



RMP
Exposure and Effects Workgroup Meeting
May 16th, 2013
San Francisco Estuary Institute
Meeting Summary

Attendees

Daniel Schlenk (UC Riverside)
 Nancy Denslow (University of Florida)
 Karen Taberski (SFBRWQCB)
 Rob Lawrence (USACE)
 Beth Christian (SF RWQCB)
 Brian Ross (USEPA)
 Michael Fry (Pacific Island Fish and
 Wildlife Service)
 Brian Anderson (UCD Granite Canyon)
 Harry Ohlendorf (CH2M Hill, Inc.)
 Mike Kellogg (SFPUC)

Mike Connor (EBDA)
 Steve Bay (SCCWRP)
 Alvina Mehinto (SCCWRP)
 Meg Sedlak (SFEI)
 Ellen Willis-Norton (SFEI)
 Rebecca Sutton (SFEI)

Call-In

Ananda Ranasinghe (SCCWRP)
 Joseph Gorsuch (CDA Inc.)
 Derek Muir (Environment Canada)
 Arleen Feng (ACCWP)

I. Introduction and Goals for Today's Meeting [Meg Sedlak]

Meg Sedlak opened the meeting by listing the meeting goals including: 1) updates on 2012 activities, 2) check-ins on planned 2013 activities, and 3) prioritizing 2014 special studies. Meg provided a quick update on current EEWG deliverables. The report on Cu's effect on the olfactory nerve in seawater-phase salmon is now available. In 2013, David Baldwin (NOAA) is examining the effects of Cu in intermediate salinities. The study on PAH in flatfish was completed, but the results were hard to interpret because of high control mortality.

Meg noted that \$50,000 is allocated for 2014 EEWG special studies. Currently, the budget for the number of studies being proposed is greater than the current allocation. There is a chance that funds from the RMP reserve could be used. Meg asked the workgroup members to rank the studies based on their technical feasibility and stakeholder needs. Meg mentioned that Don Weston (UC Berkeley), an EEWG adviser, has stepped down from the EEWG.

II. Update on 2013 Studies: Bioanalytical Tools [Nancy Denslow]

Nancy Denslow presented her results from the first several months of the year one bioanalytical tools study (2013). Nancy began her presentation by stating the chemicals recommended for monitoring in CA waters were ones with estrogenic activity including: Bisphenol A, Estrone, 17-beta estradiol, Galaxolide (HHCb), and p-Nonylphenol. The study goal is to link in vitro

bioassay effects with higher order effects (e.g. tissue or population level effects). Molecular bioassays are sensitive at low chemical exposure levels; therefore, linkages to higher order effects can be made at low concentrations. Silversides (*Menidia beryllina*) will be used to identify effects in the early life stage and juvenile stage bioassay, with growth and survival as the two endpoints. The chemicals' effect on *Menidia beryllina*'s gene expression and growth and development will be examined.

Illumina sequencing/assembly was used to obtain the four top genes associated with reproduction and development. Two high throughput assays, Estrogen Receptor alpha (ER- α) and Androgen Receptor (AR) binding assays, were used to determine the effect of the chemicals on gene expression. Nancy explained how the two molecular assays (supplied by InVitrogen) worked. She noted that the commercial InVitrogen assay was used because European assays require licenses to use the cell lines and the cells must be cultured. Fluorescence resonance energy transfer (FRET) was used to determine the effects of increasing chemical concentrations. The standard curves generated from the ER- α and AR assays were used to determine the estrogen activity and androgen activity, respectively, in blind water samples (that were part of a larger round robin study with Australian and US researchers). The blind samples included secondary effluent receiving various types advanced treatment (e.g. membrane filtration; RO; ozonation & biologically activated carbon filtration) drinking water inlet/outlets; stormwater; and a blank. Nancy found estrogen activity in secondary treated effluent sample, but not in a stormwater sample. On the other hand, there was minimal androgen activity in all the blind water samples.

