The effects of particle size and shape and animal health on toxicity test results using the amphipod *Eohaustorius estuarius*.

Estimated Cost: $79,820 (Effects assessed in summer only)
$119,140 (Effects assessed in winter and summer)
Oversight Group: Exposure and Effects Work Group
Proposed by: Marine Pollution Studies Laboratory, UC Davis (Brian Anderson/Bryn Phillips) and Southern California Coastal Water Research Project (Steven Bay)

Background

The 10-day whole sediment toxicity test protocol for the amphipod *Eohaustorius estuarius*is one of the principal tests recommended for toxicity monitoring in California. Several studies have shown this species is appropriate for this application, and this is the benchmark test used in regional monitoring programs in southern California and the San Francisco Estuary. Due to concerns about limitations of methods to determine causes of persistent moderate toxicity in field sediments and the relative influence of non-contaminant factors on amphipod survival, two recent workshops sponsored by the San Francisco Estuary Institute's Regional Monitoring Program (RMP) identified specific attributes of *E. estuarius* that require additional research. Among a list of non-contaminant factors considered, the relative impacts of grain size, particle shape, and test animal condition were identified as possibly important factors affecting amphipod survival.

As part of the initial evaluation of *E. estuarius* as a test species, Dewitt et al. (1989) assessed survival of *E. estuarius*in 42 uncontaminated field sediment samples from Puget Sound, Washington and Oregon. These authors reported that "*E. estuarius*showed little sensitivity to sediments of different grain sizes: mean survival was 92.4% in sediments with ≥80% silt-clay content and 96.7% for coarser sediments." Environment Canada (1998) published grain size recommendations for the 10d test with *E. estuarius.* Tay et al. (unpublished study described in Environment Canada, 1998) found mean survival was 74% in mixtures with 57% clay and 99% fines. Based on these experiments, they established tolerance limits of <90% coarse grained sediment, and <70% clay. The Environment Canada (1998) 10-day guideline states that "test materials with ≥ 70% clay must not be used in a 10-day sediment toxicity test with *E. estuarius*".UC Davis conducted similar experiments using mixtures of sand and field-collected reference mud that was comprised of silt and clay. The field reference material was sieved through a 75 µm screen then mixed with sand to give sediments with 10 – 90% fines. *E. estuarius*10-day survival was ≥85% in sediments with ≤70% fines. Survival was 57% in sediment with 90% fines (Marine Pollution Studies Laboratory-Granite Canyon unpublished data). In addition to these studies, analyses of data from the RMP and elsewhere have shown that survival of *E. estuarius* in field sediments is negatively correlated with percent fine grained sediment, and with percent clay in sediment.Based on the preponderance of evidence, the effect of clay was prioritized for further study by participants of the two RMP workshops.

The toxicity workshops also identified the possible interaction of seasonal differences in amphipod health and their ability to tolerate fine-grained sedimentsas a high priority topic for investigation. This is based on evidence suggesting sediment toxicity in San Francisco Bay is greater in winter, and the possibility that increased winter toxicity is related to variability of the health of field collected amphipods.Seasonal changes in amphipod fitness related to nutrition, senescence, or reproductive activity have been suggested as the reason for such variations in sensitivity to San Francisco Bay sediments. The workshop participants also recommended measurement of amphipod lipid content as an indicator of animal condition. Prior studies using *E. estuarius* have shown a correspondence between tissue lipid content and changes in sensitivity to toxicants related to amphipod condition.Measurement of amphipod lipid content may provide an valuable tool for interpreting the results of future sediment toxicity surveys, but information on the seasonal changes in this parameter and its association with changes in amphipod sensitivity to stressors is needed. Combining seasonalmeasurements of tissue lipid with studies of the sediment particle size effects on *E. estuarius* survival will provide the information needed to evaluate the usefulness of lipid measurements in toxicity testing.

A United State Geological Survey characterization of suspended sediments in the San Francisco Estuary found that water column suspended sediments contained three clay mineral types: illite, montmorillonite (=smectite), and chlorite+kaolinite (Knebel et al., 1977). Samples were collected in the spring, summer, fall and winter seasons and covered the northern and southern reaches of the estuary. The results demonstrated that waters in the northern reaches of the estuary were dominated by chlorite+kaolinitevia inputs from the Sacramento-San Joaquin river systems. Kaolinite originates from the decomposition of granite. Illite dominates the southern reach of the estuary where clay minerals are re-suspended from the estuary floor by tidal currents. Illite originates from the decomposition of micas and feldspars. Samples of the estuary sediments (bedded, not suspended sediments) showed that sediments in the northern and southern reaches were dominated by chlorite+kaolinite clays, which comprise a somewhat larger size fraction than illite clays (Knebel et al. 1977).

