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. After aeration is stopped, the *Artemia* are given time to settle and are then emptied into a light box for collection. Collected *Artemia* are aerated, counted, and used as organism food, as 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 enough *Artemia* for 2-3 chronic definitive bioassays. Note: Two funnels are typically set up for 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 the EPA acute method (EPA 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 to increase yield, if necessary.

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

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3.12. Petri dish

3.13. 1 mL fixed volume pipette

3.14. 0.2 mL fixed volume pipette

3.15. *Artemia nauplii Culture and Feeding* logbook

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 *Artemia* are needed for food, collect ASW from the carboy containing filtered ASW into a beaker or flask. Pour 1600-1800 mL of ASW into a 2 L separatory funnel or, if using a 1 L separatory funnel, use approximately 800 mL of ASW.

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 *Artemia* contamination of the ASW carboy.

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

5.2.1. If *Artemia* are to be used to feed mysids in acute or chronic tests, add 1ml of Selcon™ fatty acid concentrate to each funnel used.

5.2.2. If bacterial growth in test chambers fed with *Artemia* is an issue, refer to Section 8.2. 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 the lab, (room B246; which is temperature controlled) if necessary, 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, and analyst's initials in the *Artemia nauplii Culture and Feeding* logbook.

5.6. For morning feedings, remove aeration device and let *Artemia* settle for at least 5 but no more than 10 minutes on the counter.

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

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5.6.2. It is advised to cover the *Artemia* funnels with a towel to help them settle to the bottom.

5.7. Add 1 L of ASW to the side of the light box with the light. (For Sections 5.7 through 5.15, refer to **Figure 1** at the end of this document.)

5.8. With the divider down, turn the stopper of the funnel to drain the settled *Artemia* into the dark side of the light box.

5.9. Return the funnel to the funnel holder and let the *Artemia* once again settle to the bottom. After another 5 minutes, repeat step 5.8. If necessary, return the funnel to the funnel holder again to let the *Artemia* settle to the bottom again and repeat step 5.8.

5.9.1. Typically, after two rounds of letting the *Artemia* settle to the bottom of the funnel (and then emptying into the light box), the water 'runs clear' indicating there are no more *Artemia* left to empty.

5.10. 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.

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

5.11. Place a towel over the box and wait (~15 minutes) for the *Artemia* to swim to the lighted side.

5.12. Empty the "48-hour" *Artemia* jar and rinse with DI water.

5.13. Pour contents of "24-hour" *Artemia* jar into the "48-hour" jar and rinse the "24-hour" jar with DI water.

5.14. Place the new "48-hour" jar on shelf B-4, aerate, and add ASW to fill the jar approximately 2/3 full.

5.14.1. 48-hr old *Artemia* can be used to feed culture organisms but should not be fed to test organisms.

5.15. Using the siphon tube, siphon out the concentrated *Artemia* from the light box into the 1 L jar labeled "*Artemia* 24-hours" to approximately 2/3 of the way full and aerate.

5.16. Count the *Artemia* as directed in Section 6 of this SOP. Record the count in the *Artemia Culture and Feeding Log.*

5.17. Feed organisms as necessary and record "date used", "organism fed", "amount of *Artemia* in 0.2 mL", and "time used" in the *Artemia nauplii Culture and Feeding* Logbook.

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

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5.17.2. If there appears to be a nutritional problem in organisms fed exclusively *Artemia* nauplii, refer to Section 8.3. in the QC section of this SOP.

5.18. After feeding, return "24-hour" jar to shelf B-4 and resume aeration.

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

5.20. Start another batch of *Artemia* in funnel(s), as needed.

6. COUNTING *ARTEMIA* NAUPLII

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

6.1.1. Swirl the *Artemia* in the 1L "24-hour" jar to homogenize before counting.

6.2. Pour 100mL of the *Artemia* from the "24-hour" jar into a 100mL graduated cylinder. Then, pour the *Artemia* from the 100mL cylinder into a 60 µm mesh sieve and rinse them with DI water.

6.3. Concentrate the rinsed *Artemia* into a 50-mL beaker by slowly adding DI to the sieve, letting most of the water drain, and pouring the concentrated *Artemia* from the sieve into the 50mL beaker.

6.4. Continue rinsing the sieve with DI water and collecting the *Artemia* / water mixture until you reach approximately 25 mL.

