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NAS PENSACOLA
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UNIVERSITY OF FLORIDA RISK ASSESSOR RESPONSE TO TECHNICAL REVIEW OF THE 22 FEBRUARY 2013 DRAFT SAMPLING AND ANALYSIS PLAN WETLAND SEDIMENT SAMPLING OPERABLE UNIT 16 SITE 41 DATED SEPTEMBER 2012 NAS PENSACOLA FL
07/24/2013
NAVFAC SOUTHERN

These responses have been revised in accordance with discussions and agreements in the June 7, 2013, Team Conference Call.

The Department has reviewed the Navy's Responses to the Department's and the Department's contracted University of Florida risk assessors' Comments on the Draft Sampling and Analysis Plan (SAP), Wetland Sediment Sampling, Operable Unit 16, Site 41, Naval Air Station Pensacola, dated 22 February 2013 (received by e-mail on 22 February 2013), submitted by Resolutions Consultants. I have attached comments from the Department's University of Florida risk assessors on the responses to their previous comments on the Draft Wetlands Sediment Sampling and Analysis Plan to this letter. I have also attached Ligia Mora-Applegate's response memo.

As far as my comment on the Draft Sampling and Analysis Plan, the Navy has responded that:

The chronic tests recommended by the reviewer may be appropriate for sites in the initial phases of investigation, but since the RI has been completed and finalized using shorter durations (7 to 28 day toxicity tests), it is critical that the same test organisms and durations be used to ensure that consistent decisions be made during the FS process. The 14-day acute toxicity tests proposed for both test organisms will provide survival as an assessment endpoint, although with *Hyalella azteca,* growth will be measured and may be evaluated as a secondary sublethal assessment endpoint.

While the Department acknowledges the Navy's point about having consistent data that are comparable to each other with respect to species organism and length of the tests, the Department believes there is no reason that both acute and chronic toxicity testing, in accordance with our original request, cannot be conducted. The acute toxicity testing should be conducted to meet the Navy's criteria so as to provide data that can be directly compared with previous results in order to support decision-making with survival as a risk management criteria. Chronic toxicity testing needs to be conducted to fill a data gap from the original investigation and ecological risk assessment. That data gap is the evaluation of chronic toxicity that can determine other than lethal effects on organisms, such as reproduction, that may also be incorporated into risk management decisions.

reproduction endpoints if warranted based on comparison of sediment chemistry data to the criteria identified in the SAP (Background, PRGs, PELs) including number of samples with exceedances, number of chemicals that exceed, spatial distribution of samples with exceedances, and magnitude of exceedances. The sediment chemistry data will be presented to the Pensacola Partnering Team with proposed toxicity sample locations before collection. Final toxicity sample locations will be discussed and agreed upon by the Pensacola Team before collection. The decision rules for toxicity testing are presented on Worksheet #11 in the SAP. The standard operating procedures for the chronic tests from Hydrosphere are attached to these responses.

At your request, I have reviewed the Responses to comments on the September 2012 Draft Sampling and Analysis Plan (SAP), for the Wetland Sediment Sampling, Operable Unit 16 Site 41, at the Naval Air Station in Pensacola. The responses were prepared by the Navy and are dated 22 February 13.

The Navy combined the Wetlands at the NAS Pensacola Facility into a single Operable Unit (OU16), Site 41. Site 41 encompasses approximately 81 wetlands or wetland complexes, both tidal and nontidal, that are within the base boundary. These wetlands are either palustrine or estuarine and drain into Bayou Grande or Pensacola Bay.

In general I am in agreement with the Navy responses except that we strongly recommend once again that additional parameters such as iron in wetland 4D and DDT in wetland 6 need to be tested.

In addition and as previously discussed, I would like to emphasize that the Probable Effect Levels (PELs) should only be used as a not-to-exceed values, and delineation of contaminants in sediments in the wetlands must be done to the Threshold Effect Levels (TELs) as applicable.

Response:
Because the Navy is currently preparing a Focused Feasibility Study Report and subsequently a Record of Decision Amendment for OU 1, The Navy proposes to transfer Wetlands 1B, 3, 4D, 15, and 18 A/B from OU 16 to OU 1. All investigations associated with these wetlands will now be performed as part of OU 1. In addition, the Navy will transfer Wetlands 5A, 6/7, and 64 complex from OU16 to OU2. The Navy agrees to collect three surface water samples at each of the sampled wetlands for the same constituent list as the sediment. For the OU 1 wetlands, surface water samples will only be analyzed for iron as requested in the U. S. EPA comment. The collection of surface water samples will be addressed in an update to the OU 16 UFP-SAP. Wetland W2 will be included in the OU 16 sampling effort for sediment.

crosses under the road. The Navy will add two sediment samples to Wetland 7 where Wetland 6 and Wetland 7 are contiguous.

The RI report and risk assessment are complete; therefore, the screening level TELs are not appropriate for this phase of the investigation. In addition, as stated in Approach to the Assessment of Sediment Quality in Florida Coastal Waters, "These guidelines are intended to be used as one tool in a toolbox of companion interpretive approaches..." and that the TELs and PELs "should not be used in lieu of water quality criteria, nor should they be used as sediment quality criteria". Moreover, use of PELs as not-to-exceed values is not appropriate, since empirical data from the site has been and will be used to calculate PRGs, as recommended by the Florida Sediment Quality Guidance.

The Navy agrees to provide comparison of the detected concentrations to site-specific PRGs, PELs, and background concentrations for assessment and discussion by the Team. Final remedial goals for OU16 will be based on the findings of this current investigation.

We have reviewed at your request responses to our comments on the *Draft Sampling and Analysis Plan (SAP), Wetland Sediment Sampling, Operable Unit 16, Site 41, Naval Air Station Pensacola, Pensacola, Florida.* Our comments were provided to you in a letter dated 26 November 2012. The responses to these comments provided on behalf of the Navy are contained in document dated 22 February 2013.

To enable you to follow the discussion regarding our comments, we have reproduced each original comment and the Navy response below. Following each, we have made a follow-up comment.

Comment 1:

Original Comment: In the final Remedial Investigation (RI; August 2005), iron was listed as a contaminant of potential concern (COPC) in surface water and sediment for Wetland 4D. During a site visit on 20 September 2012, it was noted that iron continues to be a concern for this wetland. We recommend that proposed additional sampling in Wetland 40 include iron to better determine the extent of iron contamination in sediment and surface water.

Navy Response: The Navy agrees that iron floc is observed in Wetland 3; however, Wetland 3 is being addressed under OU1 and Wetland 3 is not part of this investigation. Iron was not identified at Wetland 4D for further sampling in sediment in the SAP. This finding was based on the COC refinement presented as Appendix A in the Feasibility Study Report. A site-specific no observed effects concentration (NOEC) for freshwater wetlands of 246,000 milligrams per kilogram (mg/kg) was calculated for iron based on site-specific results in Wetland 3 (041M0302) at a location with no lethal or sublethal toxicity. The NOEC was discussed in the March 2012 meeting. Concentrations of iron reported in sediment are less than the NOECs, and therefore, iron was eliminated for further consideration. As discussed during partnering meetings, in the memorandum and in the response above, the scope of the memorandum was limited to sediment sampling, so surface water and other media were not discussed in the memorandum and are beyond the scope of this follow-up work for the Feasibility Study, unless information indicates that partnering team decisions regarding those media should be reconsidered.

not exclude iron. Consequently, separately analyzing samples for iron was not proposed.

As discussed during partnering meetings and in the memorandum, the scope of the memorandum was limited to sediment sampling, so surface water and other media were not discussed in the memorandum and are beyond the scope of this follow-up work for the Feasibility Study.

Follow-up Comment: The intent of our comment was to encourage further evaluation of iron in Wetland 4D based upon observations during the September 2012 site visit, as well as the discussions held during the March 2012 meeting. Observations of iron floc in Wetland 4D appear to be inconsistent with a conclusion of no toxicity, at least in some areas. This could perhaps be addressed by toxicity testing if properly conducted and inclusive of samples from areas with the highest iron/iron floc.

Response 1:

Because the Navy is currently preparing a Focused Feasibility Study Report and subsequently a Record of Decision Amendment for OU1, The Navy proposes to transfer Wetlands 1B, 3, 4D, 15, and 18 A/B from OU16 to OU1. All investigations associated with these wetlands will now be performed as part of OU1. The collection of surface water samples and possible toxicity testing in Wetlands 3 and 4D will be addressed in the OU1 UFP-SAP.

Comment 2:

Original Comment: During a Partnering Meeting on 27-28 March 2012, field verification was proposed for Wetland 6 to determine if additional sampling for DDT is necessary (Appendix A). A site visit on 20 September 2012 verified fish and piscivorous birds are present in this wetland. Further sampling to delineate the extent of contamination appears necessary to determine whether DDT is of concern to higher trophic levels species foraging in Wetland 6.

Navy Response: As stated in the Final Remediation Investigation Report, November 2007, Wetland 6 was eliminated from further sampling during the Phase III investigation because it is a channelized ditch within the NAS Pensacola storm water drainage system which receives continual impacts from storm water and is actively maintained by base maintenance personnel. As shown on Figure 11-1 from the Remedial Investigation Report, storm water from across the southeastern portion of NAS Pensacola discharges to Wetland 6.

In the May 2012 Partnering meeting, a participant was concerned with the source of DDT. Total DDT was detected above its basewide concentration of 110 ppb at only 2 of 12 locations.

041M060301. The fate and transport analysis for Wetland 6 did not indicate that OU6 soil or groundwater was a source of the DDT in sediment. Detected DDT concentrations are not indicative of a spill and are likely from routine spraying of pesticides along the ditch. Food chain models do not indicate a risk to upper trophic level receptors from DDT.

The concern for sampling was to identify a source and evaluate potential migration rather than ecological risk. The Navy has cleared this partially lined and channelized ditch, and clearing activities to maintain the ditch will be performed by base personnel as needed to maintain flow in the future. Consequently, the Navy disagrees with adding Wetland 6 to the SAP.

Follow-up Comment: Based upon field observations during the September 2012 visit, Wetland 6 certainly *appears* to be habitat for a number of fish species and piscivorous birds. If it is considered viable habitat for management purposes, then we maintain that better characterization of contaminants in this wetland is needed. If not, then issue is moot.

Response 2:

The Navy agrees to collect a sediment sample near the weir feature (northern portion of Wetland 6) where Wetland 6 crosses under the road. This proposed sampling area is between Wetland 5B and the Wetland 64 complex. The Navy will add two sediment samples to Wetland 7 where the wetlands are contiguous.

