October 29, 2014

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Re: Shipyard Creek: Benthic Test Results with Amphipods

Dear Paul,

The discussion below briefly summarizes the results of the benthic amphipod tests conducted on the Shipyard Creek samples and an evaluation of the effect of grain size on amphipod sensitivity.

Summary of the *Leptocheirus plumulosus* benthic test results:

Mean survival in the *Leptocheirus* control was 91%; meeting the test acceptability of ≥90%. Survival in the reference was 97%, suggesting that the reference site sediment was suitable for *Leptocheirus* survival. The project samples SYC14-TB-1, SYC14-TB-2, and SYC14-AC had mean survival values of 70%, 52%, and 61%, respectively. These values were >20% less than the reference mean survival of 97%, indicating that these samples would exceed the LPC for disposal acceptability and would not be deemed acceptable for aquatic disposal.

When validating the results of sediment toxicity tests results it is useful to evaluate the potential contribution of non-contaminant related effects to any observed toxicity. These contributors include factors such as ammonia present in the porewater or overlying water, the amount of total organic carbon (TOC) in the sediments, and the potential effect grain size may have had on the test organisms. These concepts are discussed in detail below.

All test samples were treated with ammonia reduction procedures achieved through daily water exchanges prior to and during the solid-phase testing conducted with this species. The porewater ammonia was sufficiently reduced in these samples below levels predicted to result in mortality with this species (60 mg/L total ammonia). Consequently, ammonia was unlikely to be a primary contributor to the reduced survival observed. Total organic carbon levels within the Shipyard Creek samples was sufficiently high in quantity to support amphipod survival. The sample with the lowest TOC was the reference (0.084%) and resulted in 97% survival suggesting that the availability of food should not have been a contributing factor to the reduced survival observed in the project sediments.

Sediment grain size is another important factor in evaluating benthic test performance for amphipods. The amount of fine-grained sediment (percentage of silts and clays) has been used as an indicator of grain size for selecting test species (PSEP 1995). Sediments dominated by a high percentage fines have been correlated with poor survival in free-burrowing amphipod species. The percentage fines in the Shipyard Creek test sediments ranged from 75.8 to 99.0 % fines (silt and clay), whereas the percentage of fine sediments in the reference sediment was 20.2 % fines. The reference sample (20.2% fines) resulted in 97% mean survival while the project samples with percentage fines ranging from 75.8 to 99.0% resulted in mean survivals of 52% – 70%. Barring the presence of significant contaminants of concern in the project composite samples, these results suggest that the grain size may have been a significant contributor to the observed toxicity.

In addition to the broad category of percent fines, another useful tool is understanding what proportion of the fines are comprised of the smallest fraction of clays (passing through a 0.0013 mm sieve). These small particles have been shown to elicit physical stress to certain species of amphipods by preventing proper tube construction, interfering with movement, or binding to and clogging respiratory surfaces of the animals. The TB samples contained 42.5 – 45.6% of the finest fraction of clays (<0.0001 mm), while the AC sample had 32.9%. The finest fraction of the percent fine material represents almost half of the total % fines content. The presence of these very fine grained sediments may have been a contributing factor to the observed mortalities.

A method for addressing the confounding factor of sediment grain size may be to use an alternative approved amphipod species that is more adapted to very fine grain size range. The amphipod species, *Ampelisca abdita,* is the typically considered the most tolerant species for sediments containing a large amount of fine- grained materials while being sensitive to contaminant-related effects. If the negative biological effects observed are solely related to grain size, there should be a marked enhancement in survival of *Ampelisca* exposed to the project sediments.

Table 1 below summarizes the results of the *Leptocheirus* test.

Table 1. Shipyard Creek benthic *Leptocheirus plumulosus* results.

