the light box with DI water to remove cysts and salt water.

5.18. Start *Artemia* in funnel as needed.

6. DATA ARCHIVAL

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

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

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

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

6.2.2.1. 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.).

6.3. Record culture preparation in the *Culture Records for Artemia* logbook.

7. QUALITY CONTROL

7.1. *Artemia* are cultured as needed to feed test organisms. Cysts are set up approximately 24 hours before nauplii are needed for food. In-house tests suggest that hatching doesn't begin or is insignificant prior to twelve hours after setup. Thus, the 1300 setup time in the *Artemia* culture book is arbitrary.

7.2. If bacterial growth in test chambers fed with *Artemia* is an issue:

7.2.1. Place the cysts in a beaker with 100 mL of DI water.

7.2.2. Add 10 mL of bleach and mix well.

7.2.3. After 3 minutes, rinse cysts onto a 100-120 µm screen and rinse several times with DI water.

7.2.4. Place rinsed cysts into the funnel.

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7.3. Should a nutritional problem develop in organisms that are fed exclusively *Artemia nauplii*, information from EPA Gulf Breeze suggests that 10 mL of concentrated *Isochrysis* algae added to the cysts should resolve the problem. Roto-Rich® can also be used as a nutritional supplement. Fatty acid enhancement such as Selco® can also be used. Follow instructions on package.

7.4. Funnels are placed in a water bath to maintain the water/cyst temperature at ~25 ºC since the compressed air temperature is extremely low during the winter months.

8. SAFETY/HAZARDOUS WASTE MANAGEMENT

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

8.2. Refer to Bureau Quality Manual for sample disposal http://www.dep.state.fl.us/labs/library/lab_sops.htm.

8.3. Refer to the Laboratory Health and Safety Manual and Hazardous Waste Management https://www.fldepnet.org/content/dear/bureau-laboratories-safety-documents.

8.4. Read MSDS information for reagent handling and disposal.

9. REFERENCES

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

9.2. DEP SOP TA-01.05. Decapsulation of Artemia cysts.

9.3. DEP SOP TA-01.07. YCT (Yeast, Cereal Leaves, Flake-Fish Food, or Trout Chow) and Artemia Suitability Tests for NELAC D.2.8(i).

9.4. DEP SOP TA-02.00. Feeding Test-Organism Cultures.

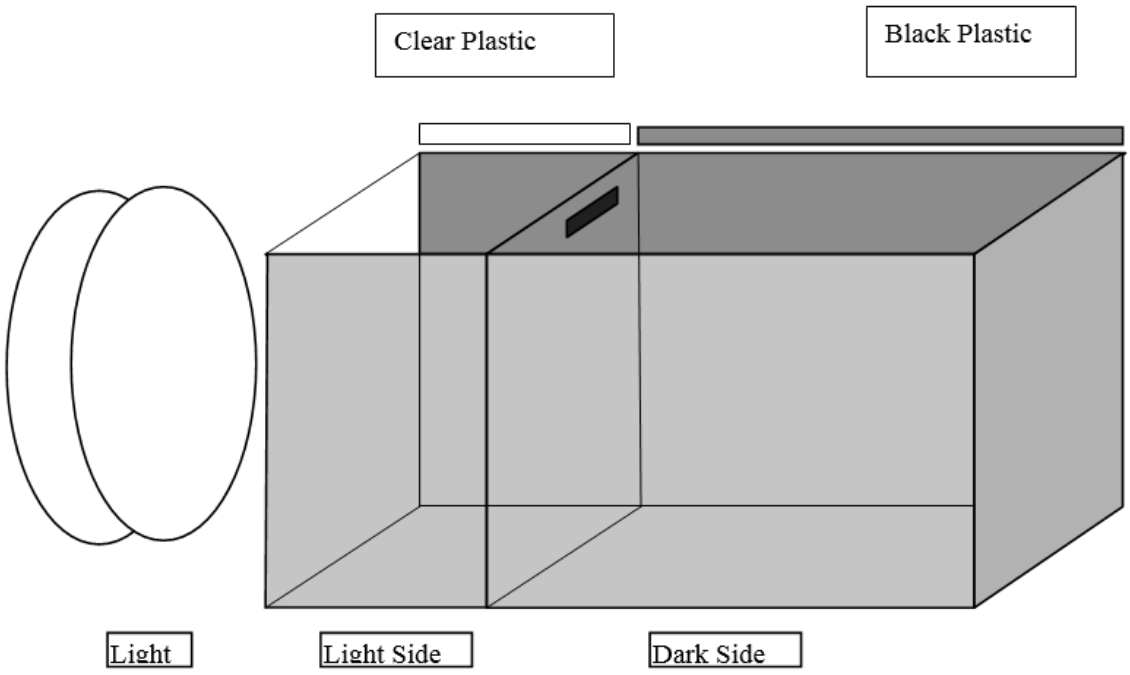
9.5. DEP SOP TA-03.02. Preparation of Artificial Seawater (ASW).

9.6. QA Manual Health and Safety Manual

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10. TABLES, DIAGRAMS

Figure 1. Lightbox for Artemia Separation.



APPENDIX OF CHANGES

07/24/2008	Added Scope and Application, Summary of the Method, References and Appendix of Changes.
04/29/2013	Reformatted SOP.
02/09/2015	Formatted for 508 compliance.
11/04/2015	Updated links.

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