Receiving and Maintaining Purchased Test Organisms

1. SCOPE AND APPLICATION

1.1. This procedure is used to ensure that required bioassay test organisms that are not cultured in-house are in the laboratory and ready for use in bioassay testing. Tests covered by this procedure are BSY-AS-FFS, BSY-AS-MMY, BSY-AS-MFS, BSY-CS-FFS, BSY-CS-MMY, BSY-CS-MFS, BSY-AD-FFS, BSY-AD-MMY, BSY-AD-MFS, BSY-CD-FFS, BSY-CD-MMY, and BSY-CD-MFS.

2. SUMMARY OF THE METHOD

The supplier is contacted and organisms (*Pimephales promelas*, *Cyprinella leedsi*, *Americamysis bahia* and *Menidia beryllina*) are ordered as needed in advance of scheduled bioassay tests. Organisms are received in the laboratory, removed from the shipping packages and placed into appropriately labelled Carolina dishes. Parameters are measured on the "shipping" water and recorded in the *Organism Log-in Benchsheets* logbook. Holding water parameters are adjusted as needed to meet the bioassay test requirements. Organisms are fed and monitored (mortality and condition) prior to use in tests.

Note: Orders can be modified until noon of the day shipped (preferably three days before shipping as a professional courtesy).

2.1. DEFINITIONS

2.1.1. Good condition: less than 5% mortality

2.1.2. Fair condition: between 5% and 10% mortality

2.1.3. Poor condition: >10% mortality

2.1.4. Conditionally Usable: mortality <10% and abnormal behavior returns to normal behavior after minimum of two hours acclimation in culture lab. Mortality >10% with no other observable issues.

2.1.5. Unusable: behavior that does not return to normal after acclimation period; organisms that appear to be physically damaged are unusable regardless of behavior

3. SUPPLIES AND EQUIPMENT

3.1. Permanent marker (black, fine point)

3.2. Labeling tape

3.3. 200-ml specimen jars (1 per culture)

3.4. Large plastic Carolina dish (one per culture received)

3.5. Aeration device (air tubing and air stone or pipette) - 1 each per Carolina dish

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3.6. *Artemia nauplii*, < 24 hours old (SOP TA-01.03 and TA-01.05)

3.7. Wash bottle filled with deionized water

3.8. Scissors

3.9. Digital thermometer

3.10. Materials as needed to measure alkalinity and hardness

3.11. *Organism Log-in Benchsheets* logbook

3.12. Accumet AR10 pH Meter (S/N AR93312105) – Calibrate and use per SOP TA-06.23

3.13. YSI Model 58 DO Meter (S/N 90H018262 – Calibrate and use per SOP TA-06.04

3.14. Seven Multi Conductivity/salinity Meter (S/N 1225117049) – Calibrate and use per SOP TA-06.25

3.15. HACH kits for measuring ammonia in freshwater and saltwater sample. See SOP TA-06.35

4. REAGENTS AND CHEMICALS

4.1. Synthetic Moderately Hard Water (MHW) (for *P. promelas* and *C. leedsi*) – prepared per SOP TA-03.15

4.2. Artificial Seawater (ASW) (for *A. bahia* and *M. beryllina*) – prepared per SOP TA-03.02

5. PREPARATION

5.1. Use label tape and marker to label Carolina dishes (1 per received culture) with *species name*, test type (*acute* or *chronic*) and DEP *batch number*.

Note: The DEP batch number is the date of receipt (label ex. *P. promelas* acute 02/01/17)

Note: All measurements and observations are to be recorded in the *Organism Log-in Benchsheets* logbook. Organism age and supplier batch information will be on the paperwork supplied with the shipment. Ensure that the organisms are the correct age for the scheduled tests.

5.1.1. Acceptable organism age for scheduled tests:

Test	Acute	Chronic
C. dubia	<24 hours old	<24 hours old
P. promelas	1-14 days old	<48 hours old
C. leedsi	1-14 days old	<48 hours old
A. bahia	1-5 days old	7 days old
M. beryllina	9-14 days old	7-11 days old

5.2. Use label tape and marker to label the specimen jars as in 5.1.

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5.3. Place a bag of organisms in the appropriately labeled Carolina dish. Carolina dishes should be placed next to the sink.

5.4. Use the scissors to cut the top from each bag, leaving 3-4 inches of bag above the water level.

5.5. Pour approximately 150 mL of water from each bag into the corresponding specimen jar. Avoid pouring any organisms out during this process.

