RMP Exposure and Effects Workgroup Meeting

May 16th, 2013
San Francisco Estuary Institute
First Floor Conference Room
4911 Central Avenue, Richmond
10:00am-3:10 pm

Lunch will be provided

DRAFT AGENDA

1.	**Introductions and Goals for Today's Meeting** The goals for today: • Provide updates on San Francisco Bay EEWG activities • Review special study proposal ideas for 2014	10:00 Meg Sedlak
2.	**RMP Planning Overview** A brief general overview of the latest on priorities and plans in the RMP, and explanation of how the this meeting fits into the planning process.	10:10 Jay Davis
3.	**Update on 2013 Studies: Bioanalytical Tools** (Update on Phase I and Plans for Phase II; Attachment) In 2013, the RMP began funding a two-year study to develop bioanalytical tools for evaluating endocrine disrupting compounds. Dr. Denslow will give an update on the project and the plans for the second year of funding.	10:20 Nancy Denslow
4.	**Update: 2012/ 2013 plans for the Mesohaline** (Update on Phase I and plans for Phase II; Attachment) In 2012, this study standardized the existing taxonomic data bases with benthic data and associated ancillary parameters, chemical contaminant, and sediment toxicity data. It also refined the habitat definitions and conducted a calibration and validation exercise. Plans for 2013 include developing the benthic indices, assure independence of the variables and conduct calibration and validation exercise.	11:10 Eric Stein
5.	**Update: 2011/2012 Hotspot Study** (Attachment) The RMP conducted a pilot study to re-evaluate two Bay hotspots – Mission Creek and San Leandro Bay. A sediment quality objectives (SQO) assessment was undertaken.	11:40 Ellen Willis-Norton
	LUNCH	12:10

6.	**Special Studies for 2014: Impacts of Dredging on Benthic Habitats** (Attachment) The dredging community is interested in better understanding the impact of dredging on benthic habitats and potential effects to fish.	12:40 Korie Schaeffer
7.	**Special Studies for 2014: Developing a Reference Site for Dredge Materials** (Attachment) Prior to disposal of dredged materials in the Bay, the sediment undergoes extensive chemical and toxicity testing. This study would help to define reference conditions in the Bay and a reference site to which Dredgers could compare their samples.	1:10 Rob Lawrence/ Brian Ross
8.	**Special Studies for 2014: Understanding Causes of Moderate Toxicity in the Bay** (Attachment) In 2012, the RMP and SCCWRP staff convened a workshop to discuss causes of moderate toxicity in the San Francisco Bay. A number of study ideas were hypothesized from this meeting and two possible projects will be discussed.	1:40 Steve Bay/ Brian Anderson
9.	**Closed Door Session**	2:10
	Wrap up and adjourn	3:10

San Francisco Estuary Institute

RMP Joint Meeting of Exposure and Effects and Emerging Contaminants Workgroups

May 15th-16th, 2012
San Francisco Estuary Institute

DRAFT Meeting Summary

In Attendance (May 15th, 2012):

Eva Agus (EBMUD)
Brian Anderson (UC Davis)
David Baldwin (NOAA Northwest Marine Fisheries)
Steve Bay (SCCWRP)
Mike Connor (EBDA)
Joe Dillon (NOAA Southwest Marine Fisheries)
Eric Dunlavey (City of San Jose)
Melanie Harrison (NOAA Southwest Marine Fisheries)
John Incardona (NOAA Northwest Marine Fisheries)
Michael Kellogg (SFPUC)
John Kucklick (NIST)
Naomi Feger (SFB RWQCB)
Arleen Feng (ACCWP for BASMAA)
Jennifer Field (Oregon State University)
Michael Fry (Pacific Island FWS)
Keith Maruya (SCCWRP)
Derek Muir (Environment Canada)
Harry Ohlendorf (CH2M HILL)
Dan Schlenk (UC Riverside)
David Sedlak (UC Berkeley)
Steve Weisberg (SCCWRP)
Don Weston (UC Berkeley)
Ian Wren (SF Baykeeper)
Rachel Allen (SFEI)
Jay Davis (SFEI)
Susan Klosterhaus (SFEI)
Lester McKee (SFEI)
Meg Sedlak (SFEI)
Don Yee (SFEI)

Via Telephone:

Richard Grace (AXYS)

1) Introductions and Review of the Agenda

Meg Sedlak reviewed the agenda for the day, and the group conducted introductions, noting that participants from both the Exposure and Effects workgroup (EEWG) and Emerging Contaminants workgroup (ECWG) were in attendance. In particular, the EEWG panel members Michael Fry, Harry Ohlendorf, Dan Schlenk, Steve Weisberg, and Don Weston, as well as the ECWG panel members Jennifer Field, Derek Muir, and David Sedlak were in attendance for the afternoon discussion of bioanalytical tools.

2) 2012 Special Study Update: Copper and the Olfactory Nerve of Salmon

Arleen Feng noted that existing storm water permits require an update on the progress of the Copper (Cu) effects on salmon study by this summer. Meg Sedlak noted that the Water Board has been kept abreast of the status of this project and the delays that were caused as a result of internal NOAA policies. Ms. Sedlak requested that David Baldwin provide a short summary on the progress to date by July 1.

David Baldwin gave an update of studies to date regarding the effects of Cu on the olfactory nerve of salmon. In freshwater, previous studies have shown that Cu impacts salmonid olfactory systems at levels detected in the environment. However, it is unclear if these levels are applicable for Cu in the Bay, since salmon are at a different life stage in the estuarine environment, the increased salinity may affect the copper effect, and dissolved organic carbon (DOC) may mitigate the binding strength of Cu by increased ligand complexation. Therefore, the RMP funded a study to investigate the olfactory toxicity of Cu to seawater phase salmon. Preliminary investigations are suggesting that Cu is much less toxic in seawater (1000 µg/L resulted in little changes from control, vs. significant effects at 5 µg/L in freshwater). David Baldwin indicated that because this was a preliminary study using relatively few individuals, these results need to be confirmed this summer.

The team proposed beginning the next round of investigations with toxicity at low DOC in 32 ppt seawater using the RMP 2012 funds. Additional funding (in the form of a grant from the Copper Development Association (CDA)) was provided to pursue toxicity in brackish waters (10 ppt). If the 32 ppt studies show low toxicity, Dr. Baldwin proposed moving towards the brackish water, low DOC scenarios before continuing to pursue the full seawater cases, as higher levels of DOC are expected to decrease toxicity even further. However, if the full salinity tests show higher levels of toxicity, the group will continue to vary the DOC before moving to lower salinity cases. The RMP studies will begin in July 2012.

Dan Schlenk noted that sodium-potassium ATPase is upregulated in salmonid gills as the fish migrate from fresh water to saltwater, and that copper has a toxic effect on this enzyme. He asked if it was also increased in the olfactory system during this transition, and David Baldwin indicated that he was unsure.

Dan Schlenk asked about the fish origins and their life stage during the tests. David Baldwin indicated that the fish are raised in a hatchery, and put through smoltification. The same size and life-stage fish will be used for the 32 ppt experiments as the 10 ppt experiments. Dan Schlenk suggested that an earlier life-stage should be used for the 10 ppt experiments, as post-smolt fish would not be found in such a low salinity environment. Meg Sedlak suggested that David Baldwin, Dan Schlenk, Richard Looker, and Joe Dillon discuss this in more detail to determine the appropriate life stage of salmon to use for the low salinity experiments.

Don Weston asked about the type of DOC, and David Baldwin indicated that fulvic acid would be used in the experiments, to keep them more straightforward, although he allowed that the choice of DOC could have large implications for the effect of DOC on copper.

Action Items:

- Meg Sedlak, David Baldwin, Dan Schlenk, Richard Looker, and Joe Dillon will discuss the appropriate life stage of salmon to use for the low salinity experiments.
- David Baldwin to provide a short summary on the progress on copper studies to date by July 1.

3) Effects of PAHs on Juvenile Flatfish

John Incardona presented results from the two-year study on the effects of PAHs on the development of juvenile California Halibut. Previous experiments had established strong relationships between carcinogenic PAHs and activation of the AHR pathway in flatfish embryos The group has investigated the linkage between a mixture of PAHs found in oil spills (lower molecular weight waterbourne PAHs) and development during fish metamorphosis. For the RMP study, the group was particularly interested in the higher molecular weight PAHs that are observed in San Francisco Bay sediments. Sediments used for the RMP analysis were from Kitimat, BC, which is nearly pristine except for PAHs from coal tar pitch, mixed in different proportions with clean sediment from Puget Sound. The results showed no large differences in fish development between the control and treatment groups, although there were significant differences in growth. However, there were some laboratory difficulties, including the low temperature at which fish were kept during metamorphosis, that could affect the results.

A few pieces remain to be completed, including verifying the exposure by CYP induction, looking at impacts on the cardiac system, and confirming the growth trend. John Kucklick asked how the fish are exposed to the contaminants, and John Incardona indicated that it is likely dermal absorption, as the fish do not appear to be eating the sediments, and their food source is not contaminated. The patterns of CYP induction should reveal more about the exposure route.

Meg Sedlak indicated that the revised report would be distributed this summer, with additional results from the remaining investigations. Steve Weisberg indicated that he would be interested in performing similar studies in Southern California, as there tend to be different mixtures of PAHs in the sediments. He will speak with John Incardona about giving a seminar on these results in Southern California.

Action Items:

- Meg Sedlak will distribute the revised report with additional results to the EEWG this summer.

4) 2012 Plans for Moderate Toxicity Workshop and Mesohaline Index Development

Moderate Toxicity Workshop
Steve Bay reminded the group of the low levels of toxicity that have been seen across San Francisco Bay year after year. It is consistently observed, and though it does not indicate a severe problem, it is very difficult to understand what is causing the toxicity. RMP efforts so far to understand this have included toxicity identification evaluation (TIE) studies and contaminants of emerging concern (CEC) research, but there is not an obvious path forward. In 2011, the Steering Committee (SC) allocated funds to convene a workshop with experts from across the country to provide ideas and to develop study designs to address this issue.

The proposed workshop would be held immediately following the 2012 National SETAC meeting in Long Beach, CA and would bring together about 20 experts from across the country and many different areas of expertise, with the objectives of assessing current knowledge regarding causes of SF Bay sediment toxicity to amphipods (*Eohaustorius estuarius*) and developing testable hypotheses and study designs to investigate likely stressors. The participants would generally be experts in either stressor identification, contaminants of emerging concern, or non-contaminants stressors and *Eohaustorius* life history.

Dan Schlenk asked why the workshop was not designed as a Pellston, as first suggested by the EEWG. Steve Bay indicated that this question did not seem to require that level of investment of time, resources, and formal structure. Dan Schlenk also asked how this workshop would bring in new ideas, as the people currently proposed to be involved may be less likely to propose new angles or approaches, due to their familiarity with the issues or current level of input. He suggested including experts on nanomaterials, and other potentially relevant experts who have not been involved with this particular topic. Brian Anderson supported the idea of including experts on *Eohaustorius* natural history. Meg Sedlak indicated that the group would re-evaluate the list of proposed participants, and look for a wider range of experts. They will also send it out to the EEWG for input.

Naomi Feger pointed out that other coastal regions in California face similar problems, and would likely be interested in the results. She proposed investigating the idea of cost sharing with other regions, and Steve Weisberg indicated that he would discuss this with Chris Beegan.

Mesohaline Index Development
Steve Weisberg outlined the proposed study to develop more robust indices to assess mesohaline benthic community assemblages. The budget for this study is more than what was allocated by the SC for 2012, however it is an accurate estimate of what the investigators predict the task requires.

Mike Connor suggested that the indices be developed in collaboration with the Interagency Ecological Program (IEP), because they have been studying this and have high quality data. Mike Kellogg noted that he supported this proposal, and that it is important to develop all three of the indices.