Part One: Establishing a Dose-Response Relationship between Sediment Clay Content and Amphipod (*Eohaustorius estuarius*) Mortality

Experimental Approach

Laboratory experiments will be used to establish a dose response relationship between *E. estuarius* survival and percent chlorite+kaoliniteclay in sediment. Experiments will be conducted using two separate natural reference sediments spiked with clay. The lipid content of each batch of test animals will be measured and compared to variations in sensitivity to sediment clay content and season.

Clay-spiked sand: In the first experiments, reference sand will be spiked with increasing concentrations of chlorite+kaolinite clay, the dominant clay found in San Francisco Estuary sediment (Knebel et al., 1977). Clay purchased from a commercial supplier will be mixed with reference sand at ratios representative of those in the Estuary. After equilibration, 10day toxicity tests will be conducted with *E. estuarius*. One range-finder test will be conducted to establish the range of percent clay that inhibits amphipod survival. Two definitive experiments will then be conducted to confirm the dose-response relationship. Results of these experiments will be used to establish LC25 and LC50s for percent chlorite+kaoliniteclay in sediment. The dose response results will also be used to examine the relationship between percent clay and amphipod survival using regression analysis to calculate of the 95% lower prediction limits ofthe regression of percent clay andnumber of survivors for *E. estuarius* (after DeWitt et al., 1989). Thismay be used for statistically partitioning the effects of percent clay from contamination in sediment toxicitytests with amphipods. Approximately 30 amphipods that are representative of the animals used in the test will be preserved for lipid analysis. If feasible, individuals will be measured in order to determine the variabilityin animal condition within each test batch.

Clay-spiked reference sediment: In the second experiments, reference sediment from Castro Cove, a site in the northern estuary, will be spiked with increasing concentrations of chlorite+kaolinite clay. The particle distributions in Castro Cove sediment in 2008 were as follows: Sand = 25.36%, Silt = 57.2%, Clay = 17.44%, TOC = 0.9%. Spiking sediment from a San Francisco Estuary reference site will allow determination of LC25 and LC50s for percent clay, and confirmation of the regression relationship between sediment clay and amphipod survival determined from experiments with clay-spiked sand.Approximately 30 amphipods that are representative of the animals used in the test will be preserved for lipid analysis. If feasible, individuals will be measured in order to determine the variability in animal condition within each test batch.

All experiments will be replicated three times (clay-spiked sand x 3 + clay-spiked reference sediment x 3 = 6 experiments total). To investigate the potential for seasonal differences in amphipod health to influence their tolerance to high clay sediments, the experiments can be conducted during winter and summer (6 experiments in summer and 6 experiments in winter = 12 experiments total).**At the suggestion of the EEWG proposal reviewers, the current budget reflects costs for experiments in a single summer season (no winter tests), and a separate budget shows costs for conducting the experiments in the winter and summer.**

The results of these experiments will be used to determine the extent to which clay affects *E.estuarius* survival in 10day toxicity tests, and to investigate whether seasonal differences in amphipod health affects their response to high clay sediment. These results may be used to determine how this protocol is implemented in the RMP. One approach may be to establish the range of grain size characteristics in San Francisco Estuary sediments appropriate for testing with *E. estuarius*, following the approach used by Environment Canada. A second approach may be to use the regression relationship to establish 95% lower prediction limits which can be used to separate mortality likely due to clay effects from mortality likely due to contaminants.A third approach may be to include lipid concentration as a factor in data interpretation.

Part Two:Investigating the Relationship between Sediment Shape Characteristics and Amphipod (*Eohaustorius estuarius*) Mortality

Experimental Approach

To investigate whether clay particle shape is correlated with amphipod mortality, particle shape will be analyzed on the experimental sediments spiked with kaolin clay in Part One. In addition, particle shape will be analyzed in field sediments collected as part of Regional Monitoring Program's Status and Trends monitoring. Particle shape characteristics will follow general methods described in Tucker (1995) using the Powers (1953) grain shape classification. These methods have been adapted by Ivano Aiello at Moss Landing Marine Laboratories to allow quantification of the relative proportion of each shape category for selected samples. In this classification, particles are categorized as either "high sphericity" or "low sphericity" and within these classifications they are further classified according to their relative angularity (highly angular to well rounded; Figure 1).

Ten replicate subsample smears of each clay-spiked sand or clay-spiked reference sediment from the experiments described above will be analyzed using light microscopy. The relative proportion of each particle shape category will be quantified andthese values will be combined to provide a shape index value for each sample. This value will then be correlated with amphipod mortality to investigate whether there is a significant correlation between particle shape and amphipod mortality in the clay-spiked samples.

