Artemia nauplii Culture, Collection, and Counting

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) to be cultured. The cysts are aerated for ~24 hours. After aeration, 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 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 the number of funnels to suit laboratory needs. One funnel will hatch enough *Artemia* for 2-3 chronic definitive bioassays. Two funnels are typically set up for bioassays.

3.2. Funnel holders (Plastic beakers inside the incubator and glass jars 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 section 9.3 for the decapsulation procedure to increase yield, if necessary.

3.4. Aeration device (tubing and a 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. Mesh sieve (with 150 µm screen)

3.16. *Artemia Culture and Feeding* logbook

4. REAGENTS AND STANDARDS

4.1. Deionized Water (DI water)

4.2. Artificial seawater (ASW), filtered, prepared per SOP TA-03.02.

4.2.1. Natural seawater (NSW) can be used instead of ASW.

5. CULTURE PREPARATION

5.1. 24 hours before *Artemia* are needed, fill two separatory funnels with filtered ASW, leaving enough head space for aeration without spillage.

5.1.1. ALWAYS rinse and scrub, using labeled *Artemia* brush, the funnels with DI water prior to culturing *Artemia*.

5.1.2. Avoid contaminating the ASW carboy with *Artemia*.

5.2. Place approximately 1 heaping teaspoon of *Artemia* cysts into each funnel (adjust amount as needed based on previous yields per lot).

5.2.1. If *Artemia* are used to feed mysids (*A. bahia)*, add 1ml of Selcon™ fatty acid concentrate to each funnel.

5.2.2. If bacterial growth in test chambers fed with *Artemia* is an issue, refer to Section 9.2. in the QC section of this SOP.

5.3. Place funnels into funnel holders.

5.3.1. Funnels should be placed in incubator #1 in room B246 or somewhere that will keep them at ~25 ºC with access to aeration.

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 Culture and Feeding* logbook.

6. ARTEMIA COLLECTION

6.1. Remove the aeration device and let *Artemia* settle for 5 minutes but no more than 10 minutes on the counter.

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

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6.1.2. *Artemia* funnels can be covered with a dark towel to help them settle to the bottom.

6.2. Add 1 L of ASW to the light box and turn on the light. (For Sections 6.2 through 6.7, refer to **Figure 1** at the end of this document.)

6.3. Turn the stopper of the funnel to drain the settled *Artemia* into the dark side of the light box.

6.4. Return the funnel to the funnel holder and repeat steps 6.1. and 6.3. This can be repeated a third time if needed.

6.4.1. Typically, after two rounds of settling and draining off the *Artemia*, the water runs clear, indicating there are no more hatched *Artemia* left to empty.

6.5. Place dark towels over the entire light box and wait (~15 minutes) for the *Artemia* to swim to the lighted side.

6.5.1. It can be helpful to set the light up near the corner of the light box so that the *Artemia* concentrate in a more condensed area, making them easier to siphon out.

6.6. Empty the "48-hour" *Artemia* jar and rinse with DI water. Pour contents of "24-hour" Artemia jar into the "48-hour" jar and rinse the "24-hour" jar with DI water. Place the new "48-hour" jar on shelf B-4, aerate, and add ASW to fill the jar approximately 2/3 full.

6.6.1. 48-hr old *Artemia* can be used to feed culture organisms but should not be fed to test organisms. This is used for backup purposes only.

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

6.8. Count the *Artemia* as directed in Section 7 of this SOP. Record the count in the *Artemia Culture and Feeding* logbook.

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

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

6.9.2. If there appears to be a nutritional problem in organisms fed exclusively *Artemia* nauplii, refer to Section 9.5. in the QC section of this SOP.

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

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

6.12. Start another batch of *Artemia* as needed , see Section 5.

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7. COUNTING *ARTEMIA* NAUPLII

7.1. Swirl the *Artemia* that was collected in Section 6, in the 1L "24-hour" jar to homogenize before counting.

7.2. Pour 100mL of the *Artemia* from the "24-hour" jar into a graduated cylinder. Then, pour the *Artemia* from the cylinder into a 150 µm mesh sieve and rinse with DI water.

7.3. 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.

7.4. Continue rinsing the sieve with DI water and collecting the *Artemia*/water mixture until you reach approximately 25-30 mL.

7.4.1. 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 25 to 30 mL volume.

7.5. 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.

7.5.1. 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.

7.6. Pipette three 1 mL aliquots from the 50-mL solution onto a petri dish, keeping the three aliquots separate.

7.7. 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.

7.8. 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.

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

7.8.2. Concentrate by pouring the concentrated *Artemia* back in the sieve, this time concentrating to a lesser volume. See step 7.3 above.

7.9. Record the final volume of concentrate that was used in the *Artemia Culture and Feeding* logbook.

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8. DATA ARCHIVAL

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

8.2. 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.

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

8.2.2. 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

8.2.2.1. 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.).

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

9. QUALITY CONTROL

9.1. *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.

9.1.1. If artemia will be concentrated in the morning for use in feeding, it is recommended that the *Artemia* be set up prior to noon the previous day to ensure a high enough yield of hatched *Artemia*.

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

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

9.2.2. Add 10 mL of bleach and mix well.

9.2.3. After 3 minutes, pour cysts onto a 150 µm screen and rinse several times with DI water.

9.2.4. Place rinsed cysts into the funnel and proceed as usual.

9.3. Decapsulation procedure to enhance hatch rate, reduce effort required to separate hatched *Artemia* from cysts, and disinfect the *Artemia*.