Action Items:

- Re-evaluate the list of proposed participants for the Moderate Toxicity workshop, and send it out to the EEWG for input.
- Meg Sedlak, Naomi Feger, and Steve Weisberg will investigate the possibility of getting additional funding from other California regions.
- Look into collaborating with the IEP on mesohaline index development.

5) RMP Planning Overview

Jay Davis introduced the RMP Multi-Year Plan (MYP), and reviewed how the different pieces of the RMP fit together. He indicated that while the "top-down" guidance from the SC is the source for the funding allocations for EEWG projects, some funding was not allocated to specific studies and the SC is looking for input and ideas from the workgroups on how to use these funds over the next few years and into the future. There is therefore some potential for additional funding beyond the allocations listed in the MYP.

Meg Sedlak indicated that this plan provides an opportunity for the RMP to interact with stakeholders and with panel members to determine if there are priority questions for stakeholders, or important information gaps or recent developments, that the RMP should be investigating. Steve Weisberg noted that his priorities for the RMP are 1) nutrients; 2) sediment toxicity; and 3) emerging contaminants, including bioanalytical screening tools. Naomi Feger suggested that the potential effects of microcystin could be an important question for the RMP to address in the near future. Mike Connor suggested that the potential effects of selenium, particularly on sturgeon, may also be of interest. Harry Ohlendorf also suggested revisiting the EEPS conceptual model, and using it to reassess what we've learned about how the Bay operates. Meg Sedlak indicated that she would add this assessment to the upcoming EEPS summary report.

Action Items:

- Revisit the EEPS conceptual model, and incorporate our improved understanding of the Bay into the EEPS summary report.

6) Application of Bioanalytical Tools for CEC Monitoring

Dan Schlenk reviewed the proposed use of bioanalytical tools in monitoring for CECs. This idea is built on a similar proposal from the recycled water policy. The basic concept is to use *in vitro* biological assays to screen for effects of CECs, rather than analyzing for the CECs individually. If the correspondence between the biological assay and CEC presence is good, then this technique provides a comprehensive, low cost means for screening for presence of relevant concentrations of CECs. The technique does have limitations in its ability to link an *in vitro* response to ecological meaning, and will therefore initially be better at establishing exposure than potential for effects. Bioanalytical protocols for drinking water are scheduled to be standardized for drinking water by winter 2012.

Mike Connor asked about investigating a tissue matrix, as opposed to water, to assess the potential for effects in the contaminants that are taken up into organisms. Dan Schlenk indicated that the procedure is likely to be possible, but the process of extracting CECs from tissue in order to run the bioassay may result in introducing some matrix effects.

7) Pilot and Special Studies 2013: Bioanalytical Tools

Nancy Denslow called in to the meeting to describe a proposal for 2013 RMP funding that will link *in vitro* (cellular level) assay results with *in vivo* (organism level) end points. This proposal focuses on about half of the CECs recommended for monitoring by the statewide advisory panel for CECs in receiving waters – those that have estrogenic endpoints. The study will use *Menidia beryllina* (silverside) as the test species. In using a sensitive estuarine species that is found across the country, including San Francisco Bay, this study will draw on the existing tests developed for this species, and begin linking exposure and effects endpoints for a species that is relevant nationwide. Silverside are appropriate because they are closely related to topsmelt and the USEPA has developed regulatory endpoints for this species.

The total cost of the two-year project will be $168,000; however, only $126,000 is being requested from the RMP, as SCCWRP will be contributing $42,000 to the project. In addition, the project will occur alongside the state-funded ($800,000) project to compare commercially available bioassays in a round robin exercise to develop bioanalytical tools for drinking water. In the first year, laboratory experiments would be undertaken to model exposures; in the second year, field exposures would occur.

David Sedlak noted that the estrogen receptor and estrogenic impacts have been studied widely. It is therefore more straightforward to link bioassays with ecological effects targeting this receptor, however, it is less likely to produce new information, while some of the other CECs with different mechanisms of action might. Dan Schlenk noted that the existing database on estrogenicity will be helpful for Dr. Denslow as the project is developed and put into context. Michael Fry agreed with Dr. Schlenk that the importance of linking *in vitro* and *in vivo* responses outweighs the slightly more limited relevance of using estrogenic receptors.

Eric Dunlavey asked about the selection of WWTPs for effluent testing in year 2 of the study, indicating that the City of San Jose was unaware of this project prior to today's meeting. Nancy Denslow noted that the plants had not yet been identified and that the plants mentioned in the proposal were examples of possible plants. Mike Connor suggested that the City of San Jose WWTP is not comparable to Hyperion WWTP in Southern California, and that EBMUD or EBDA may be a more appropriate choice.

David Sedlak asked if the silverside may be stressed due to the levels of ammonia in WWTP discharge. Nancy Denslow indicated that the estrogenic compounds will be extracted from the water sample and then reconstituted into the experimental set up, to remove external factors. Jennifer Field suggested that some of the studies use whole water, to provide context and a frame of reference.

Derek Muir and Dan Schlenk also pointed out that while a lot of work is being done on fathead minnows in freshwater, little work has been conducted in estuarine waters. The array for silverside being developed in this study will build off the body of knowledge existing for fathead minnows, and define silverside as the benchmark species for estuaries.

8) Discussion of 2013 Special Study: Bioanalytical Tools

David Sedlak suggested some adjustments to the project and future directions for research. Particularly, he suggested that other matrices, such as fish tissue, may provide more relevant results, as fish tissue will reveal the contaminants that are also able to biaccumulate in organisms of interest. He also noted that this study would likely not pick up on the higher priority CECs, such as flame retardants and perfluorinated compounds.

Harry Ohlendorf suggested that this study be put in context with the ECWG strategy and how managers may use the results. David Sedlak pointed out that embarking on this study implies a long-term commitment to bioanalytical tools from the RMP. Given the state of the research to date, it will be approximately 10 years before the tools can be applied to management and monitoring. However, Dan Schlenk noted that in the long run, bioassay results will provide data, similar to Dioxin TEQ data, in the form of estrogenic equivalency quotients (EEQs). Eventually, bioassays could be used as a screen for CECs in samples, where only samples surpassing an EEQ threshold would be subjected to further analyses. Harry Ohlendorf suggested that the ECWG create a roadmap from this study to implementation of bioanalytical tools in monitoring.

While it is projected that multiple years and further studies will be required before bioanalytical tools can be implemented in monitoring, the RMP is not committing to funding the remainder of the work. Other agencies and organizations will have an interest in this work, and will be able to contribute funding. Steve Weisberg pointed out that the NSF is more likely to fund cutting edge research, and that Regional Boards and local organizations will be interested in funding the method finalization and implementation. In between, however, organizations such as the RMP will be invaluable to fund the less "cutting edge" work needed to convert good ideas into implementable monitoring tools. Given that the State Board is asking for tools that have not yet been developed (a rare occurrence), he feels confident that they will be willing to fund implementation of these tools once they are further along. Naomi Feger suggested that the study costs for 2013 be split evenly between Northern and Southern California.

Jay Davis asked about using the fathead minnow, rather than silverside, which has been studied in greater detail. However, Dan Schlenk pointed out that the fathead minnow would not reveal important effects endpoints, because it does not exist in estuarine environments. Because this approach treats organisms as a "black box", it is important to select a relevant black box – in this case, silverside.

Naomi Feger asked what the implications would be if the study was not funded by the RMP for 2013. Keith Maruya indicated that much of the momentum and ability to leverage would be lost, as it would not line up with the drinking water tool development. Steve Weisberg pointed out that the study would likely be funded eventually by other sources, but this is an opportunity for the RMP and Southern California to influence this tool development from the beginning, rather than waiting to see what other organizations, such as the State Water Board, develop, and implemented, failing a bioassay will spark essentially a TIE, to determine what is causing the detected effects.

Meg Sedlak noted that the RMP periodically provides seed funds to start a project or keep a good idea moving along. One participant noted that this project could benefit greatly from RMP funds, as few organizations would be likely to fund a study of this type. One stakeholder suggested that the RMP will likely want to get into bioanalytical tools, but not at this point in time, largely because it is focused on the immediate concerns of its regulators and dischargers, and the base work on this development is more appropriately funded by other organizations.

In a poll of participants,

- 2 participants indicated that they DID NOT support funding the bioanalytical tools study. They cited the possibility of other organizations being more appropriate to fund the work at this point in time.
- 2 participants indicated that they PARTIALLY supported funding this study. They suggested funding one year of the study, and reassessing its progress at that point in time. They also noted that the immediate application of this work is currently unclear.
- 7 participants indicated that they DID support funding this study. They recognized the uncertainties in the proposal, but noted that the science is compelling, and that this presents a valuable opportunity to contribute to development of this work. They noted that in supporting this study, the RMP should recognize that it is committing to this idea for the long term. They suggested actively pursuing other partners to contribute funding, and hypothesized that if the work is going well, others will likely want to join in.

Jay Davis indicated that this proposal would be brought to the TRC and SC for consideration, and that the various thoughts of the EEWG and ECWG participants would be relayed as well. One panel member asked that in presenting the idea to the TRC and SC, the case for how this information will eventually be used, and why it is important to investigate now, be more compelling.

Action Items:

- Revisit the list of proposed moderate toxicity workshop participants.
- Request a $50,000 allocation for 2013 to follow up the moderate toxicity workshop with a strategy for implementing new ideas and proposals.
- Clarify the goals and milestones for each of the two years of the Mesohaline Index development project.
- Explain, in a more compelling fashion, how the results of the bioanalytical tools development will be used in the long run, and why it is important to investigate now.

attempting to provide input after the tools are already in place. Other potential funders, further down the road, include the Water Environment Research Fund (WERF) and the EPA.

Arleen Feng noted that the ECWG has not yet developed a strategy for CECs in the Bay, and that it seems premature to fund a project of this magnitude without an agreed-upon strategy. She also noted that while it is worthwhile to invest in the future, some organizations such as BASMAA are still scrambling to meet permit requirements, and may not have this flexibility.

Action Items:

- Outline a projected roadmap from the bioanalytical tools special study to implementation of bioassays in monitoring. Determine how this study fits into the broader RMP CEC strategy.
- Outline potential partners, funding agencies, or possibilities to leverage funds for future progression of bioanalytical tool development.

9) Closed Door Discussion of RMP Special Studies

The stakeholders and panel members discussed their support of the proposed special studies:

1) 2012 Moderate Toxicity Workshop
2) 2012 Mesohaline Index Development
3) 2013 Bioanalytical Tools

1) Moderate Toxicity Workshop

The group agreed that the proposed list of participants needs to be revisited, and include more outside experts who may have new ideas. They also suggested that the SC allocate about $50,000 in 2013 to follow up the workshop with a strategy to implement any new ideas. This number will serve as a placeholder, and will be modified as necessary after the workshop. In moving this proposal to the SC, it is important to emphasize that the outcomes of the workshop are unknown, and there may be many options for use of this funding.

2) Mesohaline Index Development

The group approved of the proposed mesohaline index development, and particularly recognized the need to create three different indices. They asked for clarification on what will be accomplished during each of the two years.

