FINAL DRAFT WORK PLAN

MARINA DEL REY HARBOR SITE-SPECIFIC OBJECTIVE STUDY

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LIST OF ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of Variance
BLM	Biotic Ligand Model
CCC	Criterion Continuous Concentration
CIMP	Coordinated Integrated Monitoring Program
COC	chain-of-custody
CTR	California Toxics Rule
DOC	dissolved organic carbon
DQO	data quality objective
EC50	median effective concentration
fWER	final Water-Effect Ratio
LACDBH	Los Angeles County Department of Beaches and Harbors
LACPW	Los Angeles County Public Works
MdR Harbor	Marina del Rey Harbor
µg/L	microgram per liter
mL	milliliter
QA	quality assurance
QAPP	Quality Assurance Project Plan
QC	Quality Control
RWQCB	Regional Water Quality Control Board
SSO	site-specific objective
sWER	sample Water-Effect Ratio
SWRCB	State Water Resources Control Board
TAC	Technical Advisory Committee
Toxics TMDL	*Reconsideration of the Total Maximum Daily Load for Toxic Pollutants in Marina Del Rey Harbor*
USEPA	U.S. Environmental Protection Agency
WER	Water-Effect Ratio

I. INTRODUCTION

The *Reconsideration of the Total Maximum Daily Load for Toxic Pollutants in Marina Del Rey Harbor* (Toxics TMDL; Los Angeles RWQCB/USEPA 2013) includes load allocations for dissolved copper inputs to Marina del Rey Harbor (MdR Harbor). This section provides a brief overview of the regulatory background for MdR Harbor that led to the inclusion of dissolved copper water column targets in the revised Toxics TMDL and also discusses the rationale and background for using a site-specific objective (SSO) study to develop a scientifically defensible water quality criterion that accounts for site-specific conditions and focuses on the protection of aquatic life in the MdR Harbor.

1.1 Regulatory Background

In 1998, the back basins of MdR Harbor were placed on the 303(d) list for contaminants impacting sediment, fish tissue, and benthic infauna. At this time, pollutants of concern for sediment included DDT, chlordane, lead, copper, and zinc and pollutants of concern for fish tissue included those for sediment and PCBs, dieldrin, and tribuyltin (TBT). However, in 2002, changes were made to the 303(d) list; copper, lead, zinc and TBT in fish tissue, DDT in sediment, and benthic infauna degradation were delisted and PCBs in sediment for MdR back basins were newly listed. Based on the 303(d) list and its subsequent modifications, the MdR Harbor Toxics TMDL was promulgated in 2005 to address impairments associated with sediment for copper, lead, zinc, chlordane, PCBs, and toxicity and fish tissue for DDT, dieldrin, chlordane, PCBs, and fish consumption advisory (Los Angeles RWQCB/USEPA 2005). Monitoring and special studies conducted in support of the Toxics TMDL have since provided additional information regarding the spatial extent and magnitude of the impairments; the special studies include partitioning coefficient, a low detection level, storm-borne sediment pilot, sediment characterization and BMP effectiveness studies. The results have shown that dissolved copper concentrations frequently have exceeded the chronic (4-day average) criterion (also referred to as Criterion Continuous Concentration [CCC]) of 3.1 micrograms per liter (µg/L), as specified in the California Toxics Rule (CTR).

The Toxics TMDL was revised and adopted by the Los Angeles Regional Water Quality Control Board (RWQCB) in February 2014 (Los Angeles RWQCB 2014) and was subsequently approved by the State Water Resources Control Board (SWRCB) in September 2014 (SWRCB 2014). Toxics TMDL revisions were designed to take into consideration new data on the spatial extent and magnitude of sediment contamination as well as address the dissolved copper CTR exceedances in the water column. As such, the Toxics TMDL includes load allocations for dissolved copper required to ensure that dissolved copper concentrations in MdR Harbor are less than the CCC criterion in the CTR.

In SWRCB Resolution 2014-0049 (SWRCB 2014), the SWRCB recognizes that the U.S. Environmental Protection Agency (USEPA)-approved Water-Effect Ratio (WER) method may be used to derive site-specific water quality objectives and if adopted by the Los Angeles RWQCB and approved by the SWRCB

Office of Administrative Law and USEPA will supersede the current CTR CCC criterion as the water quality standard for dissolved copper in MdR Harbor. Conditional approval to conduct an SSO study for Marina del Rey Harbor was granted by the Los Angeles RWQCB in September 2017 (revised in June 2018).

1.2 Development of Site-specific Objectives

Although there are exceedances of the dissolved copper CCC in MdR Harbor, the concentration threshold necessary to protect aquatic life in MdR Harbor is uncertain. It is well known that water quality parameters (e.g., pH, dissolved organic carbon [DOC], and salinity) influence the biological availability of copper in marine water and may reduce the potential to cause toxicity to aquatic life (USEPA 1994; Di Toro et al. 2001). It is possible that site-specific parameters can also lead to an increase in bioavailability/toxicity. As such, a WER>1 indicates the environmental conditions reduce toxicity; a WER<1 indicates an increase in toxicity; and a WER=1 indicates no site-specific change to toxicity. The federal water quality criteria (from which the CTR criteria were derived) for dissolved copper were developed to be conservative in order to protect marine aquatic life in all waters of the U.S. regardless of site-specific water characteristics. Specifically, water quality criteria were developed based on laboratory studies in which filtered seawater was used, and consequently, these studies do not necessarily account for many of the physical constituents (e.g., particulate and dissolved organic matter) that may interfere with the toxicity of potential chemicals of concern, such as copper. Consequently, the USEPA has developed procedures that can be performed to develop water quality criteria that are specific and reflective of site-specific conditions, while still providing the required level of protection for aquatic life. The current water quality criteria (CCC and CMC) for copper are designed to be protective of the most sensitive species (*Mytilus sp.*). Because the WER represents an adjustment of the copper toxicity threshold in the field sample to match the toxicity of copper in laboratory water, the margin of safety intended in the original criteria is maintained.

The *Interim Guidance on Determination and Use of Water-Effect Ratios for Metals* (USEPA 1994) provides guidance for determining SSOs. This guidance includes three options: 1) the recalculation procedure; 2) the WER procedure; and 3) the resident species procedure. The recalculation procedure is intended to account for relevant differences between the sensitivities of the aquatic organisms in the national dataset and the sensitivities of organisms that occur at the site. The WER approach compares the toxicity of copper dissolved in different water types to determine an adjustment factor for the water quality standard. The resident species procedure is intended to account for differences in resident species sensitivity to biological availability and/or toxicity of a material due to variability in physical and chemical characteristics of the site water. In this study, the WER procedure is most appropriate and will be calculated based on tests with the most sensitive resident species in MdR Harbor. There are insufficient new data for the recalculation procedure and there are no threatened or endangered species, negating the need to account for additional sensitive resident species.

The Biotic Ligand Model (BLM) is another USEPA-approved approach for determining site-specific criteria for dissolved metals in freshwater environments (Di Toro et al. 2001; Santore et al. 2001). A marine version of the BLM is currently under review by the USEPA but has not yet been approved. Nonetheless, some testing in marine environments has been performed to evaluate the BLM's ability to predict toxicity at marine sites throughout the United States (Arnold et al. 2005). Results have shown that the BLM can provide an accurate prediction of copper toxicity to sensitive marine taxa in marine receiving waters and that the BLM-predicted toxicity is strongly correlated with measured toxicity. The BLM approach requires only chemical and physical water quality data as inputs and consequently is a more cost-effective and less time-consuming method than the toxicity-based WER. Because of its efficiency, the BLM may allow for the examination of a wider range of site-specific conditions than could be captured during WER studies as well as evaluation of effectiveness of various management strategies. As such, the BLM will be used as a tool to provide additional data interpretation and comparison to the toxicity-based WER results. Ultimately, any proposed SSO will be based on the toxicity-based WER data alone.

In this study, WER procedures that are consistent with the USEPA (1994) Interim Guidance[1] will be used to calculate SSOs for MdR Harbor that are scientifically defensible and protective of beneficial uses. The BLM was used during the site characterization evaluation to support the determination of environmental conditions likely to result in the lowest copper bioavailability. The results of this analysis suggested dissolved organic carbon (DOC) is an important site-specific parameter related to predicted copper toxicity. As such, additional DOC analysis has been added to the monthly TMDL monitoring program to further characterize the Harbor during the period of this study.

1.2.1 Water-Effect Ratio

The USEPA recommends calculating a WER to account for site-specific bioavailability and toxicity of contaminants (USEPA 1994). As part of a WER study, two side-by-side toxicity tests are conducted; one test uses laboratory dilution (clean) water and the other test uses site (contaminated) water. The WER is determined by calculating the ratio of the median effective concentration (EC50) values from the two tests as shown in Equation 1:

$$\text{WER} = \frac{EC50\ Site\ water}{EC50\ \text{Control or Reference}\ water} \quad (1)$$

[1]The USEPA also published a streamlined procedure for the WER development for copper in freshwater (USEPA 2001). The streamlined procedure provides simplified WER testing specific to a waterbody where a continuous point source, such as publically owned treatment works, primarily contributes to an elevated level of copper. The streamlined WER guidance is not applicable to MdR Harbor due to differences in salinity and source of copper.

The WER is then multiplied by the national or state aquatic life criterion; in this study, the CTR CCC (to represent chronic conditions) criterion and criterion maximum concentration (CMC, to represent acute conditions) will be used. Unlike in freshwater, the marine CCC and CMC are not hardness dependent.

To calculate SSOs, the WER is multiplied by the water quality criteria as shown in Equation 2:

$$\text{Chronic SSO} = \text{WER} \times \text{CCC} \tag{2}$$

$$\text{Acute SSO} = \text{WER} \times \text{CMC}$$

The WER developed in this study will be appropriate for adjustment of both the CCC and CMC, as both criteria are applied in the TMDL to the same locations within MdR Harbor.

1.2.2 Biotic Ligand Model

The BLM is a computational model used to predict metal speciation, complexation, and toxicity to aquatic organisms using site-specific water characteristics (Di Toro et al. 2001; Santore et al. 2001). The BLM was originally designed to estimate copper toxicity in freshwater fish and invertebrates; however, it has been used successfully in estuarine systems as well (Arnold et al. 2005; Chadwick et al. 2008). The BLM is based on the premise that both metal–ligand binding and metal interaction with competing cations may affect toxicity (Di Toro et al. 2001). Thus, the degree of toxicity is expected to be related to the amount of metal available to bind to the biotic ligand, the concentration of other aqueous ligands such as organic matter that can bind up the metal of concern, and the availability of other cations (i.e., calcium), which may have a protective effect.

The marine version of the BLM uses water chemistry parameters (e.g., pH, DOC, and salinity) to calculate bioavailable metals concentrations in water and metal binding affinity to biotic ligands. The BLM then predicts metal toxicity to aquatic organisms based on these calculations and outputs EC50 values.

A BLM-based WER can be calculated using the BLM-predicted EC50 outputs for both site water and control or reference (clean) water as shown in Equation 3:

$$\text{BLM-based WER} = \frac{\text{BLM}-\text{predicted EC50 Site Water}}{\text{BLM}-\text{predicted EC50 Control or Reference Water}} \tag{3}$$

BLM-based SSOs may then be calculated using Equation 2 in Section 1.2.1. The Marine Copper BLM has not yet been approved by USEPA; therefore, for the purpose of this study, toxicity endpoints generated using the BLM will be used as a point of comparison to enhance interpretation of the study results.

1.2.3 Previous Marine or Estuarine Water-Effect Ratio Studies

While WER studies have been performed in freshwater environments nationwide, only a few WER studies conducted in California marine or estuarine waters are publicly available at this time. Only two marine/estuarine WER studies in California have resulted in SSOs that were adopted by a RWQCB and approved by the SWRCB. For other studies conducted in the marine environment, the status of adoption by the relevant RWQCB is currently unknown (LWA 2006) or the goal of the study was to better understand bioavailability of copper to aquatic organisms, as in Rosen et al. (1995) and Bosse et al. (2014), but not to develop an SSO. The most relevant studies are summarized below.

1.2.3.1 Lower South San Francisco Bay (South of Dumbarton Bridge)

An impairment assessment study for copper (and nickel) was conducted for Lower South San Francisco Bay (Tetra Tech et al. 2000). WER testing was a key part of this study and was used to understand how site-specific water quality parameters affect the bioavailability and toxicity of dissolved copper within the Lower South San Francisco Bay. The blue mussel *Mytilus edulis* and the purple sea urchin *Strongylocentrotus purpuratus* were used in this testing as primary and secondary species, respectively. Samples were collected from three sites in South San Francisco Bay, and tests were conducted from January 1996 to March 1997 to understand the temporal variability in bioavailability of Lower South San Francisco Bay waters. Results of this study demonstrated WER values ranging from 2.7 to 3.5 for dissolved copper. SSOs ranging from 6.7 to 8.8 µg/L for dissolved copper were then calculated using a modified CCC of 2.5 µg/L, based on toxicity test data collected as part of the study. A proposed SSO of 6.9 µg /L was recommended by the City of San Jose, based on pooled WER results from two stations and was suggested to be protective of the most sensitive species*, M. edulis.* An SSO of 6.9 µg /L was adopted by the San Francisco Bay RWQCB in 2002 (San Francisco Bay RWQCB 2002).

1.2.3.2 San Francisco Bay (North of Dumbarton Bridge)

A WER study was conducted in San Francisco Bay in 2000/2001 for purposes of developing copper SSOs for San Francisco Bay regions north of the Dumbarton Bridge (Clean Estuary Partnership 2005; San Francisco Bay RWQCB 2007a). Sampling was conducted at 13 stations that were selected based on stations previously sampled by the Regional Monitoring Program. The study involved sampling and WER testing during two dry seasons (September 2000 and June 2001) and two wet season (January and March 2001) events. Copper toxicity tests were performed using the bivalve *M. edulis* mussel development test. Results did not demonstrate a seasonal pattern in WERs; however, differences in WERs across San Francisco Bay regions were measured and were likely due to differences in the physicochemical characteristics of water from different regions of San Francisco Bay. The geometric

mean WERs for the San Francisco Bay regions north of San Bruno Shoal (i.e., north of Oakland airport on the eastern side and north of Little Coyote Point on the western side) ranged from 2.40 to 2.49 and the geometric mean WER for the region south of San Bruno Shoal was 2.90. Based on these findings, the Basin Plan Amendment proposed chronic and acute copper SSOs of 6.0 and 9.4 µg/L, respectively, for the area north of San Bruno Shoal and chronic and acute copper SSOs of 6.9 and 10.8 µg/L, respectively, for the region south of San Bruno Shoal. These SSOs were adopted by the San Francisco Bay RWQCB in 2007 (San Francisco Bay RWQCB 2007b).

1.2.3.3 San Diego Bay Studies

Rosen et al. (2005) evaluated the bioavailability of copper to organisms in the San Diego Bay. Water samples included composite and grab samples that were collected from various locations inside the bay from 2000 to 2002. Bivalve *Mytilus galloprovincialis* and echinoderm *S. purpuratus* or *Dendraster excentricus* embryos were used in WER toxicity tests. For WER calculations, EC50s from the copper-spiked San Diego Bay water samples (from various areas of the Bay) were compared to those from toxicity tests of copper-spiked reference seawater, which was filtered (0.45 micron) coastal seawater collected from the research pier at Scripps Institute of Oceanography. Estimates of the dissolved copper WER ranged from 1.54 to 1.67. These findings of WERs greater than 1 in San Diego Bay suggest that an SSO ranging from 4.7 to 5.2 µg/L (based on the WER range above) would be protective of the organisms throughout San Diego Bay.

More recently, a study of the bioavailability and toxicity of copper was conducted in Shelter Island Yacht Basin, a marina in North San Diego Bay (Bosse et al. 2014). As part of this study, WER sampling and testing was conducted in conjunction with copper complexation capacity measurements and modeling using the marine BLM. Samples were collected at two depths (near surface and near bottom) during two sampling events, representing the wet season and the dry season. Sampling for ambient toxicity occurred at 15 to 16 stations during each event, and samples from four of these stations were spiked with copper for use in WER testing. *M. galloprovincialis* embryos were used as the test species as part of the standard mussel development test (USEPA 1995). Results of this study demonstrated slightly lower WERs in the wet season (geometric mean of 1.2 $\pm$ 0.1) than in the dry season (geometric mean of 1.5 $\pm$ 0.2) with a final dissolved copper WER for all events of 1.33. These findings suggest that an SSO of 4.11 µg/L would be protective of marine organisms in the Shelter Island Yacht Basin.

1.2.3.4 Mugu Lagoon and Lower Calleguas Creek, Ventura County

A WER study for copper was conducted for Mugu Lagoon and Lower Calleguas Creek (LWA 2006) in accordance with the USEPA (1994) Interim Guidance. However, only the results for Mugu Lagoon, which is a marine environment, are relevant to the current study and are summarized here. Samples were collected during dry weather conditions in August 2003 and January 2004 and wet weather conditions in March 2004 and April 2006. *M. edulis* were the primary test species, and the larval bivalve development

test was used to evaluate copper toxicity. The recommended WER for dissolved copper in Mugu Lagoon was determined to be 1.51, resulting in a chronic SSO established as 4.68 µg/L (LWA 2006).

1.2.3.5 Summary of Previous Water-Effect Ratio Studies

All studies summarized above have demonstrated that WER results were greater than 1. WER findings from these studies ranged from 1.33 in Shelter Island Yacht Basin to 3.5 in Lower South San Francisco Bay. SSOs estimated from these WER results range from 4.11 to 8.8 µg/L; however, to date, only the San Francisco Bay SSOs (ranging from 6.9 to 10.8 µg/L) have been adopted by the RWQCB (San Francisco Bay RWQCB 2002). These findings demonstrate that at each of these sites, a higher copper concentration than the current CTR CCC criterion of 3.1 µg/L would be protective of marine aquatic life and beneficial uses of those sites.

1.3 Previous Relevant Studies and Data in Marina del Rey Harbor

1.3.1 Dissolved Copper in Marina del Rey Harbor (Summary of CIMP data)

The Marina del Rey Coordinated Integrated Monitoring Program (CIMP) is a requirement of the Toxics TMDL in MdR Harbor. In the 2017-2018 monitoring year, wet and dry weather samples were collected. The wet weather sampling occurred at a main channel location near the back basins on January 9th and March 2nd-3rd, 2018. Both samples exhibited toxicity and the results of a Toxicity Identification Evaluation (TIE) indicated that cationic metals were likely responsible. During dry weather, the main channel station is sampled monthly and several basins are sampled on a rotating schedule with each basin sampled every other month. Dissolved copper concentrations often exceeded the water quality criterion in dry weather sampling conducted to date; however, samples usually do not exhibit toxicity.

1.3.2 DPR Copper Level Study

In 2006, the California Department of Pesticide Regulation performed a monitoring study in 23 marinas statewide, including freshwater, brackish water, and marine environments. Several samples from each marina were collected and analyzed for water quality parameters (salinity, temperature, DOC, pH, etc.), as well as total and dissolved copper and zinc, and toxicity (DPR 2009). The freshwater BLM was adapted for use with the brackish and marine samples (under the guidance of Robert Santore, Windward Environmental, Syracuse, NY) to compare predicted toxicity to observed toxicity test results. The MdR Harbor samples exhibited the highest metals concentrations of all the marinas tested. Dissolved copper concentrations ranged from 8.1-18.4 µg/L in the back basins (BB) and 8.9-16.2 µg/L in the front basins (FB), total copper concentrations ranged from 9.0-20.2 µg/L (BB) and 9.2-17.2 µg/L (FB), and dissolved zinc concentrations ranged from 33.3-59.5 µg/L (BB) and 38.2-66.6 µg/L (FB). The DOC concentrations throughout the Harbor ranged from 0.69 mg/L to 1.90 mg/L.

Of the 47 samples tested for toxicity, 8 were toxic and 7 of those were from MdR Harbor. The toxicity at these stations ranged from 4-67 percent normal-alive mussel embryos. A Toxicity Identification Evaluation was performed on two of the samples from MdRH and it indicated that a cationic metal was responsible for the toxicity. Based on the measured copper and zinc concentrations, copper was likely the cause of the observed toxicity. These results aligned with the predicted toxicity based on the BLM. Overall, the BLM results for the marine samples were in good agreement with the observed toxicity (88% success rate).

1.4 Study Objective

The objective of this study is to develop a scientifically defensible SSO for MdR Harbor that accounts for site-specific conditions and is as protective of aquatic life and the beneficial uses of MdR Harbor as the current criteria.

II. STUDY DESIGN AND METHODS

This section comprises the WER study design and includes the details of the sampling program, analytical methods, and data analysis. The overall approach is based on the USEPA (1994) Interim Guidance for determining water effects ratios for metals. As stated in this guidance, development of WERs for surface waters (e.g., bays and harbors) located away from effluent plumes (Method 2) is a more complex and variable situation than developing WERs for plume-influenced waters. Consequently, few specific requirements for study design are provided in the USEPA (1994) Interim Guidance; instead, qualitative descriptions and recommendations are provided to guide the investigator in developing the specifics of the study. Since relatively little guidance is provided for WER studies in marine waters, a technical advisory committee (TAC) has been established to provide scientific review and guidance for the SSO study.

The approach used to develop the MdR Harbor WER study design was to adhere to the conceptual approach described in the USEPA (1994) Interim Guidance and implement this approach by using methods shown to be effective in recent California WER studies. Study design and method selection is primarily based on two studies: 1) San Francisco Bay copper and nickel SSO derivation (Clean Estuary Partnership 2005); and 2) studies of copper bioavailability and toxicity in San Diego Bay (Bosse et al. 2014). The San Francisco Bay study resulted in SSOs for copper that were adopted by the regulatory authority for use in total maximum daily loads.

The key elements and sequence of the study design development are shown in Figure 1 and are described in subsequent subsections. Toxicity testing will be the primary method used to calculate WERs. Thus, selecting test species and the test method is the first step in study design (Section 2.1).

The USEPA (1994) Interim Guidance emphasizes the importance of developing a sampling design that takes into account variations in water quality likely to affect the WER. Potential sources of variability include seasonality (e.g., summer vs. winter), stormwater discharge, hydrology (tides or depth), and episodic events (e.g., plankton blooms and harbor activities). The relative importance of these factors in controlling or influencing bioavailability of copper in MdR Harbor is not well-known. A site characterization study was conducted in 2018 to document the water quality characteristics and toxicity in MdR Harbor. The results of the characterization study and critical condition determination (described in Appendix D) were used to develop the final WER study design (Section 2.2). The study design emphasizes sample collection during the conditions when the WER is expected to be lowest and the risk of copper toxicity is greatest, known as the critical condition. Each water sample will be analyzed to determine the copper toxicity EC50, copper concentration, and BLM parameters.

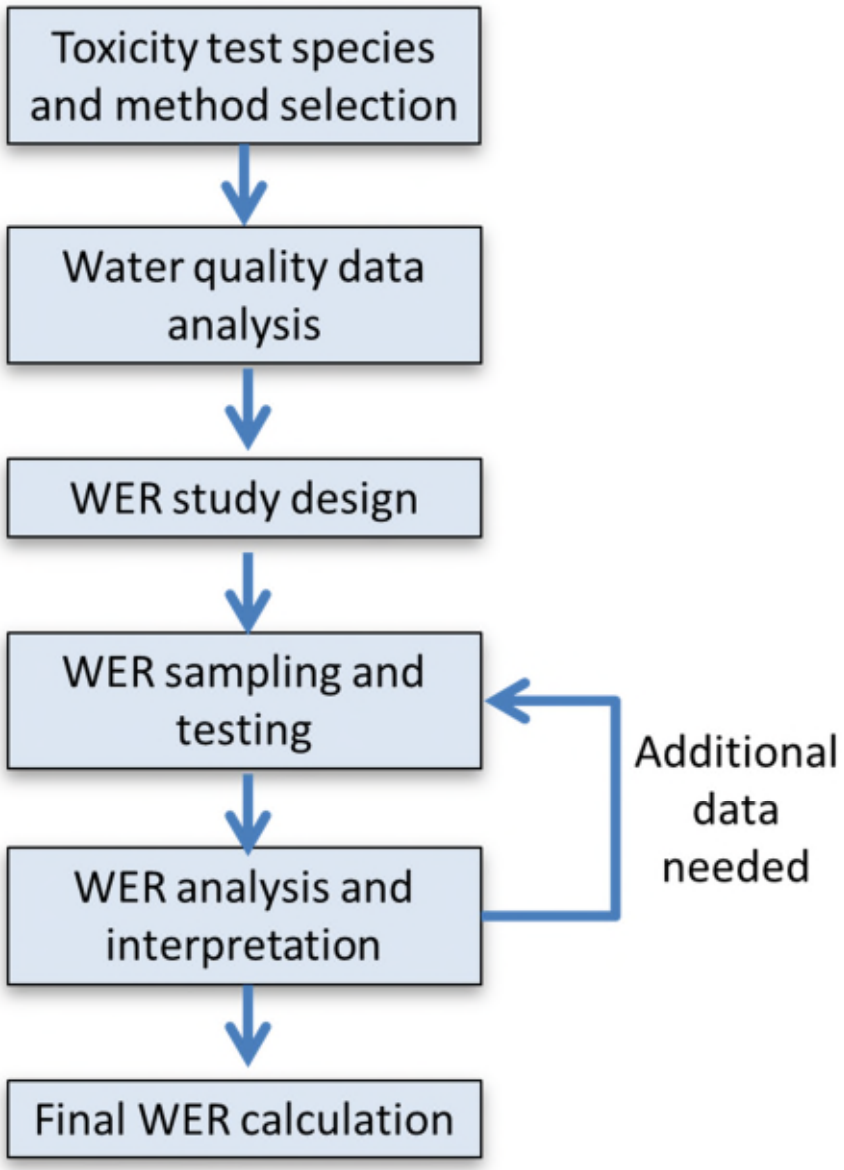

Figure 1. Study elements and process.