Comment 3:

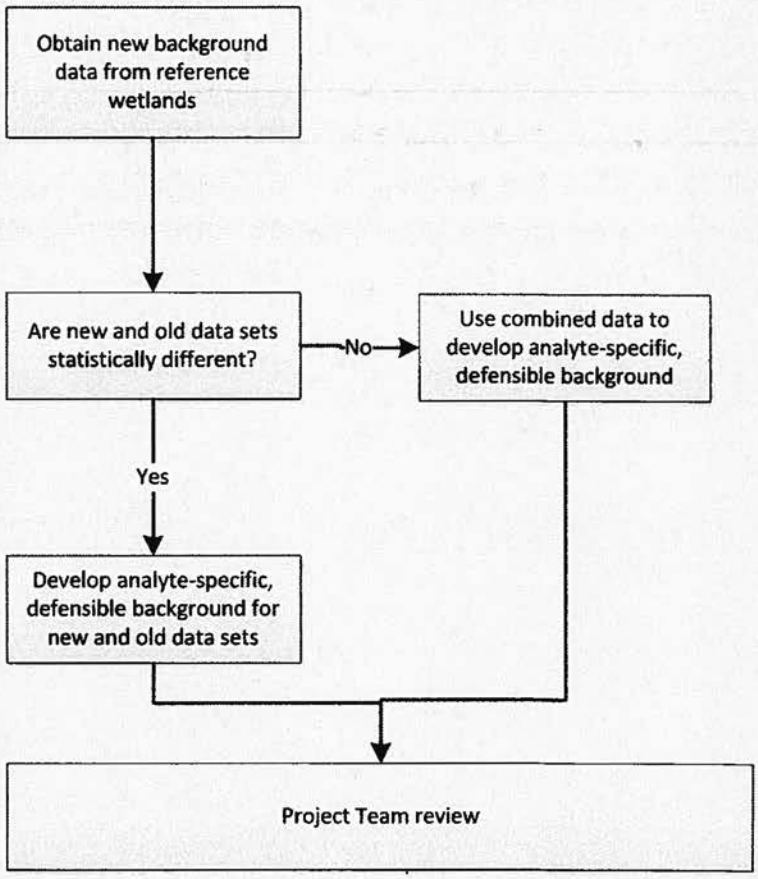
Original Comment: Worksheet 11 states that twice the mean detected concentration in the reference area will be utilized as an upper-end estimate of background concentrations at the site. The upper-end of the range of background concentrations is usually defined as the lower of twice the mean or the maximum detected concentration. This methodology prevents an overestimation of the upper limit of background that could result from a few elevated reference samples.

NAS Pensacola wetlands, including substantial input from EPA, FDEP and other stakeholders as part of the partnering process with the Navy as well as the comment and response process typically used to finalize RI Reports. Consequently, revisiting background determinations and/or comparison methods as part of this sampling and analysis plan is beyond the scope of the memorandum.

Follow-up Comment: Our comment is a reiteration of one we have made previously that the method of determining the upper limit of background is inconsistent with the approach typically used by the FDEP.

Response 3:

While the Navy appreciates and understands the approach typically used by FDEP, based on a review of all of the data and the multiple physical settings of the wetlands, the Navy intends to follow a best management approach to evaluating background. The Navy will utilize the following approach: (1) new reference data will be collected; (2) both new and old reference data will be evaluated to generate a revised background data set; and (3) the revised background data set will be used to determine whether chemicals detected at OU16 wetlands are site-related following the methods contained in Navy's Background Guidance (Guidance for Environmental Background Analysis, Volume II: Sediment, April 2003).



Original Comment: The use of PALs in Sections 11.3 and 11.5 is unclear (Worksheet 11). The document states that site-specific preliminary remedial goals (PRGs) will be reassessed using chemistry and toxicity data collected during this sampling event. However, it also states that if a PRG was calculated as part of the feasibility study (FS), the PRG from the FS will be utilized as the PAL. The PALs are then utilized to determine the extent of contamination. At the March 2012 Partnering Meeting (Appendix A), both the University of Florida and the U.S. EPA expressed concern regarding the interpretation of toxicity testing and derivation of the PRGs in the FS. It was also agreed in a Partnering Meeting on 9 May 2012 (Appendix B) that the old toxicity testing data would not be utilized for determining ecological toxicity at the site. These values should not be proposed for determining the extent of contamination in the SAP.

Navy Response: The site-specific PRGs are the no-observed effects concentration (NOEC) or the lowest observed effects concentration (LOEC) based on site-specific conditions and detected concentrations. The PRGs, reference concentrations, and other available ecological criteria were evaluated for development of the current PALs. Based on the data collected during the proposed investigation, the site-specific PRGs may be updated. The Navy agrees that the old toxicity test data will not be used with the proposed data for determining ecological toxicity at the site. Worksheet #11 will be reworded to show that new PRGs will be calculated as new data is obtained throughout the investigation.

Follow-up comment: The response indicates agreement with our (and U.S. EPA's) recommendation that the old toxicity data not be used in the development of site- specific PRGs. **This response is satisfactory.**

Comment 5:

Original Comment: The sediment screening level hierarchy (page WS 11-5) proposes to utilize the FDEP probable effect levels (PELs) for delineation purposes. Usually the threshold effect levels (TELs) are utilized for screening as well as delineation purposes. Use of the PEL for delineation could result in an average wetland contaminant concentration that exceeds the TEL.

Navy Response: An RI Report has already been developed for NAS Pensacola wetlands, including substantial input from EPA, FDEP and other stakeholders as part of the partnering process with the Navy as well as the comment and response process typically used to finalize RI Reports. Consequently, findings in the RI Report and subsequent discussions were integrated into the sampling and analysis memorandum. It was noted the TECs and TELs would not be used because this investigation is not a screening level assessment as the sites are past that stage in the

memorandum was being developed.

Follow-up Comment: Our comment was intended to address the use of the PEL for delineation purposes specifically. There are at least two potential problems with using the PEL for delineation: 1) Concentrations below the PEL can have negative impacts on benthic invertebrates, and consequently a wetland delineated using a PEL underestimates the size of the affected area; and 2) Delineation using the PEL can result in an average concentration within the delineated area that exceeds the TEL.

Response 5:

The RI report and risk assessment are complete; therefore, the screening level TELs are not appropriate for this phase of the investigation. In addition, as stated in Approach to the Assessment of Sediment Quality in Florida Coastal Waters, "These guidelines are intended to be used as one tool in a toolbox of companion interpretive approaches..." and that the TELs and PELs "should not be used in lieu of water quality criteria, nor should they be used as sediment quality criteria". Therefore use of PELs as not-to-exceed values is not appropriate, since empirical data from the site has been and will be used to calculate PRGs, as recommended by the Florida Sediment Quality Guidance.

The Navy agrees to provide comparison of the detected concentrations to site-specific PRGs, PELs, and background concentrations for assessment and discussion by the Team. Remedial goals for the OU 16 FS will be based on the analytical chemistry and/or toxicity testing results that will be obtained as part of the field work and testing planned for this investigation. Planned testing and corresponding DQOs will be documented in the SAP.

Comment 6:

Original Comment: Only three samples are proposed per reference wetland for a total of six freshwater and six estuarine reference samples. Six samples are not adequate to determine upper background concentrations with any certainty. The small number of proposed background samples is likely to result in a data set that will overestimate upper background concentrations. We recommend two additional samples per wetland for a total of ten samples per environment.

Navy Response: Two additional samples per reference wetland will be added.

Comment 7:

Original Comment: Sample 041M3306 in Wetland 33 (Figure 17-12) is the only sample proposed outside of a wetland boundary. It is unclear why a proposed reference sample does not actually lie within the boundaries of a wetland. Further explanation is necessary to clarify the placement of this sample.

Navy Response: Sample location will be changed to inside the Wetland 33 boundary.

Follow-up comment: **The response is satisfactory.**

Comment 8:

Original Comment: The duration of the proposed sediment toxicity tests is unclear. However, the draft Response to U.S. EPA Technical Comments (dated 30 July 2012) suggests the tests will be shortened to a 14-day exposure period for both Leptocheirus and Hyalella. It is important to note that 14-day toxicity testing for these species does not include reproduction. We recommend a chronic exposure period (28-60 days) to include reproductive endpoints as well as growth and survival. Reproductive endpoints may be more sensitive to some contaminants, and therefore contaminant concentrations protective of growth and mortality may not be protective of reproductive effects. Chronic reproductive endpoints are indicative of population level effects and should be evaluated unless there is evidence that reproduction is not the most sensitive endpoint for the contaminants of concern.

Navy Response: The chronic tests recommended by the reviewer may be appropriate for sites in the initial phases of investigation, but since the RI has been completed and finalized using shorter durations (7 to 28 day toxicity tests), it is critical that the same test organism and duration be used to ensure that consistent decisions be made during the FS process. The 14-day acute toxicity tests proposed for both test organisms will provide survival as an assessment endpoint, although with *Hyalella azteca,* growth will be measured and may be evaluated as a secondary sublethal assessment endpoint.

Follow-up Comment: We understand the point regarding consistency. Using 28-day tests would be consistent with testing conducted during the RI and arguably better capture reproductive endpoints than the 14-day tests proposed.

The Navy agrees to perform chronic toxicity to assess survival, growth and reproduction endpoints if warranted based on comparison of sediment chemistry data to the criteria identified in the SAP (Background, PRGs, PELs) including number of samples with exceedances, number of chemicals that exceed, spatial distribution of samples with exceedances, and magnitude of exceedances. The sediment chemistry data will be presented to the Pensacola Partnering Team with proposed toxicity sample locations before collection. Final toxicity sample locations will be discussed and agreed upon by the Pensacola Team before collection. The decision rules for toxicity testing are presented on Worksheet #11 in the SAP. The Standard Operating Procedures for the toxicity tests from Hydrosphere are attached.

Comment 9:

Original Comment: Page WS 11-4 states the PRGs in the FS were derived from the higher of the reference/background concentration, sediment screening levels, and sediment refinement levels. The PRGs were actually the higher of the reference/background, sediment screening levels, sediment refinement levels, and site-specific toxicity levels developed from sediment toxicity testing.

Navy Response: Section 11.3, page 11-4 will be reworded to state that PRGs were developed as part of the FS and the overall PRG was the highest value among the reference/background concentrations, sediment refinement levels, and site-specific NOEC or LOEC.

Follow-up Comment: **The response is satisfactory.**

Our comments above address the extent to which the proposed approach, as discussed in the responses to comments, are applicable to the problem being addressed. The document cited no references and contained no figures, tables, or numerical data or calculations. Conclusions and recommendations are implicit in the Navy responses to comments, and we have provided our comments and recommendations in the form of follow-up comments. Minor typographical errors in the presentation of our original comments and Navy responses have been corrected while reproducing them in the section above.

1.0 Introduction/Objective

1.1. The objective of a sediment test is to determine whether chemicals in sediment are harmful to or are bioaccumulated by benthic organisms. The tests can be used to measure interactive toxic effects of complex chemical mixtures in sediment.

1.2. Definitions of certain terms...

A. **Control Sediment:** Control sediment is a negative control against which comparisons (typically statistical) are made. The negative control can be an artificially prepared sediment or clean naturally occurring sediment collected from an area that has health aquatic communities associated with it. As of SOP version 4, there are no approved artificial control sediments.

B. **Reference Sediment:** These can also serve as negative control groups that can be used for comparisons. They are sediments that are collected near the study or test site. The intent is that the reference sediment is outside of the area of concern but near it, so as to have similar physical characteristic but be free of contamination.

C. **Test Sediment:** The test sediment is the material being evaluated against the control sediment and/or the reference sediment.

D. **Job Folder:** Folder containing instructions and bench sheets to conduct study. Chain-of-Custodies and test related paperwork are kept in this folder.

E. **Laboratory:** Unless stated otherwise, references in this SOP to "the laboratory" mean Hydrosphere Research, 11842 Research Circle, Alachua, FL 32615.

F. **Exposure Vessel:** This is the container, which holds the sediments, overlying waters and amphipods. Several exposure vessels containing the same test materials (sediment, water, amphipods) are referred to as "replicates".

G. **Sediment Settling Container:** The Sediment Rinse Station consists of a sink that contains the appropriate sieve and a series of Sediment Settling Containers which are 5 gallon buckets, one draining into the other, to capture the sediment and overflow rinse water. A large plastic container under the buckets provides secondary containment. The Rinse Station is located in the Utility 3 area near the back gate. Some sediment will fall into the sink during sediment washing, and it will settle on the bottom of one or more of the 5 gallon buckets. After all sediments have been washed, the sediment on the bottom of the buckets must be retrieved and placed in a sample container for return to the client.