Nancy found that primers for Silverside, which are complimentary segments of RNA needed to for DNA polymerase to initiate replication, exist for mRNAs associated with growth and development. Therefore, Illumina-HighSeq will be completed with 17 day old control and 17 β -estradiol *Menidia beryllina* fry. The Illumina sequencing measures individual mRNAs. By adding a barcode to the mRNA and converting to cDNA, the number of Estrogen Receptor alphas (receptors that are activated by estrogens) produced can be counted, providing a direct measurement of exposure. From the Illumina sequencing Nancy will also be able to look at genes that are changing based on exposure. Results from the sequencing will be shared with Richard Connon (UC Davis) who is also conducting sequencing of Silversides. During year two of the bioanalytical tools study, the same ER- α and AR assays will be conducted for San Francisco effluent. Also, the molecular endpoints will be related to higher order endpoints (e.g. growth and survival). Nancy ended her presentation by stating that the study was a highly leveraged project and the study's deliverables include a mid-term and final report.

Discussion:

Steve Bay noted that comparing the assay results in WWTP's receiving and effluent would be interesting. Harry Ohlendorf wondered if the dischargers are willing to supply effluent samples. Meg Sedlak responded that the City of San Jose is not interested in participating in the study; but, the RMP has a relationship with many South Bay plants, such as Palo Alto, and she is confident they would be willing to provide samples. Steve added that SCCWRP has a similar relationship with Southern Californian WWTPs and he is sure they would be willing to provide samples. Michael Fry asked if the treatment plants are already working to remove estrogenic compounds from their effluent. Steve replied that they are not because there is no mandate to remove estrogens. However, the treatment plants are concerned about how to remove the

compounds if the water is designated for re-use. Nancy hypothesized that the treatment plants will find that the effluent concentrations are too low to cause higher level effects in people.

Steve asked if the water samples Nancy presented were marine samples. Nancy responded that all of the samples were in freshwater. Nancy added that researchers in Switzerland are creating a mixture of estrogens found in European freshwater to see how the assays will respond. Michael asked if Nancy used transactivation assays; Dan Schlenk responded that the assays examined the activation of the receptor.

III. Update: 2012/2013 plans for the Mesohaline [Ananda Ranasinghe]

Ananda Ranasinghe presented the results from year one of the study developing benthic community conditions indices for the Bay's mesohaline environments. The rationale for the project was that the Sediment Quality Objectives' (SQOs) benthic condition line of evidence is calibrated only to one habitat, polyhaline San Francisco Bay environment. The reference benthic community condition and variations from the reference should vary according to habitat. With improved habitat definitions and additional data, benthic assessment tools for mesohaline environments can be established. Ananda described the phases and tasks for the study. Phase one included updating the benthic database, refining habitat definitions, and conducting a best professional judgment (BPJ) study to establish reference conditions.

Ananda noted that to effectively partition the data for the index development, the San Francisco Bay habitats definitions from SCCWRPs coast-wide study (Barnett et al. 2008) were spatially refined. Ananda compared the habitat assemblage definitions in the coast-wide study (n=40) to the Thompson et al. 2013 study (n=501). The mesohaline environment now extends north of Dumbarton Bridge and Suisun Bay and portions of San Pablo Bay were defined as oligohaline environments. Next steps for the project include completing the database compilation, re-doing the BPJ study to increase agreement among experts, and developing, calibrating, and validating the new index. The development and calibration of the index will include data that was not used in the BPJ study.

Discussion:

Michael Fry asked what season the samples were taken in; if they were collected during the winter, creek water inputs could affect the salinity ranges. Ananda responded that the majority of the samples were taken during the dry season. When comparing dry-season to wet-season samples, the habitat assemblages were the same unless there were very high flows. Ananda noted that the bottom of the Bay is a more stable environment than many expect; 75% of sites visited multiple times were always classified as the same habitat. Brian Ross asked if the BPJ study can be improved and if the same experts will be used. Ananda replied that he thinks the study can be improved and will use the Delphi method to improve agreement among experts. Although the experts have not been finalized, he is planning on inviting the same experts as well as additional members.

Karen Taberski asked about the study's funding; Meg Sedlak replied that funding for the second year of study was already included in the 2013 budget and that Ananda's presentation was to inform the EEWG of the study's progress. If the EEWG agrees that there has been sufficient progress, then year two of the study (2013) will be funded. Karen responded that she would like

to see the results of the BPJ study before moving forward. Meg will provide the EEWG with the BPJ study's results when they are available, likely in June.