3) Bioscreening

The group was asked to assess the technical merit of this proposal, rather than weigh it against other proposals from the ECWG. One panel member noted that this study is a high priority to the State Water Board, and that it is one of the recommendations from the monitoring chapter of both the drinking water and receiving water reports. Another panel member pointed out that the tools developed (which will likely not be ready for 10 years) will be very powerful for regional monitoring, but that the outcome of this particular study will not feed directly into the RMP. Thus, he approved of funding the proposed study only as part of a longer term commitment by the RMP to this general approach. However, he pointed out that the approach does need development, including expansion into tissue analyses and calibration to potential non-WWTP sourced endocrine disrupters. Another panel member pointed out that once they are

PS/SS: Linkage of *In Vitro* Assay Results With *In Vivo* End Points

Estimated Cost: $70,000 for 2013 (Year One). This is proposed as a two-year study; $56,000 will be requested for 2014, pending acceptable progress in Year One.
Oversight Group: Emerging Contaminants Workgroup and Exposure and Effects Workgroup
Proposed by: Nancy Denslow (University of Florida) and Keith Maruya and Steve Bay (SCCWRP)

Proposed Deliverables and Time Line

Deliverable		Completion Date
Task 1:	**Convene focus group and develop actionable plan**	**CSD + 1 month**
Task 2:	**Develop molecular biomarkers for Menidia**	**CSD + 4 months**
Task 3:	**Laboratory tests: Early life stage exposures and in vitro bioassays**	**CSD + 9 months**
Task 4:	**Field-collected sample exposures**	**CSD + 18 months**
Task 5:	**Chemical analysis of CECs**	**CSD + 21 months**
Task 6:	**Reporting**	**Mid-term (Year 1): CSD + 12 months** **Final: CSD + 24 months**

BACKGROUND

A growing number of contaminants of emerging concern (CECs) are found routinely in permitted discharges and their receiving waters. For the few CECs for which analytical methods exist, these methods are still largely in development and only some are routinely performed by commercial services laboratories. As the development and manufacture of chemicals presents an ever changing landscape, those CECs that are produced in high volumes and/or that are capable of being discharged via treated municipal or industrial wastewater effluent or stormwater runoff represent a moving target for environmental quality managers tasked with assessing and/or mitigating their potential for impact.

The CECs of most concern are those which may be potent at trace concentrations (parts per trillion range) and work as endocrine disruptors. Their presence in waterbodies may be harmful to aquatic biota inhabiting these locations. Such endocrine disrupting chemicals can interact directly with soluble hormone receptors or can interfere with the natural synthesis or metabolism of endogenous hormones and thereby impede normal function of these processes in exposed organisms. Most attention has been focused on chemicals which act as estrogens or androgens or their antagonists. Estrogens are important in brain development and programming of tissue differentiation at early time points during development (Feist and Schreck 1996; Lassiter and Linney 2007; Mandiki et al. 2005; Remage-Healey and Bass 2007; Tomy et al. 2009; Vetillard et al. 2006; Zhang et al. 2008).

In our own work, exposure of fathead minnows to concentrations of ethinylestradiol (EE2) at 2 ng/L induced pericardial/yolk sac edema (Johns et al. 2009). The estrogenic mycotoxin zearalenone (exposure range of 2-50 ng/L) also resulted in myocardial edema (Johns et al. 2009). In addition, we analyzed a limited set of gene expression changes including Vtg, which was up-regulated by the two estrogens, steroidogenic acute regulatory (StAR) protein, insulin-like growth factor 1 (IGF-1) and growth hormone (GH) which were also altered. Thus, these genes in target fish species would be viewed as critically important to include in future studies of responses to estrogenic CECs at the molecular level.

Concurrently, novel in vitro methods based on receptor binding or transactivation have been developed that are extremely sensitive to target chemicals acting with the same mode of action, including the potent endocrine disrupting CECs described above. Work is being performed to adapt these in vitro bioassays for water quality assessment and monitoring purposes. Few studies, however, link results from such *in vitro* assays with higher order *in vivo* effects which result in adversity for survival, growth, reproduction, or susceptibility to disease.

The goal of this project is to establish quantitative linkages between the *in vitro* receptor-based assays and traditional endpoints of adversity in a sensitive estuarine fish model, the common silverside (*Menidia beryllina*) which is an established EPA model for estuarine toxicity. As a demonstration, we will focus on estrogenic responses of selected chemicals of interest first in lab exposures (Year 1) followed by exposure to field-collected wastewater treatment plan (WWTP) effluent and estuarine and marine receiving waters (Year 2).

Study Objective and Applicable RMP Management Question

There is considerable concern about the presence of CECs in treated domestic wastewater that is released into the environment and in waters that are reused for irrigation. In California, many WWTPs discharge their treated waters directly into estuaries or into rivers upstream of estuaries. While there are regulations in place for monitoring specific CECs and other regulated chemicals, there is currently not much being done to discover new CECs in water or assess whether the presence of the CECs may adversely affect aquatic biota. If these experiments are successful, we should be able to start to develop methods to integrate results from *in vitro* assays into monitoring programs and determine how they relate to *in vivo* adverse effects. Results from this study will begin to enable managers to determine whether or not additional action is necessary for treated effluents that are discharged into sensitive estuarine environments. This work will not only be important for California, but also for other states that discharge domestic wastewater into marine/estuarine environments.

The objective of this effort is to develop a tool that will assist in the identification of chemicals of emerging concern that are adversely affecting biota. This study would address the following RMP management question (MQ):
MQ1. Are chemical concentrations in the Estuary at levels of potential concern and are associated impacts likely?

A: Which chemicals have the potential to impact humans and aquatic life and should be monitored?

B: What potential for impacts on humans and aquatic life exists due to contaminants in the Estuary ecosystem?

Study Plan

This study will test estrogenic chemicals that were recently recommended for monitoring in California's receiving waters by the State's Science Advisory Panel for CECs (Anderson et al. 2012), e.g. estrone (E1), bisphenol A (BPA), 4-nonylphenol (4-NP) and galaxolide (HHCB). Traditional i*n vivo* endpoints for early life stages of silversides (*M. beryllina)* will include: development, growth, and survival and for juveniles: growth, survival and biochemical endpoints such as plasma vitellogenin and hormone concentrations (Vtg, E2 and T) and hepatic gene expression for at least 5 genes per life stage. We will index estrogen equivalency concentrations required for altering higher order endpoints with biochemical responses within the fish and responses obtained with commercially available estrogen receptor (ER) transactivation assays (see also Task 3). These linkages will enable the use of *in vitro* assays as measures of both exposure and effect. The concentrations required for both *in vivo* and *in vitro* assays will be quantified to determine reference concentrations above which effects may be expected.

Tasks 1 through 3 will be completed in Year One with 2013 funds. Pending approval from the TRC and ECWG/EEWG, Tasks 4 through 6 will be completed in Year Two with 2014 funds.

Task 1: Initial meeting for project coordination. The study will be initiated by a meeting between the two laboratories to discuss the details of the experimental approach to be used and to set up the time frame for the various experiments.

Task 2: Development of molecular biomarkers for *Menidia beryllina*. There are a few molecular biomarkers already developed for *M. beryllina* including Vtg, ER alpha (esr1), ER beta a (esr3), and androgen receptor (AR), among others (Brander 2011). We will validate these assays with our own samples and also develop additional molecular assays for the following genes: IGF-1; StAR; GH; brain aromatase (cyp19b); and two genes involved in testis differentiation, anti-Mullerian hormone (amh) and doublesex and mab-3 related transcription factor 1 (dmrt1). We will get sequences for these additional genes from a high throughput DNA sequencing experiment, discussed in advance with Drs. Brander and Connon, experts involved in developing a transcriptome for Menidia. We will make all sequences we obtain accessible to these colleagues for their independent studies. The assays that we will develop will be based on quantitative reverse transcriptase polymerase chain reaction (Q-PCR) and involve the quantitative amplification of specific genes from total RNA extracted from early life stage fish or juveniles. These genes have been determined in studies of other fish to be responsive to estrogens *in vivo* (Filby et al. 2007; Ijiri et al. 2008; Johns et al. 2009; Kobayashi et al. 2003). The assays will be validated with standard curves after determining that amplification is proportional to amount of transcript present in the original sample.

Task 3: Laboratory exposures and in vitro bioassays. These experiments will be performed at the University of Florida and SCCWRP. We will divide up the exposures and each will perform a positive control. Exposures will be in the water with at least 5 concentrations of each chemical and a single concentration of 5 ng EE2/L as the positive control. The chemicals that will be tested include E1, 4-NP, BPA, and galaxolide (HHCB). Since Menidia are a sensitive species we will first use a range finding experiment to determine the LC50 and conduct our exposures at or below that level. We will then include two concentrations below and two above this value. The test chemicals will be mixed with a small volume of triethylene glycol (TEG) as a carrier to ensure the chemical gets into the water phase. Dilution water will dechlorinated tap water adjusted to 15 ppt salt (using Instant Ocean) and temperature will be controlled to 20 ^{o}C, following standardized test guidelines for early life testing (EPA, 1995).

Each lab will do two types of exposures: early life stages, where we will buy recently hatched embryos and allow them to grow under the chemical conditions described above. For these assays we will place twenty 10-d old larvae per 200 ml beaker in quadruplicate for each condition, including artificial seawater with and without carrier, the positive control and 5 concentrations of each test chemical. The exposure will extend for 7 days. Fish will be fed newly hatched brine shrimp. End points will be mortality and growth. We will also take at least 5 individuals from each beaker to determine changes in expression for the following genes: IGF-1, CYP19B, GH, dmrt1, amh.

For the Juvenile Test, we will use 50-d old fry and place 10 per 500 ml beaker under the chemical and replicative conditions described above. This period will be just before gonadal differentiation in this species, another time of vulnerability to endocrine disruptors. The fish will be exposed for 10 days in a static renewal system in 15 ppt salinity. Fish will be fed tetramin flakes and supplemented with brine shrimp. The endpoints for this assay will include measurements of whole body homogenate concentrations of Vtg, E2 and T (if the hormone evaluations are possible at this age) and hepatic measurements of gene expression for Vtg, ERα, ERβ, AR, and StAR using Q-PCR. We will also perform histopathology on the gonads to distinguish males from females and if we can find a sequence for dmrt1, we will correlate its expression with sex of the fish. In some species this gene marks genetically determined sex (Nanda et al., 2002).

For the *in vitro* assays, we will use the same final concentrations as described above, except that the chemicals will be added to the culture as 1/10 volume of a 10X solution, in order not to dilute out the nutrients required for the tissue culture. We will use the same commercial assays for estrogen receptor (ER) transactivation that will be used in a project recently funded by CA (K. Maruya, PI and N. Denslow, CoI, among others) entitled "Evaluating bioanalytical methods as screening tools for monitoring of CECs in California recycled water applications." There will be significant savings in leveraging the funded project as all assay development and validation for the in vitro assays will occur in that project. We will simply apply the assay in this one.

Expectations and Alternative Strategies. We expect that the concentrations of model compounds that we have selected will show higher order effects in survival and growth in the *in vivo* tests (at least at the higher concentrations tested). From past experiments, we are confident that these concentrations will also impact molecular endpoints within the fish resulting in alteration of gene expression. Lastly, the selected concentrations should also be potent enough to change expression of the high throughput assays. *Menidia* are expected to be as sensitive, if not more sensitive that fathead minnows to these chemicals. In the event that they have high mortality in the selected concentrations, we will repeat the experiment at lower concentrations.

Task 4: Exposure to WWTP effluent and receiving water samples and in vitro bioassays
In Year 2, we will test samples of WWTP effluent and receiving waters from two sites, one in southern California and the other in the San Francisco Bay estuary. A sufficiently large volume of treated final effluent and receiving water from each site will be filtered through a sorbing phase (e.g. C18 or Oasis HLB cartridge) to capture organic contaminants and subsequently eluted by organic solvents. A portion of each eluent will be set aside for analytical chemistry and the remainder will be shipped to one or the other of the two participating laboratories where the eluent will be reconstituted to the same proportional volume as the original sample and tested with either an early life stage assay or a juvenile assay.

One sorbing cartridge each will be processed and shipped to SCCWRP and the University of Florida. The cartridges will be eluted and then air dried in order to reconstitute test solutions to 1X, 5X and 10X the concentrations equivalent to what they were at the field site. Each solution will be tested in triplicate and we will use the 5 ng EE2/L as a positive control. We will perform early life stages and juvenile tests as described above. We will also perform in vitro nuclear receptor transactivation assays as described above, but in this case using the concentrates from the field locations.