2.0 Apparatus/Equipment/Laboratory Books

2.1. Laboratory Books

~ a complete list and location of lab books are in the front of each Laboratory Book ~

A. SOP, "Creating a Job Folder", PRO-013

B. Pen, water-proof, black ink
   1. To be used for making all records in laboratory books, logbooks and bench sheets.

C. Logbook, "Chemical & Solutions Logbook", LGB-002
   1. The chemical portion of the logbook is an electronic data base (Application: Filemaker) on the laboratory server (F:\databases\Chemical Log.fp7)
   2. New records are created here. File is printed periodically and placed in the logbook.

D. SOP, "Sample Receiving", PRO-009

E. Logbook, "Test Organism Receipt and Acclimation", LGB-015

F. SOP, "Test Organism Receipt and Acclimation", PRO-010

G. SOP, "Determination of Mass using the Sartorius Analytical Balance, Type R200D", MTR-001

H. SOP, "Randomization", PRO-036

2.2. Sampling Kit

A. Plastic Cooler, 48-quart minimum (1) (Brand: Coleman, Rubbermaid, Igloo)

B. Nalgene® Polyethylene Wide Mouth Bottles (4) (U.S. Plastic, Lima, OH ~ Stock 68082)

C. Ziplock Bag, 5" x 10" (1)
D. Clear Shipping Tape (roll)
E. Chain-of-Custody (COC) Form (1)
F. COC Seal (2)
G. Sample Bottle Labels, weather-proof (4)
H. Nylon Reinforced Tape (roll)
I. Hydrosphere Return Address Label(1)
J. FedEx Ground Shipping Label (1)
K. Shipping Label Sleeve (1)

2.3. Environmentally Controlled Water Bath

A. Fluorescent Lighting
B. Light Timer
C. Water Pump with plumbing
D. Heater, 300-watt
E. Aeration Manifold, Valved
F. Source of low pressure aeration, oil-free

2.4. Thermometer, calibrated
2.5. Sharpie Permanent Marker, Black or Colored
A. To be used for marking all sample containers and labware.
2.6. Coldroom, maintained at 0 to 6°C
2.7. Stainless Steel Beater
2.8. Drill with ½"chuck
2.9. Bucket, 5-gallon HDPE with Lid (3)

2.10. Nitex or Stainless Steel Sieves

A. ¼", 1000-µm, 800-µm, 600-µm, 425-µm, 240-µm screens

2.11. Silicone Tubing, 3/8" (~6')
2.12. Aquaculture Basin, 15-L (1 or more)
2.13. Airline Tubing (10')
2.14. Pipettes, 1-mL, for aeration (1 or more)
2.15. Sediment Rinse Container

2.16. Job Folder

A. **Bench Sheets** **for EPA Method "100.4"**

1. Sample Data
2. Survivorship
3. Water Quality
4. Overlay Water and Feedings
5. Growth
6. Reproduction

2.17. Glass Jar, 240-µm screen modified, 300-mL (12, per sample)
2.18. Stainless Steel Spoon (1 or more)
2.19. Ziploc Bag, 1-gallon, double zipper (1 or more)

2.20. Scissor (1)
2.21. Dissolved Oxygen Meter, calibrated (1) YSI, Model 58
2.22. pH Meter, calibrated (1)
2.23. Conductance Meter, calibrated (1)
2.24. Colorimeter (1) HACH, DR890
2.25. Test Strips (1 or more) HACH
2.26. Head Box Assembly
   A. This is an acrylic box fitted with…
   B. 60-cc syringes and…
   C. Stainless steel blunted needles.
2.27. Light Table
2.28. Weigh Boats, Plastic, 100-mL
2.29. Transfer Pipette
2.30. Aluminum Weigh Pans
2.31. Sediment Settling Trash Can Assembly
2.32. Table, Folding, Water-proof
2.33. Light Box
2.34. Pyrex Casserole Dish, 4-L
2.35. Forceps
2.36. Vinyl Hose with Spray Nozzle

3.0 Organisms/Reagents/Solutions

3.1. Amphipod, *Hyalella azteca.* 7 to 8-days old
   A. **Source:** In-house cultures or…
   B. **Source:** Aquatic Research Organisms, Hampton, NH, 603-926-1650
   C. **Source:** Aquatic Indicators, St. Augustine, FL, 904-829-2780

3.2. Control Sediment, Freshwater
   A. **Source:** Local, Alachua County, Florida
   B. **Source:** Aquatic Research Organisms, Hampton, NH, 603-926-1650

3.3. Freshwater
   A. Aquaculture Water

3.4. Deionized Water
   A. Reagent Grade Water · Type I

4.0 Personal Protection Equipment

4.1. No hazardous chemicals are used for the procedure, therefore; only standard laboratory safety procedures are practiced.

5.0 Procedure — Test Day

5.1. Schedule Test Event and Shipment of Sampling Kit — ~2-weeks

A. Create a Job Folder according to the SOP.
   1. Place Job folder in relevant tray on the Sample Receipt Counter in the Lab Testing Bay.

B. Sample Volume
   1. 12-replicates x 100-mLs is 1,200-mLs, however… sediment sample can vary greatly and the study may need to be repeated if it is unacceptable. The sample containers provided are 1-gallon (~3.78-L). Based on these elements the requested **sample volume should be 1-gallon.**

C. Sample Kit Shipment
   1. Determine how many sampling kits will be required. Each kit can hold 4, 1-gallon Nalgene® (or equivalent) sampling bottles and sufficient ice to preserve samples for a 24-hour period.
   2. Prepare sampling kits by including the following:
      a. Cooler containing…
         1) Clean 1-gallon Nalgene® bottles (each cooler can hold maximum of 4 bottles)
         2) Ziploc Bag. The Ziploc bag gets taped to the inside lid of the cooler but first place the following inside the Ziploc bag…
            a) Chain-of-Custody
            b) COC Seal
            c) Sample Bottle Labels
            d) Hydrosphere Return Address Label
      b. Chain-of-Custody Seal
         1) Sign & date the COC seal with water-proof black pen. Apply across the seam that's between the lid and body of the cooler.
         2) Tape the cooler closed with nylon reinforced tape. You must tape over the signed COC Seal with the tape. The tape is clear so the signed COC seal is visible.
      c. Apply the shipping sleeve containing the FedEx shipping label to the top of the cooler.
   3. Place cooler to the left of the front door to await pickup.
   4. Sign off on the Job Folder, "Shipped by" and "Shipped Date".
   5. Return Job Folder to relevant tray.
   6. Client: TVA
      a. TVA provides sample kits.

5.2. Determine where in the Laboratory the Bioassay will be Conducted — ~2-weeks

A. Determine which environmentally controlled water baths will accommodate the exposure vessels and will be able to meet the test conditions. The baths must have a pump and heater for temperature control, a source of clean, oil-free air, approximately 50 to 100 foot-candles illumination (ambient laboratory) and a 16-hour light/8-hour dark photoperiod.
B. Adjust photoperiod if necessary.
C. Adjust temperature of water bath to between 23 and 24°C. Exposure vessels typically run slightly less than bath temperature. Check water pump and verify that water is circulating. On subsequent days verify water bath temperature adjustments by checking the laboratories logbook which records the temperatures of all active baths and environmental chambers daily.

5.3. The Acquisition of *Hyalella azteca* for use in Bioassay ~ 2-weeks

A. Locate source of *H. azteca*.
B. Determine the number of amphipods required for the bioassay. The method recommends 12-replicate vessels with 10-organisms per replicate or a total of 120-amphipods per sediment sample or treatments.
C. Order 20% more than is required for the test. Mortalities may occur in shipping.
D. Have test organisms shipped priority overnight service.
E. Order amphipods so that they are as old as possible and so they arrive 2 to 3-days before the test is to be initiated. This give them time to sort themselves out (injuries during shipment may not be apparent upon arrival).

5.4. The Acquisition and Processing of Control Sediment ~ 1-week

A. Determine what the negative control(s) shall be. They may be artificial prepared or naturally occurring. They should have a high likelihood of performing adequately, as in, meeting minimal test method quality control standards.
B. Acquire control sediment approximately 1 to 2 weeks ahead of scheduled test date.
C. Control sediments may be collected by the laboratory, a contracted vendor or may be a reference site located near the test site. Refer to the Job Folder for the specific control sediments required for each test event.
D. The control sediment is to be collected in clean plastic (preferably HDPE) containers. These containers are then packed in a cooler with ice and shipped via an overnight courier to this laboratory.

E. Receipt and Processing of Control Sediment

1. **Upon arrival of control sediment…**
   a. Acquire the shipper's air bill.
   b. Inspect the packaging; ensure that the contents are intact.
   c. Note the condition of a chain-of-custody seal, if present.
   d. Open shipping container and find the paperwork describing the contents (chain-of-custody, shipping slip, etc…).
   e. Record the following on the paperwork:
      1) Temperature of the sediment.
      2) Date Received and Initial.
   f. Record the following on the sediment container:
      1) Make a record in the "Chemical & Solutions" Logbook and generate a Chemical Log number. Record this on the sediment container.
      2) Assign an 8-week expiration date and record on container.
      3) Client: TVA
         a) Record the condition of the COC seal.
         b) Scan and email completed COC to client.
   g. Refrigerate sediment in coldroom.
2. **Processing of Control Sediment**
   a. Remove control sediment from coldroom.
   b. Warm sediment to room temperature.
   c. Open sediment sample container. Do **not** pour off overlying water. During shipping and storage the sediment settles exposing some of the interstitial water. The overlying water will be mixed back into sample in the following step.

d. Homogenized the sediment by mixing with a stainless steel beater attached to a drill. This may be done in the original container (if large enough) or in another appropriate container; typically a 5-gallon HDPE Bucket.
e. The control sediment is sieved through a series of ~12" diameter Nitex® or stainless steel sieves to reduce the particle size to less than 450-µm.
   1) Wash sediment using synthetic freshwater.
   2) Sieve through ¼", 1000-µm, 800-µm, 600-µm, 450-µm screens.
f. Store sediments in a labeled 5-gallon bucket and store in coldroom. Allow washed sediment to settle for ≥2-days then draw off the overlay water with a 3/8" silicone tube.

5.5. The Receipt of Sediment and Overlay Water Samples — < 8-weeks (Sediments), < 2-weeks (Water)

A. Unless otherwise noted on the Job Folder, Sediments have an 8-week holding time and Receiving Waters have a 2-week holding time. Laboratory prepared synthetic waters have a 2-week holding time.

B. Receipt of Test Sediment

1. The receiving of the test sediment samples shall be conducted as given in the Sample Receiving SOP.
2. Points Emphasized from item 1 above.
   a. Record **Sample ID** on each container.
   b. Record **Expiration Date** (8-weeks) on each container.
3. Refrigerate sediment samples in cold room until use in testing.
4. Client: TVA
   a. Record the condition of the COC seal.
   b. Scan and email completed COC to client.

C. **Receipt of Receiving Water**

1. The receiving of receiving water samples shall be conducted as given in the Sample Receiving SOP.
2. Points Emphasized from item 1 above.
   a. Record **Sample ID** on each container.
   b. Record **Expiration Date** (2-weeks) on each container.
3. Refrigerate water samples in cold room until use in testing.
4. Client: TVA
   a. Record the condition of the COC seal.
   b. Scan and email completed COC to client.

5.6. Receipt of Amphipods — Day -3

A. Acquire the shipper's air bill.
B. Inspect the packaging; ensure that the contents are intact.
C. Open container and find shipping slip.
D. Make record in the "Test Organism Receipt and Acclimation" Logbook.
   1. Follow instructions in SOP, "Test Organism Receipt and Acclimation".
E. Fill 15-L aquaculture basin with 10-L SYNTHETIC FRESHWATER. Transfer amphipods to basin (~500 per basin).
F. Place in appropriate bath at 23°C and place on light air.
G. Water change amphipod basins at least daily over the next few days.