Sample	Initial PW Ammonia (total mg/L)	% Fines (silts and clays)	% Clay	% Finest Fraction (<0.0013mm)	Total Organic Carbon	Mean Survival (%)
SYC14-REF	NM	20.2	3.0	2.0	0.084	97
SYC14-TB1	0.17	99.0	61.8	42.5	3.34	70
SYC14-TB2	8.43	99.0	61.7	45.6		52
SYC14-AC	0.51	75.8	43.6	32.9	2.33	61

Summary of the *Ampelisca abdita* results for Shipyard Creek:

Based on the discussion above, and in consultation with ANAMAR, the Shipyard Creek sediment composites were subsequently evaluated using the amphipod *Ampelisca abdita*. The 10-day benthic test with *Ampelisca* resulted in 98% survival in the control sample; meeting the acceptability criterion of ≥90%. Survival in the reference was 97%, suggesting that the reference site sediment was suitable for amphipod survival. The project samples TB-1, TB-2, and AC resulted in mean survival of 97%, 98%, and 96%, respectively. These values were not >20% less than the reference mean survival and would not exceed the LPC for aquatic disposal acceptability.

As with the *Leptocheirus* test, the test sediments (SYC14-TB1, SYC14-TB2, and SYC14-AC) evaluated in the *Ampelisca* test were also treated for elevated ammonia through daily water exchanges prior to and during the solid-phase testing. The porewater ammonia was sufficiently reduced in these samples below levels predicted to result in mortality with this species (<30 mg/L total ammonia) and significant toxicity was not observed in any of the test treatments. Differences in the initial porewater ammonia concentrations between the *Leptocheirus* and *Ampelisca* tests are possibly the result of several factors that may have included: the amount of time samples were in storage prior to testing, variability between aliquots, and the duration of daily water exchanges conducted prior to the initiation of testing. Due to the potential of holding time expiration, the test did not undergo the ammonia reduction procedure for the number of days as was conducted for *Leptocheirus*; however, this did not have a negative impact on the significance *Ampelisca* of the test results.

Table 2 below summarizes the results of the *Ampelisca* test.

Table 2. Shipyard Creek benthic *Ampelisca abdita* results.

Sample	Initial PW Ammonia (total mg/L)	% Fines (silts and clays)	% Clay	% Finest Fraction (<0.0013mm)	TOC	Mean Survival (%)
SYC14-REF	2.63	20.2	3.0	2.0	0.084	97
SYC14-TB1	9.49	99.0	61.8	42.5	3.34	97
SYC14-TB2	8.50	99.0	61.7	45.6		98
SYC14-AC	4.87	75.8	43.6	32.9	2.33	96

Amphipod Sensitivity to Grain Size - Literature Review and Discussion:

The discussion below summarizes a brief history of the development of bedded-sediment toxicity testing utilizing estuarine and marine amphipods. The line-of-evidence below seeks to present current state of knowledge regarding grain size sensitivity in amphipods and reinforce the role this may play in sediment evaluations.

The benthic tests with amphipods utilize a variety of different species that inhabit different types of substrate. The incorporation of specific species into the regulatory framework included many factors such as selecting those species that are easily obtained from the wild or cultured, some level of documented sensitivities to contaminants of concern, and providing a regional representation of benthic organisms around the coastal United States. The results of the benthic tests conducted on the Shipyard Creek samples utilized *Leptocheirus plumulosus* and *Ampelisca abdita,* both approved species under the USACE and USEPA guidance that have been deemed sensitive organisms that are useful in evaluating potential dredged materials. The selection of which amphipod species to use for a particular sediment evaluation program can involve, but is not limited to, the salinity of porewater, the regional specificity outlined in the regulatory program, and the grain size of the materials being evaluated. Historical data on amphipod life history and the conditions of where these organisms are found in the wild help inform which candidate species may be the most appropriate for sediments with characteristics within their tolerance range.

The effects of grain size on amphipod sensitivity has been addressed (either directly or anecdotally) through the test method development activities, the production of regulatory guidance documents, and the presentation of independent research in peer-reviewed journal publications, technical memos, and programmatic clarification papers. Through the history of toxicity test method development, there has long been a recognition that grain size can play a significant role in organisms sensitivity either by not being able to support survival, growth, and reproduction or by acting as a stressor that may have an additive effect on overall test observations. Despite this recognition that grain size can is an important driving factor in amphipod survival, there is a lack of specific detail in many of the promulgated regulatory guidance documents produced by the USACE and USEPA. In regards to the estuarine amphipod *Leptocheirus plumulosus*, it has commonly been perceived that this species is tolerant of a wide range of grain sizes; however, there is evidence (as summarized below) that grain sizes in the extremes may have a negative effect on the observed biological responses.