A similar approach will be used to establish the dominant shape characteristics of sediments in representative San Francisco Estuary samples collected as part of the RMP sampling. Particle shape will be analyzed in the 27 RMP sediments from north, central and southern estuary reaches collected as part of routine RMP monitoring. Particle shape characteristics will be correlated with amphipod mortality. This information will be used to determine the extent to which clay shape affects amphipod survival, and whether this should be considered as part of the implementation of particle size limits when using *E. estuarius* in sediment monitoring programs.

Part Three:Lipid Assay Method Developmentand Measurement of Seasonal Variation

Experimental Approach

Published methods for a micro scale colorimetric assay for tissue lipids will be adapted for use with individual amphipods. The assay is conducted in a 96 well plate format, which provides rapid and cost efficient analyses. Standardized methods for extraction and analysis of amphipods will be developed, and detection limits of the assay will be determined.Monthly samples of *E. estuarius* will be obtained from the collection site in Newport, Oregon. Samples and corresponding environmental data (e.g., water temperature and salinity) will be provided through collaboration with the commercial supplier of the test animals (Northwestern Aquatic Sciences). The weight, length, and percent lipid of up to 30 individuals per sample will be measured. The mean and standard deviation of the percent lipid will be used to document seasonal variation.

Budget

Note: The top budget reflects costs of conducting experiments in the summer season only. The bottom budget reflects costs of conducting the experiments in summer and winter.

Summer Testing Only			
Task*	Laboratory	Season	Cost
Effect of Sand Spiked with Clay	MPSL-Granite Canyon	1	19,460
Effects of Reference Sediment Spiked with Clay	MPSL-Granite Canyon	1	19,460
Grain Size Analysis	Aiello – Moss Landing		2,400
Grain Shape Analysis	Aiello – Moss Landing		5,000
Field sampling	MPSL-Granite Canyon		3,000
Seasonal Lipid Analysis	SCCWRP		19,900
Reporting	MPSL-Granite Canyon		10,600
Total			79,820
Winter Testing Only			
Task*	Laboratory	Season	
Effect of Sand Spiked with Clay	MPSL-Granite Canyon	2	38,920
Effects of Reference Sediment Spiked with Clay	MPSL-Granite Canyon	2	38,920
Grain Size Analysis	Aiello – Moss Landing		4,800
Grain Shape Analysis	Aiello – Moss Landing		6,000
Seasonal Lipid Analysis	SCCWRP		19,900
Reporting	MPSL-Granite Canyon		10,600
Total			119,140

Figure 1.

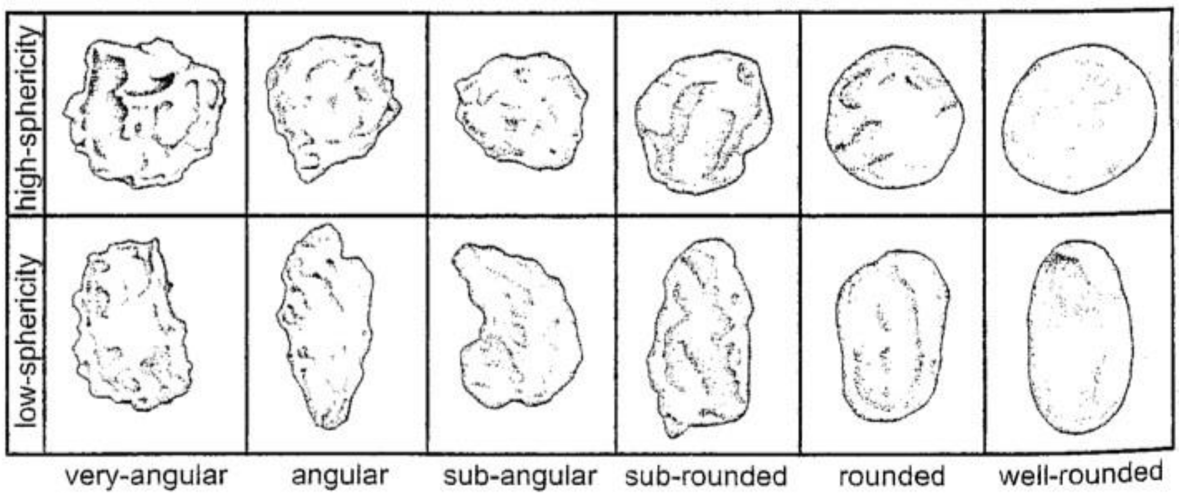

Figure 5.5 – Power's (1953) classification of grain roundness for grains displaying low sphericity and high sphericity. [From Tucker, 1995.] [Copyright © 1995 Blackwell Publishers, reproduced with permission.]

References

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