5.7. Other Pre-Test Preparations — Day -2

A. Job Order and Data/Bench Sheets

1. Locate Job Folder. Check for completeness.
2. Review information/instructions on Job Folder and Bench Sheets. Report any discrepancies to the laboratory manager.
3. Record Job Number on all bench sheets.
4. **Remember:** Corrections are made by drawing a single line through the error, initial and date correction, record correct data next to correction or, if note enough room, footnote and record correct data in "Notes and Comments" section at the bottom of the bench sheet.

B. Apparatus and Equipment Review

1. Review the list in Section 2.0. Verify that all are present, clean and in acceptable working order.
2. **Exposure Vessels, 300-mL Jars**
   a. The bioassay is conducted in 300-mL glass jars. Each sediment sample will be divided into 12 identical exposure vessels or replicates. Label each exposure vessel with Sample Type and Replicate designation (The 12-replicates are designated alphabetically "A" through "L").

5.8. Preparing Sediments for use in Bioassay — Day -1

A. Control Sediment

1. Retrieve sediment sample from cold room.
2. Warm sediment sample to room temperature.
3. ***Note:** Sediment should have already been processed per section 5.3 above.*
4. Open sediment sample container. Do **not** pour off overlying water. The overlying water will be mixed back into sample.
5. Homogenize the processed sediment (settling is likely to have occurred during storage in cold room) by mixing with stainless steel beater attached to a drill.
6. Pour or scoop sediment into a 1-gallon double Ziploc bag. Cut off a corner of the bag and squeeze sediment into appropriate exposure vessel. Fill to the 100-mL mark. Repeat for remaining replicates.
7. Place exposure vessels into testing water bath. Randomize locations. Record Template number.
8. Return unused portion to original container and refrigerate sediment in cold room.

B. Test Sediment

1. Retrieve sediment sample from cold room.
2. Warm sediment sample to room temperature.
3. Open sediment sample container. Do not pour off overlying water. During shipping and storage the sediment settles exposing some of the interstitial water. The overlying water will be mixed back into sample in the following steps.
4. Homogenized the sediment by mixing with a stainless steel beater attached to a drill. This may be done in the original container (if large enough) or in another appropriate container (HDPE Bucket).
5. If needed, the test sediment is press sieved through a series for Nitex® or stainless steel sieves to reduce the particle size. The method discourages sieving sediment samples. However, large particulate matter will make retrieving test organisms at the end of the study extremely difficult. Test organisms may be lost during test take down if large debris is not removed from the sediment sample during test setup.
   a. Client: TVA
      1) Ash samples do not typically need to be sieved.

6. Scoop sediment into appropriate exposure vessel using a stainless steel spoon. If sediment is fluid enough then it may be easier to "squeeze" it in. Pour or scoop sediment into a 1-gallon double Ziploc bag. Cut off a corner of the bag and squeeze sediment into appropriate exposure vessel. Fill to the 100-mL mark. Repeat for remaining replicates.
7. Place exposure vessels into testing water bath. Randomize locations. Record Template number.
8. **Pore Water Quality**
   a. Transfer 10-mLs of sediment to each of 2, 15-mL centrifuge tubes for each sample.
   b. Centrifuge samples for 30-minutes at ½ speed.
   c. Measure Salinity and Ammonia of supernatant.
   d. **Make a record on the "Pore Water Ammonia & Salinity" bench sheets.**
9. Return unused portion to original container and refrigerate sediment in cold room.

C. The Exposure Vessel and associated sediments are now ready to have overlay water added to them.

5.9. Preparing Overlay Waters for use in Bioassay — Day -1

A. Overlay water can either be laboratory prepare synthetic freshwater or receiving water. Receiving waters are typically taken from upstream of the test sediment sample or a nearby body of water. In either case the receiving water is to be taken from a "clean" location. Refer to Job Order to determine what overlay water is to be used.

B. **Preparation of Synthetic Freshwater**
1. Synthetic Freshwater is prepared according to the EPA reference method listed in section 9.

C. **Preparation of Receiving Water**
1. Retrieve water from cold room (unless it has just arrived and is still in cooler).
2. Warm water sample up to room temperature (~23°C).
3. **Make a record on the "Sample Data" bench sheet.**
   a. Record sample ID.
   b. Measure initial water quality and record.
      1) Measurements taken are dissolved oxygen (DO), pH, conductance, total ammonia (TAN), alkalinity, hardness, total residual chlorine (TRC).
      2) Adjust DO if supersaturated by light aeration. Receiving waters should not have an oxygen demand. If DO is <4.0-mg/L, do not use and contact the laboratory manager immediately.
   c. Record the receiving waters expiration date. This is 2-weeks unless otherwise noted on the Job Folder.

D. **Addition of Overlay Water to Exposure Vessel**
1. Assemble Head Box Assembly (HBA)
   a. Some syringes may not be used. Fit those with syringe plungers so that the syringe will not fill with water.
2. Wet screens of exposure vessels. This will prevent the exposure vessels from overflowing. Dry screens can prevent water from draining out of the exposure vessels due to surface tension.
3. Place the HBA over exposure vessels.
4. Calculate total volume of overlay water required to fill all of the exposure vessels present under HBA.
5. For the initial filling of the test vessels add ~20% to the above volume.
6. Fill HBA with overlay water.

E. The exposure vessels will now be allowed to settle overnight. Test initiation (Day 0) will be tomorrow.

5.10. Initiation of Bioassay — Day 0

A. There are several steps required to initiate the bioassay. The overlay water in the exposure vessels must be partially replenished. Water quality must be checked and recorded. Water quality must also be acceptable in order to support the unstressed survival of the amphipods for the duration of the bioassay. Amphipods are introduced to the exposure vessels once these parameters are deemed to be acceptable.

B. **Prepare Water for Renewal of Overlay Water**

1. If Overlay is **Synthetic Freshwater**, skip ahead to C.
2. If Overlay is **Receiving Water**…
3. Retrieve water from cold room (unless it has just arrived and is still in cooler).
4. Warm water sample up to room temperature (~23°C).
5. **Make a record on the "Sample Data" bench sheet.**
   a. Record sample ID.
   b. Measure initial water quality and record.
      1) Measure dissolved oxygen (DO), pH and conductance. These are measured with each days use.
      2) Measure total ammonia (TAN), alkalinity, hardness, total residual chlorine (TRC). These are only measured with the samples first use.
      3) Adjust DO if supersaturated by light aeration. Receiving waters should not have an oxygen demand. If DO is <4.0-mg/L, do not use and contact the laboratory manager immediately.
   c. Record the receiving waters expiration date (from the sample container).

C. **Measure Water Quality**

1. Retrieve one of the sediment sample sets from the water bath (a "set" is all of the replicates, "A" through "L" of a given sediment sample) and place them on a light table.
   a. Take measurements directly in each exposure vessel.
   b. **Make a record on the "Temperature" and "Dissolved Oxygen" bench sheets.**
      1) Measure and record Temperature and DO from one replicate.
      2) This is done daily for the duration of the test.
   c. **Make a record on the "pH", "Conductance", "Hardness", "Alkalinity" and "Ammonia" bench sheets.**
      1) Measure and record pH, Conductance, Hardness, Alkalinity and Ammonia from one replicate.
      2) This is done on day 0, day 28 and day 42.
2. ***Important*** Be certain that all water quality parameters are within prescribed method specifications. If not, contact the laboratory manager.

D. **Renewal of the Overlay Water**

1. Assemble Head Box Assembly (HBA)
   a. Some syringes may not be used. Fit those with syringe plungers so that the syringe will not fill with water.
2. Wet screens of exposure vessels. This will prevent the exposure vessels from overflowing. Dry screens can prevent water from draining out of the exposure vessels due to surface tension.
3. Place the HBA over exposure vessels.
4. Calculate total volume of overlay water required to fill all of the exposure vessels present under HBA.
5. Fill HBA with overlay water.
6. Repeat above process for remaining exposure vessels.

7. **Make a record on the "Renewals and Feedings" bench sheet**
   a. Record the overlays ID# on the bench sheet.

E. Addition of Amphipods to Exposure Vessels

1. Retrieve basin containing amphipods from water bath and place on a counter or light table.
2. Siphon off most of the water in the basin. This will make it easier to count out the amphipods.
3. Set out weigh boats on the table. Using a transfer pipette add some of the synthetic freshwater from the basin into each weigh boat. Then carefully count out 10-amphipods into each boat.
4. Bring out 8 randomly selected exposure vessels from the testing water bath.
5. Gently wash amphipods from the weigh boat into the exposure vessel.
6. Be certain that all amphipods break through the surface tension of the overlay water. Any floating amphipods are gently nudged into the water by using the transfer pipette and dropping overlay water onto the floating amphipod.
7. Once all vessels have had 10-amphipods added to them they are returned to the testing water bath.
8. Repeat steps 3 through 7 above for remaining sediment sample test vessels.
9. **Make a record on the "Survivorship" bench sheet**
   a. Record the initiation of the test.

F. Initial Amphipod Weights

1. Archive 80-amphipods for dry weight determination.
2. Number aluminum weigh pans. One pan per 20-amphipods.
3. Determine weight according to the SOP, "Determination of Mass using the Sartorius Analytical Balance, Type R200D".
4. **Make a record on the "Growth" bench sheet.**
   a. Record pan number, tare weights, total weight, number of specimens per pan, final dry weight, date final dry weight.

G. Feeding Exposure Vessels

1. Test Vessels are fed 1-mL of F-CD. F-CD is a feed used for *Ceriodaphnia* cultures.
2. **Make a record on the "Renewals & Feedings" bench sheet**
   a. Record the Feed ID#.

5.11. Daily Renewal, Observations and Water Quality... ~ Mornings ~ **Days 1 through 28**

A. On renewal days prepare fresh water for replenishing overlay water in the morning and evening. Make observations on mortalities, remove mortalities and record on bench sheets. Record water quality on bench sheets.

B. **Prepare Water for Renewal of Overlay Water**

1. If Overlay is **Synthetic Freshwater**, skip ahead to C.
2. If Overlay is **Receiving Water**...
3. Retrieve water from cold room (unless it has just arrived and is still in cooler).
   a. Retrieve enough for morning and evening renewals.
4. Warm water sample up to room temperature (~23°C).
5. Place water sample in bath at 23°C.
6. **Make a record on the "Sample Data" bench sheet.**
   a. Record sample ID.
   b. Measure initial water quality and record.
      1) Measure dissolved oxygen (DO), pH and conductance. These are measured with each days use.
      2) Measure total ammonia (TAN), alkalinity, hardness, total residual chlorine (TRC). These are only measured with the samples first use.
      3) Adjust DO if supersaturated by light aeration. Receiving waters should not have an oxygen demand. If DO is <4.0-mg/L, do not use and contact the laboratory manager immediately.
   c. Record the receiving waters expiration date (from the sample container).

C. **Measure Water Quality of the Exposure Vessels**

1. Retrieve one of the sediment sample sets from the water bath (a "set" is all of the replicates, "A" through "H" of a given sediment sample) and place them on a light table.
   a. Take measurements directly in each exposure vessel.
   b. **Make a record on the "Temperature" and "Dissolved Oxygen" bench sheets.**
      1) Measure and record Temperature and DO from one replicate.
      2) This is done daily for the duration of the test.
2. ***Important*** Be certain that all water quality parameters are within prescribed method specifications. If not, contact the laboratory manager.

