Special Study Proposal: Assessing the Effects of Clay on the Amphipod *Eohaustorius estuarius*

Summary: This study will confirm results of the 2014 Regional Monitoring Program (RMP) Special Study showing that sediment clay causes size specific effects on the amphipod *Eohaustorius estuarius*. Experiments with field sediments from the San Francisco Estuary will be used to corroborate laboratory experiments conducted in 2014 which showed that larger amphipods were less tolerant of kaolin clay. These results have the potential to inform policy regarding the use of this species in monitoring clay-rich sediments. These experiments may result in a revision of the toxicity testing protocol to use smaller test organisms to minimize the confounding effect of clay on toxicity test results.

Estimated Cost: $30,000

Oversight Group: EEWG

Proposed by: Brian Anderson (UC Davis)

PROPOSED DELIVERABLES AND TIMELINE

Deliverable	***Due Date***
Task 1. Project Management (write and manage sub-contract, track budgets)	September-November 2015
Task 2. Analyze data, select sites and conduct field sampling	Winter 2015
Task 3. Laboratory analysis; QA/QC	Winter 2015-2016
Task 4. Draft/final report/protocol recommendation	March 2016

Background

The 10 day survival toxicity test with the amphipod *Eohaustorius estuarius* (U.S. EPA, 1994) is the primary sediment test protocol used in the Regional Monitoring Program and the State Water Resources Control Board's Sediment Quality Objective (SQO) program (Beegan, 2009). Historical data have indicated that the mortality of this species correlates with the clay content of sediments. Based on the recommendations of two RMP workshops convened to investigate causes of moderate toxicity in the Estuary, a series of laboratory experiments were completed in 2014 to investigate the effects of kaolin clay on *E. estuarius*. Kaolin is the dominant clay in the Estuary. The results of these experiments showed that smaller amphipods were more tolerant of kaolin than larger amphipods. These results were confounded by the fact that dose-response experiments with sand-spiked kaolin mixtures did not exhibit strict monotonic decreases in amphipod survival. Analyses of clay concentrations suggested that clay was agglomerating (=flocculating) in the kaolin concentrations higher than 70%. This resulted in increasing silt concentrations in the treatments $\geq$70% kaolin (silt = particle sizes >4um to $\leq$63 um), and therefore reduced effects of clay (particles < 4 um). The flocculation phenomenon may have been due to the use of pure kaolin clay in these experiments.

To confirm the size-specific effect of clay on *E. estuarius*, confirmatory experiments are recommended using estuarine reference sediments having high clay content. Appropriate reference sites will be identified using existing RMP data. These will be sites with high clay concentrations, but low concentrations of anthropogenic contaminants. Once identified, high clay sediments from three reference sites will be collected and these will be mixed with reference sand to give a range of clay concentrations using the same procedures described in the 2014 kaolin tests (Anderson et al., 2015). Sediments will then be tested with small, medium and large size classes to confirm whether smaller amphipods are more tolerant of clay than larger animals. If small amphipods are demonstrated to be more tolerant of high clay reference sediments, these results will inform policy regarding use of *E. estuarius* in future RMP monitoring, as well as the use of this species in the SWRCB SQO program.

Study Objectives and Applicable RMP Management Questions

This study will provide confirmatory evidence that high clay in sediment inhibits *E. estuarius* in sediment toxicity tests and that clay effects are more pronounced in larger amphipod individuals. The study will address one RMP Management Question as it relates to one of the RMP and SQO monitoring indicators:

1) Are chemical concentrations in the Estuary at levels of potential concern and are associated impacts likely?

1.d. What contaminants are responsible for observed toxic responses?

The objective of the study is to define the tolerance range of three different size classes of *E. estuarius* to sediment clay concentrations, and confirm whether small amphipods are more tolerant of clay than large amphipods.

Approach

Experiments will follow methods used in the 2014 experiments where reference sand was spiked with increasing concentrations of kaolin clay and exposed to three size classes of field caught *E. estuarius* (Anderson et al., 2015). Rather than kaolin, the current experiments will use reference sediments with high clay content. Reference sediments are defined as San Francisco Estuary sediment having low contaminant concentrations based on sediment quality guideline quotient values (e.g., Effects Range Median quotient value (ERMQ) $\leq$0.11). These will be identified through screening of the most recent existing RMP data from sediments monitored by the RMP Status and Trends program. Three sites will be identified based on the following criteria: ERMQ $\leq$ 0.11; sediment clay content $\geq$90%.

The field sediment will then be hand mixed with #60 reference sand (0.25 mm mesh size) at the following ratios: 0% (sand only), 10%, 30%, 50%, 70%, 90%, and 100% field sediment. Sediment from the amphipod collection site (home sediment) will also be tested as a control. All samples will be tested with small, medium, and large size classes of *E. estuarius* collected from the Oregon field site and pre-sorted by the amphipod supplier, Northwest Aquatic Sciences. As in the previous experiments, field animals will be wet sieved onto a 1 mm

screen, then visually sorted into small, medium, and large cohorts for testing. All tests will conducted when sufficient densities of amphipods in each size class are present at the collection site (likely winter 2015-2016).

Analyses of variance with post hoc Dunnett's tests will be used to determine significant differences among amphipod responses in different concentrations of field sediment, and also among different size classes of amphipods ($\alpha = 0.05$). Sediment grain size analyses will be conducted using laser diffraction analysis as well as the pipet method, as described previously (Anderson et al., 2015).

If results of these experiments are consistent with the previous laboratory tests with kaolin, they will provide corroboratory evidence that smaller amphipods are more tolerant of clay than larger animals. If so, this will likely lead to a revision of the standard 10 day test protocol using *E. estuarius* to restrict testing of high-clay sediments using larger animals and recommend using only the smallest individual amphipods (<1 mg) to minimize the confounding effect of clay on test results.

Budget

The proposed budget for the study is $30,000. This includes review of existing RMP data to identify candidate reference sites, collection of sediment from three reference sites, dose-response experiments from each reference sites using three size classes of amphipods, grain size analyses, and data analysis and final reporting.

Table 1. Budget summary.

Task	**Laboratory**	**Cost**
Task 1 Site selection and sampling		
(a) Data screening and site selection	MPSL – Granite Canyon	$350.00
(b) Sediment sampling	MPSL – Granite Canyon	$3,150.00
Task 2 Laboratory Experiments		
(a) Size-specific effects of sand-spiked high clay field sediments	MPSL – Granite Canyon	$19,460.00
Task 3. Grain size analysis	Aiello Moss Landing	$2,400.00
Task 4. Data analysis and reporting	MPSL – Granite Canyon	$4,640.00
Total Costs		**$30,000.00**

Reporting

A draft fact sheet summarizing the approach, analyses and results of the study will be submitted to the EEWG and TRC. Upon receipt and incorporation of comments, a final recommendation for revision of the 10d sediment protocol using *E. estuarius* for high clay sediments will be issued.

References

Anderson, B.S., Phillips, B.M., Voorhees, J.P., 2015. The effects of kaolin clay on the amphipod *Eohaustorius estuarius*. San Francisco Estuary Institute.

Beegan, C., 2009. Water Quality Control Plan for Enclosed Bays and Estuaries - Part 1 Sediment Quality. State Water Resources Control Board. Sacramento, California.

U.S. EPA, 1994. Methods for assessing the toxicity of sediment-associated contaminants with estuarine and marine amphipods. EPA/600/R-94/025. Office of Research and Development, Washington D.C.