Expectations and Alternative Strategies.
Based on the results from Task 2 in year 1, we may adjust the concentrations of the positive controls and we may adjust the concentrations of the reconstituted test solutions. If we find that Menidia are more sensitive to the chemicals than we anticipated, all concentrations will be diluted by 10 to get in the range where we do not see more than 10% mortality. Under these conditions, we expect that all endpoints will be viable and that we will be able to compare estrogenic equivalencies across experiments. We may not see much with field waters for androgenic changes, as most of the CECs that target this axis react as anti-androgenic chemicals.

Task 5: Chemical analysis of CECs
Estrone (E1) and EE2 will be measured by ELISA following the methods of Huang and Sedlak (2001). Galaxolide (HHCB), bpA and 4-NP will be measured by GC-MS after extraction and derivatization as described in Ligon et al. (2008). Samples of sufficiently large volume will be collected to ensure the appropriate sensitivity of measurement, based on the range of treatments (lab) and expected receiving water concentrations. An equivalent amount of chemical as evaluated for the in vivo assays will also be assessed for the in vitro assay.

Task 6: Reporting
We plan to submit a mid-term progress report at the end of year 1 and a final report at the end of year 2. We expect that we will be able to derive relationships between the different levels of results, from the molecular high throughput assays to in vivo molecular endpoints and to in vivo higher order changes in survival, growth and development. The estrogenic and androgenic equivalencies that we will derive will help establish the usefulness of high throughput assays as a means to test the quality of estuarine water. We expect this demonstration project to show the usefulness of the approach.

Projected roadmap from this special study to implementation of *in vitro* assays in monitoring.

1. **July 2012 — Development of protocols for commercially available bioanalytical tools.** (sponsored by the State Water Board). Currently there are at least 3 commercial companies that have developed assays for nuclear hormone receptors, including InVitrogen, Biodetection Systems, and SwitchGear. We are in the process of testing the assays and will choose one system for further work. This is part of a project awarded to SCCWRP by the State Water Board (SWB). Initial studies with all three assays indicate that they work well. We will choose a method that has the best chance for implementation by end users in California.
2. **Dec 2012 – Testing of bioanalytical assays for multiple molecular pathways with concentrates extracted from water.** (sponsored by State Water Board). The assays include molecular pathways that are known to be affected by exposures to estrogens, androgens, progesterone and glucocorticoids, among other chemicals. We will use the selected assays to examine extracts of WWTP effluents and compare the results with analytical chemistry of the same extracts. We also plan to participate in a parallel project to use bioanalytical techniques to measure hormone activities in Australian WWTP effluent and surface waters (funded by WERF).
3. **April 2013 — Implement Menidia bioassays following EPA protocols.** (sponsored by RMP) Tasks 1-3 of proposed project. Determine concentrations of E1, 4-NP, BPA, and galaxolide (HHCB) that show effects *in vivo*. Compare these concentrations to those required to change expression of genes *in vivo* and with concentrations required to activate *in vitro* assays showing ER activity.
4. **July 2013 – Start Bight '13 special study utilizing in vitro assays in conjunction with other monitoring tools.** (sponsor TBD). Conduct study using southern California samples that is complementary to concurrent RMP study.
5. **April 2014 — Compare in vivo and in vitro assays for effluent and receiving water**. (sponsored by RMP). Complete tasks 4-5 of proposed project.
6. **September 2014—Integrate results of the proposed RMP *Menidia* project with results from other projects.** (co-sponsored by RMP, SCCWRP and partners, SWB, WERF) This would include other endpoints covered in the other funded studies, e.g. those that are planned in conjunction with Bight 2013, future RMP surveys, and SWAMP special studies.
7. **October 2014 – Workshop to teach bioanalytical methodology.** (sponsor TBD) Transfer of *in vitro* assay technology to public and private laboratories. This would include entities interested in applying the approach in monitoring programs.

8. **January 2015 – Start interlaboratory study to compare bioanalytical methods to current chemical analysis and *in vivo* tests.** (sponsor TBD).
9. **March2016 – Report on interlaboratory study.** If performance is deemed satisfactory, develop guidance to implement bioassays in routine monitoring.
10. **July 2016 – Start pilot implementation study.** Identify partner agencies and facilitate incorporation of in vitro assays into their monitoring programs on a limited basis.
11. **July 2017 – Assess pilot study results.** Review results from pilot study and develop plans and guidance for implementation of assay methods.

Potential partners, funding agencies, or possibilities to leverage funds for future progression of bioanalytical tool development.

1. WERF—applied research
2. NOAA – using Mussel Watch as an environmental effects platform
3. NSF – funding of basic research oriented projects
4. State Water Board – future phases for development and implementation of bioanalytical screening tools
5. Other Water Quality Stakeholders – special studies as part of regional surveys (e.g. Bight 2013; RMP) and specific discharger needs (e.g. outfall maintenance)

Budget

The scope of this study will require two years, with Year 1 devoted to obtaining molecular biomarkers for *Menidia* and defining critical endpoints of adversity in early life stage fish. Year 2 will focus on application of these endpoints to effluent and receiving water samples (see Deliverables and Time Line). We are requesting a total budget of $126,000, with $70,000 for work to be completed in 2012 and the remaining $56,000 for 2013. In addition, SCCWRP will contribute leveraged funds via two projects, one supporting the development of in vitro bioassays (or “transactivation assays”) funded by the State Water Board and scheduled for completion in mid-2013, and from an internally funded project to develop in vivo bioassays using Menidia and other estuarine marine fish species.

Project Budget

Description	Cost per Sample ($)	Total Cost Estimate ($)	Cost Estimate minus match ($)
Yr: 1			
Development of Molecular Biomarkers		22,000	**22,000**
Early life stage assay—model compounds -- 4 (SCCWRP Match – 8,000)	6,000	24,000	**16,000**
Juvenile assay – model compounds -- 4 (SCCWRP Match – 8,000)	6,000	24,000	**16,000**
Transactivation Assays -- 4	3,000	12,000	**12,000**
Analytical chemistry (SCCWRP Match 4,000)	2,000	8,000	**4,000**
Total Yr 1		90,000	**70,000**
Yr:2			
Water extracts ($6,000 in kind SCCWRP)	6,000	6,000	**0**
Early life stage assay – field sites – 4 (SCCWRP Match—8,000)	6,000	24,000	**16,000**
Juvenile assay – field sites -- 4 (SCCWRP Match 8,000)	6,000	24,000	**16,000**
Transactivation assays – 4	3,000	**12,000**	**12,000**
Analytical Chemistry – Field sites –4	3,000	**12,000**	**12,000**
Total yr 2		**78,000**	**56,000**
Total Requested Budget		**168,000**	**$126,000**
SCCWRP contribution	$42,000		

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PS/SS: Development of Benthic Community Condition Indices for Mesohaline Environments of the San Francisco Bay.

Estimated Total Cost: $125,800 (two-year study 2012 and 2013)
$50,000 has been to 2012 activities

Oversight group: Exposure and Effects workgroup
Proposed by: Eric Stein SCCWRP

Funding requested for 2013: $75,800

Introduction and Background

Benthic community assessment is often used as an indicator of ecosystem condition and has become a central element of regulatory programs such as the California's sediment quality objectives for bays and estuaries. Benthos are the indicators of choice for monitoring and assessment for several reasons, including:

- Limited mobility makes them indicative of impacts at the site where they are collected.
- Several animal phyla and classes are sensitive to impacts to their environments and can be used to differentiate certain types of effects.
- Life-histories are short enough that the effects of one-time impacts disappear within a year but long enough to integrate the effects of multiple impacts occurring within seasonal time scales.
- Living in the bottom sediments, benthos have high exposure to common anthropogenic impacts, such as sediment contamination, high sediment organic carbon, and low bottom dissolved oxygen.
- They are important components of aquatic food webs, transferring carbon and nutrients from suspended particulates in the water column to the sediments by filter feeding and serving as forage for bottom-feeding fishes.

For benthic data to be useful in a regulatory context, they must be interpreted in relation to scientifically valid criteria or thresholds that distinguish "healthy" from "unhealthy" benthic communities. While reducing complex biological data to index values has disadvantages, the resulting indices remove much of the subjectivity associated with data interpretation. Such indices also provide a simple means of communicating complex information to managers, tracking trends over time, and correlating benthic responses with stressor data.

To date, benthic indices have been calibrated and validated for two nearshore habitats in California, 1) southern California marine bays, and 2) polyhaline (high salinity) portions of San Francisco Bay. Indices have not been developed for other habitats such as the low salinity mesohaline and tidal freshwater environments. These habitats are particularly challenging because they are naturally subject to relatively broad ranges of conditions (e.g. salinity and dissolved oxygen) and hence the resident organisms are adapted to tolerate environmental stress.

These challenges can be addressed through compilation of robust data sets, careful identification of reference conditions to anchor indices and development of multiple indices that can be used to increase overall sensitivity to detect change in condition.

The objective of this project is to develop and calibrate a minimum of three benthic indices for the mesohaline environments of San Francisco Bay. To the extent possible, we will use the initial consultations with experts to provide a foundation for future work on developing an index for the tidal fresh environment. We do not anticipate that the currently available funds are sufficient for full index development. Therefore, we have divided this proposal into two phases that could be independently funded and would each produce defined products.

Study Objective and Applicable RMP Management Questions:

The objective of this effort is to develop an index for the mesohaline portions of the Bay. This study would assist in our ability to answer the following priority questions for benthos:

1. What are the spatial and temporal patterns of impacts of sediment contamination?
2. Which pollutants are responsible for observed impacts?
3. Are the toxicity tests, benthic community assessment approaches, and the overall SQO assessment framework reliable indicators of impacts?

Overall Study Approach

We will focus on the development of three indices. The first two indices are based on species composition of large numbers of species: the Benthic Response Index (BRI; Smith et al., 2001; Smith et al., 2003; Ranasinghe et al., 2009) and the River Invertebrate Prediction and Classification System (RIVPACs; Wright et al., 1993; Van Sickle et al., 2006; Ranasinghe et al., 2009). The third index will be a multimetric index (MMI) based on community measures and indicator species (e.g. the Index of Biotic Integrity; IBI; Thompson and Lowe, 2004; Ranasinghe et al., 2009).

Index development will be divided between two phases that can be independently funded and that each have independent products.

Phase 1 (to be completed in 2012 with already approved 2012 funding)

Task 1: Update database. Update the existing 2,200 sample 1992 to 2008 standardized taxonomy benthic database with recently collected benthic data and associated habitat (salinity, depth, sediment grain size distribution, and total organic carbon), chemical contaminant, and sediment toxicity data.

Task 2: Refine Habitat Definitions. Refine the San Francisco estuary and delta habitat definition scheme to facilitate the application of SQOs. The original SQO habitat boundary definitions were based on about 140 San Francisco Bay samples in a 714 sample U.S. west coast data set (Ranasinghe et al., In Press). A subsequent study based on more than 501 San Francisco Bay samples (Thompson et al., In Revision) identified potential subtle differences that should be evaluated and defined for SQO implementation. The differences include (1) potential differences in the areal definitions of existing habitats, especially at the Bay margins, and (2) potential addition of an oligohaline habitat between the mesohaline and tidal freshwater habitats in the Suisun Bay region.

Task 3: Identify and Withhold Validation Data and conduct BPJ study. For each new habitat, identify about 20 samples covering the entire range of habitat conditions for inclusion in a BPJ benthic expert study. These samples will be withheld from the calibration data set in order to assure independence of the two data sets. Experts will be asked to (1) rank the validation samples from least to most disturbed, based on species abundance and minimal habitat data, (2) assign each sample to one of four assessment categories, based on existing narrative definitions, and (3) identify sample characteristics used to rank and categorize the samples.