Action Items:

1. Meg will provide the EEWG with the BPJ study's results in June.

IV. Update: 2011/2012 Hotspot Study [Ellen Willis-Norton]

Ellen Willis-Norton presented the results from the 2011 sediment hotspot study as well as the 2011 and 2012 Status and Trends SQO results. The purpose of the hotspot study was to re-analyze two creek channel sites in Central Bay that the BPTCP listed as candidate toxic hotspots in 1998 and were subsequently added to the 303(d) list in 2002. The two sites, Mission Creek and San Leandro Creek, remain impaired 15 years after their original designations. Ellen then discussed the RMP Status and Trends sediment results; the 2011 and 2012 sediment samples were evaluated using SQO analyses. The majority of the sites were possibly impacted (54%) with widespread moderate toxicity; all of the sites had low or minimal chemical exposure.

Discussion:

Mike Connor noted that there were no new discharges into the creek and wondered what chemicals were driving high exposure at the two creek sites. Ellen responded that at Mission Creek mercury, chlordanes, PCBs, lead, zinc, and HPAHs were above their respective ERM values and in San Leandro creek mercury, chlordanes, and DDEs are above their ERM values. Ellen added that chlordanes and lead concentrations appear to be decreasing in the creeks. Karen Taberski asked Steve Bay if current use pesticides will be included in SQOs. Brian Anderson indicated that chlorpyrifos should be included in SQOs because it contributed to amphipod toxicity in the TIE conducted at Mission Creek. Dan Schlenk asked if anyone has taken the pollutants that were above the ERM and re-added them to sediment to see if the same level of toxicity was induced. Steve and Brian replied that they had not conducted that experiment.

Karen asked what benthic community indicators were used in the non-polyhaline environments. Steve answered that a modified RBI and the AMBI index is used to categorize the sites. Mike Connor asked why the benthic community condition mattered; he wondered if the health of higher trophic level species is compromised if a negative indicator species is present.

Mike Kellogg expressed concern that polyhaline calibrated benthic indices were being applied to the upper end of Mission Creek, which is a dead-end slough. He stated that he expects *Capitella capitata* complex and Oligochaeta to be found at the end of the slough. Steve and Brian agreed that is important to categorize the reference benthic assemblage correctly. Ellen noted that Mission Creek's station assessment was driven largely by high chemical exposure and high toxicity, the benthic disturbance was listed as moderated.

Brian Anderson asked whether amphipod mortality or abnormal *Mytilus* development was driving the toxicity in the creek channels; both contributed to the overall toxicity score. Brian Ross thinks that the widespread moderate toxicity in the Bay may be because of false positives for *Eohaustorius estuarius*. Steve Bay stated that 77% of the Bay was listed as possibly impacted after sampling in 2000, around 20% higher than the number of 2009-2012 sites listed as possibly impacted.

V. Special Studies for 2014: Impacts of Dredging on Benthic Habitats [Korie Schaeffer]

Korie Schaeffer presented a special study idea for 2014 on behalf of the dredging community. Korie stated that the impacts of dredging are unknown for San Francisco Bay biota. The proposed study seeks to understand if the quality of benthic habitat is degraded in dredged fish-foraging areas. The goal of the study is to determine if dredging reduces the food availability for commercial, sport, or endangered fish species. Korie made clear that the study will compare dredged sites to un-dredged sites; it will not quantify how long a dredged site takes to recover. The study will be completed in three phases: 1) a literature review to understand what fish are eating and to group benthic assemblages into functional groups (based on their value to fish feeding); 2) the creation of a statistical study design; and 3) a pilot field study to ensure that the study design will answer stakeholder questions. Korie hopes the samples could be collected during the 2014 RMP sediment cruise. Korie cannot provide the EEWG with a detailed budget until a senior project lead is identified. Jan Thompson and Jim Carter are benthic community and statistics experts, respectively, and will be involved with the study; however, they do not have the time to lead the project. Overall, \$100,000 is available for the study from America's cup and the dredging community is asking for \$50,000 from the RMP to conduct the study.