D. **Renewal of the Overlay Water**

1. Assemble Head Box Assembly (HBA)
   a. Some syringes may not be used. Fit those with syringe plungers so that the syringe will not fill with water.
2. Wet screens of exposure vessels. This will prevent the exposure vessels from overflowing. Dry screens can prevent water from draining out of the exposure vessels due to surface tension.
3. Place the HBA over exposure vessels.
4. Calculate total volume of overlay water required to fill all of the exposure vessels present under HBA.
5. Fill HBA with overlay water.
6. Repeat above process for remaining exposure vessels.
7. **Make a record on the "Renewals and Feedings" bench sheet**
   a. Record the overlays ID# on the bench sheet.

5.12. Daily Renewal, Observations and Water Quality... ~ Evenings ~ Days 1 through 28

A. Renewal of the Overlay Water

1. Assemble Head Box Assembly (HBA)
   a. Some syringes may not be used. Fit those with syringe plungers so that they will not fill with water.
2. Wet screens of exposure vessels. This will prevent the exposure vessels from overflowing. Dry screens can prevent water from draining out of the exposure vessels due to surface tension.
3. Place the HBA over exposure vessels.
4. Calculate total volume of overlay water required to fill all of the exposure vessels present under HBA.
5. Fill HBA with overlay water.
6. Repeat above process for remaining exposure vessels.
7. **Make a record on the "Renewals and Feedings" bench sheet**
   a. Record the overlays ID# on the bench sheet.

B. Feeding Exposure Vessels

1. Test Vessels are fed 1-mL of F-CD after evening renewal. F-CD is a feed used for *Ceriodaphnia* cultures.
2. **Make a record on the "Renewals & Feedings" bench sheet**
   a. Record the Feed ID#.

5.13. Dry Weight Determination and Transfer of Adults to Water Only Vessels Day 28

A. The sediments are washed through a 425-µm sieve in order to collect the amphipods. The amphipods in four of the 12 replicates are counted, placed on tared weigh pans, dried & final dry weights are determined. The amphipods in the other 8 replicates are counted and transferred into water only test vessels for further evaluations.

B. Setup a Sediment Take Down Work Station

1. **Set up Work Station outside.**
   a. Sediment sample settling assembly with Eggcrate
   b. Water Proof Table
   c. Light Box
   d. 425-µm sieve
   e. Pyrex Casserole Dish
   f. Forceps
   g. Wide-bore Transfer Pipettes
      1) Cut 5-mL glass pipettes to about 3 inches. Fire polish ends.
      2) Cut plastic 3.5 mL transfer pipette at about the 2.5 mL mark.
      3) Slide plastic bulb end onto cut glass pipette.
   h. Deionized Water Hose
   i. Spray Attachment for DI water

C. Tare Aluminum Weigh Pans

1. Number aluminum weigh pans. One pan per exposure vessel.
2. Tare pans according to the SOP "Determination of Mass using the Sartorius Analytical Balance, Type R200D" (procedure number MTR-001).
3. **Make a record on the "Growth" bench sheet.**
   a. Record pan number and tare weights.

D. Measure Water Quality

1. Retrieve one of the sediment sample sets from the water bath (a "set" is all of the replicates, "A" through "L" of a given sediment sample) and place them on a light table.
   a. Take measurements directly in each exposure vessel.
   b. **Make a record on the "Temperature" and "Dissolved Oxygen" bench sheets.**
      1) Measure and record Temperature and DO from one replicate.
      2) This is done daily for the duration of the test.
   c. **Make a record on the "pH", "Conductance", "Hardness", "Alkalinity" and "Ammonia" bench sheets.**
      1) Measure and record pH, Conductance, Hardness, Alkalinity and Ammonia from one replicate.
      2) This is done on day 0, day 28 and day 42.

E. Separation of Amphipods from Sediment

1. Work with one set and then one **exposure vessel at a time**.
   a. Transport one set of exposure vessels and tared weigh pans outside to the Sediment Settlement Container.
2. For the **8 test vessels** whose amphipods are to be transferred to the **water only test vessels**…
   a. Pore contents of exposure vessel into 425-µm sieve.
   b. Carefully wash the sediment with aquaculture water to remove fine material while minimizing possible injury to the amphipods.
   c. Wash the contents of the sieve into a casserole dish.
   d. Using a wide-bore transfer pipette, transfer all of the amphipods from the dish to the appropriately marked water-only test vessel.
   e. **Make a record on the "Survivorship" bench sheet.**
   f. Record number of living Amphipods recovered.
   g. Repeat a. through f. for the remaining 7 exposure vessels in this set.

3. For the **4 test** vessels whose amphipods are to be transferred to the **tared weigh pans**…
   a. Pore contents of exposure vessel into 425-µm sieve.
   b. Flushing the vessel with the deionized water.
   c. Rinse sediment through the sieve.
   d. Transfer the contents of the sieve to a casserole dish.
   e. Using forceps or transfer pipette remove all of the amphipods from the dish and transfer to the appropriate weigh pan.
   f. **Make a record on the "Survivorship" bench sheet.**
   g. Record number of living Amphipods recovered.
   h. **Make a record on the "Growth" bench sheet.**
      1) Record number of specimens transferred to the weigh pan.
   i. Repeat a. through h. for the remaining exposure vessels in this set.

F. Repeat 5.13.D and 5.13.E for remaining sediment sets.
G. Transport empty labware and exposure vessels to the dirty labware counter.
H. Remove the water from the sediment settling container, and use a scoop to remove and transfer the "composited" sediment remaining in the container to a sample container for return to the client.

I. **Final Dry Weight Determination**

1. Determine final dry weights according to the SOP "Determination of Mass using the Sartorius Analytical Balance, Type R200D" (procedure number MTR-001).
2. **Make a record on the "Growth" bench sheet.**
   a. Record total weights and calculate final dry weights.

5.14. Daily Renewal, Observations and Water Quality... ~ Mornings ~ Days 29 through 42

A. On renewal days prepare fresh water for replenishing overlay water in the morning and evening. Make observations on mortalities, remove mortalities and record on bench sheets. Record water quality on bench sheets.

B. **Prepare Water for Renewal of Overlay Water**

1. If Overlay is **Synthetic Freshwater**, skip ahead to C.
2. If Overlay is **Receiving Water**...
3. Retrieve water from cold room (unless it has just arrived and is still in cooler).
   a. Retrieve enough for morning and evening renewals.
4. Warm water sample up to room temperature (~23°C).
5. Place water sample in bath at 23°C.
6. **Make a record on the "Sample Data" bench sheet.**
   a. Record sample ID.
   b. Measure initial water quality and record.
      1) Measure dissolved oxygen (DO), pH and conductance. These are measured with each days use.
      2) Measure total ammonia (TAN), alkalinity, hardness, total residual chlorine (TRC). These are only measured with the samples first use.
      3) Adjust DO if supersaturated by light aeration. Receiving waters should not have an oxygen demand. If DO is <4.0-mg/L, do not use and contact the laboratory manager immediately.
   c. Record the receiving waters expiration date (from the sample container).

C. **Measure Water Quality of the Exposure Vessels**

1. Retrieve one of the sediment sample sets from the water bath (a "set" is all of the replicates, "A" through "H" of a given sediment sample) and place them on a light table.
   a. Take measurements directly in each exposure vessel.
   b. **Make a record on the "Temperature" and "Dissolved Oxygen" bench sheets.**
      1) Measure and record Temperature and DO from one replicate.
      2) This is done daily for the duration of the test.
2. ***Important*** Be certain that all water quality parameters are within prescribed method specifications. If not, contact the laboratory manager.

D. **Renewal of the Overlay Water**

1. Assemble Head Box Assembly (HBA)
   a. Some syringes may not be used. Fit those with syringe plungers so that the syringe will not fill with water.
2. Wet screens of exposure vessels. This will prevent the exposure vessels from overflowing. Dry screens can prevent water from draining out of the exposure vessels due to surface tension.
3. Place the HBA over exposure vessels.
4. Calculate total volume of overlay water required to fill all of the exposure vessels present under HBA.

5. Fill HBA with overlay water.
6. Repeat above process for remaining exposure vessels.
7. **Make a record on the "Renewals and Feedings" bench sheet**
   a. Record the overlays ID# on the bench sheet.

5.15. Daily Renewal, Observations and Water Quality... ~ Evenings ~ Days 29 through 42

A. Renewal of the Overlay Water

1. Assemble Head Box Assembly (HBA)
   a. Some syringes may not be used. Fit those with syringe plungers so that the syringe will not fill with water.
2. Wet screens of exposure vessels. This will prevent the exposure vessels from overflowing. Dry screens can prevent water from draining out of the exposure vessels due to surface tension.
3. Place the HBA over exposure vessels.
4. Calculate total volume of overlay water required to fill all of the exposure vessels present under HBA.
5. Fill HBA with overlay water.
6. Repeat above process for remaining exposure vessels.
7. **Make a record on the "Renewals and Feedings" bench sheet**
   a. Record the overlays ID# on the bench sheet.

B. Feeding Exposure Vessels

1. Test Vessels are fed 1-mL of F-CD after evening renewal. F-CD is a feed used for *Ceriodaphnia* cultures.
2. **Make a record on the "Renewals & Feedings" bench sheet**
   a. Record the Feed ID#.

5.16. Record Offspring Production Days 35 and 42

A. Remove Offspring from Test Vessels and Record

1. Retrieve one of the sediment sample sets from the water bath (a "set" is all of the replicates, "A" through "H" of a given sediment sample) and place them on a light table.
2. Transfer contents of vessel to a culture dish.
3. Replenish volume in test vessel with fresh water.
4. Transfer Adults back into test vessel.
5. Count offspring and record. Discard offspring.

5.17. Termination of Test Days 42

A. Tare Aluminum Weigh Pans

1. Number aluminum weigh pans. One pan per exposure vessel.
2. Tare pans according to the SOP "Determination of Mass using the Sartorius Analytical Balance, Type R200D" (procedure number MTR-001).
3. **Make a record on the "Growth" bench sheet.**
   a. Record pan number and tare weights.

B. Measure Water Quality

1. Retrieve one of the sediment sample sets from the water bath (a "set" is all of the replicates, "A" through "H" of a given sediment sample) and place them on a light table.

a. Take measurements directly in each exposure vessel.
b. **Make a record on the "Temperature" and "Dissolved Oxygen" bench sheets.**
   1) Measure and record Temperature and DO from one replicate.
   2) This is done daily for the duration of the test.
c. **Make a record on the "pH", "Conductance", "Hardness", "Alkalinity" and "Ammonia" bench sheets.**
   1) Measure and record pH, Conductance, Hardness, Alkalinity and Ammonia from one replicate.
   2) This is done on day 0, day 28 and 42.

C. Remove Offspring from Test Vessels and Record

1. Transfer contents of vessel to a culture dish.
2. Count offspring and record. Discard offspring.

D. Determine Sex of Adults and Final Dry Weights

1. Determine the sex of the surviving amphipods and record.
2. Using forceps or transfer pipette remove all of the amphipods from the dish and transfer to the appropriate weigh pan.
3. **Make a record on the "Survivorship" bench sheet.**
4. Record number of living Amphipods recovered and their sex.
5. **Make a record on the "Growth" bench sheet.**
   a. Record number of specimens transferred to the weigh pan.

E. Repeat 5.17.B and 5.17.D for remaining sets.

F. Final Dry Weight Determination

1. Determine final dry weights according to the SOP "Determination of Mass using the Sartorius Analytical Balance, Type R200D" (procedure number MTR-001).
2. **Make a record on the "Growth" bench sheet.**
   a. Record total weights and calculate final dry weights.