Methods for determining the copper EC50 for each sample are described in Section 2.3. The chemistry and toxicity data will be reviewed and used to calculate the sWER for each sample (Section 2.4). This step will include an assessment to determine if sWER data are sufficient to support the objectives of the study. If deficiencies are present, additional sampling may be needed to resolve them. The final step in the data analysis is the calculation of the final WER (fWER, Section 2.5).

2.1 Toxicity Test Species and Method Selection

Toxicity tests will be conducted using embryos of the *M. galloprovincialis*. This species is recommended for WER calculation in the USEPA (1994) Interim Guidance and has been the primary or sole species used for WER development in recent studies in San Francisco Bay (San Francisco Bay RWQCB 2007a) and San Diego Bay (Bosse et al. 2014). *M. galloprovincialis* is an ideal organism for use in WER copper studies because of its sensitivity to copper and commercial importance. When deriving a site-specific criterion, it is desirable to use a test species whose sensitivity is near the Final Acute Value (FAV). Mussels, *Mytilus sp*. (multiple species), is the most sensitive marine species listed by EPA, with a species mean acute value (SMAV) = 6.19 µg/L. The current EPA saltwater criteria for copper are based on this value to protect this commercially important species. The sensitivity of *M. galloprovincialis* embryo development to copper is similar to that of *Mytilus sp*. The average EC50 for *M. galloprovincialis* from the three Site Characterization study events was 8.12 µg/L (Appendix D). Use of *M. galloprovincialis* helps provide a margin of safety for SSO development for two reasons:

- The current CTR criterion for copper is determined exclusively by *Mytilus sp*., a grouping that includes *M. galloprovincialis*, for protection of this commercially important species group. Using this species in the MdR Harbor SSO study will help ensure that the same level of protection is maintained.
- Mussels, including *M. galloprovincialis*, represent the most sensitive genus in the national saltwater copper toxicity database. They are not only a good surrogate for invertebrate species in general (which tend to be more sensitive to copper than vertebrates) and mollusks (a phylum sensitive to copper; the third, fourth, and sixth most sensitive species in the national copper database are mollusks), but also it is a good surrogate for other sensitive saltwater aquatic animals.

The TAC agrees that *M. galloprovinicialis* is both appropriate and sufficient for the WER toxicity tests and that no additional organisms need to be tested. (Appendix F). However, if this organism proves too difficult to spawn, other sensitive test species could be considered. These include *Mytilus californianus* (California mussel) and *Crassostrea gigas* (Pacific oyster). Both of these organisms are also found in MdR Harbor and are similarly sensitive.

2.1.1 Test Method

Site water and laboratory control seawater toxicity will be measured using a 48-hour exposure of mussel embryos under standard conditions as described USEPA (1995). Test conditions are summarized in Table 1 and detailed methods are described in Appendix A. Control seawater will be obtained from a reference site in Granite Canyon, California, and filtered (0.45 micron) prior to use to remove resident organisms and particulate organic material. This reference site has been used for control water in previous WER studies in San Francisco Bay and San Diego Bay due to its previously reported acceptability for embryo-larval development tests and relatively low DOC content. Toxicity tests will be initiated within 36 hours of sample collection. Each sample/treatment will be tested using five replicates. For each replicate, approximately 250 *M. galloprovincialis* embryos will be exposed in 10 milliliters (mL) of sample for 48 hours. Samples of each treatment will be collected for chemical analysis at the beginning (total and dissolved copper; DOC) and end (dissolved copper) of the exposure period to provide a measured EC50 value.

Embryos are preserved for examination at the end of the exposure period. The preserved samples are examined using a microscope to determine the numbers of normal and abnormal surviving embryos (Figure 2). The percent of normal embryos is calculated from the count. Levels of key water quality parameters (dissolved oxygen, pH, salinity, and temperature) and control performance will be evaluated to assess test batch acceptability and organism condition. The salinity range has been adjusted from 30 ± 2 g/kg (USEPA guidance) to 32 ± 2 g/kg to reflect the local environmental conditions and reduce the need to dilute the site water to adjust salinity, which would alter the DOC and copper concentrations. The test will be considered acceptable if three criteria are met: 1) mean normal development in the controls must be at least 90%; 2) mean survival in the controls must be greater than 50%; and 3) the percent minimum significant difference must be less than 25%. The results of copper reference toxicant tests will be compared to past results in order to evaluate sensitivity of test organisms (EC50 should be within two standard deviations of laboratory mean). Standard statistical methods will be used to calculate the copper EC50 (concentration causing 50% reduction in percent normal-alive) for each sample type. EC50 will be expressed in terms of measured dissolved copper concentration.

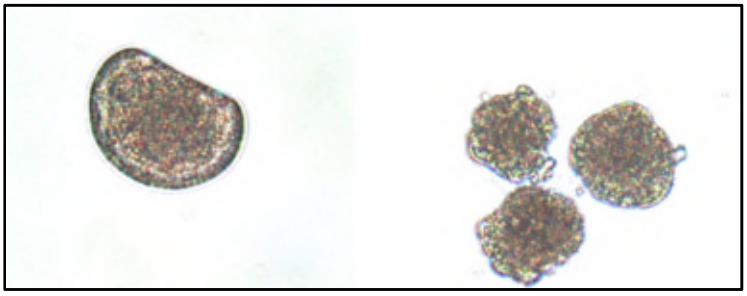

Figure 2. Normally developed (left) and abnormal mussel embryos (images courtesy of Sanitation Districts of Los Angeles County)

Table 1. Summary of test conditions for the 48-hour mussel embryo development test

Test Species	*Mytilus galloprovincialis*
Test Procedures	USEPA/600/R-95/136
Age/Size Class	Embryo
Endpoint	Normality of development and survival
Test Type/Duration	Acute static non-renewal/48 hours
Sample Storage Conditions	4°C, dark, minimal head space
Holding Time	≤ 36 hours
Control	Filtered natural seawater (from Granite Canyon, California)
Salinity Adjustment	Hypersaline brine
Water Quality Parameters	Temperature 15 ± 1°C
	Dissolved oxygen ≥ 4.0 mg/L
	Salinity 32 ± 2 g/kg
	pH 7.5 to 8.3
Photoperiod	16 hours light, 8 hours dark
Test Chamber	22 mL glass shell vials
Replicates/Sample	5
No. of Organisms/Replicate	250
Exposure Volume	10 ml
Aeration	None
Feeding	None
Water Renewal	None
Reference Toxicant	Copper chloride
Test Acceptability Criteria	Control mean normal development[1] ≥ 90%
	Control mean survival > 50%
	Percent minimum significant difference < 25%

Notes:
g/kg = grams per kilogram
mg/L = milligrams per liter
mL = milliliters
USEPA = U.S. Environmental Protection Agency
[1] Applied to surviving control embryos

2.2 Water-Effect Ratio Study Design

The study design is based on the conceptual approach outlined in the USEPA (1994) Interim Guidance and includes key design elements used in three recent WER studies conducted in California. The USEPA (1994) Interim Guidance recommends that WER analyses be conducted over a range of conditions so that the results are representative of the variations in water quality at the site. The guidance also states that the study should include multiple stations distributed over a minimum of three separate sampling events that include different seasons and locations.

The USEPA (1994) Interim Guidance recommendations have been implemented in different ways in recent California studies. For the Los Angeles River and tributaries total maximum daily load WER study, a preliminary study design was developed that included six sampling events that were distributed among three seasonal conditions: summer dry weather, winter dry weather, and winter wet weather (Steering Committee 2014). This sampling design was informed by prior studies using the BLM and refined on the basis of initial study results. The WER study for San Francisco Bay (north of Dumbarton Bridge) used a study design that was modeled after ongoing regional water quality monitoring programs (Clean Estuary Partnership 2005). Station locations were selected to match those used in other monitoring programs and represent variations in water depth and harbor region. Two sampling events were conducted in each of two seasons: wet and dry. WER analyses conducted in Shelter Island Yacht Basin were based on only two season-specific sampling events: the summer dry season and the winter wet season following a major storm event (Bosse et al. 2014). This study also examined spatial variation by distributing stations along a transect from the head to the mouth of the basin and investigated variation related to depth by collecting samples near the surface and just above the bottom at each station. Among these studies, the size, morphology, and hydrology of the Shelter Island Yacht Basin study site is the most similar to that of MdR Harbor.

2.2.1 Station Locations

The station locations for the study are a subset of 11 candidate stations used in previous monitoring surveys (Figure 3, Table 2). These stations include nine locations used for metals analysis in the MdR Harbor TMDL Coordinated Integrated Monitoring Program (CIMP; Weston 2014), consisting of one station in each of the harbor's eight basins (MdRH-A through H) and one station at the end of the main channel (MC1). These stations were augmented by adding two additional main channel stations, located near the harbor entrance and near the mid-point of the channel. Some of these stations are located

near the major stormwater outfalls into the Harbor (Figure 4). All 11 stations were sampled in 2018 in a site characterization study to document variations in harbor water quality associated with factors such as urban runoff, boat density, water circulation, and shipyard activities. Co-location of the stations with the CIMP will increase the comparability of data between the two programs.

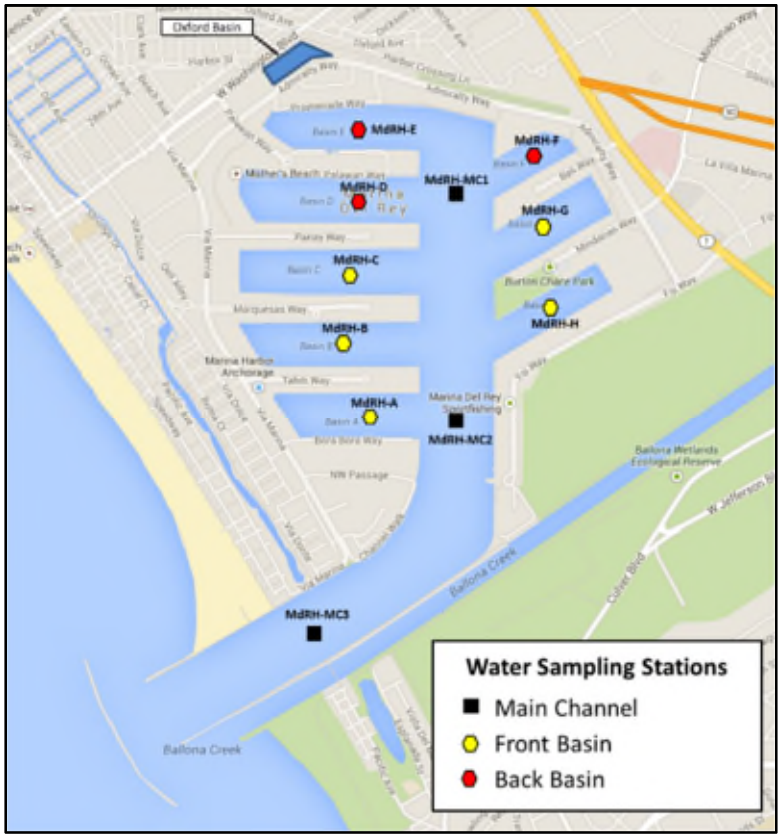

Figure 3. Site characterization sampling stations in Marina del Rey Harbor.

Table 2. Station locations

Station ID	Description	Latitude	Longitude
MdRH-MC1	Main Channel, end	33.98054	-118.44819
MdRH-MC2	Main Channel, middle	33.97231	-118.448
MdRH-MC3	Main Channel, entrance	33.96427	-118.455
MdRH-A	Front Basin A, middle	33.97251	-118.45284
MdRH-B	Front Basin B, middle	33.97514	-118.45346
MdRH-C	Front Basin C, middle	33.97773	-118.45372
MdRH-D	Back Basin D, middle	33.98022	-118.45356
MdRH-E	Back Basin E, middle	33.98301	-118.45338
MdRH-F	Back Basin F, middle	33.98198	-118.44502
MdRH-G	Front Basin G, middle	33.97939	-118.44435
MdRH-H	Front Basin H, middle	33.97635	-118.44409

Figure 4. Storm drain and outfall locations (adapted from Figure 1-1 in the Marina del Rey Coordinated Integrated Monitoring Program document, February 29, 2016). In addition to the outfalls indicated on the map, stormwater enters the MdRH main channel from Ballona Creek and the Grand Canal.

2.2.2 Site Characterization

Three water quality surveys were conducted in 2018 to characterize variations in Harbor water quality for parameters affecting copper toxicity (Table 3). One event occurred in March, the day after a rain event resulting in 1.1 inches of precipitation. The other two events (May and September) represented dry weather conditions in the Harbor. Water samples were collected from the surface and near bottom during the first two events, and from the surface only during the third event. Each sample was analyzed for parameters required to apply the BLM (pH, salinity, temperature, DOC), as well as total and dissolved copper, chlorophyll, and toxicity (mussel embryo development test).

Table 3. Water quality survey events.

Event	Date	Description	Precipitation (in)	Depth[1]
1	3/23/2018	Winter, wet weather	1.1	S, B
2	5/21/2018	Spring, dry weather	0	S, B
3	9/10/2018	Summer, dry weather	0	S

[1] S = Surface; B = Bottom

Seasonal and spatial variations in DOC were observed among the sampling events (Table 4). Seasonally, DOC was highest on average and more variable during event 1 (wet weather), compared to the two dry weather events. Discharge of stormwater runoff containing high concentrations of organic material to harbor surface water is the likely cause of this pattern. This hypothesis is supported by generally lower DOC concentrations in event 1 bottom water samples, compared to surface samples. Little difference in surface and bottom DOC concentrations were observed during dry weather (event 2).

A spatial pattern in DOC concentration was apparent for each sampling event. The lowest DOC concentrations were always observed at stations in the front basins (A) or in the main channel, close to the harbor mouth (MC3). Locations of the highest DOC were more variable but were frequently located in the back basins of the Harbor. This spatial pattern is likely related to circulation patterns within the Harbor, with sites having greatest mixing with relatively low DOC offshore water (front basins, main channel) having lower DOC concentrations.

Chlorophyll content of the water also varied spatially, with higher concentrations usually present in the back basins. Increases in Chlorophyll (a measure of biological productivity) was also correlated with elevated DOC.

Table 4. Water quality survey results for dissolved organic carbon.

			DOC (mg/L)		
Event	**Description**	**Depth**	**Average**	**Lowest**	**Highest**
1	Winter, wet weather	S	1.1	0.88 (MC3)	1.41 (A)
1	Winter, wet weather	B	0.94	0.78(MC3)	1.12 (H)
2	Spring, dry weather	S	0.77	0.54 (A)	1.0 (D)
2	Spring, dry weather	B	0.76	0.44 (MC3)	0.95 (MC1)
3	Summer, dry weather	S	0.84	0.74 (MC3)	1.02 (H)

The water quality results indicate that the critical condition, when water quality characteristics provide the greatest relative copper bioavailability, is likely to occur in winter or spring dry weather, when lower DOC concentrations are present.

Based on the 2018 water quality results, a subset of five stations is recommended for WER analysis (Figure 5), with composite samples being collected from three locations in each of the four selected basins (see 2.2.3 Sampling Design). These stations represent locations where DOC concentrations are likely to be lowest (main channel station 3, front basins A and B), as well as locations where DOC and copper concentration are likely to be high (back basins E and F). Additionally, these locations encompass many of the major stormwater outfalls in the Harbor (Figure 4) and should provide representative results during wet weather sampling. Sampling these stations at multiple times throughout the year is expected to represent variations in water quality factors controlling copper bioavailability throughout the Harbor, as well as encompassing the critical condition during each sampling event. The complete report detailing site characterization and critical condition determination is included in Appendix D.

Figure 5. Proposed stations for WER analysis.

2.2.3 Sampling Design

Six sampling events are proposed for WER calculation (Table 5). The events will be distributed over an approximately 12-month period to capture major seasonal variations in water quality. Most of the sampling events (4, or5 events) will occur during dry weather, when the critical condition is expected to be present. The specific time of the sampling will be determined by seasonal conditions. The sampling plan incorporates the three environmental factors expected to have the greatest influence on copper bioavailability: harbor location (e.g., mixing with coastal water), season, and stormwater discharge. The actual number of sampling events conducted may vary, depending on the results of the first five events. The variability in WER values for the first five sampling events will be reviewed, in consultation with the TAC, to determine the need for additional sampling events. All samples will be collected at 1 m below the water surface. In addition to the WER sample events, LACPW will collect and analyze DOC samples during their monthly TMDL monitoring program (CIMP) for one year.

To ensure spatial representativeness, the samples collected from the four basin locations will be composites of the end, middle, and mouth of the basin (Figure 5). For example, three samples will be collected and pooled together from MdRH-A (e.g., A-1:end, A-2:middle, and A-3:mouth).

The timing of each sampling event will be selected to represent potential temporal variations in water quality associated with stormwater runoff and tides. Sampling will be conducted during both wet and dry weather events. For an event to be considered "wet", there must have been at least 0.5 inches of rain in the preceding 24 hours and the samples must be taken within 24 hours from the end of the storm. Effects of tidal variation will be documented by conducting dry weather sampling during both spring tides (high variation between low and high tide) and neap tides (low difference between low and high tide). During dry weather, each of the five locations will be sampled twice in one day: once during flood tide and once during ebb tide. These two samples will be composited together such that one final sample per station is collected for chemical and toxicity analysis. The compositing over space and time during each sampling event is intended to increase the representativeness of the data for describing conditions in the Harbor.

Table 5. Proposed water-effect ratio sampling event matrix.

			Summer	**Winter**	
	Tide Type		**Dry Weather**	**Dry Weather**	**Wet Weather**
Event	**Spring**	**Neap**	**April – October**	**November – March**	**November – March**
1	X		X		
2	X			X	
3		X	X		
4		X		X	
5	NA	NA			X
6*	TBD	TBD	TBD	TBD	TBD

Notes:
Wet weather sampling is not dependent on tide type. Sampling event characteristics to be determined based on results of previous events.
NA = not applicable
TBD = to be determined
*The details of Event 6 will be based on review of data from the prior events and discussion with stakeholders and the TAC.

The tide type and tidal cycle at the time of sampling may affect the degree of mixing of harbor water with offshore coastal water, and thus water characteristics such as dissolved organic carbon concentration. Previous studies in bays have shown that the WER is strongly influenced by the water

circulation and degree of mixing with coastal water (Tetra Tech et al. 2000). Variations in both tidal stage and relative change in tide level will be considered in planning the sampling events.

Seasonal dry weather sampling is consistent with the design used in previous WER studies. Variations in temperature, plankton abundance, DOC production, and runoff inputs are expected to be associated with these seasons. Two sampling events are planned for each season, with each event representing a different phase of the tidal cycle.

One sampling event during wet weather (following substantial rainfall, ≥0.5 in) is proposed to confirm preliminary findings that indicate relatively low copper bioavailability during this time. The magnitude of the influence of stormwater discharges on copper bioavailability in MdR Harbor is likely to be variable. Depending on the amount of local precipitation and tides, stormwater enters the harbor via discharge from the Oxford Flood Control Basin to Basin E, from a portion of the Ballona Creek discharge plume that is reflected into the main channel by the breakwater, from the Grand Canal, and from multiple storm drains throughout the harbor complex (Figure 4). The impact of stormwater discharge on the WER should be captured by the chosen station locations. In Shelter Island Yacht Basin, lower WERs were obtained for the wet weather sampling event (Bosse et al. 2014). For MdR Harbor, at least one sampling event will be conducted shortly after a qualifying rain event to evaluate the influence of wet weather conditions on the WER and ambient toxicity. Qualifying criteria for sampling will include local precipitation of at least 0.5 inch and an antecedent dry period of at least 3 days.

2.2.4 Parameters to be Analyzed

Several water quality parameters (e.g., pH, temperature, dissolved oxygen, and salinity) will be measured using probes in the field at the time of water sampling (Table 6). Grab samples of water will be collected 1 m below the water surface at each station for measurement of DOC, metals, and toxicity. Concentrations of both copper and zinc will be measured, as both metals may be elevated in harbors and contribute to ambient toxicity. Zinc concentrations in MdR Harbor are not expected to exceed water quality standards but may be a partial contributor to variations in Harbor water toxicity. Inclusion of zinc in this study will facilitate a greater capability to interpret ambient toxicity test results.

Table 6. Analytes for WER study

Analyte	Occasion of Measurement		Analysis Method	Use
	Field	Laboratory		
pH	X		Probe	BLM
Temperature	X		Probe	BLM
Salinity	X		Probe	BLM
Dissolved Oxygen	X		Probe	Water quality
Dissolved Organic Carbon[1]		X	Instrument	BLM
Total Copper		X	ICP/MS	Water quality
Dissolved Copper		X	ICP/MS	Water quality
Total Zinc		X	ICP/MS	Water quality
Dissolved Zinc		X	ICP/MS	Water quality
Toxicity		X	Laboratory Test	Ambient toxicity

Notes:
BLM = Biotic Ligand Model
[1] DOC characterization by spectrophotometry will be conducted on selected samples.

2.2.5 Sample Collection and Processing

Methods for water sample collection and processing are described in Appendix A. Briefly, a peristaltic pump fitted with Teflon-lined tubing will be used to collect water samples and fill plastic bottles specific for each analyte type (Table 7). Samples for measurement of DOC and dissolved metals will be filtered in the field within 15 minutes of collection using plastic syringes fitted with 0.45-micron filters. A “clean hands/dirty hands” technique will be employed during sampling and filtering to prevent contamination of the samples. All samples will be placed in coolers with wet ice for temporary storage.

Sampling equipment will be pre-cleaned prior to the sampling event. The pump system will be flushed with site water prior to use at each station. A new filter apparatus will be used for each station.

Table 7. Volumes and containers for field samples.

Analysis Type	Volume (mL)	Container Type/Size
Dissolved organic carbon	50	Pre-combusted glass vial
Dissolved metals	50	Polypropylene tube
Total metals	50	Polypropylene tube
Toxicity	1,000	HDPE bottle

Note:
mL = milliliters

2.2.6 Documentation of Chain-of-custody

Samples are considered to be in one's custody if they are in the custodian's possession or view or retained in a secured place. The documents used to identify samples and to document possession include the chain-of-custody (COC) records and the field form. COC procedures will be used for all samples throughout the collection and analytical process. COC procedures will be initiated during sample collection. A COC record will be provided with each sample group. Each person who has custody of the samples will sign the form to ensure that the samples are not left unattended. COC forms will be signed by the person transferring samples custody. Additional information regarding COC and a copy of the COC form can be found in the Quality Assurance Project Plan (QAPP; Appendix B).

2.2.7 Analysis Methods

The methods for chemical analysis of the samples are described in the Appendix B. The methods have been selected to provide reporting limits below the levels expected in MdR Harbor (Table 8). Metal analysis will be conducted according to USEPA Method 1640 for trace elements in water, using inductively coupled plasma mass spectrometry. In this procedure, trace elements are pre-concentrated based on their reductive precipitation by sodium tetrahydroborate; iron and palladium are added to samples to aid co-precipitation of metal borides and to enhance the precipitation of metals coming out in the elemental form.

Table 8. Chemistry and toxicity analysis methods and reporting limits.

Analyte	Method Detection Limit (µg/L)		Reporting Limit (µg/L)		Analysis Method
	Total	Dissolved	Total	Dissolved	
Organic Carbon	NA	50	NA	500	EPA 9060a
Copper	0.025	0.15	0.025	0.15	USEPA 1640 – FePd
Zinc	0.025	0.15	0.025	0.15	USEPA 1640 – FePd
Toxicity	NA	NA	NA	NA	USEPA 1995

Notes:
µg/L = micrograms per Liter
NA = not applicable
SM = Standard Method
USEPA = U.S. Environmental Protection Agency

Dissolved organic carbon will be analyzed using EPA Method 9060a for the analysis of total organic carbon by combustion or oxidation. With this method organic carbon in a sample will be converted to carbon dioxide by catalytic combustion or wet chemical oxidation. The carbon dioxide formed can be measured directly by an infrared detector or converted to methane and measured by a flame ionization detector. The amount of carbon dioxide or methane is directly proportional to the concentration of carbonaceous material in the sample.

Ambient toxicity in the water samples will be measured using the 48-hour mussel embryo development test (Section 2.1). The toxicity test results from the unmodified samples from each dose-response test (no added copper spike) will provide insight to the level of ambient toxicity in MdR Harbor. MdR Harbor sample toxicity will be compared to the laboratory control (filtered seawater from reference site).

2.2.8 Biotic Ligand Model Analyses

The BLM is a chemical speciation model that can be used to predict the adverse effect levels of metals as a function of water chemistry. A freshwater version of the BLM for copper has been developed and approved by the USEPA for use in developing site-specific water quality criteria (Santore et al., 2001). For this study, the marine BLM for copper in saltwater developed by Robert Santore (Version 3.16.2.41 from Windward Environmental, LLC), which is currently under review for use by the USEPA in setting water quality criteria, will be used. Previous research provides a more in-depth description of the model parameters and equations, and its applications to both freshwater and marine systems (Di Toro et al. 2001; Santore et al. 2001; Arnold et al. 2005; Chadwick et al. 2008).

Application of the BLM requires the input of four water chemistry parameters from the site: salinity, temperature, pH, and DOC. Using chemical speciation data of the different components in seawater, the BLM will be used to predict the $EC50_{BLM}$; the concentration of dissolved copper needed to produce an adverse effect on 50% of developing mussel embryos in samples of both site water and laboratory control seawater. The predicted EC50 values will be used to calculate the BLM predicted WER, defined as the site water $EC50_{BLM}$ divided by the control water $EC50_{BLM}$ (see Equation 3 in Section 1.2.2).

2.2.9 Sampling Quality Assurance/Quality Control

Multiple quality assurance (QA) samples will be collected and processed in the field. QA samples include travel blanks, field banks, field duplicates, and matrix spikes (Table 9). One of each QA sample type will be collected during each sampling event. Furthermore, samples of tubes, syringes, filters, and bottles from every new manufacturing lot will be sent to the analytical laboratory for blank analysis.

Table 9. Description of quality assurance sample types for field sampling.