Discussion:

Steve Bay asked why the field work portion is considered a pilot study. Korie answered that the goal of the field work was to determine if the sample variability was the same as expected and if the design was informative before completing a full study. Steve replied that if the pilot study was intended to determine the benthic community variability between dredged sites, then previously collected data could be analyzed. Mike Connor asked what percent of the Bay was dredged; Brian Ross responded that 2-3% of the Bay is dredged, but not all of the sites are dredged every year. Mike stated that it is likely the RMP has collected sediment samples at dredging locations. Mike also suggested restructuring the RMP S&T sampling design to ensure a portion of the sampling locations are dredged areas. Arleen Feng responded that changing the sampling design would require discussions on whether the dredged sites would be in addition to or in lieu of current sampling locations.

Steve added that Ananda Ranasinghe has analyzed benthic community condition in both RMP and non-RMP San Francisco Bay samples; Steve similarly assumes that some of the sample locations were in dredged areas. Additionally, Ananda has already worked to reconcile inter-annual variation. Brian Ross asked if Ananda combined benthic species based on their functional group; Steve replied that Ananda did not because he was asking a different question. Ellen Willis-Norton and Meg Sedlak will work with Ananda to compare sampling locations and dredging sites.

Mike noted that the RMP also has fish contaminant data; the study could use the data for fish that spend time in dredged areas. Brian Ross responded that the study is not looking at fish contamination, it is examining the health of fish foraging habitat. Harry Ohlendorf stated that the home range of fish included in the study should be determined because it affects whether the fish forage in dredged areas. Karen Taberski noted that Kathryn Hieb at CDFW may be able to provide information on the fish species.

Steve asked why a benthic recovery study is not being conducted. Korie replied that a recovery study may take 10 years to complete and that the study will not definitively answer whether fish foraging habitats are negatively affected by dredging. Mike Connor asked whether Korie was asking the EEWG to support funding for the study, or asking the EEWG to support SFEI completing and funding a portion of the study. Meg replied that if needed, SFEI could help conduct and fund the study and it would be likely that SFEI will subcontract with an agency, such as USGS, to help complete certain aspects of the study.

Action Items:

2. Ellen Willis-Norton and Meg Sedlak will work with Ananda to compare sampling locations and dredging sites.

VI. Special Studies for 2014: Developing a Reference Site for Dredge Materials [Brian Ross]

Brian Ross summarized a proposal put forth by the dredging community to establish a sediment reference site for Bay dredging. Brian explained because the disposal site is used as the reference site, the reference site becomes increasingly contaminated with multiple disposals. Additionally, because there are four possible disposal sites in the Bay, the dredged sediment spreads out across the entire Bay. Therefore, Brian stated that there should be one reference sediment, which is representative of the Bay, for dredgers to compare their sediment quality against. Having one reference site will also save dredgers time and money; if enough testing is completed at the reference site, a database can be created for dredgers to utilize rather than continuously sampling at the reference site.

Brian then described the process for choosing a reference site. The two main requirements are the site must have greater than 85% amphipod survival and be fine-grained. A preliminary screening of potential sites was completed using 1993-2012 RMP sediment samples. Three potential reference sites were identified during the screening. Brian Ross asked the EEWG to support the inclusion of two of the former RMP sites (one in San Pablo and one in Central Bay) on the 2014 RMP sediment cruise. Most of the study's budget is dedicated to completing toxicity tests at the two potential reference sites that would be conducted for the following species: 1) *Ampelisca abdita*, 2) *Rhepoxynius abronius*, 3) *Eohaustorius estuarius*, and 4) a benthic Polychaete.

Discussion:

Brian Anderson asked if the reference site would be used year round and if there would be seasonal differences in the site's sediment quality. Brian Ross responded that the dredging companies are aware of seasonal differences and typically sample during the dry season, but he would like the reference site to still have about 85% amphipod survival in the wet season. Brian Anderson asked about the protocol if the reference site is ever below 85% survival. Brian Ross responded that there would be two options: 1) the dredgers can compare the dredged sediment toxicity to the control toxicity or 2) the dredged sediment can be compared to the Alcatraz Environs. Steve Bay noted that the two potential reference sites have been sampled only once or twice; he suggested looking at nearby RMP stations to ensure that all of the nearby sites have greater than 85% survival.