6.0 Quick Summary

6.1. No summary currently prepared.

7.0 Acceptability of Data

7.1. Minimum mean **control survival 80% at 28 days.**

8.0 General References

8.1. Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates, Second Edition (EPA 600/R-99/064, March 2000)

8.2. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition, (EPA-821-R-02-012, October 2002)

9.0 Signatures

9.1. Signature, Title of the Approving Authority and Implementation Date.

A. _____ _____ ___/___/___.

9.2. Each person conducting the SOP should read the following statement. Print and sign if you agree.

"I have read and understand this Standard Operating Procedures as it relates to my job responsibilities. I further understand that the Standard Operating Procedures are an integral part of Hydrosphere Research's operations. I agree to follow all procedures outlined in the Standard Operating Procedures."

9.3. Printed **Name, Signature, Date and Training ETW** of persons conducting this procedure:

A. _____ _____ ___/___/___ _____·ETW

B. _____ _____ ___/___/___ _____·ETW

C. _____ _____ ___/___/___ _____·ETW

D. _____ _____ ___/___/___ _____·ETW

E. _____ _____ ___/___/___ _____·ETW

F. _____ _____ ___/___/___ _____·ETW

G. _____ _____ ___/___/___ _____·ETW

H. _____ _____ ___/___/___ _____·ETW

I. _____ _____ ___/___/___ _____·ETW

J. _____ _____ ___/___/___ _____·ETW

K. _____ _____ ___/___/___ _____·ETW

L. _____ _____ ___/___/___ _____·ETW

9.4. Annual Review (signature and date)

A. _____ ___/___/___.

B. _____ ___/___/___.

C. _____ ___/___/___.

D. _____ ___/___/___.

E. _____ ___/___/___.

10.0 SOP Postings and Revisions

10.1. Postings

A. Laboratory Posted Location(s) of SOP's.

1. _____

2. _____

3. _____

10.2. Revision Checklist

- ☐ Retrieve and Dispose of Posted Copies. Initial _____
- ☐ Create New Revised SOP Initial _____
- ☐ Train Personnel on "new" SOP Initial _____
- ☐ File Electronic Version of Retired SOP Initial _____
- ☐ File Paper Version of Retired SOP Initial _____

11.0 Examples of Relevant Datasheets or Documents

11.1. None are currently included.

1.0 Introduction/Objective

1.1. The objective of a sediment test is to determine whether chemicals in sediment are harmful to benthic organisms. The tests can be used to measure interactive toxic effects of complex chemical mixtures in sediment.

1.2. Definitions of certain terms…

A. **Control Sediment:** Control sediment is a negative control against which comparisons (typically statistical) are made. The negative control can be an artificially prepared sediment or clean naturally occurring sediment collected from an area that has healthy aquatic communities associated with it. As of SOP version 0, there are no approved artificial control sediments.

B. **Reference Sediment:** These can also serve as negative control groups that can be used for comparisons. They are sediments that are collected near the study or test site. The intent is that the reference sediment is outside of the area of concern but near it, so as to have similar physical characteristic but be free of contamination.

C. **Test Sediment:** The test sediment is the material being evaluated against the control sediment and/or the reference sediment.

D. **Overlay Waters:** This water that covers the exposure sediments during testing. Overlay water are either laboratory prepared synthetic seawaters or natural waters. Natural waters are typically from a receiving water body such as an estuary or marine water body.

E. **Job Folder:** Folder containing instructions and bench sheets to conduct study. Chain-of-Custodies and test related paperwork are kept in this folder.

F. **Laboratory:** Unless stated otherwise, references in this SOP to "the laboratory" mean Hydrosphere Research, 11842 Research Circle, Alachua, FL 32615.

G. **Exposure Vessel:** This is the container, typically a glass beaker or jar, which holds the sediments, overlying waters and the amphipods. Several exposure vessels containing the same test materials (sediment, water, amphipods) are referred to as "replicates".

H. **Sediment Settling Container:** The Sediment Rinse Station consists of a sink that contains the appropriate sieve and a series of Sediment Settling Containers which are 5 gallon buckets, one draining into the other, to capture the sediment and overflow rinse water. A large plastic container under the buckets provides secondary containment. The Rinse Station is located in the Utility 3 area near the back gate. Some sediment will fall into the sink during sediment washing, and it will settle on the bottom of one or more of the 5 gallon buckets. After all sediments have been washed, the sediment on the bottom of the buckets must be retrieved and placed in a sample container for return to the client.

2.0 Apparatus/Equipment/Laboratory Books

2.1. Laboratory Books

~ a complete list and location of lab books are in the front of each Laboratory Book ~

A. SOP, "Creating a Job Folder", PRO-013

B. Pen, water-proof, black ink
   1. To be used for making all records in laboratory books, logbooks and bench sheets.

C. Logbook, "Chemical & Solutions Logbook", LGB-002
   1. The chemical portion of the logbook is an electronic data base (Application: Filemaker) on the laboratory server (F:\databases\Chemical Log.fp5)
   2. New records are created here. File is printed periodically and placed in the logbook.

D. SOP, "Sample Receiving", PRO-009

E. Logbook, "Test Organism Receipt and Acclimation", LGB-015

F. SOP, "Test Organism Receipt and Acclimation", PRO-010

G. SOP, "Determination of Mass using the Sartorius Analytical Balance, Type R200D", MTR-001

H. SOP, "Randomization", PRO-036

2.2. Sampling Kit

A. Plastic Cooler, 48-quart minimum (1) (Brand: Coleman, Rubbermaid, Igloo)
B. Nalgene® Polyethylene Wide Mouth Bottles (4) (U.S. Plastic, Lima, OH ~ Stock 68082)
C. Ziplock Bag, 5" x 10" (1)
D. Clear Shipping Tape (roll)
E. Chain-of-Custody (COC) Form (1)
F. COC Seal (2)
G. Sample Bottle Labels, weather-proof (4)
H. Nylon Reinforced Tape (roll)
I. Hydrosphere Return Address Label(1)
J. FedEx Ground Shipping Label (1)
K. Shipping Label Sleeve (1)

2.3. Environmentally Controlled Water Bath

A. Fluorescent Lighting
   1. **Note:** Photoperiod is 16L;8D
B. Water Pump with plumbing
C. Heater, 300-watt
D. Aeration Manifold, Valved
E. Source of low pressure aeration, oil-free

2.4. Thermometer, calibrated
2.5. Sharpie Permanent Marker, Black or Colored
   A. To be used for marking all sample containers and labware.
2.6. Coldroom, maintained at 0 to 6°C
2.7. Stainless Steel Beater
2.8. Drill with ½"chuck
2.9. Bucket, 5-gallon HDPE with Lid (3)

2.10. Nitex or Stainless Steel Sieves

A. ¼", 2-mm, 1000-µm, 800-µm, 600-µm, 450-µm screens

2.11. Silicone Tubing, 3/8" (~6')
2.12. Aquaculture Basin, 15-L (1 or more)
2.13. Airline Tubing (10')
2.14. Pipettes, 1-mL, for aeration (1 or more)
2.15. Sediment Rinse Container

2.16. Job Folder

A. Bench Sheets
   1. "Sample Data"
   2. "Survivorship"
   3. "Dissolved Oxygen"
   4. "pH"
   5. "Temperature"
   6. "Salinity"
   7. "Feedings & Renewals"
   8. "Growth"
   9. "Growth Rate"

10. "Reproduction"

2.17. Beaker or glass jar, 1,000-mL (7 per sample, 5-reps for test exposure and 2 dummy vessels for pore-water ammonia)
2.18. Stainless Steel Spoon (1 or more)
2.19. Ziploc Bag, 1-gallon, double zipper (1 or more)
2.20. Scissor (1)
2.21. Dissolved Oxygen Meter, calibrated (1) YSI, Model 58
2.22. pH Meter, calibrated (1)
2.23. Salinity Meter, calibrated (1)
2.24. Colorimeter (1) HACH, DR890
2.25. Test Strips (1 or more) HACH
2.26. Water Baffling Assembly
2.27. Light Table
2.28. Weigh Boats, Plastic, 100-mL
2.29. Transfer Pipette
2.30. Sediment Settling Assembly
2.31. Table, Folding, Water-proof
2.32. Light Box
2.33. Pyrex Casserole Dish, 4-L
2.34. Forceps
2.35. Vinyl Hose with Spray Nozzle
2.36. Centrifuge Tubes, 15-mL
2.37. Centrifuge, Bucket with test tube inserts.
2.38. Blender
2.39. Jars, 32 ounce with lids

3.0 Organisms/Reagents/Solutions

3.1. Amphipod, *Leptocheirus plumulosus* neonates that are < 48 hour old. These animals are typically those that are sieved through a 600-µm sieve and retained on a 250-µm sieve.
   A. **Source:** In-House Cultures
   B. **Source:** Aquatic Research Organisms, Hampton, NH, 603-926-1650
   C. **Source:** Aquatic Biosystems, Ft. Collins, CO, 970-484-5091

3.2. Control Sediment, Saltwater
   A. **Source:** Control Sediments are either material acquired from the vendor supplying the test organisms or material collected from a clean reference site that has been determined to be suitable for testing. In both cases the control materials are natural occurring sediments.

3.3. Synthetic Saltwater (SSW)
   A. Typically this would be synthetic seawater (SSW) made from ultra-pure water (18.2 MOhm) and Tropic Marin™ sea salts prepared according to the EPA's "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms", Fifth Edition, October 2002, EPA-821-R-02-012.
   B. Acclimation and control waters are prepared at **either 5‰ or 20‰** and allowed to equilibrate overnight under aeration before use.
      1. The tests overlay water salinity with either be 5‰ or 20‰ depending on the sediment sample's pore water salinity. If the salinity of the pore water is 1 to 10‰, then the overlay water will be 10‰. If the salinity of the pore water is >10 to 35‰, then the overlay water salinity will be 20‰.

3.4. Reagent Grade Water · Type I

3.5. Tropicmarin Synthetic Seasalts
3.6. Aquatox Flake Fish Feed
3.7. Staining Solution
   A. Prepare by adding 45 mgs Rose Bengal to 1 gallon of denatured alcohol.

4.0 Personal Protection Equipment

4.1. No hazardous chemicals are used for the procedure, therefore; only standard laboratory safety procedures are practiced.

5.0 Procedure **Test Day**

5.1. Schedule Test Event and Shipment of Sampling Kit **~ 2-weeks**

A. Create a Job Folder according to the SOP.
   1. Place Job folder in relevant tray on the Sample Receipt Counter in the Lab Testing Bay.

B. Sample Volume
   1. 5-replicates x 175-mLs is 875-mLs, however… sediment sample can vary greatly and the study may need to be repeated if it is unacceptable. The sample containers provided are 1-gallon (~3.78-L). Based on these elements the requested **sample volume should be 1-gallon**.

C. Sample Kit Shipment
   1. Determine how many sampling kits will be required. Each kit can hold 4, 1-gallon Nalgene® (or equivalent) sampling bottles and sufficient ice to preserve samples for a 24-hour period.
   2. Prepare sampling kits by including the following:
      a. Cooler containing…
         1) 4, clean 1-gallon Nalgene® bottles (each cooler can hold maximum of 4 bottles)
         2) Ziploc Bag. The Ziploc bag gets taped to the inside lid of the cooler but first place the following inside the Ziploc bag…
            a) Chain-of-Custody
            b) COC Seal
            c) Sample Bottle Labels
            d) Hydrosphere Return Address Label
      b. Chain-of-Custody Seal
         1) Sign & date the COC seal with water-proof black pen. Apply across the seam that's between the lid and body of the cooler.
         2) Tape the cooler closed with nylon reinforced tape. You must tape over the signed COC Seal with the tape. The tape is clear so the signed COC seal is visible.
      c. Apply the shipping sleeve containing the FedEx shipping label to the top of the cooler.
   3. Place cooler to the left of the front door to await pickup.
   4. Sign off on the Job Folder, "Shipped by" and "Shipped Date".
   5. Return Job Folder to relevant tray.