Budget, Schedule, and Deliverables

The main products of Phase 1 would be the database of organisms and their associated habitats and the results of the BPJ study, which would allow the designation of "good" vs. "bad" locations based on taxonomic composition. At the conclusion of Phase 1, we would also produce a general roadmap for next steps in constructing the benthic indices.

The total cost to complete the Phase 1 tasks would be $35,323 (Table 1). We anticipate the work would take approximately 3-4 months to complete depending on quality of the data sets and the availability of the expert panel for the BPJ exercise.

Table 1. Phase 1 Budget

Task	Description	Total
1	Update Database	6,894
2	Refine Habitat Definitions	2,219
3	Identify and Withhold Validation Data and conduct BPJ study	26,209
Total		35,323

Phase 2 (to be initiated with 2012 funds; however, bulk of the funding is being requested from 2013 funds)

Task 4: Develop and Calibrate Benthic Indices. Develop and calibrate indices based on relative site conditions using the standard statistical methods appropriate for each index.

Task 5: Assure Independence of Indices and Habitat Factors. Each calibrated benthic index will be tested for independence from habitat variables such as salinity, sediment grain size distribution, sample depth, latitude, longitude, and total organic carbon. While it is generally accepted that current models of benthic response do not discriminate between chemical contamination and other sources of stress (Borja et al., 2003) this approach will ensure that indices are not driven by habitat factors. The goal is an index responsive to benthic community condition, rather than being driven by one or two habitat factors such as sediment grain size distribution or sample depth.

Task 6: Calculate Benthic Index Values. Benthic index values will be calculated for the validation samples, recently acquired data, and new indices for previously acquired data by applying formulae developed during index calibration.

Task 7: Evaluate and Validate Benthic Indices. The BPJ study results and index values calculated by applying formulae developed by index calibration will be used to validate the indices, establish threshold index values for assessment category assignment, and select the most accurate index or combination of indices for application of SQOs.

Task 8: Prepare Summary Report/Journal Article: Results of the study will be written up for publication to a peer-reviewed journal. The journal manuscript will serve as the final project deliverable.

Budget, Schedule, and Deliverables

The main products of Phase 2 would be validated benthic indices for the mesohaline habitat and the journal manuscripts that document their development and testing process.

The total cost to complete the Phase 2 tasks would be $90,508 (Table 2**); $75,800 is being requested for work in 2013.** We anticipate the work would take approximately 6 months to complete following the completion of Phase 1.

Table 2. Phase 2 Budget

Task	Description	Total
4	Develop and Calibrate Benthic Indices	26,354
5	Assure Independence of Indices and Habitat Factors	8,785
6	Calculate Benthic Index Values	8,785
7	Evaluate and Validate Index	20,459
8	Prepare Journal Article/Project Report	26,126
Total		90,508

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PS/SS: Establishing a Reference Site for Bioassays

Estimated Cost: $ 27,000
Oversight Group: Exposure and Effects Work Group
Proposed by: Brian Ross (USEPA), Beth Christian (SF RWQCB), Ellen Willis-Norton, and Don Yee

Background

The Section 404(B)(1) of the Clean Water Act states that dredged materials may not be disposed of in U.S. waters if the discharge results in significant degradation of the aquatic ecosystem (Clean Water Act of 1972). The joint USEPA/USACE Inland Testing Manual (ITM) details testing requirements for evaluating dredged materials to ensure compliance with Section 404 of the Clean Water Act (EPA/USACE 1998). The ITM calls for a comparison of dredged material quality to that of the disposal site sediment. But it also notes that multiple discharges at a disposal site could adversely impact the point of comparison, and that adoption of a reference sediment that is unimpacted by previous discharges of dredged material will result in a more scientifically sound evaluation of potential individual and cumulative contaminant-related impacts. Identification of a common sediment reference site for the Bay will also benefit dredgers because they will no longer need to repeatedly test sediment at multiple disposal sites.

The goal of this study is to identify and potentially adopt a sediment reference site that can be compared to all San Francisco Bay dredged materials. The reference sediment should be characteristic of the Bay (e.g. fine-grained) and should be unimpacted by previous dredged material discharges and nearby industry. RMP staff will work with Brian Ross (USEPA) and Beth Christian (SF RWQCB) to locate a Bay reference site between Central and Suisun Bay in which benthic organisms thrive (consistently > 85% amphipod survival) and ambient sediment chemistry thresholds are not exceeded.

Study Objective and Applicable RMP Management Question
The objective of this effort is to develop a reference site to aid in the interpretation of toxicity bioassay results in San Francisco Bay. This study would address the following RMP management question (MQ):

MQ1. Are chemical concentrations in the Estuary at levels of potential concern and are associated impacts likely?

- A: Which chemicals have the potential to impact humans and aquatic life and should be monitored?
- B: What potential for impacts on humans and aquatic life exists due to contaminants in the Estuary ecosystem?

Approach
This effort will involve the following steps:

1. Survey previous RMP Status and Trends sediment sites to determine if there are RMP sampling stations characterized by fine grains, amphipod survival that is greater than 85 percent, and low pollutant concentrations.

2. Choose one or two sites that fit the conditions described above and add the sites to the RMP's biennial Status and Trends sediment cruise. Toxicity tests at the potential reference sites should be conducted for the following species: 1) *Ampelisca abdita*, 2) *Rhepoxynius abronius*, 3) *Eohaustorius estuarius*, and 4) a benthic Polychaete.

3. If the percent survival is above 85 for the amphipods, work with Brian Ross and Beth Christian to determine what additional work may be required to adopt the location as a Bay-wide reference site. If the site is adopted, create and maintain an online database with the reference site's toxicity and chemistry listed to serve as a comparison to dredged sediment.

4. The RMP will produce a final report describing the rationale behind selecting the Bay reference site. The report will also present the toxicity test and sediment chemistry results for the reference sediment.

Schedule

To be most cost-efficient, this study will augment the summer 2014 RMP Status and Trends sediment cruise (July/August). Depending on the site(s) and the difficulty of obtaining a sample, we estimate that it could take an additional half day of the cruise. Samples will be collected using the same sampling equipment as the RMP S&T samples and shipped to RMP laboratories. Lab results are anticipated to be available in December 2014. A short summary report will be prepared by April 2015.

Budget

Fieldwork and ship time	$1,000
Laboratory Analysis	$18,000
Data formatting and analysis	$2,500
Report	$5,500
Total	**$27,000**

References

Clean Water Act of 1972, 33 U.S.C.§1251 et seq. (2002). Retrieved from http://epw.senate.gov/water.pdf

EPA/USACE. 1998. Evaluation of dredged material proposed for discharge in waters of the U.S. – Testing manual. EPA-823-B-98-004, Washington, D.C.

Preliminary Stage 1 Results:

Figure 1: Previously sampled RMP S&T sites with fine-grained sediment, low PCB, PAH, and Hg concentrations, and greater than 85 percent *Eohaustorius estuarius* survival

Item 7 Developing a Reference Site

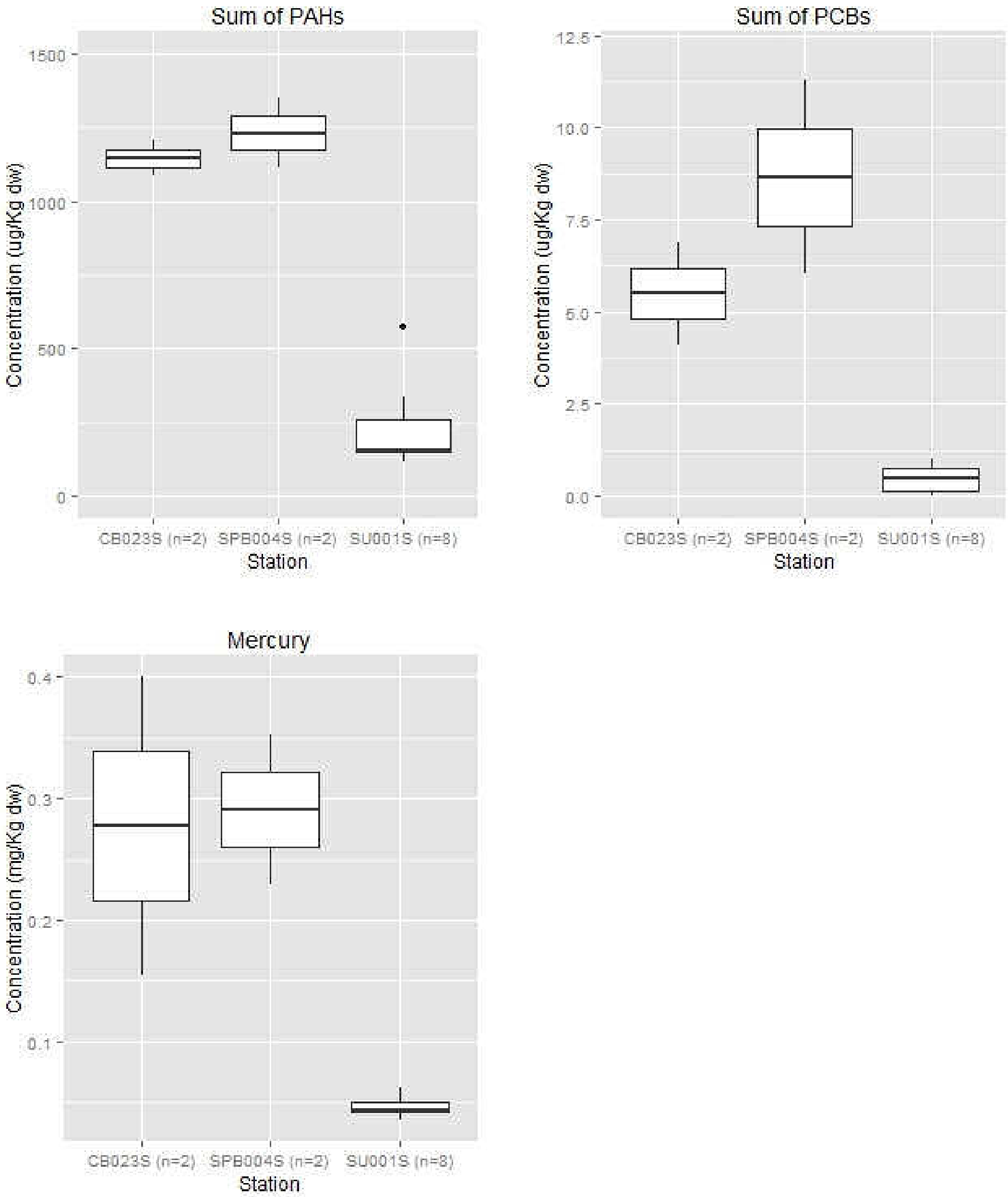

Figure 2: Sediment concentrations of PAHs, PCBs (sum of 40), and mercury in the three potential reference site locations

Stations	CB0023S	SPB004S	SU001S
% Survival (range)	88-91	93	88-96
% fines (range)	55-88	70-80	20-100
mean depth (m)	7.5	9.3	6.2

Table 1: Range of *Eohaustorius estuarius* percent survival, range of percent fines, and mean depth at the three potential reference sites

Workshop on Causes of Sediment Toxicity in California Marine Waters

Sponsored by the San Francisco Estuary Institute
Conducted by the Southern California Coastal Water Research Project and UC Davis Department of Environmental Toxicology
Long Beach, CA November 16, 2012

Summary

A one-day workshop (Workshop II) was held on November 16, 2012 in Long Beach to assist in the identification of the factors responsible for sediment toxicity in San Francisco Bay. This workshop provided a follow-up to an initial stressor identification workshop (Workshop I) held in April 2010 in Oakland. Twenty-two scientists (Table 1) with expertise in ecology, toxicology, chemistry, geology, and ecological risk assessment participated in the discussions. This document summarizes the key Workshop II activities, discussions, recommendations and post-workshop comments.