VII. Special Studies for 2014: Understanding the Causes of Moderate Toxicity in the Bay [Steve Bay and Brian Anderson]

Steve Bay presented three proposals for studies that will investigate the cause of moderate sediment toxicity in the Bay. Steve stated that the studies are important because sediment toxicity results are used for management decisions and the cause of the moderate toxicity is largely unknown. Since 2010, two workshops have been held to identify the stressors that are likely causing moderate toxicity in the Bay. Workshop participants listed the following stressors as having the potential to trigger amphipod toxicity: 1) grain size/shape and clay size, 2) shell debris, 3) predator animal interactions, 4) *Eohaustorius* health and acclimation, 5) cations (Mn, Mg, Fe, and Ca), and 6) other pesticides (e.g. fipronil and neonicotinoids). Steve discussed the three study proposals that resulted from the workshop recommendations. The first study will determine the seasonal variation in *Eohaustorius* lipid content as a measure of animal condition. The second study will examine the influence of fine sediment (including shape) on amphipod mortality. Steve noted that the first and second study proposals could be done simultaneously. The third study includes analyzing RMP sediment monitoring data to find associations between potential stressors and sediment toxicity. The estimated budget for the three studies is shown below:

Study Proposal	Estimated Cost
Seasonal Variation in Toxicity Test Organisms	\$30,000
Influence of Fine Sediments on Amphipod Mortality	\$84,000
Analysis of RMP Sediment Monitoring Data	\$50,000

Discussion:

Mike Connor asked if sediment could be spiked with chemicals that weren't identified as high priority stressors (mainly CECs) to ensure they are not causing toxicity. Brian Anderson responded that an infinite number of mixtures would need to be tested to answer Mike's question. Karen Taberski noted that the same level of toxicity existed before CECs were considered a priority in the Bay, indicating that the stressor may not be a contaminant.

Dan Schlenk asked if the cost of the study that will examine the effect of fine sediment could be reduced. Brian Anderson responded that for \$50,000 the experiment could be conducted for dry season samples. However, Brian thinks that the effect of fine sediment may vary with the reproductive cycle of the amphipods and he is interested in including the effect of seasonality. Dan then asked about the methods used to characterize sediment size; he suggested using a method similar to the one used to determine the size of nanomaterials. Steve responded that the method Dan described is different from the one currently being proposed. Dan also asked about the size variability between amphipods. Brian Anderson responded that only the moderately sized animals are used in toxicity tests. Steve noted that size of the animals could be compared with lipid content to determine if there is a correlation. Michael Fry suggested completing the studies that address the effect of lipid content and fine sediments before analyzing RMP sediment data for more information.

Mike Connor stated that determining the cause of moderate toxicity is a substantial task for a small monitoring program. He suggested using the *Ampelisca* amphipod, rather than searching for the cause of toxicity in *Eohaustorius*, even if *Ampelisca* is less sensitive. Brian Ross stated

that *Eohaustorius* is the perfect test animal if the cause of moderate toxicity is established; thus, he thinks the study proposals are worth pursuing. Mike asked if toxicity was even correlated with benthic community condition and chemical exposure. Brian Anderson responded that despite the uncertainty, both lines of evidence were correlated with toxicity. Mike then asked Steve if he thought the studies would succeed in determining the cause of moderate toxicity. Steve did not have an exact answer, but said that SQOs currently assume that amphipod toxicity is solely due to chemical exposure. If this is not true, then the conclusions regarding the Bay's health may need to be revised and could alter management decisions. Karen suggested that funding for the study could come from discontinuing the collection of S&T benthic community samples in the oligohaline. Benthic sample collection in the mesohaline could also be discontinued if the BPJ exercise did not work out.. She argued that if the results are not understood, then collecting the same data every year is not useful. Steve responded that similar strategies to fund special studies have been employed by regulatory monitoring programs in southern California.

Action Items:

3. Meg Sedlak will send the EEWG the proposal for the study that will examine the influence of fine sediments on amphipod mortality.

VIII. Closed Door Session

Meg Sedlak began the closed door session by stating there are eight studies that the workgroup needs to either remove from consideration or rank based on their value to RMP stakeholders and scientific merit. The eight studies include 1) Year two of the mesohaline index development (2013 work), 2) determining the impacts of dredging on benthic habitats, 3) developing a Bay reference site for dredged materials, 4) year two of the bioanalytical tools study, 5) establishing a dose-response relationship between clay and amphipod toxicity, 6) examining the health of amphipod test species by analyzing seasonal lipid concentrations, 7) investigating existing RMP data to evaluate hypotheses for the cause of moderate toxicity, and 8) a second hotspot study of other 303 (d)-listed sites such as the two Oakland Inner Harbor hotspots, Pacific Dry-dock Yard and Fruitvale. Meg noted that there were more studies than the current allocated budget, but the EEWG's role is to rank the studies based on their scientific value and the TRC and SC will work with the budget. She also mentioned that the multi-year plan had earmarked funds for completing a benthic recovery study/ reference study.