5.2. Determine where in the Laboratory the Bioassay will be Conducted **~ 2-weeks**

A. Determine which environmentally controlled water baths will accommodate the exposure vessels and will be able to meet the test conditions. The baths must have a pump and heater for temperature control, a source of clean, oil-free air, approximately 50 to 100 foot-candles illumination (ambient laboratory) and a 16-hour light/8-hour dark photoperiod.
B. Adjust photoperiod if necessary.

C. Adjust temperature of water bath to between 25 and 26°C. Exposure vessels typically run slightly less than bath temperature. Check water pump and verify that water is circulating. On subsequent days verify water bath temperature adjustments by checking the laboratories logbook which records the temperatures of all active baths and environmental chambers daily.

5.3. The Acquisition of *Leptocheirus plumulosus* for use in Bioassay ~2-weeks

A. Locate source of *Leptocheirus plumulosus*
B. Determine the number of amphipods required for the bioassay. The method recommends 5-replicate vessels with 20-organisms per replicate or a total of 100-amphipods per sediment sample or treatments.

C. Organisms Ordered from Outside Source
1. Order 20% more than is required for the test. Mortalities may occur in shipping.
2. Have test organisms shipped priority overnight service.
3. Order amphipods so they arrive **one day** before the test is to be initiated. This give them time to sort themselves out (injuries during shipment may not be apparent upon arrival).

D. Organisms Cultured In-House
1. Two Days prior to Testing
   a. Sieve cultures through a 600-µm sieve and then through a 250-µm sieve. Those retained on a 250-µm sieve are organisms that are < 48 hours old.

E. **NOTE:** Determine the tests overlay water salinity and request LP at either 5‰ or 20‰!!!

5.4. The Acquisition and Processing of Control Sediment ~1-week

A. Determine what the negative control(s) shall be. They may be artificial prepared or naturally occurring. They should have a high likelihood of performing adequately, as in, meeting minimal test method quality control standards.
B. Acquire control sediment approximately 1 to 2 weeks ahead of scheduled test date.
C. Control sediments may be collected by the laboratory, a contracted vendor or may be a reference site located near the test site. Refer to the Job Folder for the specific control sediments required for each test event.
D. The control sediment is to be collected in clean plastic (preferably HDPE) containers. These containers are then packed in a cooler with ice and shipped via an overnight courier to this laboratory.

E. Receipt and Processing of Control Sediment
1. **Upon arrival of control sediment…**
   a. Acquire the shipper's air bill.
   b. Inspect the packaging; ensure that the contents are intact.
   c. Note the condition of a chain-of-custody seal, if present.
   d. Open shipping container and find the paperwork describing the contents (chain-of-custody, shipping slip, etc…).
   e. Record the following on the paperwork:
      1) Temperature of the sediment.
      2) Date Received and Initial.
   f. Record the following on the sediment container:
      1) Make a record in the "Chemical & Solutions" Logbook and generate a Chemical Log number. Record this on the sediment container.
      2) Assign a 6-month expiration date and record on container.

g. Refrigerate sediment in coldroom.

2. **Processing of Control Sediment**
   a. Remove control sediment from coldroom.
   b. Warm sediment to room temperature.
   c. Open sediment sample container. Do **not** pour off overlying water. During shipping and storage the sediment settles exposing some of the interstitial water. The overlying water will be mixed back into sample in the following step.
   d. Homogenized the sediment by mixing with a stainless steel beater attached to a drill. This may be done in the original container (if large enough) or in another appropriate container; typically a 5-gallon HDPE Bucket.
   e. The control sediment is sieved through a series of ~12" diameter Nitex® or stainless steel sieves to reduce the particle size to less than 240-µm.
      1) Wash sediment using synthetic saltwater.
      2) ***NOTE:*** *Adjust salinity of pore water to either 5‰ or 20‰ as determined by the client's sample.*
      3) Sieve through ¼", 1000-µm, 800-µm, 600-µm, 450-µm, 240-µm screens.
   f. Store sediments in a labeled 5-gallon bucket and store in coldroom. Allow washed sediment to settle for ≥2-days then draw off the overlay water with a 3/8" silicone tube.

5.5. The Receipt of Sediment — < 8-weeks (Sediments), < 2-weeks (Water)

A. Unless otherwise noted on the Job Folder, Sediments have an 8-week holding time and Receiving Waters have a 2-week holding time. Laboratory prepared synthetic waters have a 2-week holding time.
B. Receipt of Test Sediment
   1. The receiving of the test sediment samples shall be conducted as given in the Sample Receiving SOP.
   2. Points Emphasized from item 1 above.
      a. Record **Sample ID** on each container.
      b. Record **Expiration Date** (8-weeks) on each container.
   3. Refrigerate sediment samples in cold room until use in testing.
      a. Record the condition of the COC seal.
      b. Scan and email completed COC to client.
   4. ***NOTE:*** *Determine salinity of pore water !!!*

5.6. Receipt of Amphipods — Day -1

A. Acquire the shipper's air bill.
B. Inspect the packaging; ensure that the contents are intact.
C. Open container and find shipping slip.
D. Make record in the "Test Organism Receipt and Acclimation" Logbook.
   1. Follow instructions in SOP, "Test Organism Receipt and Acclimation".
   2. Check Salinity and Temperature.
E. Fill 15-L aquaculture basin with 10-L SSW at either 5‰ or 20‰. Transfer amphipods to basin (~500 per basin).
F. Place in appropriate bath at 25°C and place on light air.
G. Water change amphipod basins at least daily.

5.7. Other Pre-Test Preparations — Day -2

A. **Job Order and Data/Bench Sheets**
   1. Locate Job Folder. Check for completeness.

2. Review information/instructions on Job Folder and Bench Sheets. Report any discrepancies to the laboratory manager.
3. Record Job Number on all bench sheets.
4. **Remember:** Corrections are made by drawing a single line through the error, initial and date correction, record correct data next to correction or, if note enough room, footnote and record correct data in "Notes and Comments" section at the bottom of the bench sheet.

B. Apparatus and Equipment Review

1. Review the list in Section 2.0. Verify that all are present, clean and in acceptable working order.
2. **Exposure Vessels, 1,000-mL Beakers**
   a. The bioassay is conducted in 1,000-mL beakers or glass jars. Each sediment sample will be divided into 5 identical exposure vessels or replicates. Label each exposure vessel with Sample Type and Replicate designation (The 5-replicates are designated alphabetically "A" through "E").

5.8. Preparing Sediments for use in Bioassay — Day -1

A. Control Sediment

1. Retrieve sediment sample from cold room.
2. Warm sediment sample to room temperature.
3. *Note: Sediment should have already been processed per section 5.3 above.*
4. Open sediment sample container. Do **not** pour off overlying water. The overlying water will be mixed back into sample.
5. Homogenize the processed sediment (settling is likely to have occurred during storage in cold room) by mixing with stainless steel beater attached to a drill.
6. Pour or scoop sediment into a 1-gallon double Ziploc bag. Cut off a corner of the bag and squeeze sediment into appropriate exposure vessel. Fill to the 175-mL mark. Repeat for remaining replicates.
7. Place exposure vessels into testing water bath. Randomize locations per Template. Record Template number.
8. Return unused portion to original container and refrigerate sediment in cold room.

B. Test Sediment

1. Retrieve sediment sample from cold room.
2. Warm sediment sample to room temperature.
3. Open sediment sample container. Do not pour off overlying water. During shipping and storage the sediment settles exposing some of the interstitial water. The overlying water will be mixed back into sample in the following steps.
4. Homogenized the sediment by mixing with a stainless steel beater attached to a drill. This may be done in the original container (if large enough) or in another appropriate container (HDPE Bucket).
5. If needed, the test sediment is press sieved through a series for Nitex® or stainless steel sieves to reduce the particle size. The method discourages sieving sediment samples. However, large particulate matter will make retrieving test organisms at the end of the study extremely difficult. Test organisms will likely be lost during test take down if large debris is not removed from the sediment sample during test setup. Sieving down to 1 to 2-mm is recommended.
6. Scoop sediment into appropriate exposure vessel using a stainless steel spoon. If sediment is fluid enough then it may be easier to "squeeze" it in. Pour or scoop sediment into a 1-gallon double Ziploc bag. Cut off a corner of the bag and squeeze sediment into appropriate exposure vessel. Fill to the 175-mL mark. Repeat for remaining replicates.
7. Place exposure vessels into testing water bath at 25 ± 1°C. Randomize locations per Template. Record Template number.

8. **Pore Water Quality**
   a. Transfer 10-mLs of sediment to each of 2, 15-mL centrifuge tubes for each sample.
   b. Centrifuge samples for 30-minutes at ½ speed.
   c. Measure Salinity and Ammonia of supernatant.
   d. **Make a record on the "Pore Water Ammonia & Salinity" bench sheets.**
   e. **Note:** Salinity tolerance for *L. plumulosus* is 0 to 34‰.

9. Return unused portion to original container and refrigerate sediment in cold room.

C. The Exposure Vessel and associated sediments are now ready to have overlay water added to them.

5.9. Preparing Overlay Waters for use in Bioassay — Day -1

A. Overlay water can either be laboratory prepare synthetic saltwater (SSW) or receiving water. Receiving waters are typically taken from upstream of the test sediment sample or a nearby body of water. In either case the receiving water is to be taken from a "clean" location. Refer to Job Order to determine what overlay water is to be used.

B. **Preparation of SSW**
   1. SSW is prepared according to the EPA reference method listed in section 9.

C. **Preparation of Receiving Water**
   1. Retrieve water from cold room (unless it has just arrived and is still in cooler).
   2. Warm water sample up to room temperature (~25°C).
   3. **Make a record on the "Sample Data" bench sheet.**
      a. Record sample ID.
      b. Measure initial water quality and record.
         1) Measurements taken are dissolved oxygen (DO), pH, salinity, total ammonia (TAN), alkalinity, hardness, total residual chlorine (TRC).
         2) Adjust salinity to 5 or 20‰ if necessary. Do so either by diluting with Reagent Grade Water · Type I or salting the water with Tropic Marin Sea Salts.
         3) Adjust DO if supersaturated by light aeration. Receiving waters should not have an oxygen demand. If DO is <4.0-mg/L, do not use and contact the laboratory manager immediately.
      c. Record the receiving waters expiration date. This is 2-weeks unless otherwise noted on the Job Folder.

D. **Addition of Overlay Water to Exposure Vessel**
   1. Place Water Baffling Assembly in an exposure vessel.
      a. This device is designed to allow the addition of water with minimally disturbing the sediment in the exposure vessel.
   2. Pour water to exposure vessels. Fill to 900-mLs (overlay volume is 725-mLs).

E. The exposure vessels will now be allowed to settle overnight. Test initiation (Day 0) will be tomorrow.

5.10. Initiation of Bioassay — Day 0

A. There are several steps required to initiate the bioassay. The overlay water in the exposure vessels must be partially replenished. Water quality must be checked and recorded. Water quality must also be acceptable in order to support the unstressed survival of the amphipods for the duration of the bioassay. Amphipods are introduced to the exposure vessels once these parameters are deemed to be acceptable.