Background Presentations

Steve Bay gave an introduction to Workshop II and distributed handouts of the presentations. He described the genesis of the workshop and its primary goal: to understand what is causing the pervasive moderate toxicity in San Francisco Bay and improve stressor identification methods.

Monitoring programs have observed toxicity in California embayments. Southern California Coastal Water Research Project (SCCWRP) and the Regional Monitoring Program (RMP) have observed similar trends. Toxicity in the San Francisco Bay (Bay) is mostly moderate (i.e., less than 50% mortality to test organisms), and there are not many hotspots. Because the Bay is one of the largest on the west coast (80% of the embayment area in California), this waterbody is a priority for stressor identification studies.

The goal of Workshop II was to assess what is known about Bay sediment toxicity, develop research designs to improve our knowledge regarding stressors, and address priorities that are feasible for the Bay. The workshop participants were selected to provide a diverse set of perspectives and expertise. The focus of the workshop was on factors causing mortality to the amphipod *Eohaustorius estuarius*. The workshop was intended to provide an updated, improved, and refined list of potential stressors in the Bay. The workshop also provided a forum for discussion of additional data analyses or experimental data needed to help interpret the toxicity results.

A presentation by Tom Mumley provided the regulatory background. San Francisco Bay is a significant estuary, but highly urbanized with a significant amount of human stress. Water quality has greatly improved over the years, including remediation of several major sediment hotspots. Sediments in the Bay affect the health, diversity, and abundance of organisms, and can greatly affect how contaminants are transported. Many ecological resources depend upon a benthos-based food web, so protecting sediment quality is a high priority for environmental managers.

Tom Mumley provided an overview of sediment quality regulation. The California Water Code (Section 13390) identified sediment contamination hotspots with studies by the Bay Protection and Toxic Cleanup Program (BPTCP) and established standards for the protection of sediment quality through development of Sediment Quality Objectives (SQOs). The SQOs are narrative standards that protect benthic communities and human health from impacts related to sediment pollutants. Procedures to assess sediment quality include a multiple lines of evidence (MLOE) triad, which facilitates site categorization. The triad classifies sites from unimpacted to clearly impacted, with most sites in the Bay categorized as possibly impacted based on moderate toxicity and legacy chemicals. Existing methods can identify some contaminant and non-contaminant stressors and sources. Cleanup and abatement can be accomplished through permitting and regulation.

Monitoring and assessment of Bay sediment quality is accomplished through the RMP. The RMP budget is about $3 million per year, with about $1 million assigned to special studies, and about $300,000 designated for sediment-related questions. On-going questions regarding sediment quality include the causes of sediment toxicity, identification of contaminants that require remediation, and identification of the sources of contaminants that may require abatement. **Tom Mumley** also suggested the possibility that some forms of sediment contamination in the Bay may be something we are going to have to live with.

Tom Mumley issued a challenge to the workshop participants: “The cause of moderate sediment toxicity in The Bay cannot be identified - prove me wrong”. If sediment toxicity stressor identification is not a solvable problem, should the RMP continue to provide resources for this issue? Additional questions and observations included: How specific do we need to be in terms of identifying the cause(s) of sediment toxicity (for example, identification of a specific metal vs. pyrethroids as a class of pesticides)? The sites in the Bay that have been identified as moderately impacted are largely driven by toxicity and not chemistry. How many causes of toxicity are we looking for?

Discussion:
David Moore did not think we should start with the presumption of guilt, and wanted to re-phrase the question as “what is the cause of amphipod mortality” and not “what is the cause of toxicity”. This statement was intended to distinguish between contaminant and non-contaminant causes of mortality.

A presentation by **Brian Anderson** provided background on the sediment toxicity test protocol for *E. estuarius*, patterns of toxicity in the Bay, and previous stressor identification research.

Eohaustorius occurs in the sandy beach habitats as a free burrower. The amphipod is mesohaline and a detritivore. Sediment toxicity tests with this species use a well-vetted protocol based on DeWitt (1989) and formally adopted by the EPA in 1994. The organisms are collected intertidally at Beaver Creek, Oregon (more recently Yaquina Bay, Oregon) and shipped to testing labs. They are wild-caught and not cultured. **Brian Anderson** provided a short summary of non-contaminant factors that could influence test results (e.g., reference toxicant, acclimation, life cycle).

Selection of this species for toxicity testing was based on a rigorous comparison of methods by BPTCP. The amphipod tests had good controls and the organism was responsive to contaminants and gradients (particularly compared to *Ampelisca* and *Neanthes*, which were non-responsive). The State Water Board's SQO program performed a similar exercise and arrived at the same conclusion.

Toxicity in the Bay shows some seasonality in the magnitude of toxicity, and also demonstrates spatial patterns, with higher toxicity at sites in the margins of the Bay. The likely contaminants of concern are organic chemicals. Other stressors are unknown (such as algal toxins), and there are potentially non-contaminant factors (ammonia and sulfide are not a factor in The Bay, but grain size may be). Historically, about 30% of the samples in the Bay have been toxic. In 2001, the RMP included comparisons to *Ampelisca* (which did not show toxicity). Matching toxicity to benthos is difficult based on uncertainty regarding relative disturbance of mesohaline bethic community assemblages. There is some concordance between toxicity and benthos, but only about half of the sites have good benthic tools, and about half of those had favorable comparisons (e.g., if toxicity was present then the benthos was degraded).

Observed seasonal effects could be related to chemistry, but no obvious correlation is evident based on the current analyte list. Temporal responses of reference toxicants have been examined, but the data do not show a pattern suggesting seasonal variations in amphipod sensitivity. It could be assumed that the animals are more sensitive in the winter months, but **Ted DeWitt** was not sure, and recommended revisiting the original Bosworth thesis [1]. **Chris Ingersoll** suggested testing *Eohaustorius* from two populations to determine differences. **Bryan Brooks** and **Swee Teh** suggested that there may be interactive effects of physiochemical stressors (e.g., salinity and temperature) at the organism source site and that animal health might influence batch sensitivity.

Previous stressor identification studies have correlated toxicity with measured constituents. There are a lot of significant correlations, but no absolute causative factors have been identified.

DeWitt et al. (1989) evaluated grain size in 42 uncontaminated sites from Washington and Oregon and observed variable survival (60-100%) at sites with fines >70%. Environment

[1] Bosworth, W.S., Jr. 1976. Biology of the genus *Eohaustorius (Amphipoda: Haustoriidae)* on the Oregon coast. Ph.D. thesis, Oregon State University, Corvallis, OR.

Canada does not recommend use of *Eohaustorius* at sites with >80% fines. The relationship between % clay in uncontaminated sediments and survival has also been examined by the UC Davis Marine Pollution Studies Laboratory (MPSL); the data show high variability at high clay content with a significant relationship. A recommendation was made to examine relationships with only high mortality samples (e.g., <70% survival) and look at what might be special about those sites. **Jim Shine** recommended transforming the data for statistical analysis, as untransformed data violated statistical assumptions.

Brian Anderson's presentation also included results from a single MPSL grain size test and a discussion about grain size vs. grain shape (**Ivano Aiello's** work). He also discussed the shell debris observed in a number of RMP samples and summarized the results of a preliminary experiment to investigate shell hash effects.

There was a suggestion to look at pathogens. There was also a suggestion to hold the sediment in refrigerated storage and see if the toxicity changes.

Variability: High variability is occasionally found in the sediment toxicity tests, but does not seem to be associated with routine laboratory procedures. **Meg Sedlak** commented that Bay sediments look well-homogenized as a result of wind, water currents, and shallow water. **David Moore** provided an example of 30 mercury cores in a single square meter that had huge variability. **Howard Bailey** suggested there might be a nugget effect due for example to the presence of hydrocarbon balls (e.g., at Mission Creek) or mortality due to the presence of a predator in particular replicates. **Ted DeWitt** suggested the presence of homogenized organisms (anemone) might contribute to variability because of the presence of active and toxic nematocysts. The workshop participants recommended trying to match up toxicity results with records of anemones, infaunal predators, or other sediment characteristics from field sampling or laboratory notes. It was also noted that routine screening of sediments to remove predators prior to testing could eliminate this problem.

Toxicity Identification Evaluation (TIE) Results: Brian Anderson presented the TIE flow chart developed during Workshop I. Evaluations conducted to date have narrowed the list of likely stressors, but have not shown a definitive cause. **David Moore** mentioned that rare-earth metals are non-reactive and could be substituting for minerals such as calcium (perhaps we can look at ion imbalance, but would not necessarily explain variability).

Hot Spot History: Historical Bay toxic hot spots have changed over time (e.g., Castro Cove and Paradise Cove). **Tom Mumley** added some perspective on these sites: there was a clean-up action at Castro, and there are no current inputs to this site. Islais Creek and Mission Creek have combined sewer overflows with current inputs.

Mission Creek TIE Summary: An exhaustive TIE was conducted; however, no primary toxicant was identified. Ammonia removal and carbon addition reduced toxicity, and the elution of extraction media returned toxicity, but the latter provided only a qualitative result. Contaminant concentrations of potential concern were found for chlordane (later determined through dose-response experiments to not be high enough to cause toxicity), chlorpyrifos, PAHs, and to a lesser extent copper, so mixtures were identified as the cause of toxicity at this site, along with ammonia.

Workshop I Summary: There was a consensus that TIE methods were sufficient, but required some improvements. Workshop I participants prioritized contaminants and concluded that the RMP effort was one of few formal programs in country addressing the development of sediment TIE methods. The primary recommendation of Workshop I was to define the influence of grain size and shape on *E. estuarius* mortality. In addition, workshop participants identified other likely contaminants of concern, and suggested conducting dose-response experiments with priority chemicals. Finally, a second workshop was recommended to develop a research strategy to address remaining issues.

Discussion

David Moore suggested comparing the response of amphipods observed in the Bay to other locations. Are we seeing more amphipod mortality in the Bay than in other places? **Steve Bay** indicated that previous analyses had shown much similarity between the Bay and the Southern California Bight (SCB) in terms of prevalence and magnitude of toxicity, but that Bay samples tended to show greater incidence of toxicity at similar levels of chemical contamination (e.g., using SQG index scores), relative to SCB samples.

Bryn Phillips recommended assembling the amphipod acclimation records from SCCWRP and MPSL and comparing the data to reference toxicant results, overall variability in test batches, and control performance. This was recommended to address the possibility that sensitivity of wild-caught test organisms could vary depending on seasonal factors affecting populations at Oregon collection sites. **Jay Field** suggested that a routine measure of amphipod lipid content would also allow a determination of the relative health of the test organisms. In addition, he suggested that because sensitivity of amphipods to organic chemicals is likely influenced by lipid content, this would allow a method to determine whether seasonal variability in lipids is correlated to sensitivity to contaminants.

Stressors of Concern:

Steve Bay led a discussion to review and update the list of stressors of concern developed at Workshop I. Workshop II participants discussed the previous list, provided suggestions regarding additional stressors of concern, and revised the priority ranking of some types of stressors (Table 2).

Discussion

Ted De Witt would like to see condition of animals, acclimation, etc. added to list of potential stressors.

Chris Ingersoll suggested using peepers in the exposure system to better measure interstitial concentrations of ammonia and sulfide (if this is high priority issue).

Several participants recommended that a high priority be assigned to investigating physical toxicity related grain size, clay content, and particle shape. The issue of shells and smothering by oils was also discussed. However, because of uncertainty regarding the magnitude of exposure to these factors, it is not clear what should be measured. **Bryn Phillips** and **Ted DeWitt** recommended that more detailed analyses of existing grain size data and toxicity be conducted, including an emphasis on the coarse end of the spectrum. Another suggestion was made to pre-sieve samples to remove shell debris and compare to un-sieved samples.