Sample Type	DOC Volume (mL)	Total Metals (mL)	Dissolved Metals (mL)
Travel Blank	40	50	50
Field Blank	40	50	50
Field Duplicate	40	50	50
Matrix Spike Blank	40	50	50
Pump Tubing Blank	40	50	50

Notes:
DOC = dissolved organic carbon
mL = milliliters

2.3 Dose-Response Testing

All water samples will be tested for toxicity and WER calculation using test methods described in Section 2.1. A series of spiked copper treatments will be prepared and tested for EC50 determination. The spiking methods will follow recommendations in the USEPA (1994) Interim Guidance. Water from each MdR Harbor station and the laboratory control will be spiked to generate a series of copper concentrations designed to produce toxicity results ranging from no effect to complete inhibition of normal embryo development (assuming minimal ambient toxicity). Results from samples with ambient toxicity will be reviewed in consultation with the TAC to determine if a valid EC50 can be calculated for that sample.

Copper-spiked water samples for WER tests will be prepared by adding reagent grade copper salt solutions. Spiking methods and concentrations will be consistent with the USEPA (1994) Interim Guidance. Spikes will be equilibrated for 12-24 hours before testing. Both site water and control water samples will be spiked with specific amounts of copper to produce six to nine treatments that range from a dose that does not cause toxicity to a dose that causes nearly complete mortality or abnormal development. Data from preliminary tests will be used to select treatment concentrations for MdR Harbor water. Spiked control water treatments are expected to range from approximately 2 to 30 µg/L.

Toxicity test results for each copper treatment will be expressed as average percentage normal of five replicate test chambers. Control performance will be compared to test acceptability criteria and water quality specifications (Table 1) to verify data quality. The spiked copper treatments for every sample will be analyzed to verify dissolved copper concentrations. Only those treatments used in the statistical analysis to determine the EC50 will be submitted for chemical analysis.

2.4 Water-Effect Ratio Analysis and Interpretation

The USEPA (1994) Interim Guidance on calculating and interpreting results will be followed in this study. The general steps include:

- Evaluating the acceptability of each toxicity test
- Calculating the results of each test
- Evaluating the acceptability of the laboratory dilution water
- Calculating the sWERs
- Investigating the WER

Completing the first three steps and calculating copper EC50 values for each sample will use methods and criteria in accordance with USEPA (1995). Generally, the EC50 will be determined using the Trimmed Spearman-Karber method.

The sWER will be calculated as the ratio of the sample EC50 divided by the control EC50 (Section 1.2.1). The BLM predicted sWER will also be calculated for each sample for comparison purposes only. The predicted sWER is calculated using copper EC50s for the sample and laboratory control predicted by the BLM.

2.4.1 Toxicity Quality Assurance/Quality Control

The practices used by the toxicity laboratory to ensure reliable, high-quality results for the tests conducted for this project are described in the QAPP (Appendix B). The objectives for accuracy and precision involve all aspects of the testing process, including:

- Seawater sampling and handling
- Source and condition of test organisms
- Test conditions
- Instrument calibration
- Use of reference toxicants
- Record keeping
- Data evaluation

Concurrent reference toxicant tests will be conducted for each toxicity test batch to verify the sensitivity and health of the test organisms. The reference toxicant EC50 will be compared to a control chart of historical values. Water quality parameters will be monitored to ensure that they fall within prescribed limits; corrective action will be taken if necessary. All limits established for this study meet or exceed those recommended by the USEPA. All data collected or produced from these analyses will be recorded and summarized to become part of the permanent data record for this study.

In addition, samples from one laboratory water reference toxicant series will be split and analyzed by a second toxicity testing laboratory. This will provide an interlaboratory comparison of the toxicity test results to provide further quality assurance.

2.4.2 Chemistry Quality Assurance/Quality Control

Detailed descriptions of QA/quality control (QC) procedures and data quality objectives (DQOs) for the chemical analyses of samples for this project are contained in the QAPP (Appendix B) and chemistry laboratory standard operation procedures included with the QAPP. QA/QC involves all testing aspects, including:

- Method SOPs
- Calibration methods and frequency
- Data analysis, validation, and reporting
- Internal QC
- Preventive maintenance
- Procedures to ensure data accuracy and completeness

Laboratory QC results, qualifications, and exceptions will be reported. Laboratory accuracy will be indicated by analysis of matrix spikes, blank spikes, certified reference materials, and/or recovery surrogates. Certified reference materials will be used during analysis of DOC and dissolved metals concentrations. Matrix spikes will be used to assess the effects that the sample matrix (e.g., seawater) has on the accuracy of a measurement. Blank spikes will demonstrate the performance of the preparation method on a clean matrix, void of potential interferences. Precision will be determined by analysis of duplicate matrix spikes, blank spikes, recovery surrogate spikes, and duplicate test samples. Potential laboratory contamination introduced during analysis will be assessed by analyzing procedural/method blanks. Any QC samples that fail to meet the QC criteria detailed in QAPP (Appendix B) will be identified, corrective action taken, and the corresponding data will be appropriately qualified in the final report. All QA/QC records will be kept on file.

2.4.3 Water-Effect Ratio Investigation

The sWERs for different stations and events will be summarized and evaluated to determine if the results are sufficient for calculation of the fWER. These analyses will be structured to answer the following questions:

- Do the samples represent typical MdR Harbor conditions?
- Is the critical condition adequately represented?
- Is the sWER sample size and precision sufficient for calculation of the fWER?
- Are the toxicity-based and BLM predicted sWERs comparable?

Water quality (e.g., pH, DOC, temperature, and salinity) and copper concentration measurements for the field samples will be compared to values obtained in the site characterization study and TMDL monitoring to determine if the samples are representative of MdR Harbor. Statistical evaluation will include comparing sample data to the 95% confidence interval for the parameters.

Representation of the critical condition will be assessed by comparing the season and tide stage of each sampling event to the conditions used to define the critical condition. A determination will be made as to whether the goal of conducting four sampling events during the critical condition was met.

The criteria and statistical methods used to evaluate sWER sample size precision will be developed in consultation with the TAC and stakeholders during the data review process. In addition to the recommendation from the TAC, the coefficient of variation and 95% confidence intervals will be calculated for the sWER dataset (or region-specific subset) and compared to the maximum interval size desired.

Three approaches will be used to investigate the comparability of the toxicity-based and BLM predicted sWERs:

1. Summary statistics (e.g., mean, standard deviation, and range) will be compared between the two types of sWER. This analysis will indicate the overall magnitude of differences between the methods.
2. T-tests or ANOVA will be used to determine if mean sWERs are significantly different.
3. Graphical methods (e.g., scatterplots) will be used to compare pairs of individual sWERs matched by station. This analysis will indicate whether there is a pattern of consistent bias between the two WER approaches.

The results of the WER investigations described above will be reviewed to determine if data are sufficient to support fWER calculation at the desired level of precision and seasonal specificity. A minimum of three sampling events is recommended by the USEPA (1994) Interim Guidance, which is half the number of events proposed in this work plan. If the data are not sufficient, the feasibility of conducting additional sampling and analyses will be explored.

2.5 Final Water-Effect Ratio Calculation

The fWER will be calculated as the geometric mean of the group of sWERs selected for analysis, as recommended in the USEPA (1994) Interim Guidance. The geometric mean is calculated as the average of the natural log-transformed sWERs. The number and type of fWERs calculated will depend on the characteristics of the sWERs and final study objectives. For example, if statistical analyses indicate that sWERs collected in different regions of the harbor (or different seasons) are similar, then data may be pooled and a single fWER calculated. Alternatively, several fWERs may be calculated to represent important variations in critical condition or copper bioavailability (e.g., front basins vs. back basins). Similarly, if the sWERs are highly variable, use of the lowest sWER may be considered. A determination of the number and type of fWERs to be calculated will be made in consultation with the TAC and local stakeholders.

III. DATA MANAGEMENT

Data management will involve compiling data collected as part of the SSO study into standardized formats, data review, and export of field, toxicity test, and chemistry data as flat files that are accessible by Los Angeles County Public Works (LACPW). Data will be reviewed for quality and completeness, compiled, and exported in CEDEN format to LACPW.

3.1 Analytical Chemistry Data Quality Review and Management

Analytical chemistry data will be submitted by the laboratory in specified PDF and electronic data deliverable formats. Analytical data will undergo verification and validation in accordance with the QAPP (Appendix B) and final validation qualifiers will be applied and stored. A concise data validation summary will be prepared and included in the final report.

3.2 Toxicity Test Data Quality Review and Management

All toxicity test data including laboratory bench sheets and randomization sheets (listed in the QAPP; Appendix B) will be reviewed to ensure that data meet QA/QC standards specified in the standard method guidance documents. The toxicity test data review process is detailed in the QAPP and briefly described here. A determination will be made as to whether DQOs were met by assessing test acceptability criteria, reference toxicant test results, protocol deviations (i.e., water quality deviations), sample handling notes, and data completeness. Minor data quality issues, that likely do not affect the test outcome, will be noted and summarized in the final report. Database contents will be compared to bench sheets to ensure that the electronic data are complete and accurate.

3.3 Data deliverables

A draft Excel database containing data collected during the first half of the SSO study will be provided before the end of 2019. A final Excel database containing field sampling coordinates, field water quality measurements, compiled validated analytical data, and compiled toxicity summary data for the entire study will be provided along with the final report.

IV. DELIVERABLES AND REPORTING

4.1 Task Reports

Interim progress reports and data summaries will be provided as specific study tasks are completed. The format and content of these reports will vary, according to the nature of the activity. Preliminary data and reports will be made available to stakeholders when they are sent to the TAC for review. Discussion regarding changes to the work plan will occur during the quarterly TAC meetings and will be open to stakeholder participation. Reports for key tasks will include the following:

- Quarterly progress reports covering all activities
- Summary of field sampling events, including station locations and a description of deviations from the sampling plan
- Summary of water chemistry results for each sampling event
- Summary of toxicity results for each testing event
- Summary tables of WER values, BLM output, and predicted WERs
- Data validation summary

4.2 Site-specific Objective Study Report

The results of the SSO tasks will be summarized, integrated, and evaluated in a draft report. The final dataset will be provided to LACPW/Los Angeles County Beaches and Harbors (LACDBH) in CEDEN format. Laboratory reports, copies of field forms, and data validation reports will be included as appendices. At a minimum, the following will be included in the report:

- Summary of all field activities, including a description of any deviations from the approved work plan
- Locations of stations in latitude and longitude (degrees, decimal minutes)
- Project maps with actual sampling locations
- Summary of water chemistry results compared to CTR criteria
- Summary of toxicity results and WER values
- Conclusions
- Data validation summary

The draft study report (one electronic copy) will be prepared for LACPW review and comment. Following receipt of comments and revisions to the draft report, a draft final report will be prepared for review by the TAC, RWQCB, and other stakeholders. All comments will be reviewed and addressed, and a final report will be prepared and provided to LACPW (three hard copies and an electronic copy).

V. PUBLIC PARTICIPATION PLAN

Public participation will be actively sought during the SSO study. Various stakeholders including non-governmental organizations (NGOs), boaters, marina operators, Harbor lessees, and other interested parties will be invited to listen in during all TAC review meetings. In addition, two public information workshops will be conducted. The first TAC review meeting was held in December 2018 after the completion of a draft work plan and concurrent with the public work plan review. TAC and public comments (and responses) on the work plan are included in Appendices E and F.

The first public workshop will be held soon after concurrence from the Regional Board regarding the work plan is obtained. The second workshop will be held after the completion of the draft final report to explain the outcomes of the SSO study and to solicit comments from the public before finalizing the final report. All key documents from the SSO study, including the draft work plan, draft final report, and draft implementation strategy report will be available for public review for a minimum of 30 days once they are submitted to the RWQCB. Public review comments will be considered in preparation of the final documents.

VI. TECHNICAL ADVISORY COMMITTEE

A TAC has been established to provide scientific review and guidance for the SSO study. Three scientists with expertise in metal speciation, bioavailability, toxicology, ecology, and water quality modeling comprise the TAC (Table 10). The TAC members were selected based on recommendations from RWQCB staff and environmental groups. Each of the TAC members have international and national recognition as leaders in their field, extensive publication records, and a mixture of local and international experience. The TAC will provide an independent review of the study design, study results, and final report. The TAC will also provide a resource to questions or concerns from stakeholders that require the application of expert judgment. Additional background on the TAC members is provided in Appendix C.

Table 10. Technical Advisory Committee Members

Name	Affiliation	Expertise
Peter Campbell	University of Quebec, INRS, Quebec, Canada	Trace metal analysis, speciation, toxicology, bioaccumulation
Gary Cherr	Bodega Marine Laboratory, University of California, Davis, CA	Reproductive physiology, developmental biology, biochemistry, environmental toxicology
Richard F. Ambrose	University of California, Los Angeles, CA	Assessment, restoration, and remediation of coastal habitats, including wetlands and rocky intertidal. Climate change impact assessment and mitigation.

VII. IMPLEMENTATION REPORT

Following the completion of the SSO final report, an implementation report will be developed in coordination with LACPW/LACDBH and the regulatory agency in order to incorporate the SSO study results in an amendment to the Basin Plan and the Toxics TMDL.

The implementation report will include recalculations of TMDL numeric targets for dissolved copper in MdR Harbor; i.e., chronic CCC and acute CMC will be recalculated using fWERs specific to MdR Harbor. The implementation report will also include recalculation of TMDL load allocation for dissolved copper in MdR Harbor based on the recalculated CCC. In addition, the implementation report will provide analyses to support the implementation of the SSOs for dissolved copper in MdR Harbor including environmental and economic impacts, California Water Code Section 13241, anti-degradation review (as appropriate), and anti-backsliding review (as appropriate).

A draft implementation report (electronic copy) will be submitted to LACPW and the RWQCB staff for review. All comments will be reviewed and addressed accordingly. A final implementation report will be submitted to the LACPW (3 hard copies, 1 electronic copy). A copy of the final implementation report will be also submitted to the RWQCB E.O.

VIII. PROJECT SCHEDULE

A project schedule with key milestones has been developed and is provided in Table 11.

Table 11. Site-specific objective study schedule

Deliverables	Target Date[1]
Review of work plan by stakeholders	November 2018
TAC Meeting 1: Review of work plan[2]	December 2018
Submission of revised SSO Work Plan for Los Angeles RWQCB concurrence	March 2019
TAC Meeting 2: conference call to discuss work plan revisions	April 2019
Public Outreach Workshop 1: Study background and description of work plan	April/May 2019
WER Sampling and Testing	April 2019 to April 2020
TAC Meeting 3: conference call to discuss interim results of WER analyses	July 2019
TAC Meeting 4: conference call to discuss interim results of WER analyses	December 2019
TAC Meeting 5: conference call to discuss preliminary WER results	April 2020
SSO Draft Report and Implementation Draft Report	July 2020
Public Outreach Workshop 2: Presentation of report findings to stakeholders	August 2020
TAC Meeting 6: conference call to discuss TAC's review of the draft report	August 2020
TAC Meeting 7: discussion of revised SSO Final Report	September 2020
Final SSO and Implementation Reports	October 2020

Notes:
RWQCB = Regional Water Quality Control Board
SSO = site-specific objective
TAC = Technical Advisory Committee (all TAC meetings will be open to stakeholder participation)
WER = Water Effect Ratio
1 Dates are for planning purposes only; specific dates for meetings have not yet been established.
2 An orientation conference call with the TAC will be held prior to the December meeting.

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APPENDIX A
SAMPLING AND LABORATORY METHODS

MARINA DEL REY HARBOR SITE-SPECIFIC OBJECTIVE (SSO) STUDY

WATER SAMPLING METHODS

Southern California Coastal Water Research Project

February 26, 2019

TABLE OF CONTENTS

BACKGROUND

This document describes methods for the collection and field processing of water samples from Marina del Rey Harbor for site characterization, toxicity and chemical analyses. The data resulting from these analyses will be used to characterize variations in harbor water quality, calculate Water Effects Ratios for copper, and apply the Biotic Ligand Model to predict copper toxicity.

SAMPLE COLLECTION

Water samples will be collected from single or multiple depths (depending on study objective), including one meter above the sediment surface and one meter below the water surface. Provisional sample locations include 11 stations that represent the central areas of each harbor basin and portions of the main access channel (Figure 1, Table 1). Upon station occupation, sampling and processing will occur in the following order: 1) record station location and general conditions in log, 2) collect water samples, 3) process samples, 4) measure water quality parameters (grab samples or profiles), and 5) record sampling data in log.

Water samples will be collected using a peristaltic pump fitted with Teflon-lined tubing. Once the tubing intake is at depth, the pump will be turned on to allow tubing to be flushed. After flushing, two liters of site water will be collected in clean 1 liter fill bottles. Subsampling for subsequent processing of trace metals and organic carbon will come from the fill bottle. The water in the fill bottle will be swirled to homogenize the sample before transferring to the filtering apparatus or sample containers. A “clean hands-dirty hands” technique (see below) will be employed to minimize contamination of samples. Of the two liters of water that will be collected 50 ml will be filtered for dissolved metals and 40 ml more for DOC analysis. All samples will be placed in dark coolers with wet ice for temporary storage. The field crew will not add any preservatives to the samples.

Composite samples may be collected to include spatial or tidal variations in water characteristics. Spatial composite samples will be created by pooling equal volumes of water collected from three regions of a basin (e.g., end, middle, and end). The pooled sample will be subsampled and filtered as needed to generate separate samples for analysis of metals, organic carbon, chloropyll,

and toxicity. Temporal composite samples will also be created to represent potential variations in water quality associated with tidal exchange. These composites will be prepared by pooling spatial composite samples collected at two times during the sampling day: morning and afternoon. Ideally, sampling events should be scheduled so that a different tide stage (ebb or flood) is represented by each spatial composite.

In order to reduce potential contamination, sampling personnel will adhere to the following rules:

- No smoking.
- Never sample near a running vehicle.
- Do not eat or drink during sample collection.
- Do not breathe, sneeze or cough in the direction of an open sample bottle.
- Each person on the field crew will wear clean clothing that is free of dirt, grease, or other substances that could contaminate the sampling apparatus or sample bottle.

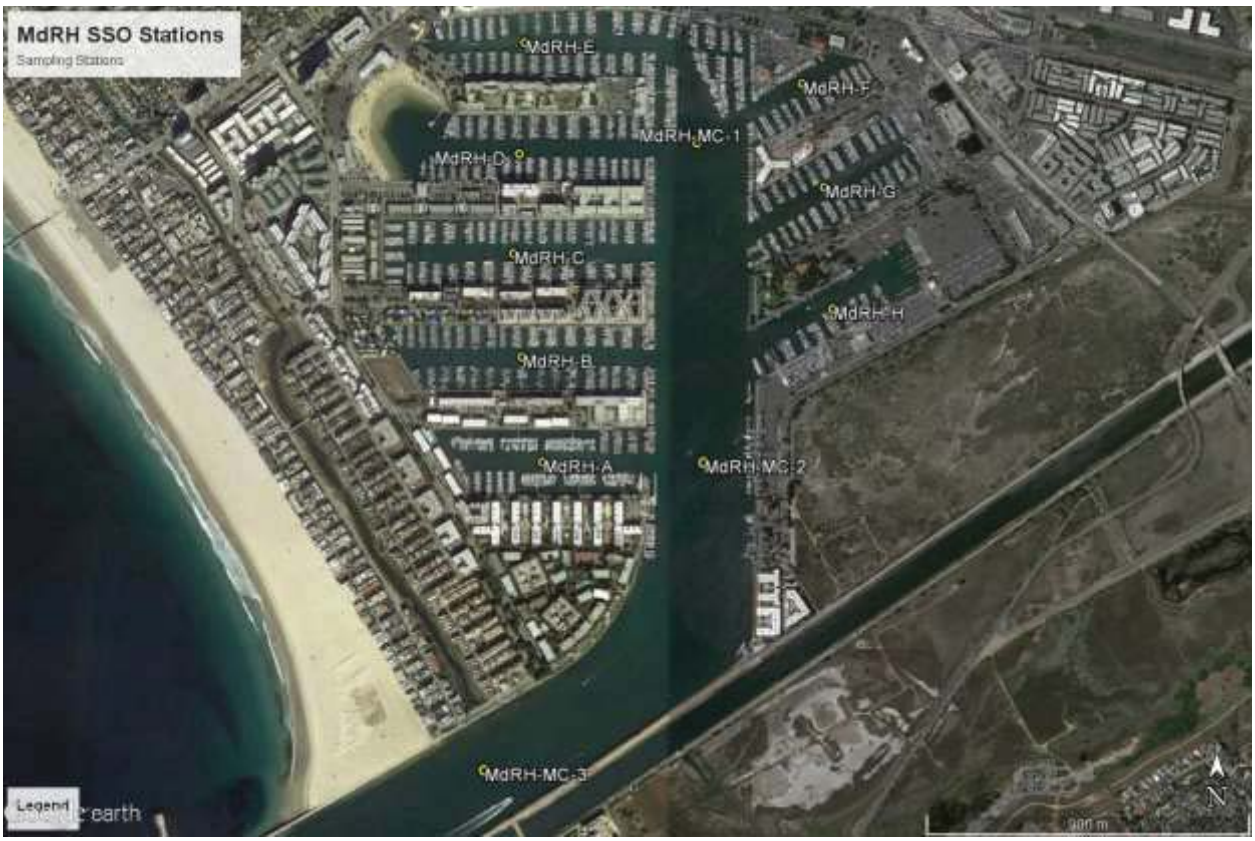

Figure 1. Sampling locations for the study.

Table 1. Coordinates for sampling locations.

Station ID	Latitude	Longitude
MdRH-MC-1	33.9805	-118.448
MdRH-MC-2	33.97231	-118.448
MdRH-MC-3	33.96427	-118.455
MdRH-A	33.97251	-118.453
MdRH-B	33.97514	-118.453
MdRH-C	33.97773	-118.454
MdRH-D	33.98022	-118.454
MdRH-E	33.98301	-118.453
MdRH-F	33.98198	-118.445
MdRH-G	33.97939	-118.444
MdRH-H	33.97635	-118.444

CLEAN SAMPLE HANDLING TECHNIQUES

To prevent contamination of samples, clean metal sampling techniques using USEPA protocols outlined in USEPA Method 1669 will be used throughout all phases of the sampling laboratory work, including equipment preparation, sample collection, and sample handling, storage, and testing. Filled sample containers will be kept on ice or refrigerated until receipt at the laboratory.

The protocol for clean metal sampling, based on USEPA Method 1669, is summarized below:

- Samples are collected in clean sample vials or bottles with any tubing specially processed to clean sampling standards.
- At least two persons, wearing clean, powder-free nitrile or latex gloves at all times, are required on a sampling crew.
- One person, referred to as “dirty hands”, opens only the outer bag of all double-bagged sample bottles.
- The other person, referred to as “clean hands”, reaches into the outer bag, opens the inner bag and removes the clean sample bottle.
- Clean hands rinses the bottle at least two times by removing the bottle lid, filling the bottle approximately one-third full, replacing the bottle lid, gently shaking and then

emptying the bottle. Clean hands then collects the sample by filling the bottle and replacing the bottle cap.

- After the sample is collected, the sample bottle is double-bagged in the opposite order from which it was removed from the same double-bagging.
- Clean, powder-free gloves should be changed whenever something not known to be clean has been touched.
- The time of sample collection is recorded on the field log sheet.

SAMPLE PROCESSING

Sample Filtration

A 60 ml plastic syringe with a 0.45 µm filter attached will be used to collect and filter the dissolved metals sample in the field. Each filter apparatus is placed in zip-lock plastic bags and double bagged for storage. The filter material will be tested for Cu and DOC contamination with a field blank. Use one syringe per station. Maintain clean sampling techniques at all times. Filter the dissolved organic carbon and dissolved Cu samples first before collecting the total metals sample. Double bag each sample container after collection and place it in wet ice for storage until delivery to the analyzing laboratory. The samples will be preserved by the analyzing laboratory.

Dissolved Organic Carbon

Remove the syringe from the storage bag. Next, remove the filter from the bag and screw it tightly onto the tip of the syringe. Fill the syringe with sample and insert the plunger. Then, put the tip of the syringe with the filter into the clean dissolved metals container and push the sample through the filter taking care not to touch the inside surface of the sample container with the apparatus.

The sample volume for dissolved organic carbon analysis needs to be 40 ml (Table 2). If the filter becomes clogged prior to generating 40 ml of sample, remove and dispose of the used filter and replace it with a new clean filter. Continue to filter the sample. When 40 ml have been collected, cap the sample vial tightly and store on ice for delivery to the analysis laboratory. This

sample vial needs to be kept in the dark to prevent sample degradation from exposure to sunlight. The analysis method and detection limits for dissolved organic carbon can be found in Table 3.

Dissolved Metals

A 50 ml sample is needed for dissolved metals analysis (Table 2). Filter the sample using the methods described above. If the filter becomes clogged prior to generating 50 ml of sample, remove and dispose of the used filter and replace it with a new clean filter. Continue to filter the sample. When 50 ml have been collected cap the sample bottle tightly and store on ice for delivery to the analysis laboratory. Filtration must occur within 15 minutes of sample collection. The analysis method and detection limits for dissolved copper and zinc can be found in Table 3.

Total Metals

The total metals sample does not need to be filtered. Using clean handling techniques, transfer 50 ml of sample to a 50 ml plastic bottle. Double bag the sample and place it on ice after collection. The analysis method and detection limits for total copper and zinc can be found in Table 3.

Table 2. Sample volumes and storage containers.

Analysis Type	Volume (ml)	Container Type
Dissolved organic carbon	40	Pre-combusted glass vial (40 ml)
Dissolved metals	50	PE tube (50 ml)
Total metals	50	PE tube (50 ml)

Table 3. Analysis methods, method detection limits (MDL) and reporting limits (RL).