B. **Prepare Water for Renewal of Overlay Water**

1. If Overlay is **SSW**, skip ahead to C.
2. If Overlay is **Receiving Water**…
3. Retrieve water from cold room (unless it has just arrived and is still in cooler).
4. Warm water sample up to room temperature (~25°C).
5. **Make a record on the "Sample Data" bench sheet.**
   a. Record sample ID.
   b. Measure initial water quality and record.
      1) Measure dissolved oxygen (DO), pH and salinity. These are measured with each days use.
      2) Measure total ammonia (TAN), alkalinity, hardness, total residual chlorine (TRC). These are only measured with the samples first use.
      3) Adjust DO if supersaturated by light aeration. Receiving waters should not have an oxygen demand. If DO is <4.0-mg/L, do not use and contact the laboratory manager immediately.
   c. Record the receiving waters expiration date (from the sample container).

C. **Measure Water Quality**

1. Retrieve one of the sediment sample sets from the water bath (a "set" is all of the replicates, "A" through "E" of a given sediment sample) and place them on a light table.
   a. Take measurements directly in each exposure vessel.
   b. **Make a record on the "Temperature" bench sheets.**
      1) Measure and record Temperature from one replicate.
      2) This is done daily for the duration of the test.
   c. **Make a record on the "Dissolved Oxygen", "pH" and "Salinity" bench sheets.**
      1) Measure and record Dissolved Oxygen, pH and Salinity from ALL replicates.
      2) This is done on day 0 and day 28.
2. ***Important*** Be certain that all water quality parameters are within prescribed method specifications. If not, contact the laboratory manager.

D. **Renewal of the Overlay Water**

1. Place Water Baffling Assembly (WBA), in an exposure vessel.
2. Siphon overlay water out of exposure vessel into a 5-gallon bucket on the floor.
3. Repeat for remaining test replicates on the table.
4. With the WBA still in one of the exposure vessel, carefully fill the exposure vessel with fresh overlay water. Fill to top (but not to overflowing!) of exposure vessel.
5. Carefully remove the WBA and move it to the next exposure vessel.
6. Check overlay volume. If necessary adjust 900-mLs (overlay volume is 725-mLs).
7. Repeat steps 4 and 5 for remaining exposure vessels.
8. Return the exposure vessels to the water bath.
9. **Make a record on the "Renewals and Feedings" bench sheet**
   a. Record the overlays ID# on the bench sheet.

E. Repeat C and D above for remaining Sediment Sample Exposure Vessels.

F. Addition of Amphipods to Exposure Vessels

1. Retrieve basin containing amphipods from water bath and place on a counter or light table.
2. Siphon off most of the water in the basin. This will make it easier to count out the amphipods.
3. Set out weigh boats on the table. Using a transfer pipette add some of the SSW from the basin into each weigh boat. Then carefully count out 20-amphipods into each boat.
4. Bring out 5 randomly selected exposure vessels from the testing water bath.
5. Gently wash amphipods from the weigh boat into the exposure vessel.
6. Be certain that all amphipods break through the surface tension of the overlay water. Any floating amphipods are gently nudged into the water by using the transfer pipette and dropping overlay water onto the floating amphipod.
7. Once all vessels have had 20-amphipods added to them they are returned to the testing water bath.
8. Repeat steps 3 through 7 above for remaining sediment sample test vessels.
9. **Make a record on the "Survivorship" bench sheet**
   a. Record the initiation of the test.
10. 20-minutes after addition of amphipods to test vessels check to ensure all animals have burrowed into sediment. Replace any animals that have not burrowed. Do not replace amphipods that are attempting to burrow but are immediately emerging as this is likely an avoidance response. Record the number of animals replaced.
11. Prepare 3 sets of 20 neonates for initial weight of the growth rate endpoint. Rinse with deionized water and dry overnight at 70°C.

G. Feeding Exposure Vessels

1. None

H. Aeration

1. Place all test vessels on light aeration.
2. The test is aerated continuously for the duration of the 28-days.

5.11. Observations and Water Quality during the test... Days 1 through 27

A. Measure Water Quality on Renewal Days... Monday, Wednesdays and Fridays

1. Retrieve one of the sediment sample sets from the water bath (a "set" is all of the replicates, "A" through "E" of a given sediment sample) and place them on a light table.
   a. Take measurements directly in each exposure vessel.
   b. **Make a record on the "Temperature", "Dissolved Oxygen", "pH" and "Salinity" bench sheets.**
      1) Measure and record Temperature, Dissolved Oxygen, pH and Salinity from ONE replicate.
2. ***Important*** Be certain that all water quality parameters are within prescribed method specifications. If not, contact the laboratory manager.

B. Observations on Renewal Days... Monday, Wednesdays and Fridays

1. Record observations of amphipod activity and condition of sediment and water in all test chambers.

C. Renewal of the Overlay Water Monday, Wednesdays and Fridays

1. Place Water Baffling Assembly (WBA), in an exposure vessel.
2. Siphon overlay water out of exposure vessel into a 5-gallon bucket on the floor.
3. Repeat for remaining test replicates on the table.
4. With the WBA still in one of the exposure vessel, carefully fill the exposure vessel with fresh overlay water. Fill to top (but not to overflowing!) of exposure vessel.
5. Carefully remove the WBA and move it to the next exposure vessel.
6. Check overlay volume. If necessary adjust 900-mLs (overlay volume is 725-mLs).
7. Repeat steps 4 and 5 for remaining exposure vessels.
8. Return the exposure vessels to the water bath.
9. **Make a record on the "Renewals and Feedings" bench sheet**
   a. Record the overlays ID# on the bench sheet.
   b. Repeat C and D above for remaining Sediment Sample Exposure Vessels.
      1) This is done on a Monday, Wednesday, Friday schedule.

D. Feeding Exposure Vessels on Renewal Days... Monday, Wednesdays and Fridays

1. Prepare a stock of Aquatox Flake Fish Feed by blending 40-grams in 1L of RGW·I for 5-minutes.
2. Assign a 2-week expiration date and refrigerate at 4°C.
3. On days 0 to 13, feed ½ mL to each replicate after renewal.
4. On days 14 to 28, feed 1 mL to each replicate after renewal.
5. Record feeding.

E. Measure Water Quality on Non-Renewal Days...

1. Retrieve one of the replicate sediment exposure vessels from each sample and place them on a light table.
   a. Take measurements directly in each exposure vessel.
   b. **Make a record on the "Temperature" bench sheets.**
      1) Measure and record Temperature from ONE replicate.

F. Aeration

1. Check aeration of test vessels. Adjust aeration were needed.
2. If any test vessels have lost aeration, make a record of which vessels lost aeration. Restore aeration to those vessels immediately.

5.12. Termination of Bioassay — Day 28

A. Termination of the bioassay occurs on the 28th day. Final water quality is taken. The sediments are washed through a 600-µm sieve in order to collect and count the adult amphipods. The sediments are then washed through a 250-µm sieve in order to collect and count the amphipods offspring.

B. On the day before the test is to be terminated, prepare aluminum weigh pans per SOP number MTR-001.

C. **Setup a Sediment Take Down Work Station**

1. **Set up Work Station outside.**
   a. Sediment Rinse Station
   b. Water Proof Table
   c. Light Box
   d. 600-µm sieve
   e. 240-µm sieve
   f. Pyrex Casserole Dish
   g. Forceps
   h. Deionized Water Hose
   i. Spray Attachment for DI water

D. **Measure Water Quality**

1. Retrieve one of the sediment sample sets from the water bath (a "set" is all of the replicates, "A" through "E" of a given sediment sample) and place them on a light table.
   a. Take measurements directly in each exposure vessel.
   b. **Make a record on the "Temperature" bench sheets.**
      1) Measure and record Temperature from one replicate.
   c. **Make a record on the "Dissolved Oxygen", "pH" and "Salinity" bench sheets.**
      1) Measure and record Dissolved Oxygen, pH and Salinity from ALL replicates.
   d. **Make a record on the "Pore Water Ammonia" bench sheets.**
      1) Measure and record Ammonia, pH and Temperature from one replicate.

E. **Separation of Amphipods from Sediment**

1. Work with one set and then one **exposure vessel at a time**.
   a. Transport one set of exposure vessels and tared weigh pans outside to the Sediment Settlement Container.
   b. Pore contents of exposure vessel into 240-µm sieve. Flushing the vessel with DI water will likely be necessary.
   c. Rinse sediment through the sieve.
   d. Locate and count the adult amphipods.
   e. **Make a record on the "Survivorship" bench sheet.**
   f. Using forceps, transfer adult amphipods to the appropriate weigh pan.
   g. Transfer remaining sediment into a 32 ounce jar.
   h. Add about 250 mLs of Staining Solution and seal. Place jars in a safe place for later scoring of offspring.
   i. Repeat a. through e. for the remaining exposure vessels in this set.
   j. Place weigh pans in oven at 70°C to dry overnight.

F. Repeat C and D above for remaining sediment sets.

G. Transport empty labware and exposure vessels to the dirty lab ware counter.

H. Remove the water from the sediment settling container, and use a scoop to remove and transfer the "composited" sediment remaining in the container to a sample container for return to the client.

I. **Final Dry Weights**
1. After pans have dried overnight, transfer to a desiccator for 20 minutes.
2. Determine final dry weights using the SOP number MTR-001.

J. **Counting Offspring**
1. Within 2 weeks, count offspring and record.

6.0 Quick Summary

6.1. No summary currently prepared.

7.0 Acceptability of Data

7.1. Minimum mean **control survival 80%** in the control sediment with **no single control having ≤60% survival.**

8.0 General References

8.1. Method for Assessing the Chronic Toxicity of Marine and Estuarine Sediment-associated Contaminants with the Amphipod *Leptocheirus plumulosus*. (EPA 600/R-01/020, March 2001)

8.2. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition, (EPA-821-R-02-012, October 2002)

9.0 Signatures

9.1. Signature, Title of the Approving Authority and Implementation Date.

A. _____ _____ ___/___/___.

9.2. Each person conducting the SOP should read the following statement. Print and sign if you agree.

"I have read and understand this Standard Operating Procedures as it relates to my job responsibilities. I further understand that the Standard Operating Procedures are an integral part of Hydrosphere Research's operations. I agree to follow all procedures outlined in the Standard Operating Procedures."

9.3. Printed **Name, Signature, Date and Training ETW** of persons conducting this procedure:

A. _____ _____ ___/___/___ _____·ETW

B. _____ _____ ___/___/___ _____·ETW

C. _____ _____ ___/___/___ _____·ETW

D. _____ _____ ___/___/___ _____·ETW

E. _____ _____ ___/___/___ _____·ETW

F. _____ _____ ___/___/___ _____·ETW

G. _____ _____ ___/___/___ _____·ETW

H. _____ _____ ___/___/___ _____·ETW

I. _____ _____ ___/___/___ _____·ETW

J. _____ _____ ___/___/___ _____·ETW

K. _____ _____ ___/___/___ _____·ETW

L. _____ _____ ___/___/___ _____·ETW

9.4. Annual Review (signature and date)

A. _____ ___/___/___.

B. _____ ___/___/___.

C. _____ ___/___/___.

D. _____ ___/___/___.

E. _____ ___/___/___.

10.0 SOP Postings and Revisions

10.1. Postings

A. Laboratory Posted Location(s) of SOP's.

1. _____

2. _____

3. _____

10.2. Revision Checklist

- ☐ Retrieve and Dispose of Posted Copies. Initial _____
- ☐ Create New Revised SOP Initial _____
- ☐ Train Personnel on "new" SOP Initial _____
- ☐ File Electronic Version of Retired SOP Initial _____
- ☐ File Paper Version of Retired SOP Initial _____

11.0 Examples of Relevant Datasheets or Documents

11.1.

A. None currently prepared for this document.