Grain size is a very complex issue, but the shell hash problem could be easily resolved. **Swee Teh** suggested that microplastics and microscrubbers could also be part of the grain size issue. These materials concentrate contaminants from ambient water and include chemical additives. Their size and shape can also cause effects. Amphipods are selective feeders and may be eating microplastics that have concentrated toxicants.

Sediment manipulation during laboratory testing was identified as another factor of potential importance. Pre-sieving sampling sites could help address impacts associated with shell hash or macrofauna, but these factors were not considered likely to account for the seasonal pattern seen in previous toxicity monitoring. .

David Moore suggested including a cost estimate in the ranking of new research or analyses.

Jay Field suggested that seasonal changes in toxicity might be due to differences in amphipod lipid content. It is likely that *Eohaustorius* has a seasonal lipid cycle, with highest lipid in the summer and lowest in the winter, as has been shown for other benthic amphipods.[2] Higher lipid content may reduce sensitivity to lipophilic contaminants (e.g., organochlorine pesticides) in

[2] Lehtonen, K. 1996. Ecophysiology of the benthic amhipiod *Monoporeia affinis* in a open-sea area of the northern Baltic Sea: seasonal variations in body composition, with bioenergetic considerations. Marine Ecology Progress Series 143: 87-98.

sediment toxicity tests. Meador (1993) observed an increased sensitivity to tributyltin in two amphipod species (*Eohaustorius* and *Rhepoxynius*) that was correlated with lower lipid concentrations.[3] Variation in contaminant sensitivity related to tissue lipid content is not likely to be detected with standard reference toxicants, which are typically trace metals.

Phytotoxins: Darrin Greenstein conducted at least one experiment with microcystin, but did not observe an effect. These toxins have been detected in sediments. Microcystin LR is available and could be tested to describe the dose response relationship. **Bryan Brooks** stated that biological, chemical, and physical factors influence the development of harmful algal blooms, but predictive models of how these factors control bloom formation are needed to understand site-specific bloom dynamics. **Swee Teh** has knowledge of bloom conditions and knows that the microcystin toxin can be present in the northern San Francisco Estuary water column. Some cyanobacteria actively release their toxins, but others do not. Cysts can also be a source of toxin exposure; they can be more toxic than the hatched organism. **Bryan Brooks** suggested examining the invertebrate literature (e.g., research on *Gammarus* or mayflies) to assess the potential for phytotoxin-related toxicity in the Bay. **Swee Teh** indicated that there is toxicity data for Bay area copepods. The 48-h LC-50 and LC-10 values for Microcystin-LR were 1.55 and 0.14 mg/L for *Eurytemora affinis*; and 0.52 and 0.21 mg/L for *Psuedodiaptomus forbesi*.[4] Dietary *Microcystis* has also been shown to cause copepod mortality and may be cause adverse impacts on Bay zooplankton.[5] The optimal conditions for bloom and toxin production are species and region specific, and there is a great amount of interaction among habitat parameters and species.

Rob Burgess suggested looking at TIE methods suitable for phytotoxins (e.g., identifying potentially useful existing methods based on chemical structures of selected toxins). **Sue Norton** suggested this is a high priority, at least for discussion purposes (try to mine the literature).

Howard Bailey suggested a higher priority be given to metals not traditionally considered to be important causes of toxicity (Mn, etc.). Examining minerals (Ca, Mg, etc.), and paying attention to ionic imbalance was also suggested. This could be accomplished by mining existing data.

Organics: Pesticides are still considered high priority. **David Moore** suggested looking at PBO (piperonyl butoxide) as an additional stressor compound. Emerging pesticides with high toxicity such as fipronil should be included in chemical monitoring, but these compounds did not account for the occurrence of toxicity in past data.

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[3] Meador, J.P. 1993. The effect of laboratory holding on the toxicity response of marine infaunal amphipods to cadmium and tributyltin. Journal of Experimental Marine Biology and Ecology 174:227-242.
[4] Ger, K.A., S.J. Teh, C.R. Goldman. 2009. Microcystin-LR toxicity on dominant copepods *Eurytemora affinis* and *Pseudodiaptomus forbesi* of the upper San Francisco Estuary. Science of the Total Environment 407:4852-4857.
[5] Ger, K.A., S.J. Teh, D.V. Baxa, S Lesmeister, C.R. Goldman. 2010. The effects of dietary *Microcystis aeruginosa* and microcystin on the copepods of the upper San Francisco Estuary. *Freshwater Biology* 55:1548-1559.

Tom Mumley stated that pyrethroids were low in the Bay, although much higher in the creeks, and that emerging chemicals would not explain past data; therefore, these should not be a high priority.

Polyaromatic hydrocarbons (PAHs) should still be listed as a high priority, although some of the data gaps have been filled. There was a suggestion to mine the chemistry data in order to try to predict toxicity using PAH toxicity models.

Polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) were considered to be of low priority because they are not expected to cause toxicity over the short term exposures used in the *Eohaustorius* sediment toxicity test. **Meg Sedlak** asked if any PCB-11 dose response studies are being performed on invertebrates. **Rob Burgess** replied that they are simply not very toxic, but **Chris Ingersoll** believed that they could cause sublethal toxicity in exposures greater than 10 days.

The influence of pharmaceuticals and personal care products (PPCPs) on sediment toxicity is very under-studied, but **Bryan Brooks** stated that some can act like pesticides in terms of cholinergic interactions. These include antihistamine pharmaceuticals. **Howard Bailey** thought this could be a possibility. **Bryan Brooks** noted that many PPCPs partition strongly to sediment and are persistent in the environment.

Other Factors: Variations in temperature and pH (e.g., ocean acidification) are not likely to be important, as they are controlled in laboratory tests; however, temperature could be a useful TIE treatment. Acclimation of test organisms and predators were identified as potentially important factors. Sediment organic enrichment of total organic carbon was considered not to be a likely cause of toxicity at the levels typically present in the Bay.

Chris Ingersoll suggested that non-optimal levels of grain size, temperature, or something else in the test system might make *Eohaustorius* temperamental. He suggested that the test temperature could be warmer and potentially more stable. **Ted DeWitt** mentioned that these critters are pretty tough considering the environment that they live in. **Brian Anderson** was concerned about temperature from the acclimation/health aspect.

David Moore asked what the dominant amphipod species in The Bay was. **Brian Anderson** responded that it was *Ampelisca* and maybe *Corophium*, but *Ampelisca* is introduced. If *Haustoriid* is used in evaluations, then *Eohaustorius* is a good choice ecologically because it is the most habitat-diverse.

Data Analysis and Research Designs:

Discussion in the afternoon focused on the development of plans to address the priority issues identified in the morning. Three types of activities were suggested by the workshop participants:

- **Analysis of existing data sets** to investigate the influence of factors related to animal condition, predation, sedimentology, and nontraditional contaminants. There is a wealth of high quality monitoring data with matched chemical, toxicity, and benthic community data that can be used to look for associations among some factors of interest. Such analyses represent "low hanging fruit" and can be accomplished rapidly and at modest cost. Multiple types of analyses were suggested, including:

  a) Compile collection/acclimation data on test animals and compare to toxicity patterns.

  b) Examine correlation of coarse grain size fraction or other sedimentological characteristics to toxicity (possibly multivariate or alternative statistical methods).

  c) Characterize presence of predators or other infauna likely to impact *Eohaustorius* survival and relate to toxicity results

  d) Examine association of other chemical analytes (e.g., Mn, Ca) with toxicity patterns. Might indicate whether ionic imbalance is a significant factor.

  e) Use various statistical methods to evaluate the association between various contaminant mixtures and toxicity. Pay particular emphasis to factors that correspond to seasonal variations in toxicity.

  f) Compare San Francisco Bay monitoring data to southern California data in order to identify similarities/differences in relationships between chemistry and toxicity.

  A work plan should be developed prior to conducting additional data analyses. The plan should identify the effects that are to be explained, as well as the seasonal and spatial scope of the analyses.

  Data sets from regions outside of the Bay (e.g., southern California, Yaquina Bay, Puget Sound) should be used to help validate cause-effect models developed from analyses of Bay monitoring data. The relationships between *Eohaustorius* survival and environmental/health factors should be broadly relevant to other regions if these factors constitute true constraints on the application of the test.

  While such analyses are likely to be productive and should help to confirm preliminary hypotheses, they may not yield definitive results due to lack of data on key parameters.

- **Sediment TIE studies**. Relatively few TIE studies have been conducted for San Francisco Bay sediments using the *Eohaustorius* test. While the low level of toxicity presents a challenge, application of modified/cost efficient TIE methods at multiple sites would help confirm existing conclusions/hypotheses regarding toxicants of concern. For example, it might be useful to focus on a limited number of TIE treatments (i.e., a targeted TIE design) that are most helpful for distinguishing between major contaminant classes and sediment characteristics.

- **Laboratory experiments and new analyses** to investigate influence of priority factors on *Eohaustorius*. Existing information is not sufficient to evaluate the role of several high priority potential stressors identified by the workshop, such as sedimentological characteristics, phytotoxins, and test animal condition. Several types of studies were suggested, but additional discussion among workshop participants is needed to develop specific research plans. Suggestions included:

    a) Conducting exposure to various fractions of natural sediments and looking for relationships between particle size/morphology and biological response (e.g., mortality, external abrasion, lacerations). Include a grain size control (or concentration series) with test batches containing sediments with high fines content.

    b) Analyses of gut contents to determine if phytotoxins or harmful algae are present

    c) Measurement of tissue lipids, stress biomarkers, or other condition measures (e.g., reburial ability to exhaustion) as an index of initial test animal condition and investigating correlation to toxicity results.

    d) Conduct interstitial water toxicity tests on a subsample of sediments that demonstrate marginal toxicity (i.e., 20–40% mortality) to assess the role of the presence of particles on toxicity

    e) Using representative commercially-available phytotoxins, conduct toxicity tests to evaluate the magnitude of toxicity to *Eohaustorius*

Next Steps

The workshop concluded with an agreement to continue discussion and development of research designs. The participants were encouraged to develop and circulate draft designs for studies/analyses among the group over the next two months. Two additional activities were planned:

- Early January: The workshop organizers will use input from the group to develop several draft study designs for discussion and refinement
- Late January: Hold a conference call among the participants to develop final recommendations.

Table 1. Participants in the November 16, 2012 workshop.

Name	Organization
Brian Anderson	UC Davis Marine Pollution Studies Laboratory
Howard Bailey	Nautilus Environmental
Steve Bay	Southern California Coastal Water Research Project
Chris Beegan	State Water Board
Bryan Brooks	Baylor University
Rob Burgess	USEPA Narragansett Laboratory
Don Cadien	LA County Sanitation Districts
Eric Chavez	NOAA National Marine Fisheries Service
Ted DeWitt	USEPA Hatfield Marine Science Center
Jay Field	NOAA Office of Response and Restoration
Darrin Greenstein	Southern California Coastal Water Research Project
Chris Ingersoll	US Geological Survey
David Moore	Weston Solutions
Tom Mumley	San Francisco Regional Water Quality Control Board
Ellen Willis-Norton	San Francisco Estuary Institute
Susan Norton	USEPA ORD National Center for Environmental Assessment
Bryn Phillips	UC Davis Marine Pollution Studies Laboratory
Meg Sedlack	San Francisco Estuary Institute
Jim Shine	Harvard School of Public Health
Karen Taberski	San Francisco Regional Water Quality Control Board
Swee Teh	UC Davis Aquatic Health Program
Josh Westfall	LA County Sanitation Districts

Table 2. Assessment of stressors of concern for San Francisco Bay sediment toxicity by workshop participants. Toxicity potential is a relative assessment of the sensitivity of response of the 10-day amphipod survival test to the stressor; magnitude of exposure is a relative assessment of the likelihood that toxic levels of the stressor are present in areas of San Francisco Bay distant from discharge sites; TIE method indicates whether currently available sediment TIE methods are likely to affect the stressor; Workshop I refers to the April 2010 TIE workshop in Oakland; Workshop II refers to the November 2012 TIE workshop in Long Beach. Bold type indicates a new or changed category. Shaded rows indicate high priority stressors.