9.3.1. Pour approximately 200 mL of ASW into the 300 mL beaker.

9.3.2. Place 2 level teaspoons of *Artemia* cysts into the beaker.

9.3.3. Aerate moderately for 1 to 2 hours.

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9.3.4. Remove the aeration device from the beaker. Use deionized (DI) water to rinse the cysts from the beaker onto the screen. Rinse the beaker with DI water.

9.3.5. Use the wash bottle (containing bleach) to rinse the cysts back into the beaker. Place the stir bar in the beaker and place the beaker on the magnetic stirrer. Stir at moderate speed (slight vortex).

9.3.5.1. ~ 50 - 100 mL of bleach are used in this process. DO NOT PROCESS LONGER THAN 3 MINUTES after bleach contacts the cysts.

9.3.6. Constantly watch the beaker for the formation of a foam layer.

9.3.6.1. Formation of foam takes ~ 30 seconds to 2 minutes. Brownish-white bubbles should appear, spread throughout the solution, and then form a layer on the surface.

9.3.7. After the foam layer forms, watch for a color change in the solution from brown to grayish orange. When the intensity of the grayish orange color appears stable (2-3 minutes), take the beaker and the *Artemia* screen to the sink (B246). Pour the solution through the screen. Use the DI water hose to thoroughly rinse the decapsulated *Artemia* that are on the screen. A bleach odor should not remain.

9.3.7.1. If in doubt about a bleach odor coming from the *Artemia*, the *Artemia* can be returned to the beaker (with about 100 mL of DI water), treated with 2-4 mL of 0.025N sodium thiosulfate, rescreened and thoroughly rinsed as above after about 30 seconds of contact.

9.3.8. Use the DI water hose to rinse the *Artemia* from the screen into the plastic cup. Use the wash bottle containing ASW to rinse the *Artemia* into the separatory funnel.

9.3.9. Place the funnel in the holder. Provide moderate aeration. Record the "date", "*Artemia* lot #", "*Artemia* batch #", and additional information as required in the *Artemia Culture and Feeding* logbook.

9.3.10. Process the *Artemia* starting at Step 6 of this SOP.

9.4. 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.

9.5. 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.

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10. SAFETY/HAZARDOUS WASTE MANAGEMENT

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

10.2. Refer to Laboratory Quality Manual for sample disposal.

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

10.4. Read MSDS information for reagent handling and disposal.

11. REFERENCES

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

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

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

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

11.5. DEP Laboratory Quality Assurance Manual

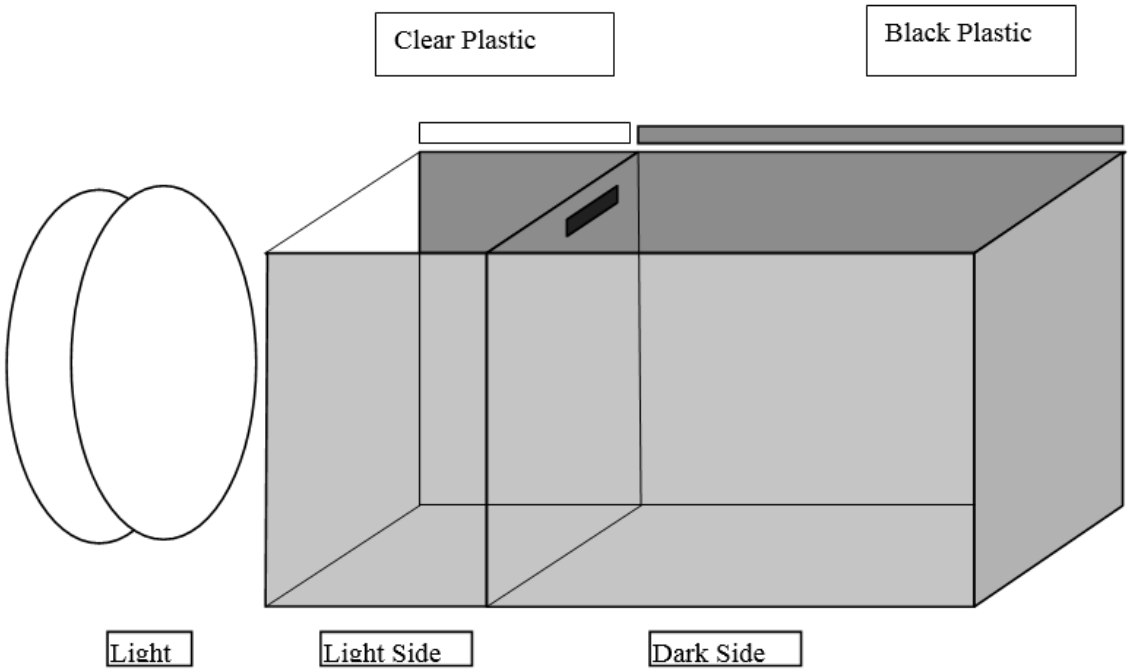
11.6. DEP Health and Safety Manual

11.7. MSDS Information Sheets

12. 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.

02/09/2015 Formatted for 508 compliance.

07/16/2025 Combined with SOP TA-01.05, that SOP is now section 9.3 of this SOP.

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