6.4.1. The total volume of *Artemia* needed depends on the concentration of *Artemia*. This can be eyeballed by an experienced analyst. If the *Artemia* yield appears to be low, aim for a lower volume that is more concentrated; if the yield looks high then aim for a higher, less concentrated volume. Historically, the lab's *Artemia* yield is high and contains the proper concentration at about 30 mL volume.

6.5. Homogenize the 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.

6.5.1. 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.

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

6.7. Using a hand lens (to magnify the *Artemia*), count the *Artemia* in each aliquot using the handheld counters. After the three aliquots have been counted, average the three, and multiply by 50. The result is the estimated amount of *Artemia* per 0.2 mL of concentrate.

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6.8. Further dilute or concentrate as needed to achieve the EPA recommended amount of *Artemia* for each test organism. The *Artemia* count should be approximately 800-1100 organisms per 0.2mL.

6.8.1. Dilute by adding a little more DI to the concentrated *Artemia* in the beaker.

6.8.2. Concentrate by pouring the concentrated *Artemia* back in the sieve, this time concentrating to a lesser volume. See step 6.3 above.

6.9. Record the final volume of concentrate that was used 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, date and initial the correction. Do not overwrite entries.

7.2.1. The corrected entry is written as near as possible to the error or 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. Do not attempt to put all information (correction and comments) into a small space

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 *Artemia* are needed for food. In-house tests suggest that hatching doesn't begin or is insignificant prior to twelve hours after setup. Thus, setup time from 6am-4pm the day before use should provide *Artemia* that are <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.

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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

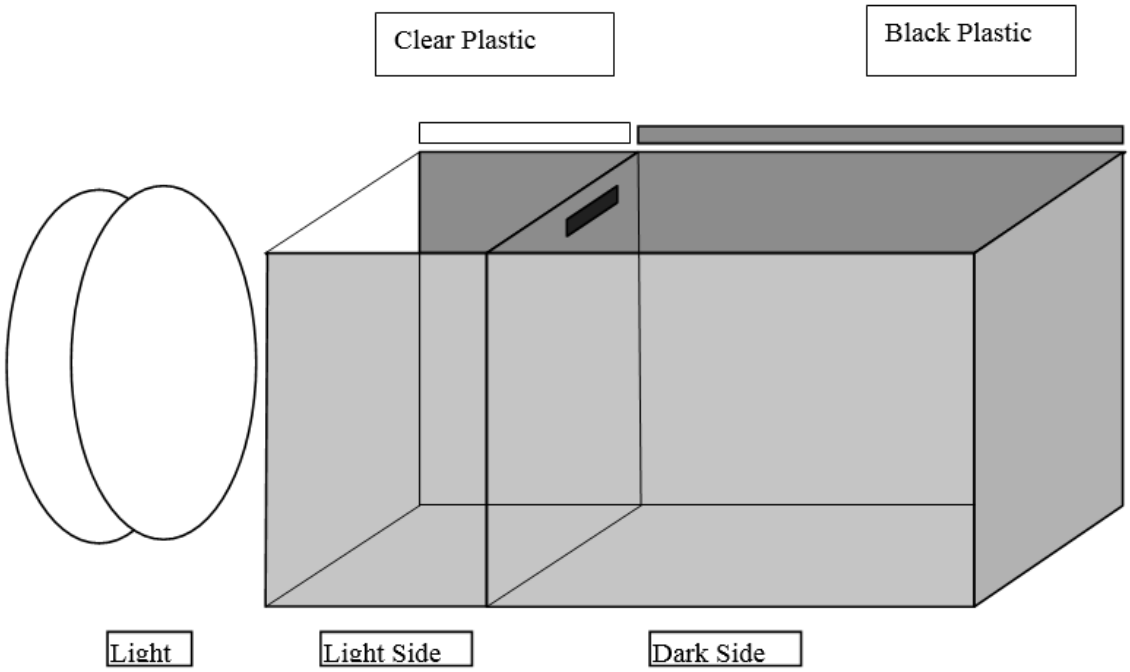
10.7. QA Manual Health and Safety Manual

10.8. MSDS Information Sheets

11. TABLES, DIAGRAMS

Figure 1. Lightbox for *Artemia* separation.

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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.
03/04/2020	Updated links and reviewed for grammatical errors.
09/23/2020	Fixed grammatical errors in Section 5.17.
09/11/2021	Updated with universal language. Updated links. Added logbook to equipment section.
09/05/2023	Updated links, added reference 10.8, and reviewed for grammatical errors and continuity.
09/04/2023	Added section 6.4 and 6.4.1 to better reflect in lab practice.

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