The development of codified test methodologies for evaluation sediment toxicity promulgated by the United States Army Corps of Engineers (USACE) and the United States Environmental Protection Agency (USEPA) build upon the 1991 document, *Evaluation of Dredged Material Proposed for Ocean Disposal: Testing Manual* (USACE 1991), also referred to as the “Green Book”. While this document summarized the framework for conducting dredge material evaluations for chemical, physical, and biological criteria, it did not provide significant details on conducting the biological tests.

Regional guidance for the New York District (NYD) published in 1992 includes one of the earlier USACE/USEPA guidance documents that collectively describes in detail the specific test methods and conditions for the sediment bioassays (USACE 1992). The recommendations in this document for conducing benthic tests suggest that *Leptocheirus* is tolerant of the full range of grain size and *Ampelisca* can be used in scenarios where the sediments contain at least 10% fines. Subsequent USACE/USEPA guidance documents promulgated in 1994, 1998, and 2008 appeared to build on the same source material without contributing any additional detail. These included the documents: *Methods for Assessing the Toxicity of Sediment-associated Contaminants with Estuarine and Marine Amphipods* (USACE 1994), *Evaluation of Dredged Material Proposed for Discharge in Waters of the U.S. – Testing Manual* (USACE 1998), also referred to as the "Inland Testing Manual", and *Requirements and Procedures for Evaluation of the Ocean Disposal of Dredged Material in Southeastern U.S. Atlantic and Gulf Coast Waters* (SERIM 2008). The document published in 1994 was catered toward specifically addressing the sediment toxicity test methods utilizing benthic amphipods and attempted to discuss the life-histories of the selected amphipod species. This document also suggests that extremes in grain size, such as >95% sands may illicit excess mortality in *Ampelisca.* The Inland Testing Manual (USACE 1998) includes a discussion recognizing the value of including a "clean' reference station in the suite of bioassays that attempts to match the grain size distribution of the project materials.

This reference sediment would be included in an effort to correlate the contribution of grain size to biological effects where there is no potential contaminant of concern involved. This recommendation is not always feasible depending upon the objectives of the program; however, this concept has been incorporated as a requirement in the USACE Region X (Seattle, WA District) guidelines and promulgated through Puget Sound Estuarine Protocols (PSEP 1995).

The most recent American Standards for Testing and Materials (ASTM) method *Standard Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Estuarine and Marine Invertebrates* (ASTM E1367) has adopted much of the language detailed in the USACE/USEPA documents referenced above. Here, however, the ASTM method suggest that *Leptocheirus* is suitable for sediments "with a silt content greater than 5 % and a clay content less than 85 %", recognizing that a large proportion of fines, particularly clays, may influence the testing with this species. An excerpt from section A2.4.3 Effects of Sediment Grain Size from the ASTM method is included below.

> In some studies, *Leptocheirus* has exhibited >90 % survival in clean sediment ranging from nearly 100 % sand to nearly 100 % silt + clay (Schlekat et al., 1992 (13), USEPA 1994a (1)). However, adverse effects can occur in sediment with very high levels of clay or sand. Laboratory studies have shown significant reduction in survival when clay content exceeded 84 %, and survival, growth and reproduction were significantly reduced in 100 % sand (Emery et al., 1997 (8)). Results have been equivocal from controlled tests with mixed grained sediments (between 10 % and 90 % silt/clay). Emery et al. (1997 (8)) found an increase in growth as sediment coarseness increased up to 75 % sand…Therefore,

> *Leptocheirus* should be tested with sediment with silt/clay content between 5 % and 85 % (Table A2.1). If sediment characteristics exceed these bounds, an appropriate clean control and reference sediment should be incorporated into the test to separate effects of sediment associated contaminants from effects of particle size. (ASTM E1367)