Stressor	Toxicity Potential	Magnitude of Exposure	TIE Method	Workshop I Priority	Workshop II Priority
		Biological Products			
NH3	High	Low	Yes	Low	Low
H2S	High	Low	Yes	Low	Low
Cyanototoxins	**Unknown**	**Unknown**	**No**	**Not Discussed**	**Moderate**
Anenome nematocysts	**Unknown**	**Unknown**	**No**	**Not Discussed**	**Low**
		Sedimentological/Physical Characteristics			
Grain Size **Clay Size** **Shape**	**Uncertain**	**Uncertain**	**No**	**High**	**High**
Shells	**Uncertain**	**Variable**	**Yes**	**Not Discussed**	**High**
Smothering by oils	Uncertain	Unknown	No	Low	Low
		Ecological Factors			
Animal Interactions	**Uncertain**	**Uncertain**	**No**	**Not Discussed**	**High**
***Eohaustorius* Health & Acclimation**	**Uncertain**	**Uncertain**	**No**	**Not Discussed**	**High**
		Metals			
Cations (Cu, Zn, Cd)	Low	Low	Yes	Low	Low
“Other” cations **(Mn, Mg, Fe, Ca)**	**Uncertain**	**Uncertain**	**Unknown**	**Not Discussed**	**High**
Anions (As, Cr)	Low	Low	Yes	Low	Low

Table 2. Continued.

Organic Compounds					
Organochlorine Pesticides	**High**	**Low**	**Yes**	**High**	**Low**
Organophosphate Pesticides	High	Moderate	Yes	Low	Low
Pyrethroid Pesticides	**High**	**Moderate**	**Yes**	**High**	**Low**
Other Pesticides	High	Uncertain	Yes	High	High
Fungicides & Herbicides	Unknown (low?)	Unknown	Yes	Moderate/Low	Low
PAHs	**High**	**Moderate**	**Yes**	**High**	**Low**
PCBs	Moderate	Low	Yes	Moderate/Low	Low
PBDEs	Unknown	Low	Yes	Low	Low
PPCPs	Unknown	Unknown	No	Low	Low
Other nonpolar organic compounds	Unknown	Unknown	Yes	Unknown	Not Discussed
Mixtures	**Unknown**	**Unknown**	**Some**	**Not Discussed**	**Moderate**

Analysis of San Francisco Bay Sediment Quality Data

Project Proposal
Southern California Coastal Water Research Project

Estimated Total Cost: $50,000
Funding requested for 2013-2014: $50,000

Background

Widespread sediment toxicity has been documented in San Francisco Bay studies from 1991 to 2012. RMP monitoring has shown that the magnitude and frequency of sediment toxicity is greater in the winter wet season than in the summer dry season, which suggests that storm water inputs are an important factor. The causes of the toxicity have not been determined, which limits the development of management plans to improve sediment quality.

Analysis of existing monitoring data to evaluate the influence of sedimentology, predation, seasonal effects, and non-traditional contaminants on *Eohaustorius estuarius* was a primary recommendation of a workshop investigating the cause of sediment toxicity in San Francisco Bay that was conducted in November, 2012. The workshop was sponsored by SFEI and included scientists from throughout the United States with expertise in ecology, toxicology, chemistry, geology, and ecological risk assessment. The 2012 workshop provided an updated list of potential sediment quality stressors in San Francisco Bay, as well as suggestions for data analyses to determine the cause of sediment toxicity.

This proposal, including collaboration with NOAA (Jay Field), implements the high priority workshop recommendations by combining and analyzing high quality multidisciplinary RMP monitoring data.

Study Objective

The objective of this project is to investigate the associations between sediment toxicity to *E. estuarius* and high priority factors identified by the 2012 workshop. The factors to be addressed include:

- Sedimentology: Sediment grain size distribution, sediment grain size classes (gravel, sand, silt, and clay), clay size, grain shape, and presence of shells and shell hash;
- Interactions with predatory and non-predatory benthic organisms;
- Metals such as Mn, Mg, Fe, and Ca, and current use pesticides.

Approach

The overall study approach includes:

1. Combining existing RMP sediment monitoring data for sites having matching *E. estuarius* toxicity, sediment chemistry, and benthic macrofaunal species abundance data;
2. Supplementing these data with additional information on sediment characteristics and test animal handling;

3. Applying spatial and temporal filters to reduce the influence of confounding factors; and
4. Statistical analysis to describe associations between *E. estuarius* toxicity and sediment chemistry, benthic macrofauna, and habitat factors.

Task 1: Data Preparation

Sediment chemistry, toxicity, benthic macrofauna, and habitat data (e.g., sediment grain size distribution, sediment texture, presence of shell hash, and salinity) data from RMP studies from 1993-2012 will be compiled, standardized, and integrated into a database for use in this project. Previous work by SCCWRP, SFEI, and NOAA has already compiled much of the data, but additional work is needed to merge datasets, incorporate additional constituents, and locate supplemental data. Supplemental data include field records of sediment characteristics, toxicity organism handling information, and high resolution sediment particle size (laser diffraction analysis). Inclusion of supplemental data will be contingent on the availability of electronic records from other agencies (e.g., Applied Marine Sciences, UC Davis, Moss Landing Marine Labs).

Task 2: Data Analysis

a. **Data review and filtering.** The compiled data will be reviewed for availability of data for priority factors identified by the workshop. Spatial and temporal filters will be applied prior to analysis to reduce the effects of confounding factors. Spatial filtering may include analyzing the data in (a) regional segments to minimize potentially confounding salinity, flow, and benthic community composition effects, and (b) at stations where sediment toxicity to *E. estuarius* has varied substantially over time. Temporal filtering may include segmenting the data by season to facilitate distinguishing the influence of priority factors from seasonal effects.
b. **Regression analyses.** The relationship between specific chemical constituents and amphipod mortality will be determined using logistic regression analysis modeling (LRM). Analyses using several different LRM variations will be conducted on selected data subsets identified in Task 2a. Model fit and performance will be evaluated to select the best-performing regression model for each parameter. The LRM results will be compared between data subsets and also with LRMs obtained in previous analyses for CA and other locations. LRM analyses will be conducted in collaboration with Jay Field of NOAA at minimal cost to the project.
c. **Priority stressor analyses.** Associations and specific hypotheses between sediment toxicity and priority stressors will be investigated for the data subsets established in Task 2a, using parametric and non-parametric statistical methods. Non-parametric methods are based on rank order, and are more sensitive to non-linear relationships such as step functions than parametric statistics, which are often confounded by extremely large or extremely small values.

Task 3: Final Report

A final report will be prepared that presents conclusions based on the analysis results and recommendations for future analytical activities, if appropriate. The report will document key results, and the data and methods used to obtain them.

Schedule

Task/Product	Date*
1. Data preparation	3 months
2a. Data review and filtering	6 months
2b. Regression analyses	9 months
2c. Priority stressor analyses	9 months
3. Final report	11 months

*: Dates are relative to project initiation

Task Summary Budget

Task	Description	Total
1	Data preparation	13,371
2	Data analysis	25,536
3	Final report	10,954
Total		**49,860**

Line Item Budget

Line Item	Category	Total Days	Total Costs
1.0	Total Personnel	47.0	46,360
2.0	Supplies		
3.0	Travel		3,500
4.0	Capital Expenditures		
5.0	Equipment Rental		
6.0	Boat Charter		
7.0	Other		
8.0	Contractual		
9.0	Subcontract Administration Fee		
	Total		**49,860**

Seasonal Variation in Toxicity Test Organisms

Project Proposal
Southern California Coastal Water Research Project

Estimated Total Cost: $30,000
Funding requested for 2013/2014: $30,000

Introduction and Background

Widespread sediment toxicity to the amphipod *Eohaustorius estuarius* has been documented throughout the San Francisco Estuary since the early 1990s. Some of this toxicity is associated with variations in season, sediment particle size, and sediment organic content (Anderson *et al.* 2007). One hypothesis to account for this association is that seasonal variations in test animal condition, possibly due to water quality at the animal collection site or reproductive cycles, may influence the sensitivity of this species to environmental stressors, such as the fine sediments present in San Francisco Bay. The *E. estuarius* used for toxicity testing are collected from an estuary in Oregon that undergoes substantial changes in temperature and salinity throughout the year. Several studies have documented seasonal changes in the sensitivity of field collected amphipods to contaminants. Seasonal changes in amphipod fitness related to nutrition, senescence, or reproductive activity have been suggested as the reason for such variations in sensitivity.

Toxicity identification workshops held in Oakland in April 2010 and Long Beach in November 2012 recommended additional research to investigate the influence of changes in *E. estuarius* fitness and sensitivity to fine sediments on San Francisco Bay sediment toxicity results. The 2012 workshop also recommended the analysis of amphipod lipid content as a measure of seasonal changes in animal condition. Further, changes in lipid content may influence the sensitivity of *E. estuarius* to contaminants as a result of altering the internal distribution of nonpolar contaminants (Meador 1993). Amphipod lipid content is not routinely measured as part of sediment toxicity testing, so little information is available to determine the magnitude of variation in lipids in *E. estuarius* and whether such variation is likely to influence toxicity results for San Francisco Bay. The goal of the proposed study is to determine whether seasonal changes in *E. estuarius* fitness are a likely contributor to the variations in sediment toxicity observed in the RMP.

Study Objectives

This study will measure tissue lipid content in samples of *E. estuarius* from multiple experiments to accomplish the following objectives:

- Determine the seasonal variation in lipids among field-collected individuals
- Evaluate the influence of lipid variation on amphipod sensitivity to fine sediment

Approach

Task 1: Lipid assay method development

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.

Task 2: Measurement of seasonal changes in lipid

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.

Task 3: Comparison of amphipod lipid and test sensitivity

Samples of *E. estuarius* from multiple toxicity tests conducted throughout the year will analyzed to determine if there is an association between variations in lipid and changes in sensitivity to sediment stressors. It is anticipated that samples will be obtained by collaboration with UC Davis (MPSL) on studies of the effect of clays on amphipod survival. Animals from at least three different test batches will be analyzed and the data compared to clay effect thresholds (e.g., NOEC or LC 20).

Task 4: Final report

A draft report describing the results of Tasks 1-3 will be produced for review. A final report will be produced that addresses the comments received.

Literature Cited

Anderson, B.S., J.W. Hunt, B.M. Phillips, B. Thompson, S. Lowe, K.M. Taberski and R.S. Carr. 2007. Patterns and trends in sediment toxicity in the San Francisco Estuary. Environmental Research 105:145-155.

Meador, J.P. 1993. The effect of laboratory holding on the toxicity response of marine infaunal amphipods to cadmium and tributyltin. Journal of Experimental Marine Biology and Ecology 174:227-242.

Item 8: SS Seasonal Variation in Organisms

Schedule

Task/Product	Date*
1. Method development	3 months
2. Seasonal lipid analysis	10 months
3. Comparison of lipid and sensitivity	10 months
4. Draft final report	11 months

Dates are relative to project initiation

Task Summary Budget

Task	Description	Total
1	Assay method development	9,595
2	Analysis of seasonal variation in lipid	10,234
3	Comparison of lipid and toxicity responses	4,592
4	Final report	5,564
Total		29,986

Line Item Budget

Line Item	Category	Total Days	Total Costs
	Total Personnel	38.5	27,786
2.0	Supplies		1,800
3.0	Travel		400
4.0	Capital Expeditures		
5.0	Equipment Rental		
6.0	Boat Charter		
7.0	Other		
8.0	Contractual		
	Total Contractual		
9.0	Subcontract Adm fee		
	Total		29,986