Analyte	MDL (µg/L)		RL (µg/L)		Analysis Method
	Total	Dissolved	Total	Dissolved	
Organic Carbon	NA	55	NA	550	EPA 9060a
Copper	0.15	0.15	0.15	0.15	EPA 1640 – FePd only
Zinc	0.15	0.15	0.15	0.15	EPA 1640 – FePd only

FIELD MEASUREMENTS AND OBSERVATIONS

Field measurements will be collected, and observations will be made at each sampling site after a sample is collected. All field measurement results and field observations will be recorded on a log sheet similar to the one presented in Figure 2. Field measurements will include either a depth profile or single depth measurement of dissolved oxygen, temperature, salinity, and pH. Measurements will be collected using aYSI multi-probe meter at approximately 0.5 m intervals. Prior to each sampling event, water quality probes will be calibrated using fresh calibration solutions. For all constituents a two-point calibration will be used. After each calibration, the sensor will be checked to verify the accuracy is within 10% of the known value of a standard solution. The calibration process will be repeated until the accuracy is verified.

Figure 2. Sampling log.

MdRH Water Daily Sampling Log

Date_____ Crew_____

Station_____ Latitude_____ Longitude_____

Time at Start_____ Time at Finish_____

Visual Water Description_____

Picture Numbers_____

pH_____ Salinity_____

Temperature_____ Dissolved Oxygen_____

Other Notes_____

QUALITY ASSURANCE / QUALITY CONTROL

Quality assurance and quality control (QA/QC) measures will be followed to assure high data quality (Table 4). Field QA/QC for this project includes the following:

Tubing blanks. The use of tubing blanks is intended to test whether contamination is introduced from the collection tubing. Samples of Milli-q water that has passed through the tubing while connected to the pump will be collected for total and dissolved metals as well as DOC analysis. These samples will serve as a blank for the tubing, collection vials, and filtration system prior to field collection. These QA samples will be collected and analyzed once per sampling event. Samples will be collected using clean sampling techniques.

Field blanks. The use of field blanks is intended to test whether contamination is introduced from sample collection and handling, sample processing, or the sample containers. For this blank, laboratory water is processed in the field in the same matter that all the other field samples are processed, excluding the pump tubing. Field blanks will be analyzed for total and dissolved copper and dissolved organic carbon. Clean sampling techniques will be used to process these samples. One field blank sample will be processed per sampling event.

Field duplicates. The use of field duplicates is intended to test the precision of sample collection. Field duplicates will be analyzed for all chemistry constituents. Clean sampling techniques will be used to minimize sample contamination. One field duplicate sample will be processed per sampling event. The station to collect the field duplicate will be chosen during each sampling event.

Travel blanks. Travel blanks are plastic bottles that contain laboratory water to test if contamination is introduced to the samples by the laboratory or transportation methods. Travel blank bottles will be provided by the analytical laboratory and taken into the field at time of sample collection. This bottle will be opened during the time it takes to collect and process one station. They will be returned to the analytical laboratory with the other field collected samples. The analytical laboratory will analyze this water sample for total Cu. Dissolved copper is not

analyzed as this will be reflected in the field blank. One travel blank sample will be processed per sampling event.

Matrix Spike Blanks. Additional water samples will be collected for the analysis of matrix spike samples. The matrix spike sample provides information on the extraction efficiency of the method on the sample matrix. Clean sampling techniques will be used to process these samples. One matrix spike blank sample will be processed per sampling event.

Table 4. Quality assurance sample types and volumes. One of each QA sample type will be collected and analyzed for each sampling event.

Sample Type	DOC (ml)	Total Metals (ml)	Dissolved Metals (ml)
Travel Blank	na	50	na
Field Blank	40	50	50
Field Duplicate	40	50	50
Matrix Spike Blank	0	50	50
Pump tubing Blank	40	50	50

na: not applicable

Chain-of-custody procedures for this project include the following:

- Proper labeling of samples.
- Use of chain-of-custody (COC) forms for all samples.
- Prompt sample delivery to the laboratory.

All aspects of the sample collection process, including generating field logs at each site and chains of custody (COC) forms, will be documented and tracked. COC forms will accompany all water samples to the laboratory for analysis. SCCWRP will retain a copy of all COCs. Physis will document and track all aspects of sample receipt, analyses, and reporting.

Laboratory QA/QC for this project includes the following:

- Use of the lowest available method detection limits (MDLs) for trace elements.

- Analysis of method blanks and laboratory duplicates.
- Use of matrix spikes (to test analytical accuracy) and matrix spike duplicates (to test analytical precision).
- Routine analysis of standard reference materials and method blanks.

Sample Vial and Bottle Labeling

Each sample will have a waterproof paper label affixed to the container and will be labeled at the time of collection. The following information will be recorded on the container label at the time of collection (Figure 3):

- Project name
- Sample identification
- Date of sample collection
- Analysis to be performed

Project: **MDR SSO**
Station: **MdRH-E**
Sample #: 1
Analysis: Total Copper
Laboratory: Physis
Date: March 3, 2019

Figure 3. Sample label example.

SAMPLE DELIVERY

Samples will be stored and transported at 4±2°C. Water samples will be provided to the toxicity and chemistry testing laboratories on the same day that sample collection process is completed. The individual sample containers containing the marine water samples for chemical analysis will be picked up by the analytical chemistry laboratory for analysis. Contacts for the field or laboratory coordinators are shown in Table 5. Each sample must be accompanied by a COC form (Figure 3).

Table 5. Agency contacts.

Coordinator	Agency	Contact Name	Email	Phone
Field	SCCWRP	Dario Diehl	dariod@sccwrp.org	714 755-3212
Toxicity	SCCWRP	Ashley Parks	ashleyp@sccwrp.org	714 755-3216
Chemistry	Physis	Rich Gossett	richgossett@physislabs.com	714 602-5320

REFERENCES

USEPA. April 1995. Method 1669: Sampling Ambient Water for Trace Metals at EPA Water Quality Criteria Levels. EPA 821-R-95-034.

Southern California Coastal Water Research Project
3535 Harbor Blvd. Suite 110
Costa Mesa, CA 92626
(714) 755-3200

Chain of Custody

Date _____ Page _____ of _____

Sample Collection By: _____			Project Name: _____				Project Number: _____
Sample ID	Date	Time	Matrix	Container Type	Number of Containers	Comments	Analysis

Relinquished By		Relinquished By		Relinquished By	
(Signature)	(Date)	(Signature)	(Date)	(Signature)	(Date)
(Printed Name)	(Time)	(Printed Name)	(Time)	(Printed Name)	(Time)
(Company)		(Company)		(Company)	
Received By		**Received By**		**Received By**	
(Signature)	(Date)	(Signature)	(Date)	(Signature)	(Date)
(Printed Name)	(Time)	(Printed Name)	(Time)	(Printed Name)	(Time)
(Company)		(Company)		(Company)	

Figure 3. Chain of custody.

PHYSIS

TERRA FAUNA FLORA AQUA AURA

ENVIRONMENTAL LABORATORIES, INC.

STANDARD OPERATING PROCEDURE

for EPA Method 1640

DETERMINATION OF TRACE ELEMENTS IN SALINE WATER BY PRECONCENTRATION AND INDUCTIVELY COUPLED PLASMA - MASS SPECTROMETRY

REVISION # 1 Effective date: June 15, 2010

Approved by:

Name	Title	Phone	Signatures	Date
Misty Mercier	Laboratory Director			
Rhonda Moeller	Quality Manager			

METHOD 1640:

EXTRACTION OF TRACE METALS IN SEAWATER AND SEDIMENT

REFERENCES:

Battelle Pacific Northwest Laboratories, Marine Sciences Laboratory. Standard Operating Procedure MSL-M-034-01. APDC Extraction of Metals in Seawater.

Bloom, N.S. and Crecelius, B.A. 1984. Determination of Silver in Seawater by Coprecipitation with Cobalt Pyrrolidine Dithiocarbamate and Zeeman Graphite-Furnace Atomic Absorption Spectrometry, *Analytica Chimica Acta*, 156, pp.139-145.

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U.S. Environmental Protection Agency, Office of Water Engineering and Analysis Division (4303): Method 1640.

EPA Methods for Chemical Analysis of Water and Wastes, Method 200.8, Revision 5.4 (May, 1994); NOAA Sampling and Analytical Methods of the National Status and Trends Program, Volume III, (1993)

1.0 SCOPE AND APPLICATION

1.1 This method provides procedures for the extraction and preconcentration of dissolved and particulate elements from aqueous samples using chelation and precipitation for subsequent analysis by inductively coupled plasma mass spectrometry (ICP-MS). It includes stringent quality control (QC) and sample handling guidelines necessary to avoid contamination and ensure the validity of analytical results

during sampling and analysis. The method contains QC procedures that will ensure that any possible contamination will be detected when blanks accompanying samples are analyzed.

Table 1.

ANALYTE	SYMBOL	AMU	CASRN
Aluminum	(Al)	27	7429-90-5
Antimony	(Sb)	123	7440-36-0
Arsenic	(As)	75	7440-38-2
Beryllium	(Be)	9	744-38-2
Cadmium	(Cd)	111	7440-43-9
Chromium-total	(Cr)	52	7440-47-3
Cobalt	(Co)	59	7440-48-4
Copper	(Cu)	63	7440-50-8
Iron	(Fe)	56 or 57	7439-89-6
Lead	(Pb)	206, 207, or 208	7439-92-1
Manganese	(Mn)	55	7439-96-5
Mercury	(Hg)	202	7439-97-6
Molybdenum	(Mo)	98	7439-98-7
Nickel	(Ni)	60	7440-02-0
Selenium	(Se)	82	7782-49-2
Silver	(Ag)	107	7440-22-4
Thallium	(Tl)	205	7440-28-0
Tin	(Sn)	118	7440-31-5
Titanium	(Ti)	48	
Vanadium	(V)	51	7440-62-2
Zinc	(Zn)	66	7440-66-6

Table 1. Target analytes in seawater samples extracted from both the APDC and iron-palladium procedures. The CASRN is the Chemical Abstract Services Registry Number.

Table 2.

PARAMETER	METHOD DETECTION LIMIT (ug/L)	ACCEPTANCE RANGE (%)	REPORTING LIMIT (ug/L)
Aluminum (Al)	0.01	52-149	0.025
Antimony (Sb)	0.01	44-97	0.025
Arsenic (As)	0.01	71-112	0.025
Barium (Ba)	0.5	70-130	1

Beryllium (Be)	0.005	62-113	0.01
Boron (B)	0.5	70-130	1
Cadmium (Cd)	0.005	69-100	0.01
Calcium (Ca)	0.5	70-130	2
Chromium (Cr)	0.005	85-133	0.01
Cobalt (Co)	0.005	75-124	0.01
Copper (Cu)	0.005	72-108	0.01
Iodine (I)	0.5	70-130	1
Iron (Fe)	0.01	35-97	0.025
Lead (Pb)	0.005	56-116	0.01
Lithium (Li)	0.01	70-130	
Magnesium (Mg)	5	70-130	10
Manganese (Mn)	0.005	64-120	0.01
Mercury (Hg)	0.005	68-117	0.01
Molybdenum (Mo)	0.005	59-125	0.01
Nickel (Ni)	0.005	68-118	0.01
Potassium (K)	5	70-130	10
Selenium (Se)	0.01	55-110	0.025
Silver (Ag)	0.005	66-125	0.01
Sodium (Na)	5	70-130	10
Strontium (Sr)	0.01	70-130	0.025
Thallium (Tl)	0.005	66-92	0.01
Tin (Sn)	0.005	68-110	0.01
Titanium (Ti)	0.005	95-143	0.01
Vanadium (V)	0.005	95-140	0.01
Zinc (Zn)	0.005	62-108	0.01

Table 2. The Approximate detection limits, acceptance range, and reporting limits for the target analytes in aqueous samples.

2.0 SUMMARY OF METHODS

2.1 An aliquot of a well-mixed, homogeneous sample is accurately measured for sample processing. Target metals are chelated out of the aqueous sample using ammonium pyrrolidine dithiocarbamate (APDC). The chelated precipitate is filtered onto a membrane filter and then digested in a nitric acid solution.

2.2 An aliquot of a well-mixed, homogeneous seawater sample is accurately measured for sample processing. Target metals are co-precipitated out of the sample using borohydride Iron-Palladium reductive precipitation. The precipitate is centrifuged out of the seawater matrix. The metal-containing pellet is then digested with nitric acid.

2.3 An aliquot of sample is diluted from 10-100 times before it is acidified using HNO3 until the pH is <2. The diluted, acidified sample must sit for a minimum of 16 hours before it can be analyzed.

2.4 The sample is then analyzed using Inductively Coupled Plasma Mass Spectrometry (ICPMS) by pumping the sample through a nebulizer producing a fine spray. An argon carrier gas atomizes the sample which is ionized and detected with a mass spectrometer. Qualitative identification is based on the mass to charge ratio for each element. It is recommended that samples be analyzed within 1 day of digestion.

3.0 PREVENTION OF INTERFERENCE

3.1 Samples may be contaminated by numerous routes. Contamination by trace metals can occur due to the use of metallic or metal-containing labware (e.g., talc gloves which contain high levels of zinc), containers, sampling equipment, reagents, and reagent water during sampling. Contamination also results from improperly cleaned and stored equipment, labware, and reagents, as well as from atmospheric inputs such as dirt and dust.

3.2 The avoidance of contamination can be achieved by carrying out the following procedures:

3.2.1 Clean sample containers, nucleopore filters, and filtration apparatus with acid and rinse with Milli-Q water. Upon cleaning, store the labware in clean zip-type bags and place them in a plastic box.

3.2.2 Keep samples and glassware covered when possible.

3.2.3 Ensure all materials that come into contact with the sample are nonmetallic. Only the following materials should come into contact with samples: fluoropolymer (FEP, PTFE), conventional or linear polyethylene, polycarbonate, polypropylene, polysulfone, or ultrapure quartz. All materials that will directly or indirectly contact the sample must be cleaned or must be known to be clean and metal free before proceeding.

3.2.4 Minimize exposure of sample to an uncontrolled atmosphere.

3.3 ICPMS Interferences

3.3.1 Chemical interferences are characterized by molecular compound formation, ionization effects and solute vaporization effects. These

effects are not usually pronounced with the ICPMS technique due to the high temperature of the torch.

3.3.2 Isobaric interferences are caused by isotopes of different elements which form singly or doubly charged ions of the same nominal mass-to-charge ratio and which cannot be resolved by the mass spectrometer. All elements determined by this method have one isotope free of isobaric elemental interference except Selenium-82, which has isobaric interference from the Krypton impurities in the Argon gas supply. This interference can be minimized by using high purity argon. All data must be corrected by measuring the signal from another isotope of the interfering element and subtracting the appropriate signal ratio from the isotope of interest.

3.3.3 Wing overlap interferences may occur when a small ion peak is being measured adjacent to a large one. The potential for these interferences should be recognized and the spectrometer resolution adjusted to minimize them.

3.3.4 Polyatomic interferences are caused by ions consisting of more than one atom which have the same nominal mass-to-charge ratio as the isotope of interest, and which cannot be resolved by the mass spectrometer. The ions may be formed in the plasma or interface system from support gases or sample components. Most of the common interferences have been identified and are listed within the instrument data system along with the elements affected. All data shall be corrected by measuring the signal from another isotope of the interfering element and subtracting the appropriate signal ratio from the isotope of interest. Equations for the correction of the data are presented below in Table 3.

Table 3.

ELEMENT	MASS	EQUATION
Vanadium	51	(51)*1 - (53)*3.127 + (52)*0.353
Arsenic	75	(75)*1 - (77)*3.132 + (82)*2.736 - (83)*2.761
Selenium	82	(82)*1 - (83)*1.0087
Molybdenum	98	(98)*1 - (99)*.0146
Cadmium	111	(111)*1 - (108)*1.073 + (106)*0.764

3.3.5 Physical interferences are effects associated with the sample nebulization and transport processes. Properties such as the change in viscosity and surface tension can cause significant inaccuracies, especially in samples that may contain high dissolved solids and/or acid concentrations. These interferences are greatly

reduced in this procedure by the use of mass flow controllers for the control of the argon flow rate, the use of a peristaltic pump for sample introduction, and the use of internal standards.

4.0 SAFETY

4.1 It is mandatory to wear a laboratory coat, closed-toe shoes, and safety glasses in the Laboratory. Gloves shall be worn while working with samples and acids.

4.2 All steps involving the use of concentrated acids shall be performed in a fume hood.

4.3 Material Safety Data Sheets (MSDS) are on file and available at all times to personnel using hazardous materials. It is the responsibility of everyone using these materials to be familiar with the potential hazards to the chemicals in their work area. If the analyst is uncertain of the potential hazards of specific chemicals, contact a supervisor prior to using these chemicals.

4.4 Extreme caution, awareness and knowledge of the location and safe use of fire extinguishers, eye wash fountains, and safety showers are required.

4.5 Personnel performing this procedure shall be instructed in the safe use of acids, the requirements for protective equipment, and acid spill cleanup procedures.

5.0 APPARATUS AND MATERIALS

5.1 APDC procedure

5.1.1 250 ml polyethylene screw cap extraction bottles (Nalgene)

5.1.2 Teflon forceps

5.1.3 Polycarbonate filters; 47 mm, 0.45 µm pore size (Nucleopore)

5.1.4 15 ml polyethylene screw cap centrifuge tubes

5.1.5 Sonicator with heated water bath maintained at 65 ± 2 ^{o}C

5.2 Iron-palladium procedure

5.2.1 50 ml polycarbonate tapered centrifuge tubes with caps.

5.2.2 Centrifuge system

5.2.3 Sonicator with heated water bath maintained at 65 ± 2 ^{o}C

6.0 REAGENTS

6.1 APDC

6.1.1 Cobalt nitrate stock solution, 2000 mg/L- Dissolve 2.0 g cobalt metal (Fisher Scientific, certified) in 950 ml of Milli-Q water and 50 ml HNO_3 (Optima)

6.1.2 Cobalt nitrate, 200 mg/L – Dilute 10 ml stock solution (6.1) to 100 ml with Milli-Q water.

6.1.3 Ammonium Pyrrolidine Dithiocarbamate (APDC), 2% solution (Fisher Scientific)- Dissolve 2.0 g APDC in 100 ml Milli-Q water. Store solution at 4 ^{o}C; but use at room temperature.

6.1.4 Nitric acid 10%, ultrapure (Optima, Fisher Scientific)- Mix 10 ml HNO_3 into 90 ml Milli-Q water.

6.1.5 Milli-Q water

6.2 Iron-palladium

6.2.1 Nitric acid 20%, ultrapure (Optima, Fisher Scientific)- Mix 20 ml HNO_3 into 80 ml Milli-Q water.

6.2.2 Pure iron and palladium solution made 1:1, 1000 µg/ml (SPEX).

6.2.3 Ammonium hydroxide, concentrated, ultrapure (Optima, Fisher Scientific).

6.2.4 Sodium borohydride, 5% (Fisher Scientific)- Dissolve 0.5 g sodium borohydride in 10 ml Milli-Q water. A fresh solution is made on day of extraction.

6.2.5 Ammonium Pyrrolidine Dithiocarbamate (APDC), 2% (Fisher Scientific)- Dissolve 2.0 g APDC in 100 ml Milli-Q water. Store solution at 4 ^{o}C; but use at room temperature.

6.2.6 Milli- Q water

6.3 Reagent water-Water that is free from the metal(s) that would potentially interfere at the MDL for the metals listed in Tables 1 and 2. The water is prepared by distillation, deionization, reverse osmosis, anodic/cathodic stripping voltammetry, or other techniques that remove the metals and potential interferants.

6.4 Standard Stock Solutions- purchased from a reputable commercial source (Claritas ppt, SPEX CertiPrep Inc; Plasma Cal, SPC Science).

7.0 CALIBRATION AND MAINTENANCE OF THE ICPMS

7.1 Trace metal concentrations are determined by comparing the response of a known standard obtained from a certified source traceable to NIST.

7.2 An initial calibration curve is performed on the instrument covering the expected range of concentrations in the samples before each batch of samples is run and every 12 hours during sample analyses.

7.2.1 Two different commercially available standard solutions are run at three or more concentrations. The calibration standards are diluted to the appropriate levels of the operating range using reagent water containing 1% (v/v) nitric acid.

7.2.2 The standard solutions are prepared the day the samples are run. All calibration solutions are spiked with 1 ml of internal standard solution containing 1000ng/ml of Rhodium and Thulium.

7.3 The response factor is computed as follows:

$$RF = \frac{A_s \times C_{is}}{A_{is} \times C_s}$$

A_s: height or area of the response at the m/z for the analyte
C_{is}: concentration of the internal standard in the solution
A_{is}: height or area of the m/z for the internal standard
C_s: concentration of the analyte in the standard or blank solution

7.3.1 Compute the mean RF for each analyte using the individual response factors at each concentration.

7.3.2 If the RF value range is constant (<20%), the RF value is assumed to be invariant and thus used for calculations. If the range varies significantly, the results can be used to plot a calibration curve of response ratios, A_s/A_{is} vs. RF.

7.4 Calibration Verification- Initial calibration verification is performed immediately following calibration. The ICPMS is adjusted until verification criteria are met. After the criteria are met, the blanks and samples may be analyzed.

7.5.1 A second-source calibration standard at a mid-level concentration is analyzed before running the samples and then again every 20 samples.

7.5.2 Using the mean RF value, the percent recovery of each metal is obtained using the calibration curve in the initial calibration.

7.5.3 Compare the recovery of each metal with the corresponding limit for the calibration verification in Table 4. The response for the initial calibration can be used for the blanks and the samples if all of the metals meet the accepted criteria. If a value fails to meet the acceptance range, the system's performance is unacceptable for that compound. The problem should be identified and amended, or a new calibration check standard should be prepared and the test repeated.

Table 4.

METAL	CALIBRATION VERIFICATION
Arsenic	85-115
Cadmium	85-115
Copper	85-115
Lead	85-115
Nickel	85-115
Silver	85-115
Zinc	85-115

Table 4. Quality control acceptance criteria for performance tests for freshwater and effluent samples in EPA method 1640. All specifications are represented as percents. The specifications for cadmium, copper, lead and nickel were calculated from validation conducted on ambient, freshwater samples.

7.5 Tuning solution- this solution is used for mass calibration and instrument tuning before analysis. The solution is composed of the same stock solutions that are used to obtain the calibration curve. Internal standards are not added to this solution.

7.6 Continuing calibration verification (CCV)- Aliquots of multi-element stock standard is added to an aliquot of reagent water. The CCV is treated as a

sample and digested as the other samples, when applicable. The internal standards are added to the CCV as well.

7.7 An instrument maintenance schedule is maintained for the Hewlett Packard 4500 ICPMS. Dates and initials are recorded in a notebook located near the instrument.

7.7.1 The instrument is serviced by the manufacturer at least once per year.

8.0 QUALITY CONTROL

8.1 QA/QC records are maintained to document the quality of data generated. If any element falls outside the designated range, that element has failed the acceptance criteria. Failure to meet the stated requirement shall require that corrective action be taken to eliminate the problem prior to the analysis of any samples. Samples from the batch being analyzed at the time the failure is detected shall be reanalyzed after the corrective action has been taken. A batch is defined as 20 or less samples. If any sample cannot be reanalyzed, the result for that element shall be flagged and a detailed report is included with the result.

8.1.1 **Lab Blanks-** Two process blanks (reagent blanks), where Milli-Q water is treated as a sample, are run with each batch of samples (15 or less samples). The process blanks are used to assess if there is any internal contamination in the instrument. No element shall be detected at greater than 3 times the method detection limit. A rinse blank is used to flush out the instrument between samples to avoid contamination between samples.

8.1.2 **Field Blanks-** At least one field blank consisting of distilled water in a similar container as the sample container is transported to the sampling site. The blank is exposed to the environment while the actual samples are being collected.

8.1.3 **Matrix Spikes-** A matrix spike and matrix spike duplicate shall be analyzed with each batch of samples to determine precision for each element. A control chart is generated to document the precision. The relative standard deviation for all elements combined shall be within 15% and no single element shall be greater than 20% for those elements that are greater than 10 times the method detection limit.

8.1.4 **Duplicate Samples-** Each sample is extracted and analyzed in duplicate. If the duplicates are not in agreement, then the sample is re-extracted and reanalyzed.

8.1.5 **CRM/LCM-** Certified reference materials and/or lab control materials shall be analyzed with each batch of samples to evaluate accuracy for each element. The reported value shall be within 15% of the true value.

8.1.6 **Initial Calibration Check-** Prior to analyzing any samples, an initial calibration of the instrument is performed with each batch of samples (15 or less). This calibration shall be within 15% of the initial calibration curve (see Section 7.2).

8.1.7 **Internal Standards-** Internal standards shall be added in known amounts to blanks, calibration standards, continuing calibration verification solutions, and samples to compensate for instrumental drift. Elements that may be used are presented in Table 5. Relative response factors are used to correct responses of the target analytes.

Table 5. Internal Standards

INTERNAL STANDARD	MASS
Scandium (Sc)	45
Yttrium (Y)	89
Rhodium (Rh)	103
Terbium (Tb)	159
Thulium (Tm)	169
Bismuth (Bi)	209

9.0 SAMPLE COLLECTION, PRESERVATION AND HANDLING

9.1 Sampling personnel are required to wear clean, nontalc gloves at all times when handling sampling equipment and sample containers.

9.2 Before samples are collected, all sampling equipment and sample containers are cleaned by soaking in a 10% nitric acid solution for a minimum of 24 hours followed by five rinses with Milli-Q water. The bottles are capped and individually double-bagged and placed in a clean plastic box.

9.3 Sample bottles are opened only to collect the seawater sample and to add acid preservative. Samples are preserved (at the sampling site or upon

return to the lab) with 1 ml of Optima concentrated nitric acid per liter of sample that will bring the pH ≤ 2.

9.3.1 Samples must be acidified within 48 hours of sampling at 4 ± 2 ^{o}C until acidified.

9.3.2 Once acidified, the samples must sit for at least 48 hours allowing the acid to completely dissolve the metals absorbed on the walls of the container.

