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 given time to settle and are then emptied into a light box for collection. Collected nauplii are aerated and counted then used as organism food when needed. Culture information is recorded in the *Artemia nauplii Culture and Feeding* logbook.

3. EQUIPMENT AND SUPPLIES

3.1. Two separatory funnels (1 L or 2 L can be used)

3.1.1. Adjust number of funnels to suit laboratory needs. One funnel will hatch sufficient nauplii for 2-3 chronic definitive bioassays.

3.2. Funnel holder (Plastic beakers for inside the incubator and glass jars for holding the funnels outside the incubator)

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

3.8. Hand lens

3.9. Handheld counter, 2

3.10. 100 mL Graduated Cylinder

3.11. 50 mL Graduated Cylinder

3.12. Petri dish

3.13. 1 mL fixed volume pipette

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3.14. 0.2 mL fixed volume pipette

4. REAGENTS AND STANDARDS

4.1. Deionized Water

4.2. Artificial seawater (ASW), filtered, 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

5. CULTURE PREPARATION

5.1. 24 hours before nauplii are needed for food, collect ASW from the carboy containing filtered ASW into a beaker or flask and pour 1600-1800 mL of ASW into the 2 L separatory funnel; or, approximately 800 ml of ASW into a 1 L separatory funnel

5.1.1. ALWAYS rinse the funnels and ASW collection vessel with deionized water prior to collecting ASW.

5.1.2. Avoid artemia cyst or nauplii contamination of the ASW carboy.

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

5.2.1. If nauplii are to be used to feed mysids in acute or chronic tests, add 1ml of Selcon™ fatty acid concentrate to the funnel(s).

5.2.2. 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,” “ASW Batch used,” “date used,” “Organism Fed,” “Amount of Artemia in 0.2mL,” and “time used” in the *Artemia nauplii Culture and Feeding 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 on the counter.

5.6.1. Settling for more than 10 minutes may kill *Artemia* due to potential DO issue.

5.6.2. Make sure to cover the *Artemia* funnels with a towel to help them settle to the bottom.

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**.)

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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 should not be fed to test organisms.

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. Fill the 24-hour jar containing *Artemia* approximately with ASW (about 2/3 full), aerate.

5.16. Count the *Artemia* as directed by Step 6 of this SOP and record the count in the *Artemia Culture and Feeding Log.*

5.17. Feed the appropriate organisms.

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

5.17.2. 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.

5.18. After feeding, return jar to tray B-2 and resume aeration.

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

5.20. Start *Artemia* in funnel as needed.

6. COUNTING *ARTEMIA NAUPLII*

6.1. Prepare *Artemia nauplii* cultures as stated in Step 5 of this SOP.

6.1.1. Be sure cultured *Artemia* are homogenized in the 1L chamber before counting.

6.2. Remove a 100-mL aliquot of *Artemia* solution from the chamber labeled "24 Hr."

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6.3. Pour the *Artemia* (6.2) onto a 60 µm mesh screen, rinse with DI water and concentrate the rinsed *Artemia* into a 10-mL beaker.

6.3.1. Be sure organisms are concentrated to a volume of 10 mL.

6.4. Homogenize the 10 mL of concentrated *Artemia* and pipette 0.2 mL of concentrated *Artemia* into a 50-mL graduated cylinder and use DI water to bring the solution to a total volume of 50-mL.

6.4.1. Be sure the 50-mL solution is well mixed, as the *Artemia* will quickly move to the bottom of the cylinder and skew the measurement.

6.5. Pipette three 1 mL aliquots from the 50-mL solution onto a petri dish, being sure to keep the three aliquots separate.

6.6. Using a hand lens (to better see the *Artemia*), count the *Artemia* in each aliquot using the handheld counter to tally the count. After the three aliquots have been counted, average the three counts and multiply this number by 50. This will give you an estimated amount of *Artemia* per 0.2 mL of concentrate.

