Adapting to Supply Challenges and Species Substitution for the 10-Day Amphipod Bioassay

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Introduction

For the 10-day amphipod bioassay test the DMMP and TCP procedures include a suite of three organisms (*Eohaustorius estuarius*, *Ampelisca abdita*, and *Rhepoxinius abronius*) to allow for flexibility in selecting the most appropriate available species. This includes factors such as tolerance to specific grain size distributions and salinity. Additional factors such as seasonal availability of field collected amphipods as well as logistical constraints (supplier availability, weather, shipping interruptions) may also affect availability.

These species are field collected organisms and are subject to seasonal reproductive cycles that affect the availability of animals appropriate in size and age for testing. This is most extreme with *A. abdita* which has an overwintering population that sees a high production of offspring in the Spring and a die-off of adults (March – June, depending on the population). Historically there have been two populations on the Atlantic and Pacific coasts of the US that have slightly overlapping reproductive windows (Table 1).

Since 2021 the supply of the Pacific population of *A. abdita* has not been available to laboratories. The historical collection site in San Rafael Bay (an embayment in the San Francisco Bay Area, California) has been reported to have experienced a decline in population that has made field collection unfeasible. Possible reasons for decline may be related to macroalgae blooms [green tides] resulting in sediment surface smothering and changes in water quality characteristics in the collection area. Additionally, *R. abronius* does not have a reliable supply source and has been rarely used over the past decade. With a reduction in supply options leaving two species (*E. estuarius* and *A. abdita*) collected each by a single supplier, this provides a challenge to completing testing programs within holding times.

Table 1. Summary of Amphipod Species Availability Based on Seasonal Reproductive Cycles:

Species	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Ampelisca abdita (Pacific)												
Ampelisca abdita (Atlantic)												
Eohaustorius estuarius												
Rhepoxinius abronius												
Leptocheirus plumulosus	Cultured/Available Year-Round											

Green = available

Gold = available, but care must be taken with selection; fringe months (Mature adults reproducing, not present, and/or offspring coming into size)

Orange = not available

Problem Identification

Bioassays are often performed within a tiered framework where testing is triggered based on the results of chemical analyses. The holding time for initiating bioassay in 8 weeks from the time of sediment collection. A chronic bottleneck for achieving holding time milestones is that chemical analysis and review can take 5-7 weeks. This leaves laboratories a short window of time (1-3 weeks) to coordinate testing and obtain field collected test organisms. Based on the challenges listed above, this often does not leave enough time for laboratories to troubleshoot if supply disruptions or organism health concerns arise and rarely leaves enough holding time to repeat tests, if necessary. Therefore, we require an alternative species option to enhance flexibility and ensure project success.

Proposed Issue Solution

Leptocheirus plumulosus is a benthic amphipod species that has been in use for sediment bioassays since the inception of formalized test methods. During the early PSEP/PSSDA program development Leptocheirus was not adopted into the formal species toolkit; however, *L. plumulosus* has been used on a case-by-case with Agency approval for recent dredging and cleanup projects. This species is also listed in the Biological Testing Toolbox within the Sediment Evaluation Framework (RSET 2018; Appendix D.). The utilization of this species is comprehensively documented in both national and regional guidance materials, a selection of which includes:

- Evaluation of Dredged Material Proposed for Discharge in Waters of the U.S.- Testing Manual (Inland Testing Manual) (USACE/USEPA. 1998)
- Guidance for Performing Tests on Dredged Material Proposed for Ocean Disposal (New York District) (USACE/USEPA. 2016)
- Southeast Regional Implementation Manual (SERIM) (USACE/USEPA. 2008)
- Methods for Assessing the Chronic Toxicity of Marine and Estuarine Sediment-associated Contaminants with the Amphipod *Leptocheirus plumulosus* (USEPA. 2001)

In order to address the issues identified above, the DMMP/TCP agencies propose to allow the flexibility to use the amphipod *Leptocheirus plumulosus* when the primary species options are not available for field collection or not in a healthy condition suitable for testing. Any proposed species substitutions for the amphipod bioassay must be coordinated with the DMMP/TCP agencies, prior to testing.

Rationale

L. plumulosus is a subtidal estuarine amphipod native to the Atlantic coast of the US from Cape Cod, MA. to northern Florida. It is found in oligo- or mesohaline habitats in the wild (0.5 – 16 ppt)) has been shown to have a wide salinity tolerance in laboratory studies (ranging from 0 – 32 ppt). The sediment type it is most associated with in the wild is fine-grained with a high proportion of particulate organic material, although it can also be found in fine sand with some organic content. *L. plumulosus* creates loose tubes in the sediment and it feeds on particles that suspended in the water column and on the sediment surface. This behavior is in contrast to *A. abdita* which constructs mucous cemented sac-like tubes and primarily filter feeds from inside the tube.