9.3.3 The sample must have a pH<2. If the pH of the samples is >2, more acid will be added and they must sit for 16 hours. The pH must be verified to be <2 before analysis.

9.4 With each sample set, preserve a field blank, a method blank and a CCV (continuing calibration check) in the same way as the sample(s).

10.0 PROCEDURE

10.1 APDC METHOD

10.1.1 Before a sample can be processed, the sample pH should be verified/adjusted to pH ≤ 2. Do not measure the pH directly from the original sample; rather, pour a small aliquot of sample into a separate container to verify the pH.

10.1.2 Transfer a 200 ml aliquot from a well-mixed, acid preserved sample to a pre-calibrated polyethylene bottle.

10.1.3 To the 200 ml sample add 1 ml of 200 mg/L cobalt nitrate solution. Cap the bottle and mix by shaking. Let the solution stand for 2 minutes.

10.1.4 Remove the cap, add 1 ml of 2% APDC and re-cap the bottle and shake gently for 1 minute. This mixture is set aside to react for a minimum of 30 minutes.

10.1.5 The mixture is filtered through an acid cleaned 0.45 µm Nucleopore filter using an acid cleaned Millipore vacuum filtration system. The Nucleopore filter is handled with Teflon forceps.

10.1.6 The empty polyethylene bottle that once contained the sample is subsequently rinsed with 5 ml of Milli-Q water (acidified to pH 2.0 with Optima HNO_3) and this is filtered through the Nucleopore filter.

This is repeated 2 times. Finally, the Millipore vacuum filtration cup is rinsed with an adequate amount of acidified Milli-Q water to ensure any particles sticking to the sides are rinsed onto the filter. This final step also ensures that all seawater matrix has been rinsed through the filter.

10.1.7 The filter containing the filtrate is removed from the filtration system and folded into quarters ensuring no contact is made with the filtrate. This is then inserted into a 15 ml acid cleaned centrifuge tube.

10.1.8 Add 2 ml of 10 % Optima nitric acid into the centrifuge tube containing the filter using a clean pipette.

10.1.9 The tube containing the filter is placed in a sonicator with a water bath maintained at 65 ± 2 ^{o}C and allowed to digest for 2 hours.

10.1.10 Dilute sample (with filter) by adding 8 ml Milli-Q water, re-cap and allow to cool. The sample is now ready for analysis by ICP-MS.

10.1.11 The extracts can be stored at 4 ± 2 ^{o}C until analysis; however, they should be analyzed as soon as possible after the extraction.

10.1.12 Internal standards are added just prior to analysis by ICP-MS.

10.2 IRON-PALLADIUM METHOD

10.2.1 Before a sample can be processed, the sample pH should be verified/adjusted to pH ≤ 2. Do not measure the pH directly from the original sample; rather, pour a small aliquot of sample into a separate container and verify the pH.

10.2.2 Transfer a 50 ml aliquot from a well-mixed, acid preserved sample to an acid cleaned 50 ml polycarbonate centrifuge tube.

10.2.3

10.2.4 To the 50 ml sample add 0.5 ml 1:1 1000 µg/ml of Fe/Pd solution with a clean pipette.

10.2.5 Next, add 0.3 ml ammonium hydroxide to the sample and mix by shaking.

10.2.6 Then add 0.5 ml 5% $NaBH_4$ solution, 0.25 ml 2% APDC solution and mix by shaking. This sample is set aside to react for a minimum of 1 hour.

10.2.7 After a minimum of 1 hour, the sample is centrifuged at 2500 rpm for 30 minutes. Upon completion, the seawater matrix is carefully decanted and discarded.

10.2.8 To the remaining pellet, add 1 ml 20% Optima nitric acid with a clean pipette.

10.2.9 The centrifuge tube is placed in a sonicator with a water bath maintained at 65 ± 2 ^{o}C and allowed to digest for 2 hours or until all precipitate is dissolved.

10.2.10 After the digestion is complete and the extract(s) have reached room temperature, the sample(s) can be stored at 4 ± 2 ^{o}C until analysis by ICP-MS; however, they should be analyzed as soon as possible. Internal standards can be added just prior to analysis by ICP-MS.

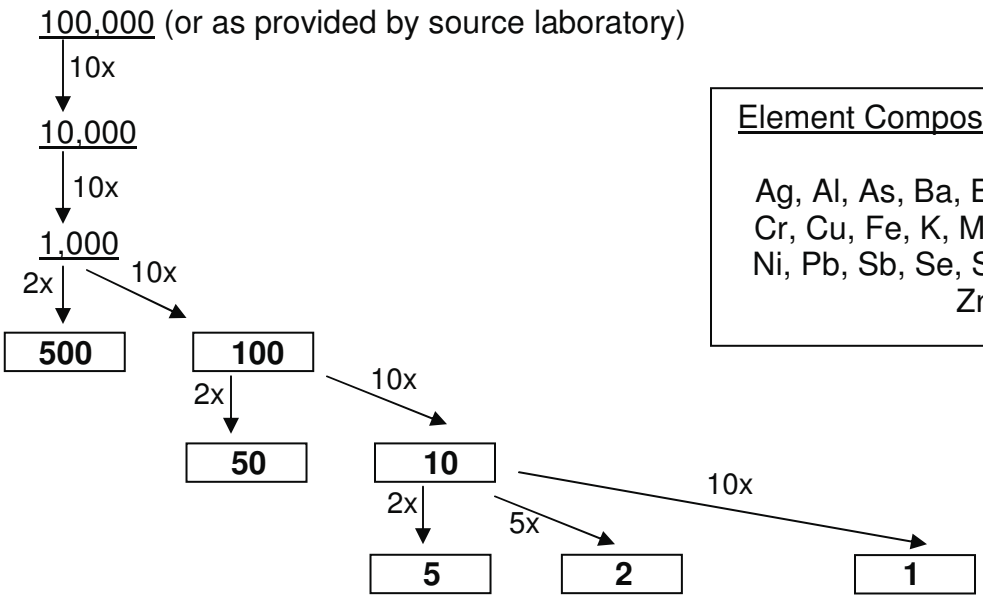
10.3 Sample Analysis- Standards are prepared by serial dilutions before each run on the ICPMS. The pipettes and autosampler tubes used for analysis and standards are calibrated before standards are run. Pipettes are calibrated by weighing 1.000 ml of DI water (=1.000 gram) and calibration marks on the autosampler tubes are checked.

10.3.1 Use volumetric flasks and calibrated pipettors to make calibration standards by diluting 10 mL of a commercially prepared stock solution to 100 mL. The standard source laboratory and lot number of each of the standards used for each ICPMS run are recorded in the laboratory notebook.

10.3.2 Mix standards by inverting and shaking a minimum of 10 times. A dilution stock of 2% HNO_3 and 1% HCl is used for all dilutions. Prepare 5 concentrations of calibration standards ranging from the method detection limit to at or above the maximum expected concentration in the sample. The standards in the boxes below are used in the calibration curves(see diagrams on pages 16 and 17).

GENERAL CALIBRATION MIX (ng/ml) DILUTIONS

FOR LOW CONCENTRATION SAMPLES

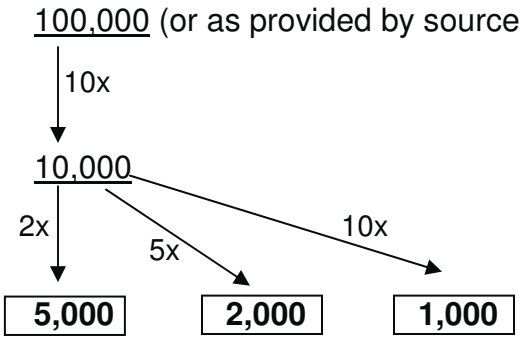


Element Composition of Low Mix:

Ag, Al, As, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Sn, Sr, Ti, Tl, V, Zn

GENERAL CALIBRATION MIX (ng/ml) DILUTIONS FOR HIGH CONCENTRATION SAMPLES

(continuation of calibration mix of low concentrations)

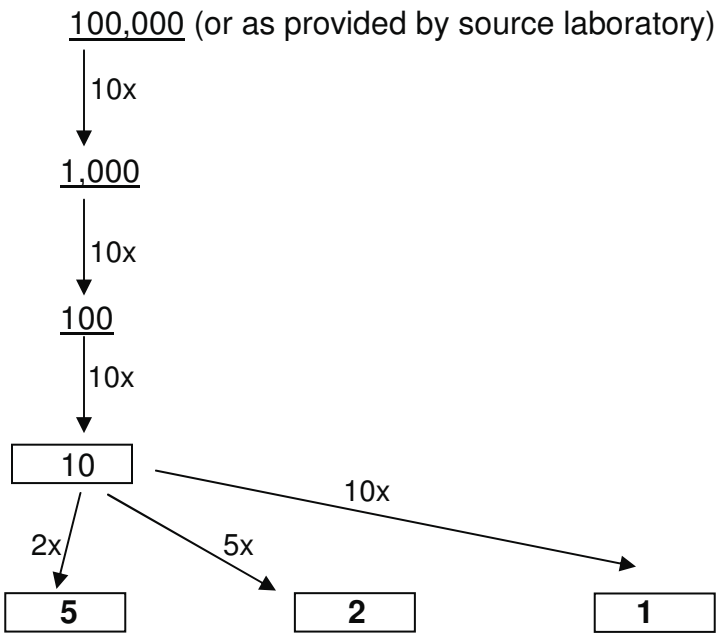


Element Composition of High Mix:

Al, Ba, Ca, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Sr, Ti, V, Zn

CALIBRATION DILUTION FOR MERCURY STANDARDS (ng/ml)

_____



10.3.3 Instrument parameters are stored in the computer program that operates the ICPMS. These parameters are listed in Table 7.

10.3.4

Table 7. Instrument Parameters

PARAMETER	SETTING
RF Power	1350 watts
Acquisition Mode	Spectrum Analysis
Detector Mode	Auto
Acquisition Points/Mass	3
Acquisition Repetitions	3
Argon Flow Rate	16 L/min
Nebulizer	Concentric
Sample Uptake Rate	0.4 mL/min
Sample Uptake Time	90 sec
Pump Stabilization Time	45 sec
Rinse Time	15 sec
Carrier Gas Flow Rate	1.26 L/min
Auxilliary Gas Flow Rate	1.0 L/min
Spray Chamber Temperature	2 °C
Sample Depth	7.5 mm[18]

10.3.5 Fill the autosampler rinse container with deionized water.

10.3.6 Empty the spray chamber drain bottle and fill to approximately 1/4 full with tap water. It is important that the drain line from the spray chamber be immersed in water to prevent fluctuations in the plasma.

10.3.7 Turn on the argon gas supply.

10.3.8 Ignite the plasma and allow a minimum of 30 minutes for stabilization.

10.3.9 Check the system operating conditions by tuning the instrument according to the parameters listed in Table 8. If parameters do not fall within these limits, retune the instrument per manufacturer's procedures. Once you are satisfied with the tune, save the parameters and print out a copy for the laboratory notebook.

Table 8 Optimal Tune Results

PARAMETER	OPTIMAL RESULT
Sensitivity for AMU 2	7,000 counts
Sensitivity for AMU 89	15,000 counts
Sensitivity for AMU 205	10,000 counts
RSD for AMU 2, 89, & 205	< 5%
Pulse to Analog Factors	100 ± 1
Doubly Charged Ions	< 3%
Oxides	< 1%
Axis	± 0.05 AMU
Peakwidth	0.65 - 0.75 AMU at 10%

10.3.10 Load the appropriate method file into the Chemstation data system. Complete the sample sequence table with the specified sample information and dilution factors. Load the samples into the autosampler according to the order listed in the sequence file. Double check to make sure the standards, blanks, and samples are in the correct autosampler position assigned in the sequence file.

NOTE: The instrument may be set for automatic shutoff at the end of the sequence by adding the following command in the last line of the sequence file:

TYPE = Keyword

KEYWORD = Command

KEYWORD COMMAND = tune ”macro`shutdown.mac’,go”
(this must be typed exactly as written here)

10.3.11 Start the analytical sequence and make sure that it is operating properly.

11.0 CALCULATIONS

11.1 For water samples, concentration factors necessary for the subsequent ICP-MS analyses are calculated by dividing the original seawater volume by the final digestate volume**.**

STANDARD OPERATING PROCEDURE FOR THE DETERMINATION OF TOTAL ORGANIC CARBON (TOC) USING THE SHIMADZU SSM-5000A

1.0. SCOPE AND APPLICATION

This protocol describes a standard procedure for the determination of Total Organic Carbon (TOC) using the Shimadzu SSM-5000A solid sample module.

2.0. SUMMARY OF METHOD

Samples are weighed in ceramic boats and inorganic carbon removed by HCl. The samples undergo oxidative combustion infrared analysis. The boats are loaded into the elemental analyzer combustion reactor tube to be completely oxidized. The CO_2 generated from the combustion is measured by an infrared detector (non-dispersive infrared analysis (NDIR)) and results in a TOC peak that is correlated to the total CO_2 concentration in the sample aliquot.

3.0. INTERFERENCE

3.1. All working surfaces and equipment should be clean and free of particles which can increase carbon levels in the samples. Use aluminum foil on all working surfaces. Use latex gloves to prevent oils from the hand from getting into samples.

3.2. Ceramic boats should be cleaned with soap and water. Soak in 2M Sulfuric acid for 10 min. Rinse with tap water, Follow with D.I. water and kiln at 900 °C for at least 20 min and store in a clean glass jar.

3.3. Weighing spatula should be cleaned with Kimwipes to prevent contamination between samples.

3.4. Check gas cylinder pressure.

4.0. APPARATUS AND MATERIALS

4.1. Apparatus and materials

4.1.1. Shimadzu TOC-VCPH/CPN and Shimadzu SSM-5000A Solid Sample Module.

4.1.2. Sartorius balance capable of weighing $\pm$ 0.01 mg .

4.1.2. Ceramic boats.

4.1.3. Fume chamber for acid exposure

4.1.4. Pasteur Pyrex borosilicate glass pipettes.

4.1.5. Mortar and pestle.

4.1.6. Forceps

4.2. Gas Supply

4.2.1. Compressed Air.

4.3. Reagents

4.3.1. Cobalt oxide catalyst (pn. 630-00566)

4.3.2. Platinum catalyst. (pn. 638-60202-01)

4.3.3. Conc. HCl trace metals grade (Baker A508-500)

4.3.4. Acetanilide Standard.

4.3.5. Cyclohexanone-2,4-dinitro-phenylhydrazone C=51.79% N=20.14%

4.3.6. PACS-1 Marine Sediment. Marine Analytical Chemistry Standard Program Institute for Environmental Chemistry, Ottawa Canada.

4.3.7. PACS-2 Marine Sediment. Marine Analytical Chemistry Standard Program Institute for Environmental Chemistry, Ottawa Canada.

4.3.8. SRM 1941b Organics in Marine Sediment. NIST

5.0. SAMPLE HANDLING AND PRESERVATION

5.1. Sample Handling

5.1.1. A chain-of-custody should be maintained as samples are received they are checked for damage and logged into the laboratory sample ID file.

5.2. Sample Storage

5.2.1. Store sediment samples in clean whorl pack bags or glass jars.

5.2.2. Store sediment and filter samples in a freezer at –20 °C until analyzed.

6.0. PROCEDURE

6.1. Sediment samples

6.1.1. Homogenize by stirring wet sediment with a glass rod.

6.1.2. Take at least 10g of sub-sample and freeze in beaker. Also determine moisture content if necessary (see chap. 14).

6.1.3. Freeze dry at least 16 hours.

6.1.4. Grind sediment sample with mortar and pestle into a fine homogeneous powder. Store sample in a clean, labeled 5 ml. vial.

6.1.5. If soot carbon values are needed oxidize about 1 g of sample at 375° C for 24 h in a glass Petri dish placed in a muffle furnace. Treat oxidized sample for soot carbon as normal TOC sediment samples (incl. HCl treatment)

6.1.6. Weigh about 40-50 mg. from each vial into a numbered clean ceramic boat and place in the holding container. Record weight and number of ceramic boat for each sample. Reduce the impact of external contaminants by choosing a sample amount that will produce a result close to the upper limit of the instrument (about 30mg C)

6.1.7. Go to 6.3.

6.2. Filter paper samples from TSS filtration apparatus. (2.5 cm diameter)

6.2.1. Use entire filter with sample.

6.2.2. Record weight of filtrate on each filter as determined from TSS analysis.

6.2.3. Place the filter into a numbered glass petri dish and record number for each sample.

6.2.4. Use clean dry filters as blanks and process as unknown samples. (subtract blank % value from unknown sample % value to determine final sample % value)

6.2.5. Go to 6.5.

.

6.3. Prepare calibration standards and controls.

6.3.1. Five Acetanilide standards are used to determine the calibration curve.

Acetanilide Standard #1	0.5 mg
Acetanilide Standard #2	2.5 mg
Acetanilide Standard #3	5.0 mg

6.3.2. One set of Acetanilide and Cyclohexanone samples is processed for every batch of 10 samples.

Acetanilide Control	4-5 mg
Cyclohexanone Control	4-5 mg

6.4. Add ~3-4 drops of 6N HCl to sediment or filter samples to remove inorganic carbon.

6.4.1. Do **not** treat PACS-1 and PACS-2 with HCl. Treat SRM 1941b with HCl.

6.4.2. Do not expose Acetanilide standards or Cyclohexanone to HCl.

6.4.3. Treat soot carbon samples with HCl.

6.5. Place under hood for several hours until HCl dries then place samples in drying oven set at 50° C for 24 hrs. to remove CO_2 produced from the reaction of HCl and inorganic carbon.

6.6. Enter samples into program file.

6.7. Instrument.

6.7.1. Turn TOC-VCPH/CPN and SSM-5000A instruments on.

6.7.2. Select TOC-ControlV Ver. 1.05 icon on desktop.

User: System
Password: TOC6001

6.7.3. Select sample table editor.

6.7.4. Highlight SSM5000A for TOC use.

6.7.5. Under file heading select [New] follow with [sample run] SSM 5000A choice.

6.7.6. Insert calibration curve [SSM_cal_2013] into sample table. Edit calibration points manually with new weights of standard.

Conc.: 71.09%
Weight: 0.5, 2.5, 5.0 mg

6.7.7. Insert sample. Use method SSM_method_2013.met.

6.7.8. Open compressed air valve. Under instrument heading select [connect]. Follow selecting [use settings on PC]. TOC-VCPH/CPN instrument pressure will read 200 kPA and SSM pressure will read 200 kPA. SSM carrier gas pressure should be set at 0.5 L/min. SSM temp will start to rise to 900 °C and TOC-VCPH/CPN instrument to 680 °C. Check background monitor for baseline position, fluctuation and noise before starting first sample.

6.7.9. Under instrument heading select [start]. The screen will prompt "press [repeat] to start another replicate or [next] to continue the sample sequence". Select [next]. Select [start] again and enter weight of sample. Insert the sample into the SSM holding position, wait 2 minutes for ambient CO_2 to clear from system. In some cases it may take near 5 minutes for ambient CO_2 to clear from system. Before running samples run blank to determine at what point in time ambient CO_2 peak is appearing. Slide sample into combustion chamber and press [start]. Open sample window to view sample peak profile. It will take approximately 2 minutes for a peak to begin (if ambient CO_2 is present a peak will form before the 2 minute mark). Sample run will end approximately after 5-6 minutes at which point a prompt will appear to pull sample boat back to the cooling position and finally sample change position. Repeat for each sample.

7.0. QUALITY ASSURANCE/QUALITY CONTROL

7.1. Control Samples

7.1.1. One set of Acetanilide and Cyclohexanone samples is processed for every batch of 10 samples.

Substances	Theoretical Values %C
Acetanilide	**71.09**
Cyclohexanone	**51.79**

7.2. Sample Replicate

7.2.1. One or more replicate samples are processed for each batch of 10 samples. The samples chosen should contain detectable amounts of TOC.

7.3. Standard Reference Material (SRM)

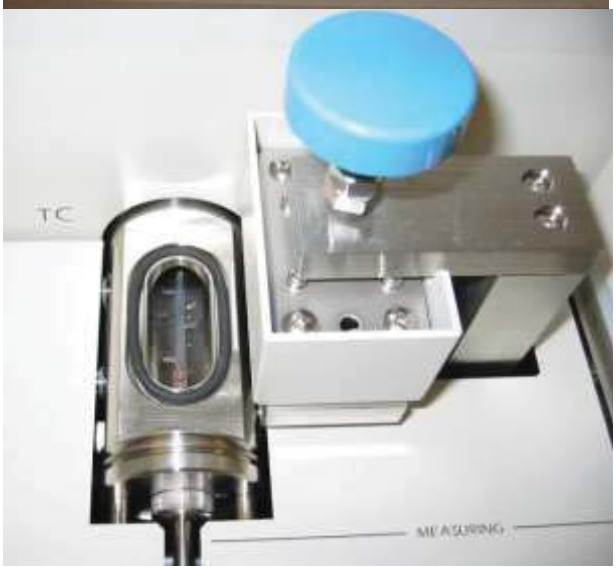
7.3.1. A standard reference material (SRM) obtained from the Marine Analytical Chemistry Standard Program Institute for Environmental Chemistry should be processed for every batch of 10 samples.

Substances	**Values**	
	%C	**%TOC**
PACS-1	**3.69 ± 0.11**	
PACS-2	**3.3**	
SRM 1941b	**3.3**	**2.99 ± 0.24**

8.0. SAFETY

8.1. Analyst should wear gloves during sample preparation to prevent sample and chemical contact with the skin.

8.2. Analyst should read the MSDS for all the chemicals and reagents used and follow all safety recommendations outlined in the MSDS.


TC
MEASURING

Southern California Coastal Water Research Project Toxicology Laboratory
Standard Operating Procedure for Mussel Embryo Development Test

I. Overview

This method estimates the toxicity in aqueous samples by a 48 hour exposure of *Mytilus galloprovincialis* embryos. The test endpoint is normal embryo development and survival. The test is based on methods in the EPA's Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms (EPA/600/R-95/136). The purpose of this SOP is to detail the test procedure as specifically applied in our laboratory. The SOP is intended to supplement the material in the protocol, not replace it.

II. Supply Checklist

- Deep trays for use as water baths (2)
- Glass bread pan (2)
- Seawater and DIW squirt bottles
- pH, DO and conductivity meter/probes
- Graduated cylinders 50-1000 ml for making gamete and solution dilutions
- Automatic pipets 0.1 ml up to 10 ml
- Water pump
- Tubing
- Thermometer
- 250 ml, 400 ml and 1 L beakers (several)
- Inverted microscope
- Counter, 2 unit
- Sedgwick-Rafter counting chamber
- Perforated plunger to fit 250 ml, 400 ml and 1 L beakers
- Nitex screening 100 µm or smaller openings
- Razor blades
- Eppendorf Pipet tips (100 µl, 1 ml and 10 ml)
- Shell vials with translucent caps, 5 dram
- Formalin, 30% borax buffered (see recipe below)
- Dispenser for formalin to repeatedly deliver 1 ml
- Pasteur pipets and bulbs (both 5 ¾ and 9 in)
- Scintillation vial racks (plastic for exposure, cardboard for storage)
- Spawning and gamete calculation data sheet
- Glass or Fiberglass aquaria tanks (3)
- Air pump
- Pairing knife.
- Air stones
- UV Light –to pass seawater through

III. Animals Collection and Culturing

Adult Bivalves (*Mytilus galloprovincialis*) are obtained from a commercial supplier. Set up glass aquaria in the cold room. To each tank add about six inches of seawater the day before the mussels arrive. Temperature shock may cause the animals to spawn; therefore once you have received the mussels, the animals should be acclimated to the cold room by opening the travel cooler. After about two hours of acclimation, transfer the mussels equally among the tanks, and add air stones.

The seawater should be changed everyday. Mussels can remain in holding under optimal conditions up to eight weeks from receiving date. No food is given to the mussels while in holding. Water quality measurements (pH, ammonia, DO and salinity) should be made on the system on a weekly basis.

IV. Test Design

Summary of test conditions

Type: Static non-renewal
Salinity: 32 ± 2 g/kg
Temperature: 15 ± 1 °C
Duration: 48 hours
Endpoint: normality of development and survival
Exposure volume: 10 ml
Test containers: 29.35 x 55 mm (5 dram) glass shell vial with snap cap.
Lighting: Ambient laboratory
Photoperiod: 16 hours Light and 8 hours Dark
Salinity adjustment: Hypersaline brine
Dilution water: natural seawater (activated carbon and 0.45 µm filtered)
Water Quality: DO, pH, salinity and ammonia (optional)
Reference toxicant: concurrent with each experimental batch, ammonia chloride or copper chloride

Exposures should be conducted in 5 dram glass shell vials. The vials should be vigorously rinsed with DIW and allowed to dry before use. Vials should be labeled and randomly distributed in vials racks (based on our experiment set-up randomization program).

The sample volume is 10 ml per replicate, with 4 replicates per concentration. Include an additional 5 vials of 32 ‰ seawater to determine the actual embryo density. After the samples are in the vials, the vials should be placed in the 15 ºC room for at least ½ hr before starting the exposure. The vials should be kept covered with parafilm whenever possible from the time of labeling through the end of the exposure to prevent cross contamination and evaporation.

V. Sample Handling

Care should be taken during sample preparation and dilution that cross contamination of glassware used for the samples and for the gametes does not occur. The exposure vials should be covered at all times to prevent contamination.

Samples having a salinity of less than 30 ‰ should be adjusted using hypersaline brine. To make the brine, first place a glass container (usually a 1 L beaker or 1 Gal jar) of seawater in a freezer for at least 18 hr. Remove the container from the freezer and allow the ice to thaw at room temperature. During the thawing process, occasionally pour off the thawed brine to a clean beaker. When the salinity of the brine is close to the desired level, or the volume needed is achieved, final dilution of the brine to the desired level should be made using seawater. The salinity of the brine used for sample adjustment should never exceed 80 ppt, as higher levels have been known to cause toxicity. When testing samples that have no saline content (stormwater, sewage effluent, etc) it is usually desirable to make the brine at 64 ‰ so that a 50:50 mixture of sample and brine has a final salinity of 32 ‰. We have found that brine may be stored in the refrigerator for up to a week.