5.6. Measure the temperature, pH, conductivity, salinity (marine species only) and dissolved oxygen of the water in the specimen jars and record in the *Organism Log-in Benchsheets* logbook. Set the jars aside for later measurements of alkalinity and hardness (ammonia measurements are optional but typically done to determine ammonia toxicity if there is high mortality).

5.7. Return to the Carolina dish, carefully lift the bag (containing the organisms) from one side and gently transfer the organisms and water into the Carolina dish. Use deionized water to rinse any organisms remaining in the bag into the dish.

5.8. Take the dishes containing the organisms to the light table. Observe the organisms, noting the general condition. Count and remove any dead organisms. Check organisms and remove dead organisms immediately prior to loading tests. Organisms received that are to be used the following day are to be checked and dead organisms removed upon receipt, prior to the end of the work day (~1500-1600 hours) and immediately prior to being used in tests. Record observations (number dead and date/time checked) in the *Organism Log-in Benchsheets* logbook.

5.9. Place the dishes containing the organisms on Culture Tray C2. Feed the organisms from 1 to 2 mL of *artemia* nauplii (adjust quantity as needed). Organisms should be ready to use approximately 2 hours after feeding. Organisms should not be used < 2 hours after initial feeding without supervisor approval.

5.10. Place an aeration device (air stone or pipette) into each dish and gently aerate.

5.11. Scan the supplier's receipt page and save the file to \\FLDEP1\DEAR\Labs\Lab5\Biology\FYI Materials & Reports\FYI3 Reports\#0 Put Organism Receipts Here

Note: The file name should be [date on receipt] *space* [Supplier Name]. Example: 12-12-17 MBL

5.11.1. Make a copy of the supplier's receipt. Place the original receipt on the Biology Administrator's desk and place the copy in the filing cabinet in room 216N.

6. QUALITY CONTROL

6.1. To correct raw data entries, place a single line through the incorrect or illegible entry (do not overwrite it), write the corrected entry near the error, date and initial the

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

7. SAFETY/HAZARDOUS WASTE MANAGEMENT

7.1. Refer to the FDEP Laboratory Health and Safety Plan

7.2. Read MSDS information for reagent handling and disposal.

7.3. Use proper safety equipment including the lab hood or chemical ventilation system, a lab coat, safety glasses, a respirator and latex gloves (or a suitable substitute if allergic to latex) when working with chemicals and unprocessed samples.

8. REFERENCES

8.1. FDEP Laboratory Health and Safety Plan, https://www.fldepnet.org/content/dear/bureau-laboratories-safety-documents

8.2. DEP SOP TA-03.15, *Preparation of Synthetic Moderately Hard Freshwater*

8.3. DEP SOP TA-03.02, *Preparation of Artificial Seawater (ASW)*

8.4. DEP SOP TA-01.03, *Artemia Nauplii Culture*

8.5. DEP SOP TA-01.05, *Decapsulation of Artemia cysts*

8.6. DEP SOP TA-06.04, *YSI Model 58 Dissolved Oxygen Meter Calibration and DO Measurement*

8.7. DEP SOP TA-06.08, *Measuring Total Alkalinity and Hardness*

8.8. DEP SOP TA-06.35, *MeasuringTotal Ammonia using HACH Ammonia Nitrogen Pocket Colorimeter II Test Kit*

8.9. DEP SOP TA-06.23, *Calibration and Use of Accumet Model AR10 pH Meter*

8.10. DEP SOP TA-06.25, *Calibration and Use of Mettler SevenMulti for Measuring pH, Conductivity, Salinity and TDS*

8.11. DEP SOP TA-07.13, *Salinity Adjustment of Samples: Toxicity Bioassay*

APPENDIX OF CHANGES:

07/25/2008	Added Scope and Application, Summary of the Method, References and Appendix of Changes
02/06/2015	Formatted for 508 compliance.
03/01/2015	Reformatted the SOP.
02/03/2016	Updated link and moved it to the reference section. Re-organized sections per SOP LB-001
02/01/2017	Clarified acceptable age of organisms shipped to the lab.

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12/05/2017 Returned definitions to SOP. Update Ammonia SOP.

11/16/2018 Updated SOPs. Added information on supplier's receipt. Changed font from Times New Roman to Calibri.

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