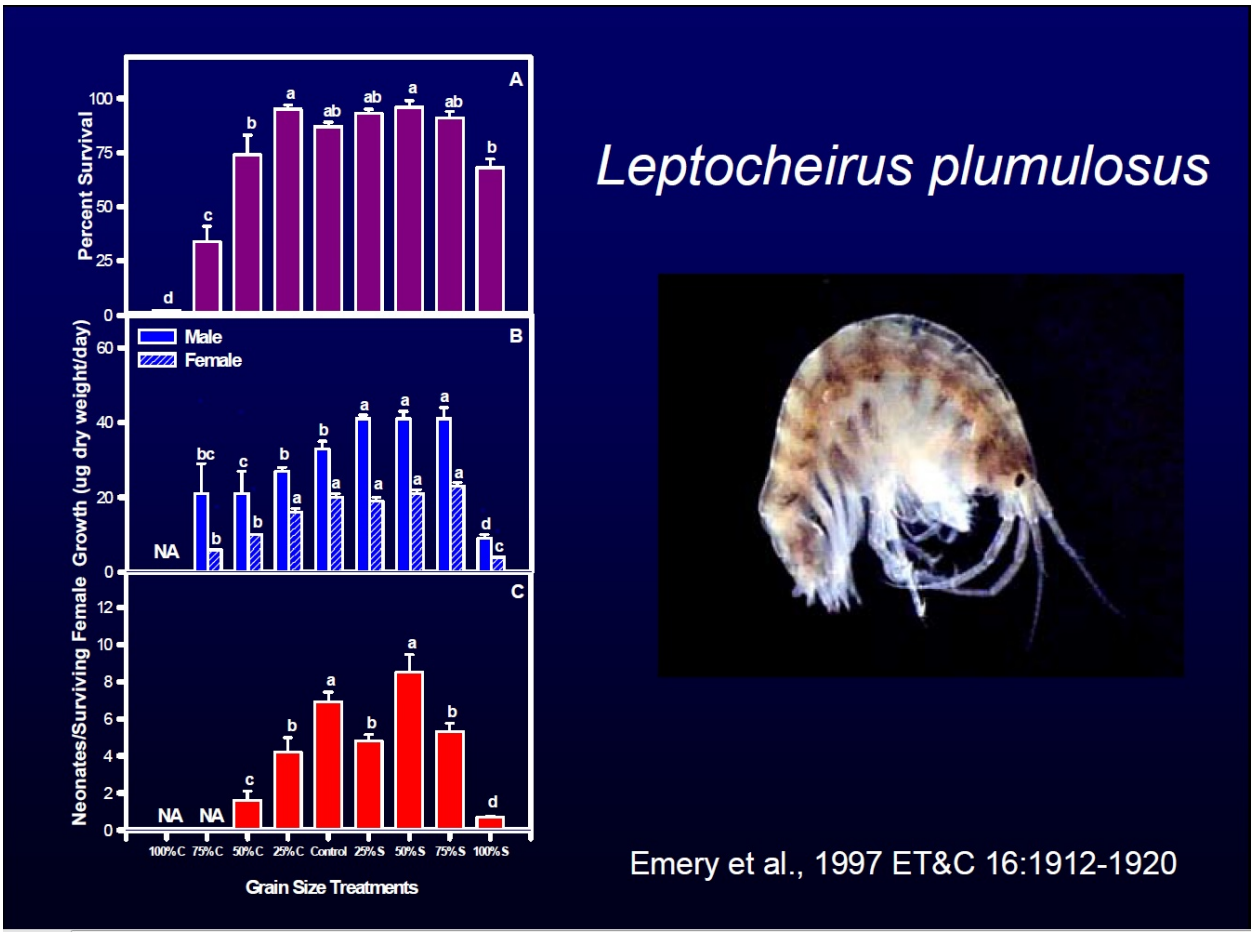
As noted above, the development of sediment test methods has included a recognition that there are many factors, including grain size that may influence the results of sediment toxicity test. Alan Kennedy et al. in the journal article *A Comparison of Acute and Chronic Toxicity Methods for Marine Sediments* (Kennedy et al. 2009) reiterates:

> Endpoint response to sediments is not necessarily indicative of contaminant effects. Several factors may complicate the interpretation of sediment tests, including uncertainty (Vorhees et al., 2002) and confounding factors (e.g., grain size, ammonia, sulfides, indigenous animals) (Postma et al., 2002). Grain size, for instance, is a well-known confounding factor in sediment tests (U.S. EPA, 1994; U.S.EPA/ACE, 2001) that can cause amphipod mortality by physical or energetic stress when outside species-specific tolerance ranges (Emery et al., 1997).