Water quality measurements are made at the beginning and end of the testing time. Separate sub-samples for water quality analysis of each test sample or dilution should be taken at the time the samples are prepared. Samples should be measured for pH, DO and salinity. Ammonia analysis should be considered optional.

VI. Reference Toxicant

Each test of field or laboratory samples should include a concurrent reference toxicant exposure to ammonia. Copper can be used as an alternative reference toxicant. The reference toxicant exposure should include a control (0 µg/L) and five concentrations of ammonia.

The ammonia concentrations are prepared with ammonium chloride. The ammonia concentrations tested should be 0, 2.0, 4.0, 6.0, 8.0 and 10.0 mg/L. First prepare a stock solution of 1000 mg/L ammonia with 0.297g of NH_4Cl and 100 mL DIW. Then use the stock solution to achieve these concentrations by adding 0.2, 0.4, 0.6, 0.8, and 1.0 mL of stock solution to seawater to make 100 ml of each concentration. An ammonia sample will also be measured from each concentration on day 0 in order to calculate the actual total and unionized ammonia concentrations. An extra vial for each concentration should be included at test initiation for water quality analysis at test termination.

The copper reference toxicant concentrations are prepared by first making a stock solution of 10,000 µg/L copper. This stock solution consists of 0.0268 g $CuCl_2 \cdot 2 \cdot H_20$ in 1 L DIW. A working stock is prepared by diluting 10 ml of stock solution into 90 ml of seawater to produce a concentration of 1,000 µg/L. These concentrations are achieved by

adding 0.45, 0.65, 0.95, 1.39, 2.04, and 3.00 mL of working stock to seawater to make 100 ml of each concentration. An approximately 40 ml sample of the highest concentration should be saved in a plastic container for copper concentration verification. This sample should be preserved by adding two drops of concentrated, redistilled nitric acid then storing it in the refrigerator.

VII. Test Procedure

A. Before Spawning Mussels

Fill about half full with 32‰ seawater two deep trays and heat with an aquarium heater to 20 ºC. Place both bread pans and the pump with tubing in one of the trays. With seawater, rinse about fifteen 250 ml beakers and fill with 75 mL of seawater at 15 ºC.

Gently scrap off the barnacles and other encrusting organisms with a pairing knife from twenty mussels. Then rinse animals with 32 ‰ seawater.

B. Mussel Spawning

Place the animals into bread pans in the 20 ºC seawater bath. Turn on the water pump so that there is flow in each pan. Note initial time of mussel addition, look for spawning mussels, after 30 min. stop the pump. Wait 15 min. If no spawning occurs place the mussels in a 15 ºC, 32 ‰ seawater bath for 15 min. then start the process again. At least two animals of each sex with good gamete quantity and quality are necessary.

C. Gamete Collection

When individual animals are observed shedding gametes, remove them from the pan. Rinse each animal individually thoroughly with 32 ‰ seawater and place in their own 250 mL beaker that has enough seawater to cover the animal at 15 ºC.

Early in the spawning process, using a clean Pasteur pipet mix up the eggs in the beaker from one female and transfer about 0.5 ml of egg solution to the rafter cell. Check the eggs on the microscope at 100X power. Greater than 90% of the eggs should be round, of average size, not clumped, and not containing germinal vesicles. If the eggs appear to be of good quality, add a very small amount of sperm to the eggs in the Rafter cell. Watch for motility of the sperm and the ability to fertilize. Continue checking so that all of the males and females are tested in this manner.

D. Egg Counting

Allow the eggs of the females that were deemed to be in good condition to settle to the bottom of their collection beakers. Pour off most of the water from each

beaker, then pour the remaining water with the eggs through the 100 um nitex screen into a 1 L beaker. After adding the eggs from all the "good" females, bring the water level in the beaker up to about 600 ml. Allow the eggs to resettle (about ½ hr. After the eggs have settled, again pour off most of the water, then again pour the eggs through the nitex into a clean 1 L beaker. Again bring the water up to about 600 ml.

Put 9 ml of seawater into each of two scintillation vials, labeled A and B. Using the perforated plunger mix the egg solution well and take a 1 ml sample and place it into vial A. Mix vial A well and take 1 ml sample from it and place in vial B. Mix vial B well and place a 1 ml sample onto the Rafter cell. Count all of the eggs on the Rafter cell on a microscope a 100X. If total count is less than 30, then use vial A for counting. Record the count in the appropriate place on the egg and sperm count form. Take a second sample from vial B and count. Record the second count. If the two counts are within 20% calculate the mean. If the counts are not within 20%, count one more sample before calculating the mean. The egg density target should be about 5000-8000 eggs/ml. This is a stock solution, so if the egg density is higher or lower it is ok; just use the actual value when calculating the embryo density. Density must not be less than 1500. If the density of the eggs is less than 1500, let the eggs settle and decant excess water. Recount the eggs as described above.

E. Sperm suspension

Filter high quality sperm through a 100 um nitex screen into one beaker and make a note as to which animals were used on the mussel spawning data sheet.

F. Trial fertilization test

A trial fertilization must be performed with each spawning event. A series of sperm dilutions will be performed to achieve final sperm to egg ratio. Use a 10 mL pipet with the tip cut off to place10 mL of egg suspension into three scintillation vials. Add 0.1, 0.3, and 1.0 mL of sperm suspension using pipets. Let these solutions sit for 1.5 –2.5 hours in the lab. Transfer about 0.5 ml of egg solution to the rafter cell. Check the eggs on the microscope at 100X power. Fertilized eggs will have a single polar body, a very small clear circle attached to an egg, or they will have multiple cells that look like Mickey Mouse ears. Use the ratio of egg to sperm that has the lowest amount of sperm to achieve >90% fertilization.

While the eggs are being fertilized, finish the egg counts and determine the eggs/mL concentration. (See Mussel Spawning Datasheet)

To calculate the sperm suspension volume necessary to add to the egg solution, take the volume of the egg suspension prepared in section D and multiply by the sperm to egg ratio determined in the trial fertilization.

G. Test Initiation

Add sperm to eggs (embryo suspension), and use the perforated plunger to mix the suspension. Adjust the embryo suspension density to 1500 – 3000/ ml. Our target density and volume for the embryo suspension is 2500 embryos/mL in 300mL of 32 ‰ seawater. (See Mussel Spawning Datasheet) Achieve this by measuring out the needed amount of embryo stock solution and add 15 °C seawater to a total value of 300mL. Use the perforated plunger to mix the suspension. Cover the beaker with parafilm and set aside until ready to use (do not let stand for more than one hour).

On the mussel spawning record form record the time that you will add the embryo solution to the first vial. Using the perforated plunger, continually agitate the embryo solution while adding 0.1 ml to each exposure container. Be careful to insure that the embryo solution is added to the liquid in the exposure containers and does not contact the side of the vials first. Record the time that you finish the embryo addition. Recover the vials with the parafilm. Record the temperature at which the exposure is being performed.

The 5 additional vials of seawater will serve as the initial embryo density sub-samples. One mL of 30% borax buffered formalin will be added to each vial within minutes of the embryo solution addition. These will be used to determine the survival in the controls and the other treatments. Record the counts on the embryo count form. Calculate the actual embryo density by averaging the 5 sub-samples.

48 hours after the start of the addition of embryos, transfer the racks of exposure vials to the Biology Lab. Terminate the test by adding 1 ml of 30% borax buffered formalin to each vial. This should be done inside a fume hood. The formalin should be dispensed from the re-pipettor. Secure a snap cap on each vial and give the vial a quick swirl to insure that the formalin is evenly distributed. This task is made easier with two people; one adding the formalin and the other capping and swirling the vials.

VIII. Microscopic Evaluation

The samples can be evaluated whenever convenient. There is not a known maximum holding time for preserved samples.

The samples are evaluated by placing the entire vial in a small petri dish and placing this over the objective port on the stage of the inverted microscope. The embryos are easily viewed at 100 X. Start at the top of the vial and move across to the opposite side, scoring

all "D" shape embryos as normal and those without the "D" shape as abnormal. Move the stage down one field of view and make another complete pass of the vial; continue this process until the entire vial has been counted. Record the results on the mussel embryo development examination data sheet and put a colored dot on the cap to designate it as counted.

IX. Data Analysis

There are three endpoints that can be analyzed. One endpoint is the percent normal. In this case the number of normal embryos is divided by the total number of normal and abnormal embryo present in a vial then multiplied by 100. A second endpoint is percent normal alive, which is the number of normal embryos present in the vial divided by the mean of the initial count multiplied by 100. The third endpoint is percent alive. In figuring the percent alive one assumes that if embryos are present, no matter what condition, then they are alive. To calculate percent alive sum of both the normal and the abnormal embryos and divide by the mean of the initial count of embryos multiplied by 100.

Enter the endpoint data into the Excel spreadsheet by container number. The means and standard deviations are calculated automatically by the spreadsheet. For each experiment, run an ANOVA and Dunnett's test using Toxstat. Use a point estimation program (such as Toxstat) to calculate the EC50 using the probit method.

The reference toxicant data are similarly entered in the appropriate Excel spreadsheet. Calculate the EC50 as above and plot this value on the running laboratory control chart for this bioassay.

X. Quality Assurance

Test Acceptability Criteria

Mean normal development in the controls must be at least 90%. Mean survival in the controls must be > 50%. The percent minimum significant difference (MSD) must be less than 25%.

Reference toxicant results

The reference toxicant EC50 should fall within two standard deviations of the mean on the control chart. If the EC50 falls outside this range, results of concurrent tests should be examined carefully. The investigator should include a discussion of the significance of the exceedance in any report of the data.

Deviations from test conditions

Deviations from acceptable test conditions must be recorded (i.e. temperature out of range). Best professional judgment will be applied to determine whether the deviation was significant enough to render the results of the test questionable. The investigator should include a discussion of the significance of the deviation in any report of the data.

XI. Cleaning procedures

The exposure vials are used as shipped except that they should be vigorously rinsed with DIW and allowed to dry before use. All glassware and plastic ware used in handling the gametes or samples should be processed under the normal toxicology lab cleaning procedure to remove metals and organics.

After it is decided that the embryo samples can be discarded, the vials should be emptied into the sink under a fume hood with running water. The vials should then be rinsed once with tap water and then discarded in the trash. To prevent injuries from broken glass, it best to accumulate the discarded vials in a separate trash bag and then discard directly to the dumpster.

XII. References

USEPA, 1995. "Short-term methods of estimating the chronic toxicity of effluents and receiving water to west coast marine and estuarine organisms. National Exposure Research Laboratory, Office of Research and Development. Cincinnati, Ohio.

Mussel Spawning Data Sheet

Experiment No. _____ Animal Source _____
Date _____ Time in Culture _____
Temperature of Water Bath _____

Mussel No.	Induction	Spawn	Sex	Comments
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				

Pooled eggs from mussels _____
Pooled sperm from mussels _____

Egg Counts

Sample	Dilution	Count	Eggs/mL

For 300 mL of embryo suspension at 2500 embryos/mL use:
300 x 2500 / (counted eggs/mL) = mL of egg stock

750000 eggs / _____ eggs/mL = _____ mL of egg stock

Time of embryo addition _____

Mussel Bioassay Number_____
EMBRYO EXAMINATION

Initials	Count Date	Vial Number	Sample	Normal	Abnormal	% Normal Alive

APPENDIX B
QUALITY ASSURANCE PROJECT PLAN

APPROVAL SIGNATURES FOR THE QAPP

Emiko Innes, Los Angeles County Public Works, Contract Manager

Signature Date

Steve Bay, Southern California Coastal Water Research Project, Project Director

Signature Date

Ashley Parks, Southern California Coastal Water Research Project, Laboratory and Data Analysis Lead/Quality Assurance Officer

Signature Date

Keith Maruya, Southern California Coastal Water Research Project, Chemistry Lead

Signature Date

Wayne Lao, Southern California Coastal Water Research Project, Chemistry Technician

Signature Date

Rich Gossett, Physis Environmental Laboratories, Inc., Contract Analytical Laboratory

Signature Date

1 QUALITY ASSURANCE PROJECT PLAN OVERVIEW

This Quality Assurance Project Plan (QAPP) establishes quality assurance (QA) objectives for conducting sampling and evaluation activities described in the Work Plan. The methods and QA procedures described herein will be followed by the Southern California Coastal Water Research Project (SCCWRP) and its contractors during various sample collection and data analysis activities beginning in 2019.

Field and laboratory activities will be conducted in such a manner for the results to meet specified data quality objectives (DQOs). Guidance for QA/quality control (QC) is derived from protocols developed for the U.S. Environmental Protection Agency (USEPA) SW-846 (2007), the USEPA Contract Laboratory Program National Functional Guidelines (USEPA 2009, 2010, 2014), and the methods described in Section 3 below.

2 FIELD QUALITY CONTROL

2.1 Data Collection, Processing, and Sampling Forms

All field activities will be recorded on field forms logged by field staff. Field forms will provide a description of sampling activities, a list of sampling personnel, weather conditions, and a record of all modifications to the procedures and plans identified in this QAPP if necessary. Field information will be recorded as shown in Attachment 1.

The following forms, included as Attachment 1, will be used to record pertinent collection, processing, and sampling information:

- Chain-of-custody (COC) form
- Daily log and sampling form
- Water profiling instrument calibration form

2.2 Sample Identification and Labels

Samples will be identified with a sample identifier that specifies the waterbody or site (Marina del Rey Harbor [MdRH]), basin or station location, and sample number.

An example sample identifier for the first sample collected from Basin E, would be:

MdRH-E1

An example sample identifier for a field blank of the decontaminated sample processing equipment after sample collection of the above sample would be:

FB-20150630

An example sample identifier for a field duplicate sample collected from Basin E, would be:

MdRH-E1-FD

Each sample will have a waterproof paper label affixed to the container and will be labeled at the time of collection. The following information will be recorded on the container label at the time of collection:

- Project name
- Sample identifier
- Date of sample collection
- Analysis to be performed
- Depth

An example label would be:

Project: **MDR SSO**
Sample ID: **MdRH-E1**
Sample #: 1
Analysis: Total Copper
Laboratory: Physis
Date: March 3, 2019
Depth: Surface

2.3 Sample Custody and Shipping Requirements

Samples are considered to be in one's custody if they are in the custodian's possession or view or in a secured location with restricted access.

COC procedures will be followed for all samples throughout the collection, handling, and analysis process. The principal document used to track possession and transfer of samples is

the COC form (Attachment 1). Each sample will be represented on a COC form the day it is collected. All entries on the COC form will be made using indelible ink pen. Corrections will be made by drawing a single line through the error, writing in the correct information, and dating and initialing the change. Blank lines/spaces on the COC form will be lined-out, dated, and initialed by the individual maintaining custody.

A COC form will accompany each group of samples to the analytical laboratory. Each person who has custody of the samples will sign the COC form and ensure that the samples are not left unattended unless properly secured. Copies of all COC forms will be retained in the project files.

Each cooler containing samples for analysis will be hand-delivered to Physis Environmental Laboratories, Inc. (Physis) the same day of sample collection or the following morning. In the event that Saturday delivery is required, the field coordinator(s) will contact the analytical laboratory on Friday to ensure that the laboratory is aware of the number of samples to be transferred. Following each shipment, the field coordinator(s) will call the laboratory and verify the shipment from the day before was received and is in good condition. Samples will be packed with ice to maintain recommended storage temperatures of 4°C. Ice will be sealed in separate double plastic bags and placed in the transportation coolers. Individual sample containers will be placed in a sealable plastic bag, packed to prevent breakage, and transported in an ice chest or other suitable container. The shipping containers will be clearly labeled with sufficient information (name of project, time and date of collection, and contact person) to enable positive identification.

Upon transfer of sample possession to the analytical laboratory, the persons transferring custody of the sample container will sign the COC form. Upon receipt of samples at the laboratory, the receiver will record the condition of the samples on a sample receipt form. COC forms will be used internally in the laboratory to track sample handling and final disposition.

2.4 Field Measurement Quality Objectives

Field measurement quality objectives include calibration and measurement accuracy for measurements including dissolved oxygen, pH, temperature, or salinity. Table B-1 summarizes the measurement quality objectives for field measurements. Field QC samples will also be collected and analyzed by the laboratory as indicated in Table B-2.

3 ANALYTICAL CHEMISTRY LABORATORY QUALITY CONTROL

3.1 Summary of Analytical Methods

All sample analyses will be conducted in accordance with approved methods: USEPA 1640 for total and dissolved metals analysis and USEPA 9060afor the analysis of total organic carbon by combustion or oxidation. Metals analysis will be conducted at Physis, which is accredited under California's Environmental Laboratory Accreditation Program ELAP (CA ELAP; Certificate No. 2769). Toxicity testing and DOC analysis will be conducted by SCCWRP. The contact person for the toxicity laboratory is Darrin Greenstein, who can be reached via email at darring@sccwrp.org or by phone (714) 755-3224. The contact person for the chemical laboratory is Rich Gossett, who can be reached via email at richgossett@physislabs.com or by phone at (714) 602-5320, ext. 201.

Prior to analysis, all samples will be maintained according to the appropriate holding times and temperatures for each analysis according to Table B-3. Table B-4 presents the analytes, analytical methods, and targeted reporting limits (RLs) for the chemical testing. Each participating laboratory will prepare detailed reports in accordance with Sections 3.5.6 and 4.1 of this QAPP. Those reports will be included as an appendix in the appropriate data report.

Prior to sample analysis, the laboratory will calculate method detection limits (MDLs) and method reporting limits (MRLs) for each analyte of interest, where applicable. MRLs will be at or below the values specified in Table B-4. Results detected above the MDL and below the MRL will be reported with a "J" qualifier. If required RLs are not achieved, some modifications to the methods may be necessary. These modifications from the specified analytical methods will be provided by the laboratory when establishing the laboratory

contract and must be approved by Los Angeles County Public Works (LACPW) and the Technical Advisory Committee (TAC) prior to implementation.

When completing chemical analyses for this project, the contract laboratories are expected to meet the following minimum requirements:

- Adhere to the methods outlined in this QAPP, including methods referenced for each analytical procedure (Table B-4).
- Deliver scanned and electronic data deliverables, as specified.
- Meet reporting requirements for deliverables.
- Meet turnaround times for deliverables.
- Implement QA/QC procedures discussed in this QAPP and its tables including DQOs, laboratory QC requirements, and performance evaluation testing requirements.
- Notify the project manager of any QA/QC problems when they are identified to allow for quick resolution.
- Allow laboratory and data audits to be performed, if deemed necessary.

3.2 Analytical Laboratory QA/QC Overview

Laboratory QC procedures, where applicable, include initial and continuing instrument calibrations, laboratory control materials (LCMs), laboratory control samples (LCSs), laboratory duplicates, matrix spikes (MS), and method blanks. Table B-5 lists the frequency of analysis for laboratory QA/QC samples, and Table B-6 summarizes the DQOs for precision, accuracy, and completeness.

QC sample results from each sample group will be reviewed by the analyst immediately after a sample group has been analyzed (Table B-6). QC sample results will then be evaluated to determine if control limits have been exceeded. If control limits are exceeded in the sample group, the QA/QC Manager will be contacted immediately and corrective action (e.g., method modifications followed by reprocessing the affected samples) will be initiated prior to processing a subsequent group of samples.

3.3 Laboratory Instrument Calibration and Frequency

An initial calibration will be performed on each laboratory instrument to be used at the start of the project, after each major interruption to the analytical instrument, and when any ongoing calibration does not meet method control criteria. An initial calibration verification (ICV) will be analyzed following each initial calibration and will meet method criteria prior to sample analysis. Continuing calibration verifications (CCVs) will be performed daily prior to any sample analysis to track instrument performance. The frequency of CCVs varies with the method. For metals and inorganic methods, one CCV will be analyzed for every 10 field samples, or daily, whichever is more frequent. If the ongoing continuing calibration is out of control, the analysis will be halted until the source of the control failure is eliminated or reduced to meet control specifications. All project samples analyzed while instrument calibration was out of control will be reanalyzed. Instrument blanks or continuing calibration blanks (CCBs) provide information on the stability of the baseline established. CCBs will be analyzed immediately prior to or following CCV at the instrument for each type of applicable analysis.

3.4 Laboratory Quality Control

3.4.1 Laboratory Duplicates/Replicates

Laboratory duplicates provide information on the precision of the analysis and are useful in assessing potential sample heterogeneity and matrix effects. Laboratory duplicates are subsamples of the original sample that are prepared and analyzed as a separate sample.

3.4.2 Matrix Spikes and Matrix Spike Duplicates

Analysis of MS samples provides information on the extraction efficiency of the method on the sample matrix.

3.4.3 Method Blanks

Method blanks are analyzed to assess possible laboratory contamination at all stages of sample preparation and analysis. The method blank for all analyses must be less than the MRL of any single target analyte/compound. If a laboratory method blank exceeds this criterion for any analyte/compound, and the concentration of the analyte/compound in any

of the samples is less than five times the concentration found in the blank (10 times for common contaminants), analyses must stop and the source of the contamination must be eliminated or reduced.

3.4.4 Laboratory Control Samples

LCSs are analyzed to assess possible laboratory bias at all stages of sample preparation and analysis. The laboratory control sample is a matrix-dependent spiked sample prepared at the time of sample preparation along with the preparation of samples and MS samples. The laboratory control sample will provide information on the precision of the analytical process and, when analyzed in duplicate, will provide accuracy information as well.

3.4.5 Laboratory Control Material

Laboratory Control Materials (LCMs) are substances of the same or similar matrix to the project samples. In this study, the LCM will be Physis seawater, which will be used as a reference for background concentrations in clean, natural seawater. The LCM will be prepared and analyzed in the same manner as routine samples and in the same preparation and analytical batch. The recovery of the target analyte(s) provides information on interferences caused by the sample matrix.

3.4.6 Laboratory Deliverables

Data packages will be checked for completeness immediately upon receipt from the laboratory to ensure that data and QA/QC information requested in Section 3.5.6 are present.

3.4.7 Granite Canyon Seawater

The decision to use seawater from Granite Canyon is based on its history of having low DOC and metals concentrations as well as no ambient toxicity. These parameters will all be analyzed in the unspiked control during the WER study. Additionally, an outside contract laboratory will provide duplicate toxicity testing of this water as described in the Work Plan. No specific holding time will be implemented for this water; however, holding time will be minimized and documented. Recently collected water will be used for each test.

3.5 Data Quality Objectives and Criteria

The DQOs for this project are to ensure that data collected are of known and acceptable quality. The quality of laboratory data is assessed by precision, accuracy, and completeness. Definitions of these parameters and the applicable QC procedures are given below. Frequency of QC samples is listed in Table B-5. Applicable quantitative goals for these data quality parameters are listed or referenced in Table B-6.

3.5.1 Precision

Precision is the ability of an analytical method or instrument to reproduce its own measurement. It is a measure of the variability, or random error, in sampling, sample handling, and laboratory analysis.

In the laboratory, "within-batch" precision is measured using replicate sample or QC analyses and is expressed as the relative percent difference (RPD) between the measurements. "Batch-to-batch" precision is determined from the variance observed in the analysis of standard solutions or LCSs from multiple analytical batches.

Field precision will be evaluated by collecting blind field duplicates for chemistry samples. Field chemistry duplicate precision will be screened against a RPD of 35 percent. However, no data will be qualified based solely on field duplicate precision.

Precision measurements can be affected by the nearness of a chemical concentration to the MDL, where the percent error (expressed as RPD) increases. The equation used to express precision is as follows:

$$RPD = \frac{(C_1 - C_2) \times 100\%}{(C_1 + C_2)/2}$$

Where:

RPD = relative percent difference

C_1 = larger of the two observed values

C_2 = smaller of the two observed values

3.5.2 Accuracy

Accuracy is a measure of the closeness of a measurement to the true or expected value. Accuracy is determined by calculating the mean value of results from ongoing analyses of laboratory-fortified blanks, LCMs, and standard solutions. In addition, laboratory-fortified (i.e., MS) samples will be measured; this sample type indicates the accuracy or bias in the actual sample matrix. Accuracy is expressed as percent recover (%R) of the measured value, relative to the true or expected value. If a measurement process produces results which are not the true or expected value, the process is said to be biased. Bias is the systematic error either inherent in a method of analysis (e.g., extraction efficiencies) or caused by an artifact of the measurement system (e.g., contamination). Analytical laboratories use several QC measures to eliminate analytical bias, including systematic analysis of method blanks, LCSs, and independent calibration verification standards. Because bias can be positive or negative, and because several types of bias can occur simultaneously, only the net, or total, bias can be evaluated in a measurement.

Laboratory accuracy will be evaluated against quantitative laboratory control sample, MS, and surrogate spike recovery performance criteria provided by the laboratory. Accuracy can be expressed as a percentage of the true or reference value, or as a %R in those analyses where reference materials are not available and spiked samples are analyzed.

The equation used to express accuracy is as follows:

$$\%R = 100\% \times (S-U)/Csa$$

Where:

%R	=	percent recovery
S	=	measured concentration in the spiked aliquot
U	=	measured concentration in the unspiked aliquot
Csa	=	concentration of spike added

Field accuracy will be controlled by adherence to sample collection procedures outlined in the sample collection sections of this QAPP.

3.5.3 Completeness

Completeness is a measure of the amount of data that is determined to be valid in proportion to the amount of data collected. Completeness will be calculated as follows:

C = [(Number of acceptable data points) x 100]/ (Total number of data points)

The DQO for completeness for all components of this project is 95 percent. Data qualified as estimated because QC criteria were not met will be considered valid for the purpose of assessing completeness. Data qualified as rejected will not be considered valid for the purpose of assessing completeness.

3.5.4 Sensitivity

Analytical sensitivities must be consistent with, or lower than, the values listed in Table B-4 in order to demonstrate compliance with this QAPP. When achievable, target reporting limits specified will be at least a factor of 2 less than the analyte's corresponding target criteria.