Of the estuarine/marine amphipod species used for sediment toxicity testing, *L. plumulosus* is one of the few organisms that has been readily cultured by commercial suppliers and laboratories. The USACE Engineer Research and Development Center (ERDC) has maintained populations over many years for the purposes of research and national dredge material evaluation testing. Under laboratory cultured conditions, this organism does not experience seasonal disruptions. An inter-species comparison of test conditions for performing amphipod testing under PSEP are summarized in Table 2. Testing with *L.*

plumulosus would follow the test acceptability and interpretive guidelines for marine amphipods in the DMMP User Manual (DMMP 2021) and Ecology's Sediment Cleanup User Manual (SCUM).

The sensitivity of *L. plumulosus* to porewater ammonia is described as being similar to or more tolerant than *E. estuarius* (ASTM. 2023). Internal reference-toxicant data shared by EcoAnalysts, Inc. resulted in a mean no observed effect concentration (NOEC) of 1.3 mg/L unionized ammonia (n=21). Relevant published information regarding sulfide sensitivity specifically for *L. plumulosus* is not available. The DMMP published a clarification paper in 2015 establishing ammonia and sulfide trigger values for the PSEP bioassay test species (Inouye et al. 2015) including procedures to address. Applying the established trigger values for *E. estuarius* to *L. plumulosus* would be protective of this species. Any approaches to purging sediment prior to bioassay test initiation must be coordinated with the appropriate agencies and is typically not appropriate for programs under cleanup investigations.

Table 2. Amphipod Test Condition Summaries

Species	***Eohaustorius estuarius***	***Ampelisca Abdita***	***Rhepoxinius abronius***	***Leptocheirus plumulosus*[3]**
Life Stage Tested	Mature amphipods 3-5 mm, mixed sexes	Immature amphipods	Mature amphipods 3-5 mm, mixed sexes	Mature amphipods 2-4 mm, mixed sexes[4]
Feeding	Will not be fed	Will not be fed	Will not be fed	Will not be fed
Temperature (°C)	15 ± 1	20 ± 1	15 ± 1	25 ± 2
Salinity (ppt)	28 ± 1 or ± 1 ambient[1]	28 ± 1	28 ± 1	28 ± 1 or ± 1 ambient[1]
pH	7 – 9	7 – 9	7 – 9	7 – 9
DO (≥ 60% Saturation)	5.1 mg/L	4.6 mg/L	5.1 mg/L	4.4 mg/L
Trigger values for performing a concurrent ammonia reference-toxicant exposure (Inouye et al. 2015)				
Un-ionized Ammonia (mg/L)	0.4	0.118	0.2	0.4
Trigger values for performing purging procedures (Inouye et al. 2015)				
Un-ionized Ammonia (mg/L)	0.8	0.236[5]	0.4	0.8
Hydrogen Sulfide[3] (mg/L)	0.122	0.0094[5]	0.099	0.122

[1] Test salinity for *E. estuarius* and *L. plumulosus* may be conducted at the interstitial salinity (ambient) of the test sediments. The target test salinity should be approved by the client or regulatory agency and will vary depending upon the objectives of the testing program.

[2] Kendall and McMillan 1999

[3] Direct guidance for *L. plumulosus* is not given under PSEP guidelines; however, test conditions are similar to that of *E. estuarius* and described in other guidance documents.

[4]Size here relates to total animal length. The size of animals used for testing in the 10-day sediment exposure have been reported as those that pass through a 1mm sieve and are retained on a 0.7mm sieve.

[5]Ampelisca unionized ammonia and hydrogen sulfide limits are for overlying water, all other amphipod limits are for porewater

Clarification Summary

1. DMMP/TCP will allow the use of the amphipod *Leptocheirus plumulosus* when *A. abdita* or *E. estuarius* are not available for field collection or not in a healthy condition suitable for testing. Any proposed species substitutions for the amphipod bioassay must be coordinated with the Dredged Material Management Office, and approved by the DMMP agencies, prior to testing.
2. Ammonia and sulfide management will follow the benchmark criteria established for *E. estuarius* when utilizing *L. plumulosus*.
3. The use of multiple amphipod species may be required for projects where grain size varies greatly and crosses the sensitivity benchmarks.
4. Table 3 and 4 provide a visual guide to grain size selection criteria.

Table 3. Amphipod Grain Size Selection Benchmarks (% fines/% sand)

% Fines (Silt + Clay)	0-10	10-20	20-30	30-40	40-50	50-60	60-70	71-80	80-90	90-100
% Sand	100-90	90-80	80-70	70-60	60-50	50-40	40-30	30-20	20-10	10-0
Species										
Ampelisca abdita										
Eohaustorius estuarius										
Rhepoxinius abronius										
Leptocheirus plumulosus										

Green = tolerance
Gold = potential inference
Orange = will likely not tolerate

Table 4. Amphipod Grain Size Selection Benchmarks for % clay

% Clay	0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100
Species										
Ampelisca abdita										
Eohaustorius estuarius										
Rhepoxinius abronius										
Leptocheirus plumulosus										

Green = tolerance
Gold = potential inference
Orange = will likely not tolerate

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