1) Year two of the mesohaline index development (2013 work)

The group requested that the results of the Best Professional Judgment portion of the 2012 mesohaline work be reviewed before 2013 funds are released. The group considered the study a medium to high priority for the EEWG.

2) Determining the impacts of dredging on benthic habitats & 3) Developing a Bay reference site for dredged materials

The committee expressed reservations about the lack of a technical lead for a project. They were also concerned that phase two and three of the study were less well defined. However, this study was one of LTMS's highest priorities because they agreed to complete the study under the Programmatic EFH Agreement. The group agreed that the first year of the study could be

completed without any field work. There is a considerable amount of benthic data that already exists for the Bay; the EEWG expects that there are previously sampled sites in both dredged and un-dredged areas that could be compared for the study. The study could be separated into a two-year investigation. The first year would consist entirely of data mining and the second year would possibly involve fieldwork. America's Cup funds could be used to complete the first year of the project, which will include only data mining. The EEWG decided that once the first phase is completed, the proposal authors can request RMP funding for the second stage of the project. Brian, Meg, Beth Christian, Brenda Goeden, and Korie Schaeffer will work together to re-write the study proposal and frame it as a data mining exercise. The proposal authors will contact Ananada Ranasinghe to request benthic data.

Committee members then considered alternative funding sources for the Bay reference site study. The EEWG was unsure if the America's Cup money could be used to complete the Bay reference site study as well. The RMP or the dredging community could also contact the Bay Planning Council to leverage funding. The group ranked Bay reference site study as a high priority because of its clear goal and methodology.

4) Year two of the bioanalytical tools study

Meg asked the workgroup if the bioanalytical tools study should move forward and clarified that funding for the study is not earmarked. Most committee members ranked year two of the bioanalytical tools study as a high priority.

5) Establishing a dose-response relationship between clay and amphipod toxicity, 6) Examining the health of amphipod test species by analyzing seasonal lipid concentrations, & 7) Investigating existing RMP data to evaluate hypotheses for the cause of moderate toxicity

The workgroup then discussed the three studies presented by Steve Bay and Brian Anderson to investigate the cause of moderate toxicity in the Bay. Some members stated that establishing a dose-response relationship between clay and amphipod toxicity and examining the health of amphipods test is a good starting point for investigating the cause of moderate toxicity. Others were unsure that the studies will be able to determine the cause of moderate toxicity. The group agreed that the proposed methods for establishing a dose-response relationship between clay and amphipod toxicity are outdated. The relationship between amphipod toxicity and the size fractionation of the sediment could be identified by using a microfluidics sensor that estimates particle size and density. Steve and Brian could contact Jennifer Field, Lee Ferguson, Jeff Steevens, Steve Klaine, or Chris Vulpe to discuss the new methodology.

The EEWG agreed that combining the dose-response relationship study with the lipid analysis study is a cost-effective way to examine seasonal variation in animal health.

The SQO data mining study was a low priority for most of the workgroup. The RMP does not collect some of the data that was mentioned in the study proposal (e.g. grain shape and shell hash). Although, archived sediment samples could be used to complete that analysis.

8) A second hotspot study of other 303 (d)-listed sites such as the two Oakland Inner Harbor hotspots, Pacific Dry-dock Yard and Fruitvale

The final study idea was another hotspot study that used SQOs to analyze the condition of Fruitvale/Dry Dock. The study idea is driven by the interest in removing the site from the 303(d) list, if it is no longer impacted. The workgroup suggested holding off on the study until next year.

Action Items:

1. Brian Ross, Meg Sedlak, Beth Christian, Brenda Goeden, and Korie Schaeffer will work together to re-write study proposal and frame it as a data mining exercise. They will contact Ananada Ranasinghe to request benthic data.