The MDL is defined as the minimum concentration at which a given target analyte can be measured and reported with 99 percent confidence that the analyte concentration is greater than zero. Laboratory RLs are defined as the lowest level that can be reliably achieved within specified limits of precision and accuracy during routine laboratory operating conditions. Laboratory MDLs and RLs will be used to evaluate the method sensitivity and/or applicability prior to the acceptance of a method for this program.

The sample-specific MDL and RL will be reported by the laboratory and will take into account any factors relating to the sample analysis that might decrease or increase the RL (e.g., dilution factor, percent moisture, sample volume, or sparge volume). In the event that the MDL and RL are elevated for a sample due to matrix interferences and subsequent dilution or reduction in the sample aliquot, data will be evaluated to determine if an alternative course of action is required or possible. The sample-specific RL will be provided in the project database.

3.5.5 Field Data Management

Field data forms will be checked for completeness and accuracy by the field coordinator(s) prior to delivery to the project manager(s) and disbursement to rest of the project team. Original forms will be retained and filed in a project binder after data entry and checking are complete.

3.5.6 Analytical and Chemistry Records and Deliverables

Analytical data records will be retained by the laboratory and in the project files. For all analyses, data reporting requirements will include items necessary to complete data validation. Laboratory analytical reports will be provided in electronic format, including the scanned PDF of the report and the Electronic Data Deliverable (EDD). The analytical laboratory will be required, where applicable, to report the following:

- Project Narrative. This summary, in the form of a cover letter, will discuss problems, if any, encountered during any aspect of analysis. This summary should discuss, but is not be limited to, QC, sample shipment, sample storage, and analytical difficulties. Any problems encountered, actual or perceived and their resolutions will be documented in as much detail as appropriate. The narrative should also include final dilution volumes for all samples analyzed at a dilution in which one or more analytes is reported as not detected.
- COC Records. Legible copies of COC forms will be provided. This documentation will include the time of receipt and condition of each sample received by the laboratory. Additional internal tracking of sample custody by the laboratory will also be documented on a sample receipt form. The form must include all sample shipping container temperatures measured at the time of sample receipt.
- Sample Results. Results for each sample analyzed will be provided. The summary will include the following information when applicable:
  - Field sample identifier and the corresponding laboratory identification code
  - Sample matrix
  - Date of sample preparation
  - Date and time of analysis
  - Identification of the instrument used for analysis
  - Analytical results with reporting units identified
  - Data qualifiers and their definitions

- QA/QC Summaries. Results of the laboratory QA/QC procedures will be provided. Each QA/QC sample analysis will be documented with the same information required for sample results (see above). No recovery or blank corrections will be made by the laboratory. The required summaries are listed below; additional information may be requested.
  - Method Blank Analysis. The method blank analysis associated with each sample and the concentration of all compounds of interest identified in these blanks will be reported.
  - MS Recovery. MS recovery data will be included. The name and concentration of all compounds added, %R, and range of acceptable recoveries will be listed. The recoveries and RPD for all MS duplicate analyses will be reported.
  - Laboratory Duplicate. The RPD for all laboratory duplicate analyses will be included.
  - Laboratory Control Sample. All laboratory control sample recovery data will be included. The name and concentration of all compounds added, %R, and range of acceptable recoveries will be listed. The recoveries and RPD for all laboratory control sample duplicate analyses will be reported.

All instrument data will be fully restorable at the laboratory from electronic backup. Laboratories will be required to maintain all records relevant to project analyses for a minimum of 7 years. Data validation reports will be maintained in the project files with the analytical data reports.

3.5.7 Data Reduction

Data reduction is the process by which original data (analytical measurements) are converted or reduced to a specified format or unit to facilitate data analysis. Data reduction requires that all aspects of sample preparation that could affect the test result, such as sample volume analyzed or dilutions required, be taken into account in the final result. It is the laboratory analyst's responsibility to reduce data, which are subjected to further review by the laboratory manager, the project manager, and independent reviewers. Data reduction may be performed manually or electronically. If performed electronically, all software used must be demonstrated to be true and free from unacceptable error.

3.6 Data Validation and Usability

3.6.1 Data Review, Validation, and Verification

During the validation process, analytical data will be evaluated for method QC and laboratory QC compliance, and their validity and applicability for program purposes will be determined. Based on the findings of the validation process, data validation qualifiers may be assigned. Validated project data, including qualifiers, will be entered into the Excel project database, thus enabling this information to be retained or retrieved as needed.

3.6.2 Validation and Verification Methods

Data validation includes review for completeness and accuracy by the field coordinator(s) and laboratory manager; review by the QA/QC manager (or designee) for outliers and omissions and the use of QC criteria to accept or reject specific data. All data will be entered into the Excel project database.

Laboratory data will be reviewed and verified to determine whether all DQOs have been met and that appropriate corrective actions have been taken, when necessary. Calculations will be verified by the laboratory. The project manager or designee will be responsible for the final review of all data generated from sample analyses.

The first level of review will take place in the laboratory as the data are generated. The laboratory manager (or designee) will be responsible for ensuring that data generated meet minimum QA/QC requirements and that the instruments were operating under acceptable conditions during data generation. DQOs will also be assessed at this point by comparing the results of QC measurements with pre-established criteria as a measure of data acceptability.

Data packages will be checked for completeness immediately upon receipt from the laboratory to ensure that data and QA/QC information requested are present. A Stage 2A data quality review will be performed in accordance with EPA National Functional Guidelines (USEPA 2010, 2014) by considering the following:

- Holding times
- Method blanks
- Detection limits
- RLs

- LCS/LCMs
- MS/MSD samples

Data will be validated in accordance with the project-specific DQOs described above, analytical method criteria, and each laboratory's internal performance standards based on their SOPs.

3.6.3 Reconciliation with User Requirements

The data will be reviewed after each survey to determine if DQOs have been met. If data do not meet the project's specifications, the project manager or designee will review the errors and determine if the problem is due to calibration/maintenance, sampling techniques, or other factors and will suggest a corrective action. It is expected that the problem would be correctable by retraining, revising techniques, or replacing supplies/equipment; if not, the DQOs will be reviewed for feasibility. If specific DQOs are not achievable, the project manager or designee will recommend appropriate modifications.

4 TOXICITY TESTING LABORATORY QUALITY CONTROL

All biological tests will incorporate standard QA/QC procedures, the tests are based on methods in the USEPA's Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms (USEPA 1995). Standard QA/QC procedures include the use of negative controls, reference toxicant samples, replicates, and water quality measurements during testing.

The negative control is used to establish the health of test organisms and ensure acceptability criteria are met. Control material will consist of filtered seawater adjusted to the appropriate salinity, if appropriate. Positive control or reference toxicant tests will be used to establish the sensitivity of test organisms. The reference toxicant test median lethal concentration (LC_{50}) or median effective concentration (EC_{50}) should fall within two standard deviations of the historical mean for the laboratory, indicating sensitivity is normal.

Proper water quality conditions will be maintained for all tests to ensure that organisms do not experience undue stress unrelated to test samples. If water quality measurements are outside protocol ranges, corrective action will be taken immediately. Laboratory equipment will be maintained, and all instruments will be calibrated regularly. All laboratory work will

be documented on approved datasheets.

4.1 Toxicity Laboratory Reporting

Toxicity test reports will be retained by the laboratory and stored electronically in the project files. The laboratory will be required, where applicable, to report the following:

- Test Methods. A summary of test conditions for each test will be included. All methods should be in accordance with guidelines described in the Work Plan and other guidance or as otherwise noted in the Work Plan.
- Test Results. Results will include a summary of the following information:
  - Test dates
  - Source of control material
  - Source of organisms
  - Water quality measurements
  - Appropriate lethal or sublethal endpoint results for each species
  - LC_{50} or EC_{50}
  - Control acceptability statement
  - Summary of reference toxicant test results
- Statistical Analyses. Statistical analyses will be performed to determine the median effective concentration (EC_{50}), or the statistically derived concentration indicative of toxic effects in 50% of test organisms under specific test conditions.
- QA/QC Summaries. The results of a QC review, with any protocol deviations and corrective actions taken, will be provided.
- Raw Data. Legible copies of raw datasheets used in testing, including water quality, daily observations, and final lethal or sublethal endpoint results, will be provided.
- Reference Toxicant Test Data. Raw datasheets, statistical analyses, and control charts comparing current test results with historical test results will be provided.
- COC Records. Legible copies of the COC forms will be provided. Forms will include the time of receipt and condition of each sample received by the laboratory. Additional internal tracking of sample custody by the laboratory will also be documented on a sample receipt form. The form must include all sample shipping container temperatures measured at the time of sample receipt.

5 INSTRUMENT/EQUIPMENT TESTING, INSPECTION, AND MAINTENANCE REQUIREMENTS

5.1 Field Instruments/Equipment

In accordance with the QA program, an inventory of field instruments and equipment will be maintained. The frequency and types of maintenance will be based on the manufacturer's recommendations and/or previous experience with the equipment.

The field coordinator(s) will be responsible for the preparation, documentation, and implementation of the preventative maintenance program. The equipment maintenance information will be documented in the instrument's calibration log. The frequency of maintenance is dependent on the type and stability of the equipment, the methods used, the intended use of the equipment, and the manufacturer's recommendations. Detailed information regarding the calibration and frequency of equipment calibration is provided in specific manufacturer's instruction manuals.

All maintenance records will be verified prior to each sampling event. The field coordinator(s) will be responsible for verifying that required maintenance has been performed prior to using the equipment in the field.

5.1.1 Laboratory Instruments/Equipment

In accordance with the QA program, the laboratory will maintain an inventory of instruments and equipment and the frequency of maintenance will be based on the manufacturer's recommendations and/or previous experience with the equipment.

The laboratory preventative maintenance program, as detailed in their QA plan, is organized to maintain proper instrument and equipment performance and to prevent instrument and equipment failure during use. The program considers instrumentation, equipment, and parts that are subject to wear, deterioration, or other changes in operational characteristics; the availability of spare parts; and the frequency at which maintenance is required. Any equipment that has been overloaded, mishandled, gives suspect results, or has been determined to be defective will be taken out of service, tagged with the discrepancy noted,

and stored in a designated area until the equipment has been repaired. After repair, the equipment will be tested to ensure that it is in proper operational condition.

Each laboratory will be responsible for the preparation, documentation, and implementation of the preventative maintenance program. All maintenance records will be checked according to the schedule on an annual basis and recorded by the responsible individual. The laboratory manager (or designee) shall be responsible for verifying compliance.

5.2 Instrument Calibration

Proper calibration of equipment and instrumentation is an integral part of the process that provides quality data. Instrumentation and equipment used to generate data must be calibrated at a frequency that ensures sufficient and consistent accuracy and reproducibility.

5.2.1 Field Instrument/Equipment Calibration

Field equipment will be calibrated prior to each sampling event according to the manufacturer's recommendations using the manufacturer's standards. A calibration check will be performed at the end of the day. The equipment, calibration, and maintenance information will be documented in the instrument calibration log. The frequency of calibration is dependent on the type and stability of the equipment, the methods used, the intended use of the equipment, and the manufacturer's recommendations. Detailed information regarding the calibration and frequency of equipment calibration is provided in specific manufacturer's instruction manuals.

Equipment that fails calibration or becomes inoperable during use will be removed from service and tagged (time and date of action) to prevent inadvertent use. Such equipment will be satisfactorily recalibrated or repaired and tagged (date and time of return to service) prior to use.

5.2.2 Laboratory Instrument/Equipment Calibration

As part of their QA/QC program, laboratories perform two types of calibrations. A periodic calibration is performed at prescribed intervals (i.e., balances, drying ovens, refrigerators, and thermometers), and operational calibrations are performed daily, at a specified frequency, or

prior to analysis (i.e., initial calibrations) according to method requirements. Calibration procedures and frequencies are discussed in the laboratory's QA plan. Calibrations are discussed in the laboratory's standard operating procedures (SOPs) for analyses.

The laboratory manager (or designee) will be responsible for ensuring that laboratory instrumentation is calibrated in accordance with any specifications. Implementation of the calibration program shall be the responsibility of the respective laboratory group supervisors. Recognized procedures (USEPA, ASTM, or manufacturer's instructions) will be used when available.

Physical standards (i.e., weights or certified thermometers) will be traceable to nationally recognized standards, such as the National Institute of Standards and Technology (NIST). Chemical reference standards shall be NIST standard reference materials or vendor-certified materials traceable to these standards.

Calibration requirements for each method and respective corrective actions will be accessible, either in the laboratory's SOPs or the laboratory's QA Plan for each instrument or analytical method in use. All calibrations will be preserved on electronic media.

6 PERSONNEL TRAINING

6.1 Southern California Coastal Water Research Project

Toxicology personnel are trained in the standard toxicity test methods by the Laboratory Manager (Darrin Greenstein). A previously recorded video from the Bight 2008 intercalibration study is provided as additional training for proper embryo development identification. Prior to sample analysis, an intercalibration exercise is performed to ensure the same results are obtained from multiple technicians when counting the normal and abnormal mussel embryos in several test samples.

7 REFERENCES

USEPA (U.S. Environmental Protection Agency), 1995. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms. National Exposure Research Laboratory, Cincinnati, Office of Research and Development. USEPA 600-R-95-136. August 1995.

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USEPA, 2010. USEPA Contract Laboratory Program National Functional Guidelines for Inorganic Superfund Data Review. USEPA Office of Superfund Remediation and Technology Innovation. USEPA 540-R-10-011. January 2010.

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TABLES

Table B-1
Field Measurement Quality Objectives

Parameter	Measurement Accuracy
Salinity (g/kg)	± 0.1 g/kg
Temperature (°C)	± 0.2 °C
pH (su)	± 0.2
Dissolved Oxygen (mg/L)	± 1 %

Notes:
g/kg = grams per kilogram
mg/L = milligrams per liter
su = standard unit

Table B-2
Field Quality Control Samples

Sample Type	Dissolved Organic Carbon volume (mL)	Total Metals Volume (mL)	Dissolved Metals Volume (mL)
Travel Blank	na	50	na
Field Blank	40	50	50
Field Duplicate	40	50	50
Matrix Spike	0	50	50
Pump Tubing Blank	0	50	50

Notes:
Dissolved samples will be field filtered
mL = milliliters
na = not applicable

Table B-3
Sample Containers, Holding Times, and Preservation Methods

Parameter	Sample Size	Container Size and Type	Holding Time	Preservative
Dissolved Organic Carbon	40 mL	Pre-combusted glass vial (40 mL)	28 days	Cool/4°C; H_2SO_4[c] to pH<2
Total Metals	50 mL	Centrifuge tube (50 mL)	6 months	Cool/4°C; HNO_3 to pH<2
Dissolved metals	50 mL	Centrifuge tube (50 mL)	6 months	Cool/4°C; HNO_3[c] to pH<2 after filtration

Notes:
Dissolved samples will be field filtered mL = milliliters

Table B-4
Analytical Methods and Detection Limits

Parameter	Analytical Method	Method Detection Limit
Dissolved Organic Carbon (mg/L)	USEPA 9060a	0.055
Total and Dissolved Metals (µg/L)		
Copper	USEPA 1640	0.15
Zinc	USEPA 1640	0.15

Notes:
µg/L = micrograms per liter
mg/L = milligrams per liter
USEPA = U.S. Environmental Protection Agency

Table B-5
Frequencies for Quality Assurance/Quality Control Samples

Analysis Type	Initial Calibration	Ongoing Calibration	Duplicates	Matrix Spikes	LCS/LCM	Method Blanks	CRM
Dissolved Organic Carbon	Daily or each batch	1 per 10 samples	1 per 20 samples		1 per 20 samples	Each batch	Each batch
Total and Dissolved Metals	Daily or each batch	1 per 10 samples	1 per 20 samples	1 per 20 samples	1 per 20 samples	Each batch	Each batch

Notes:
LCM = laboratory control material
LCS = laboratory control sample
CRM = certified reference material

Table B-6
Data Quality Objectives

Parameter	Precision	Accuracy[1]	Completeness
Dissolved Organic Carbon	± 25% RPD	75-125% R	95%
Total and Dissolved Metals	± 25% RPD	75-125% R	95%

Notes:
R = recovery
RPD = relative percent difference
1 Laboratory control sample and matrix spike/matrix spike duplicate percent recovery

ATTACHMENT 1
SAMPLING FORMS

MdR Harbor Water Daily Sampling Log

Date_____ Crew_____

Station_____ Latitude_____ Longitude_____

Time at Start_____ Time at Finish_____

Visual Water Description_____

Picture Numbers_____

pH_____ Salinity_____

Temperature_____ Dissolved Oxygen_____

Other Notes_____

_____

_____

_____

Southern California Coastal Water Research Project
3535 Harbor Blvd. Suite 110
Costa Mesa, CA 92626
(714) 755-3200

Chain of Custody

Date _____ Page _____ of _____

Sample Collection By: _____			Project Name:_____				Project Number: _____
Sample ID	Date	Time	Matrix	Container Type	Number of Containers	Comments	Analysis

Relinquished By		Relinquished By		Relinquished By	
(Signature)	(Date)	(Signature)	(Date)	(Signature)	(Date)
(Printed Name)	(Time)	(Printed Name)	(Time)	(Printed Name)	(Time)
(Company)		(Company)		(Company)	
Received By		**Received By**		**Received By**	
(Signature)	(Date)	(Signature)	(Date)	(Signature)	(Date)
(Printed Name)	(Time)	(Printed Name)	(Time)	(Printed Name)	(Time)
(Company)		(Company)		(Company)	

Date: _____ Project: _____ Experiment(s): _____

pH/DO QA verification sheet

Make the appropriate quality assurance entry for each water quality parameter analyzed. The person performing the analysis for each parameter should write their initials in the space provided. Report QA measurements that do not meet the performance objectives to the QA supervisor for the test.

RPD = 100 X 2(D1 – D2)/(D1 + D2); D1 = measurement1, D2 = measurement 2

QA Supervisor Approval _____

Performance Objective

pH (YSI Pro1020): Analyst _____

Initial Calibration: Standard solutions no more than four weeks old: _____

Beginning of Run

Reading for freshly obtained lab seawater: _____ 7.8-8.2

During Run

Standard (pH 7.0): _____ 6.9-7.1

Duplicate pH measurement:

sample type: _____ 1st _____ 2nd _____ RPD _____% 3 %

Were QA objectives met? _____ If not, explain actions taken

Performance Objective

Dissolved Oxygen (YSI Pro1020): Analyst _____

Beginning of run:

Well aerated lab seawater: _____ 7.5±0.5 ppm @ 20°C

Duplicate sample measurement:

Sample type: _____ 1st _____ 2nd _____ RPD _____% 8 %

Were QA objectives met? _____ If not, explain actions taken:

Date: _____ Project: _____ Experiment(s): _____

Salinity QA verification sheet

Make the appropriate quality assurance entry for each water quality parameter analyzed. The person performing the analysis for each parameter should write their initials in the space provided. Report QA measurements that do not meet the performance objectives to the QA supervisor for the test.

RPD = 100 X 2(D1 – D2)/(D1 + D2); D1 = measurement1, D2 = measurement 2

QA Supervisor Approval _____

Performance Objective

Salinity (YSI Pro30): Analyst _____

Beginning of run:

Lab seawater: cond._____ temp_____ salinity _____ | 33 to 35 ppt

During run:

Duplicate conductivity measurement:

Sample type: _____ 1st _____ 2nd _____ RPD _____% | 5 %

Standard (Ricca at 25°C): _____ | 49.9- 50.1 mS

Were QA objectives met? _____ If not, explain actions taken:

APPENDIX C
TAC MEMBER PROFILES

Richard F. Ambrose
Professor, Department of Environmental Health Sciences, University of California, Los Angeles

(a) Professional Preparation

University of California, Irvine	Biological Sciences	B.S., 1975
University of California, Los Angeles	Ecology	Ph.D., 1982
Simon Fraser University	Ecology	Postdoc., 1983-84

(b) Appointments

1992-present	Professor (2000-present) and Associate Professor (1992-2000), Department of Environmental Health Sciences and the Institute of the Environment and Sustainability, UCLA
1998-2011	Director, Environmental Science and Engineering Program, UCLA
1985-1992	Assistant/Associate Research Biologist, Marine Science Institute, University of California, Santa Barbara

(c) Publications (Selected from 206 total, with 98 peer-reviewed journal articles)

1. Doughty, C.L., K.C. Cavanaugh, R.F. Ambrose and E.D. Stein. *In press*. Evaluating regional resiliency of coastal wetlands to sea level rise through hypsometry-based modeling. Global Change Biology.
2. Rosencranz, J.A., K.M. Thorne, K.J. Buffington, J.Y. Takekawa, R.F. Hechinger, T.E. Stewart, R.F. Ambrose, G.M. MacDonald, M.A. Holmgren, J.A. Crooks, R.T. Patton, and K.D. Lafferty. 2018. Sea-level rise, habitat loss, and potential extirpation of a salt marsh specialist bird in urbanized landscapes. Ecology and Evolution 8: 8115-8125. DOI: 10.1002/ece3.4196.
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10. Rosencranz, J.R., L.N. Brown, J.R. Holmquist, Y. Sanchez, G.M MacDonald and R.F. Ambrose. 2017. The role of sediment dynamics for inorganic accretion patterns in southern California's Mediterranean-climate salt marshes. Estuaries and Coasts. DOI: 10.1007/s12237-017-0224-3.
11. Ambrose, R.F. 2017. Managing Sea Level Rise in Coastal Wetlands: Testing Thin Layer Sediment Augmentation as an Adaptation Strategy. Proceedings of the US-Iran Symposium on Wetlands, March 28-30, 2016, in Irvine, CA. University of Arizona, National Academies, and Sharif University of Technology. pp. 149-160.
12. Mehring, A.S., B.E. Hatt, D. Kraikittikun, B.D. Orelo, M.A. Rippy, S.B. Grant, J.P. Gonzalez, S.C. Jiang, R.F. Ambrose and L.A. Levin. 2016. Soil invertebrates in Australian rain gardens and their potential roles in storage and processing of nitrogen. Ecological Engineering 97: 138-143.

13. Thorne, K.M., MacDonald, G.M., Ambrose, R.F., Buffington, K.J., Freeman, C.M., Janousek, C.N., Brown, L.N., Holmquist, J.R., Gutenspergen, G.R., Powelson, K.W., Barnard, P.L., and Takekawa, J.Y., 2016, Effects of climate change on tidal marshes along a latitudinal gradient in California: U.S. Geological Survey Open-File Report 2016-1125, 75 p. Available at: http://dx.doi.org/10.3133/ofr20161125.
14. Rosencranz, J.A., N.K. Ganju, R.F. Ambrose, S.M. Brosnahan, P.J. Dickhudt, G.R. Guntenspergen, G.M. MacDonald, J.Y. Takekawa and K.M Thorne. 2016. Balanced sediment fluxes in southern California's Mediterranean-climate zone salt marshes. Estuaries and Coasts. . Estuaries and Coasts 39: 1035-1049. DOI 10.1007/s12237-016-0077-1. Erratum published online 17 February 2016.
15. Murray, S.N., S.B. Weisberg, P.T. Raimondi, R.F. Ambrose, C.A. Bell, C.A. Blanchette, J.L. Burnaford, M.N. Dethier, J.M. Engle M.S. Foster, C.M. Miner, K.J. Nielsen, J.S. Pearse, D.V. Richards, and J.R. Smith. 2016. Evaluating ecological states of West Coast rocky intertidal communities: A best professional judgment exercise. Ecological Indicators 60: 802-814.
16. Wang, W., Y.Ding, J.L. Ullman, R.F. Ambrose, Y. Wang, X. Song and Z. Zhao. 2016. Nitrogen removal performance in planted and unplanted horizontal subsurface flow constructed wetlands treating different influent COD/N ratios. Environmental Science and Pollution Research. Pages 1-7. Published online 29 January 2016. DOI 10.100 7/s 11356-016-6115-S
17. Ferguson, D., G.N. Talavera, L.R. Hernández, R.F. Ambrose, J.A. Jay and S. Weisberg. 2016. Virulence genes among *Enterococcus faecalis* and *Enterococcus faecium* isolated from coastal beaches and potential sources in southern California and Puerto Rico. Journal of Pathogens. 7 pages. DOI 10.1155/2016/3437214.
18. Yap, T.A., M.S. Koo, R.F. Ambrose, D.B. Wake and V.T. Vredenburg. 2015. Averting a biodiversity crisis. Science 349: 481-482.
19. Ambrose, R.F. and B.K. Winfrey. 2015. Comparison of stormwater biofiltration systems in southeast Australia and southern California. WIREs Water 2: 131-146. DOI: 10.1002/wat2.1064
20. Myers, M.R. and R.F. Ambrose. 2015. Salt marsh reduces fecal indicator bacteria input to coastal waters in southern California. Bulletin of the Southern California Academy of Sciences 114: 76-88.
21. Needles, L.A., S.E. Lester, R. Ambrose, A. Andren, M. Beyeler, M.S. Connor, J.E. Eckman, B.A. Costa-Pierce, S.D. Gaines, K.D. Lafferty, H.S. Lenihan, J. Parrish, M.S. Peterson, A.E. Scaroni, J.S. Weis, D.E. Wendt. 2015. Managing bay and estuarine ecosystems for multiple services. Estuaries and Coasts 38 (Suppl. 1): S35-S48.
22. Ambrose, R.F., J.H. Dorsey, K.K. Johnston, and E.D. Stein. 2015. State of the Bay Report. "Habitat Conditions: Coastal Wetlands." Urban Coast 5(1): 59-68. Available online: http://urbancoast.org/.
23. Altstatt, J., R. Ambrose, J. Carroll, J. Coyer, J. Wible and J. Engle. 2014. Eelgrass meadows return to Frenchy's Cove, Anacapa Island: Recovery ten years after successful transplantation. Monographs of the Western North America Naturalist 7: 500-517.
24. Zimmer-Faust, A.G., R.F. Ambrose and M.N. Tamburri. 2014. Evaluation of approaches to quantify total residual oxidants in ballast water management systems employing chlorine for disinfection. Water Science and Technology 70: 1585-1593. DOI: 10.2166/wst.2014.394.
25. Grant, S.B., J-D Saphores, D.L Feldman, A.J. Hamilton, T.D. Fletcher, P.L.M. Cook, M. Stewardson, B.F. Sanders, L.A. Levin, R.F. Ambrose, A. Deletic, R. Brown, S.C. Jiang, D. Rosso, W.J. Cooper and I. Marusic. 2012. Taking the "waste" out of "wastewater" for human water security and ecosystem sustainability. Science 337: 681-686.
26. Peterson, C.H.,S.S. Anderson, G.N. Cherr, R.F. Ambrose, S. Anghera, S. Bay, M. Blum, R. Condon, T.A. Dean, M. Graham, M. Guzy, S. Hampton, S. Joye, J. Lambrinos, B. Mate, D. Meffert, S.P. Powers, P. Somasundaran, R.B. Spies, C.M. Taylor, R. Tjeerdema, and E.E. Adams. 2012. A Tale of Two Spills: Novel Science and Policy Implications of an Emerging New Oil Spill Model. Bioscience 62: 461-469.
27. Willette, D.A. and R.F. Ambrose. 2012. Effects of the invasive seagrass Halophila stipulacea on the native seagrass, Syringodium filiforme, and associated fish and epibiota communities in the eastern Caribbean. Aquatic Botany 103: 74-82.
28. Coffman, G.C., R.F. Ambrose and P.W. Rundel. 2011. Wildfire promotes dominance by the invasive Giant Reed (*Arundo donax*) in riparian ecosystems. Biological Invasions 12: 2723-2734.
29. Stein, E.D., D. Ackerman, T. Hogue, S. Lopez, R. Ambrose and V. Chan. 2010. Effect of global climate change on southern California coastal wetlands. Report to the State of California Department of Water Resources.
30. Rothenberg, S.E., M.E. Kirby, B.W. Bird, M.B. DeRose, C. Lin, X. Feng, R.F. Ambrose and J.A. Jay. 2010. The impact of over 100 years of wildfires on mercury levels and accumulation rates in two lakes in southern California, USA. Environmental Earth Sciences 60: 993–1005.