6.7. Further dilute or concentrate as needed to achieve the EPA recommended amount of *Artemia* for each test organism.

6.8. Record the final used volume of concentrate in the *Artemia nauplii Culture and Feeding logbook*.

7. DATA ARCHIVAL

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

7.2. 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.

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

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

7.2.2.1. 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.).

7.3. Record culture preparation and counts in the *Artemia nauplii Culture and Feeding logbook*.

8. QUALITY CONTROL

8.1. *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.

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Thus, setup time from 0600 – 1800 the day before use should provide nauplii < 24 hours old for use in feeding test organisms.

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

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

8.2.2. Add 10 mL of bleach and mix well.

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

8.2.4. Place rinsed cysts into the funnel.

8.3. Proactive use of a fatty acid supplement such as Selcon™, concentrated *Isochrysis* algae (information from EPA Gulf Breeze), or a rotifer food such as Roto-Rich® can enhance the nutritional value of artemia nauplii.

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

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 Laboratory Quality Manual for sample disposal.

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

9.4. 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.

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

10.3. DEP SOP TA-01.07. YCT (Yeast, Cereal Leaves, Flake-Fish Food, or Trout Chow) and Artemia Suitability .

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

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

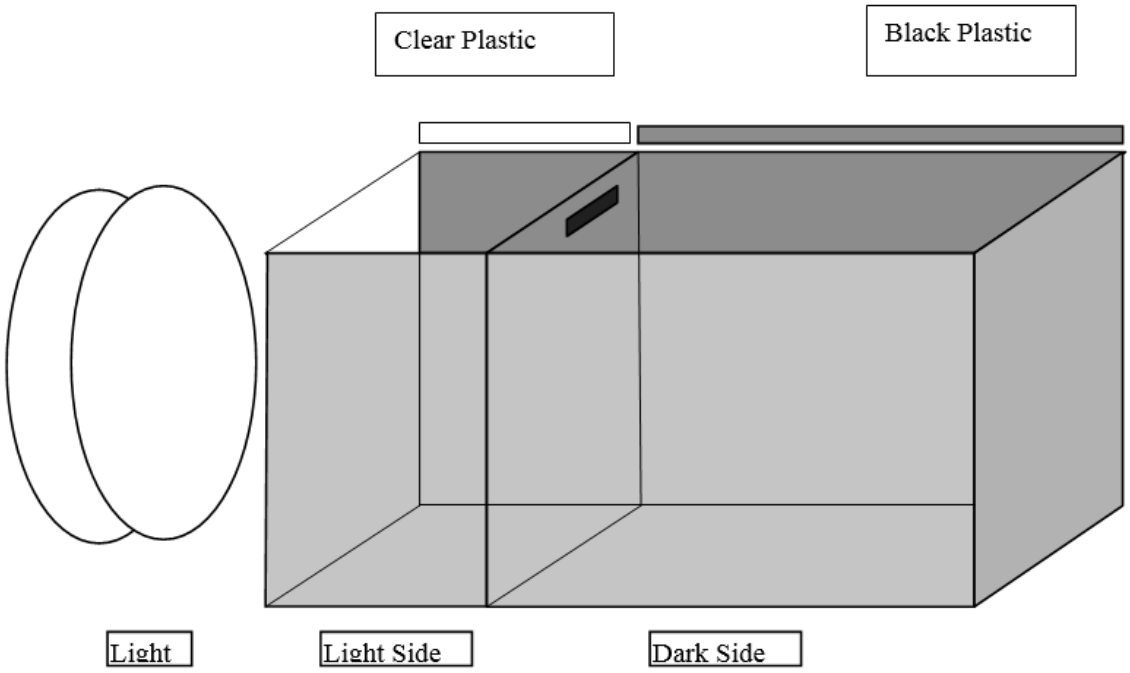
10.6. Laboratory Quality Manual.

10.7. QA Manual Health and Safety Manual

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11. 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.
03/29/2018	Added Counting *Artemia nauplii,* Updated hyperlinks, Updated Equipment section.
03/07/2019	Reviewed for grammatical errors.

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