Several of the references above cite the laboratory studies by the USACE Engineer Research and Development Center (ERDC) (former Waterways Experiment Station) that were conducted during the development of a 28 day survival, growth and reproduction test with *Leptocheirus* sought to explore the influence of confounding factors on organism sensitivity, which included an evaluation of the effect of grain size. The results of this study were summarized in the journal article *Development of a Chronic Sublethal Sediment Bioassay Using the Estuarine Amphipod Leptocheirus plumulosus (Shoemaker)* (Emery et al. 1997). The sediment treatments evaluated in this study were fabricated utilizing mixtures of natural sediment, kaolinite clay, and clean beach sand. A series of nine grain sizes were created ranged from 100% clay to 100% sand. The results of this study indicated that that “extremely fine grained” material (>75% Clay) had a significant negative effect on the survival, growth, and reproductive success of *Leptocheirus* exposed for 28 days. These effects were also observed under coarse grained conditions (>75% sand). The authors defined “clays” as particles less than 0.0039mm. The amount of clays in the shipyard creek samples using this delineation resulted in 94.2, 94.6, and 63.8% “clay” for samples SYC14-TB-1, SYC14-TB-2, and SYC14-AC. The proportion of clay in samples SYC14-TB-1, SYC14-TB-2 correlate with where significant toxicity was observed with *Leptocheirus* in the Shipyard Creek sediment analysis. While sample SYC14-AC does not exceed this hypothetical range (>75% clay), it was potentially in the range where the grain size could have acted as a stressor and influenced the significance of the test results.

The excerpt of PowerPoint presentation (Figure 1) that was created by Todd Bridges (USACE-ERDC) was adapted from the Emery et al. document. This shows that even at 50% “clay”, there appeared to be some level of negative effects observed on *Leptocheirus* survival, growth, and reproduction.

Figure 1. Slide adapted from Emery et al. data (Attributed to Bridges, T.)



Additional research into the effects of confounding factors on toxicity continues to be explored to this day, through independent research and regulatory investigations. In particular, the San Francisco Estuary Institute (SFEI) has recently coordinated a study to evaluate the effects of fine sediment particles on the survival of the amphipod *Eohaustorius estuarius*, a similar free-burrowing estuarine amphipod to *Leptocheirus*. *Eohaustorius* is also considered to be tolerant of a wide range of grain sizes, but evidence suggests that sediments with grain size distributions in the extremes are capable of acting a stressor. The interest in this study suggests that further research in this matter is underway and that it is of concern to the method development framework. The report on this study in pending.

Summary

The effects observed in the amphipod tests conducted on the Shipyard Creek samples resulted in a marked improvement in survival between the *Ampelisca* and *Leptocheirus* tests. Barring the presence of significant contaminants of concern in the project composite samples, these results suggest that one species was more suited for the grain-size distribution in the materials evaluated. Both the high proportion of % fines (silts and clays) and the unusually large proportion of the finest fraction of clays may have been sufficient to cause stress and subsequent mortality with the free-burrowing amphipod *Leptocheirus*. This finding is supported by the evidence discussed above regarding the sensitivity of *Leptocheirus* to extremes in grain size (predominantly clay or predominantly sand). The mechanism for clay particles acting as a stressor to *Leptocheirus* appears to be a physical interaction that prevents normal burrowing and feeding behavior and (more importantly) the adhesion of clays to the respiratory structures of the animal, essentially clogging their ability to breathe. The high amount of fines appear to present less of a challenge to the tube-building amphipod *Ampelisca*, which exhibits a life-strategy adapted to predominantly fine grain sediments.

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Sincerely,

Brian Hester
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