31. Myers, M.R. and R.F. Ambrose. 2009. Differences in benthic cover inside and outside marine protected areas on the Great Barrier Reef: influence of protection or disturbance history? Aquatic Conservation: Marine and Freshwater Ecosystems 19: 736-747.
32. Smith, J.R., P. Fong and R.F. Ambrose. 2009. Spatial patterns in recruitment and growth of the mussel Mytilus californianus (Conrad) in southern and northern California, USA, two regions with differing oceanographic conditions. Journal of Sea Research 61: 165-173.
33. Willette, D.A. and R.F. Ambrose. 2009. The distribution and expansion of the invasive seagrass Halophila stipulacea in Dominica, West Indies, with a preliminary report from St. Lucia. Aquatic Botany 91:137-142.
34. Smith, J.R., P. Fong and R.F. Ambrose. 2008. The impacts of human visitation on mussel bed communities along the California coast: Are regulatory marine reserves effective in protecting these communities? Environmental Management 41: 599-612.
35. Rothenberg, S.E., R.F. Ambrose and J.A. Jay. 2008. Mercury cycling in surface water, pore water and sediments of Mugu Lagoon, CA., USA. Environmental Pollution 154: 32-45.
36. Pankratz, S., T. Young, H. Cuevas-Arellano, R. Kumar, R.F. Ambrose and I.H Suffet. 2007. The ecological value of constructed wetlands for treating urban run-off. Water Science and Technology 55: 63-69.
37. Tetreault, I. and R.F. Ambrose. 2007. Temperate marine reserves enhance targeted but not untargeted fishes in multiple no-take MPAs. Ecological Applications 17: 2251-2267.
38. Swenson, D.P. and R.F. Ambrose. 2007. A Spatial Analysis of Cumulative Habitat Loss in Southern California under the Clean Water Act Section 404 Program. Landscape and Urban Planning 82: 41-55.
39. Miller, A.W., G.M. Ruiz, M.S. Minton and R.F. Ambrose. 2007. Differentiating successful and failed molluscan invaders in marine ecosystems. Marine Ecology Progress Series 332: 41-51.
40. Raimondi, P., R. D. Sagarin, R. Ambrose, C. Bell, M. George, S. Lee, D. Lohse, C.M. Miner, and S. Murray. 2007. Consistent frequency of color morphs in the sea star Pisaster ochraceus (Echinodermata: Asteriidae) across open-coast habitats in the northeastern Pacific. Pacific Science 61: 197-206.
41. Sagarin, R.D., R.F. Ambrose, B.J. Becker, J.M. Engle, J. Kido, S.F. Lee, C.M. Miner, S.N. Murray, P.T. Raimondi, D.V. Richards, C. Roe. 2007. Ecological impacts on the limpet Lottia gigantea populations: human pressure over a broad scale on islands and mainland intertidal zones. Marine Biology 150: 399-415.
42. Smith, J.R., R.F. Ambrose, and P. Fong. 2006. Long-term change in mussel (*Mytilus californianus* Conrad) populations along the wave-exposed coast of California. Marine Biology 149: 537-545.
43. Smith, J.R., R.F. Ambrose, and P. Fong. 2006. Dramatic declines in mussel bed community diversity: Response to climate change? Ecology 87: 1153-1161.
44. Forrester, G.E., B.I. Fredericks, D. Gerdeman, B. Evans, M.A. Steele, K. Zayed, L.E. Schweitzer, I.H. Suffet, R.R. Vance and R.F. Ambrose. 2003. Correspondence between field-measured growth rates of fish from several California estuaries and the inferred toxicity of multiple sediment contaminants. Marine Environmental Research 56: 423-442.
45. Raimondi P., M. Wilson, R. Ambrose, J. Engle and T. Minchinton. 2002. Continued declines of black abalone along the coast of California: Are mass mortalities related to El Niño events? Marine Ecology Progress Series 242: 143-152.
46. Miller, A.W. and R.F. Ambrose. 2000. Optimum sampling of patchy distributions: Comparison of different sampling designs in rocky intertidal habitats. Marine Ecology Progress Series 196: 1-14.
47. Murray, S.N., R.F. Ambrose, J.A. Bohnsack, L.W. Botsford, M.H. Carr, G.E. Davis, P.K. Dayton, D. Gotshall, D.R. Gunderson, M.A. Hixon, J. Lubchenco, M. Mangel, A. MacCall, D.A. McArdle, J.C. Ogden, J. Roughgarden, R.M. Starr, M.J. Tegner and M.M. Yoklavich. 1999. No-take reserve networks: Protection for fishery populations and marine ecosystems. Fisheries 24: 11-25.
48. Palmer, M.A., R.F. Ambrose and N.L. Poff. 1997. Ecological theory and community restoration ecology. Restoration Ecology 5: 291-300.

(d) Synergistic Activities

Southern California Wetlands Recovery Project Science Advisory Panel; California Coastal Commission's Science Advisory Panels for the SONGS and Poseidon Mitigation Programs; Santa Monica Bay National Estuary Program Technical Advisory Committee; California Ocean Protection Council Science Advisory Team; California Marine Life Protection Act Initiative (South Coast) Science Advisory Team (2008-9); U.S. Army Corps of Engineers Environmental Advisory Board (2007-13); Science Advisory Committees for Malibu Lagoon Restoration (2003-5), Ballona Wetland Restoration (2005-9), Santa Clara River Estuary Restoration, Bunker Point Artificial Reef, Ormond Beach Wetland Restoration, Aliso Creek Estuary Restoration, Tijuana Estuary Restoration, San Elijo Lagoon Restoration.

CURRICULUM VITAE

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(phone +1-418-654-2538; fax +1-418-654-2600)
E-mail: campbell@inrs.ca

BIRTH/CITIZENSHIP May 4, 1943 / Canadian

MARITAL STATUS Married, 3 children

ACADEMIC QUALIFICATIONS

BSc (Honours): Bishop's University, 1963
Chemistry / Physics

PhD: Queen's University, 1968
Organic / Organometallic Chemistry

AWARDS

2011	Distinguished Visiting Scientist award, CSIRO Land and Water, Australia
2010	Honorary doctorate, Université de Bordeaux I, Bordeaux, France
2006	Excellence in Review Award, Environmental Science and Technology Journal, American Chemical Society, Washington, DC, USA
2002	Elected Fellow, Royal Society of Canada
2001	Canada Research Chair, Metal Ecotoxicology
2001	Synergy Award for Innovation, Research and Development Partnerships, Natural Sciences and Engineering Research Council (NSERC) and Conference Board of Canada, co-winner with colleagues from McMaster University.
2000	Chandler-Misener Award, International Association for Great Lakes Research (jointly with Dr. M.R. Twiss)
1998	Ministère de l'Éducation nationale, de l'Enseignement supérieur et de la Recherche (MENESR, France), Accueil de chercheurs étrangers en France, Bourse de Haut Niveau
1994, 1986	Prix d'excellence en recherche, Institut national de la Recherche scientifique
1989	Association canadienne française pour l'avancement des sciences
	Prix Michel Jurdant (Environmental science): Co-winner with Dr. A. Tessier
1968 - 1970	National Research Council (Canada), postdoctoral fellowship
1964 - 1967	National Research Council (Canada), postgraduate scholarship
1963 - 1964	Woodrow Wilson Fellowship

EXPERIENCE

2016- **Emeritus Professor**, Université du Québec, Institut national de la recherche scientifique, INRS-Eau

1983 -2015 **Professor**, Université du Québec, Institut national de la recherche scientifique, INRS-Eau

1978-1982 **Director**, Université du Québec, Institut national de la recherche scientifique, INRS-Eau

1970-1978 Université du Québec, Institut national de la recherche scientifique, INRS-Eau, **Associate Professor**, 1973-78; **Assistant Professor**, 1970-73

1968-1970 Monash University, Department of Chemistry, Melbourne, Australia, **Postdoctoral Fellow** and Lecturer

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1999-2004 Metals in the Environment Research Network (MITE-RN), **Research Director** and **Principal Investigator** (www.mite-rn.org)

2005-2009 Metals in the Human Environment Research Network (MITHE-RN), **Aquatic Theme Co-Director** (www.mithe-rn.org)

2011-2012
2004-2005 CSIRO Land and Water Directorate, Environmental Contaminants Division, Adelaide Laboratory, Waite Institute, Urrbrae, South Australia, **Visiting Scientist** (sabbatical leave)

1998 Université de Bordeaux I, Laboratoire d'écophysiologie et écotoxicologie des systèmes aquatiques (LEESA), Arcachon, France, **Visiting Professor** (sabbatical leave)

1997-1998
1990-1991 CSIRO Centre for Advanced Analytical Chemistry, Lucas Heights Research Laboratories, Menai, New South Wales, Australia, **Visiting Scientist** (sabbatical leave)

1982-1983 Stanford University, Department of Civil Engineering, Environmental Engineering and Science Section, Stanford, California, USA, **Visiting Professor** (sabbatical leave)

1976-1977 Freshwater Biological Association, River Laboratory, East Stoke, Dorset, U.K., **Visiting Scientist** (sabbatical leave)

RESEARCH INTERESTS:

Biogeochemistry of metals in the aquatic environment: current research topics include elements of analytical chemistry (development and refinement of methods to determine metal speciation), geochemistry (identification of factors controlling metal speciation in natural waters) and ecotoxicology (development of predictive models relating the biological response elicited by a metal to its speciation in the external medium). Metals of current interest include Al, Ag, Mn, Cd, Cu, Hg, Pb, Zn + Pd, Pd, La, Ce; target organisms include freshwater algae, rooted aquatic plants, benthic invertebrates, and fish. The biological responses of interest are metal bioaccumulation within the target organism, metal detoxification (transcriptome; proteome), and metal-induced changes in physiology and growth.

COMMITTEES (current)

- International Copper Association, International Lead Zinc Research Organization, Nickel Producers Environmental Research Association - Ecotoxicity Technical Advisory Panel (ETAP), Member 2000- …
- International Scientific Council, Centre d'Écotoxicologie et Toxicologie Environnementale de Rovaltain, France, Member 2009-…; Chair 2014-…

- Co-editor, Cambridge Environmental Chemistry Series, Cambridge University Press, UK, 1988- .
- Editorial Board, Journal "*Revue des Sciences de l'Eau*" 1993- …
- Editorial Board, Journal "*Environmental Chemistry*", 2004-… .

PAST COMMITTEES (partial list)

- Agence nationale de la Recherche, France, Comité Écosystèmes et Santé, Member, 2008-2010; Comité de sélection, BIOADAPT, 2011-2013
- Agence nationale de la Recherche, France), Comité sectoriel, Écosystèmes et Développement durable, Member 2010-2013.
- Society of Environmental Toxicology and Chemistry (SETAC), Advisory Group on Metals, Member 2000-2006.
- Environment Canada / Health Canada Chemical Management Plan (CMP) Science Committee, Member 2013-16 (http://www.chemicalsubstanceschimiques.gc.ca/plan/sc-cs/index-eng.php)
- Environment Canada, Categorization of inorganic substances on the Domestic Substances List (Canadian Environmental Protection Act), Inorganic Working Group, Member, 1999-2004.
- Canadian Foundation for Innovation, University Research Development Fund Committee, Member, 1999-2002.
- Natural Resources Canada, Aquatic Effects Technology Evaluation programme, Technical Committee; Member, 1995-98.
- Health Canada, Expert Steering Committee, Aluminum and animal neurotoxicity study, Member, 1999-2008.

- Natural Sciences and Engineering Research Council of Canada (NSERC), Advisory Committee on University-Industry Grants (ACUIG), Co-Chair, 2015-17.
- Natural Sciences and Engineering Research Council of Canada (NSERC), Advisory Committee on University-Industry Grants (ACUIG), Member, 2006-2009.
- Natural Sciences and Engineering Research Council of Canada (NSERC), Advisory Group on Interdisciplinary Research (AGIR), Member, 2000-2008.
- Natural Sciences and Engineering Research Council of Canada (NSERC), Committee on Collaborative Research Initiatives; Member 1991-95; Chair 1993-95.

- Natural Sciences and Engineering Research Council of Canada (NSERC), Interdisciplinary Grant Selection Committee; Member, 1988-1991; Chairman, 1989-1990.

- Fonds pour la Formation de Chercheurs et l'Aide à la Recherche (FCAR), Comité de sélection pour l'équipement de recherche, Member 1999-2000, Chair 2000-01.
- Fonds pour la Formation de Chercheurs et l'Aide à la Recherche (FCAR), Comité du programme <Centres de recherche>; Member 1986-1988; Chair 1987-1988.

- National Research Council of Canada (NRCC), Associate Committee on Hydrology; Member, 1985-1991; Executive committee, 1987-1990
- National Research Council of Canada (NRCC), Associate Committee on Scientific Criteria for Environmental Quality; Member, 1987-1991; Member, Water Subcommittee, 1983-1985; Member, Manganese working group, 1984-1986; Co-chairman, Working group on the bio-availability of metals in sediments, 1984-1988.
- National Research Council of Canada (NRCC), Committee on Marine Analytical Chemistry; Member, 1986-1989.

- Royal Society of Canada, Global Climate Change Program, Committee on Acid Precipitation; Member, 1989-92.
- Royal Society of Canada / U.S. National Academy of Sciences / Mexican Academia de la Investigacion Cientifica: Joint Committee on Acid Precipitation; Member, 1981-1985.

- International Joint Commission (Canada/U.S.A.), Great Lakes Science Advisory Board; Member 1985-1987.

- Hydro-Québec, Environmental Advisory Committee, 1988-1990.

- Canadian Association for Water Pollution Research and Control (CAWPRC), Executive, 1978-1982; 1983-90; Vice-president, development, 1989-1990.

CONFERENCES (partial list)

- Co-Chair, Symposium on Metallomics – Metal speciation in living cells, Canadian Society for Chemistry, May 2013, Quebec.
- Co-Chair, Symposium on Speciation and Bioavailability of Metals, Canadian Society for Chemistry, June 2011, Montreal.
- Co-Chair, Symposium on Site-specific Community-Based Risk Assessments for Metals, Society for Environmental Toxicology and Chemistry (SETAC), World Congress, August 2008, Sydney, Australia.
- Co-Chair, Symposium on Environmental Risk Analysis for Metals, Society for Environmental Toxicology and Chemistry (SETAC), Annual Meeting, November 2006, Montreal.
- Chair 4e Colloque annuel, Chapitre St-Laurent, Qualité de l'Environnement – concepts et outils, June 2000, Quebec
- Co-chair (with Dr. R.J. Allan), International Symposium on the Fate and Effects of Toxic Chemicals in Large Rivers and their Estuaries, October 1988, Quebec.

- Chair, International Symposium on Trace Metal Speciation, Canadian Society for Chemistry, June 1987, Quebec; Organizer, Scientific Programme, Analytical Chemistry Division, 70th Canadian Chemical Congress, June 1987, Quebec.
- Chair, International Symposium on Reservoir Ecology and Management (UNESCO/Hydro-Québec / James Bay Energy Society), June 1981, Quebec.

RECENT CONSULTATIONS (partial list)

- Natural Resources Canada, CANMET, Mine Environment Neutral Drainage (MEND) program, Report on *How to Assess Possible Biological Effects of Sub-Aqueous Disposal of Reactive Mine Tailings – Literature Review and Recommended Tools and Methodologies*, 2017-present.
- Grand Council of the Cree, Oujé-Bougoumou Cree Community. Co-chair of the Steering Committee overseeing the ecological risk assessment of metal mining activities in the traditional Cree territory near Chibougamau, Quebec, 2003-present.
- Rio Tinto Fer et Titane Inc. Geochemical characterization of the interstitial water and sediments of Lake Petit-Pas, Charlevoix, Quebec, 2008-2009; 2017-present.
- National Defence Canada. Evaluation of the bioavailability of silver present in drainage waters from the Munitions Experimental Test Centre, Nicolet, Quebec, 2007.
- Government of New Caledonia ("Province du Sud"). Member of the Expert committee formed to evaluate the environmental impacts of the effluent from the projected Goro nickel mine, 2006-2008.
- Broken Hill Proprietary (Melbourne, Australia). Member of Peer Review Committee formed to assess the effects of the Ok Tedi copper mine on the downstream environment, Papua New Guinea, 1997-2001.

MEMBERSHIP IN PROFESSIONAL SOCIETIES

Canadian Society for Chemistry (CSC), Ottawa

Association for the Sciences of Limnology and Oceanography (ASLO)

Society of Canadian Limnologists (SCL)

Society of Environmental Chemistry and Toxicology (SETAC)

PUBLICATIONS SUMMARY (Web of Science Researcher ID: H-4348-2011)

Articles in refereed journals	205
Publications in conference proceedings	36
Book chapters	23
Refereed reports	3
Technical reports	52
Edited volumes	2
Invited communications	41

CURRICULUM VITAE: Gary N. Cherr

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Education and Training:
B.A. Biology, Sonoma State University, 1979
Ph.D. Zoology, University of California, Davis 1984
National Institutes of Health Postdoctoral Fellow, Department of Obstetrics and Gynecology, School of Medicine, University of California, Davis, 1983-1986.

Areas of Specialization: reproductive physiology, reproductive and developmental biology and toxicology, sperm cell physiology, embryo defense mechanisms, biochemistry and cell biology of environmental stress, endocrine disruption, environmental toxicology, oil spill impacts

Current Appointments:
Director (2009-present), UC Davis Bodega Marine Laboratory
Professor (1999-present), UC Davis Departments of Environmental Toxicology and Nutrition

Professional Experience:
Acting Director, Bodega Marine Laboratory, 2007-2008
Research Biologist (1993-1999), Bodega Marine Laboratory and Dept. Environmental Toxicology, University of California, Davis, 1999

Service: *University of California*
Study Section Member for Tobacco-Related Disease Research Program, Office of the President of the University of California, 1990
Steering Committee for University of California at Santa Barbara, Southern California Education Initiative, U.S. Department of Interior, 1990-1994
California Sea Grant College Program Advisory Board Member, 2003-present
California Sea Grant College Program Advisory Board Chair, 2005-present
University of California Marine Council, 2007-2008
Executive Board, Cooperative Institute for Marine Ecosystems and Climate (NOAA and UC San Diego), 2011-2015.

Service: *Non-University*
Science Advisory Board, San Francisco Bay Estuary Project, 1988-1990
Biomonitoring Science Advisory Board, Department of Ecology, State of Washington, 1991-1995
Advisor, US Regional Marine Research Board, Pacific Northwest Region, Washington State Sea

Grant College Program, 1992-1993
Protocol Review Committee, Water Resources Control Board, State of California, 1994-2000
Technical Review Panel, Department of Ecology, State of Washington, 1998
Toxicity Identification Evaluation Review Committee, US EPA, 1999-2000
Organizer of 2000 Western Regional Developmental Biology Conference, Society for Developmental Biology, Bodega Marine Laboratory
Exxon Valdez Oil Spill Trustee Council Science Panel, 2006-present
State of California Resources Agency Science Sea Grant Advisory Panel, 2007-2010
Technical Advisory Committee, Coastal Conservancy Creosote Removal Project, 2014-present

Selected Publications (150 total):

Cherr, G.N. and Wallis H. Clark, Jr. 1982. Fine structure of the envelope and micropyles in the eggs of the white sturgeon, *Acipenser transmontanus* Richardson. *Development Growth and Differentiation* 24:341-352.

Cherr, G.N. and W.H. Clark, Jr. 1984. An acrosome reaction in sperm from the white sturgeon, *Acipenser transmontanus*. *Journal of Experimental Zoology* 232(1):129-139.

Cherr, G.N. and W.H. Clark, Jr. 1985. Gamete interaction in the white sturgeon, *Acipenser transmontanus*: A morphological and physiological review. *Environmental Biology of Fishes* 14(1):11-22.
Also in: *North American Sturgeons: Biology and Aquaculture Potential*, F. Binkowski and S. Doroshov (eds.), Dordrecht: Dr W. Junk: pp 11-22.

Cherr, G.N. and W.H. Clark, Jr. 1986. Induction of the acrosomal reaction in sperm from the white sturgeon, *Acipenser transmontanus*, pp 235-249, In: J.L. Hedrick (ed.) *The Molecular and Cellular Biology of Fertilization*, Plenum Publishing Corporation, New York, New York.

Cherr, G.N., J. Shenker, C. Lundmark and K.O. Turner. 1987. Toxic effects of selected bleached kraft mill effluent constituents on the sea urchin sperm cell. *Environmental Toxicology and Chemistry*, 6:561-569.

Cherr, G.N., J. Shoffner-McGee, and J.M. Shenker. 1990. Methods for assessing fertilization and embryonic/larval development in toxicity tests using the California mussel (*Mytilus californanus*). *Environmental Toxicology and Chemistry*, 9:1137-1145.

Shenker, J.M., and G.N. Cherr. 1990. Toxicity of zinc and bleached kraft mill effluent to larval English sole (*Parophrys vetulus*) and topsmelt *(Atherinops affinis*) *Archives of Environmental Contamination and Toxicology*, 19:680-686.

Cherr, G.N. 1990. Toxicity tests for *Mytilus californianus* (California mussel). In: *Proceedings of A Workshop on Culture and Toxicity Testing of West Coast Marine Organisms.* G.A. Chapman, ed., U.S. Environmental Protection Agency Publication pp. 89-94.

Cherr, G.N., R.G. Summers, J.D. Baldwin, and J.B. Morrill. 1992. Preservation and visualization of the sea urchin blastoceolic extracellular matrix. *Microscopy Research and Technique*, 22:11-22.

Baldwin, J.D., M.C. Pillai, and G.N. Cherr. 1992. The response of sea urchin (*Strongylocentrotus purpuratus*) embryos to aqueous petroleum wastes includes the expression of a high molecular weight glycoprotein. *Marine Biology*, 114: 21-30.

Pillai, M.C., J.D. Baldwin, and G.N. Cherr. 1992. Early development in an algal gametophyte: Role the cytoskeleton in germination and nuclear translocation. *Protoplasma*, 170:34-45. DOI: 10.1007/BF01384455

Yanagimachi, R., G.N. Cherr, M.C. Pillai, and J.D. Baldwin. 1992. Factors controlling sperm entry into the micropyles of salmonid and herring eggs. *Development, Growth, Differentiation*, 34:447-461.

Higashi, R.M., G.N. Cherr, J.M. Shenker, J.M. Macdonald, and D.G. Crosby. 1992. A Polar high molecular mass constituent of bleached kraft mill effluent is toxic to marine organisms. *Environmental Science and Technology*, 26:2413-2420.

Higashi, R.M., G.N. Cherr, C.A. Bergens, T.W-M. Fan, and Crosby, D.G. 1992. Toxicant isolation from a produced water source in the Santa Barbara Channel. In: *Produced Water: Technological/Environmental Issues and Solutions*. J. P. Ray and F.R. Engelhardt, eds. Plenum Press, New York. pp. 223-233.

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APPENDIX D

Marina del Rey Harbor Site-Specific Objective Study
Critical Condition Summary Report

February 26, 2019

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INTRODUCTION

The USEPA interim guidance for water effect ratio (WER) determination emphasizes the importance of using a sampling design that considers variations in water quality likely to affect the WER (USEPA 1994). EPA has not developed a generalized specific study design for WER studies at large sites such as MdRH. Rather, conceptual guidance for design development is provided:

> *"Each design has to be formulated individually to fit the specific site. The design should try to take into account the times, locations, and depths at which the extremes of the physical, chemical, and biological conditions occur within the site, which will require detailed information concerning the site."*

Potential sources of variability include seasonality (e.g., summer vs. winter), presence of stormwater discharge, hydrology (tides or depth), and episodic events (e.g., plankton blooms, harbor activities). The relative importance of these factors on WERs in Marina del Rey Harbor (MdRH) is not known.

Included within the combination of various site characteristics is the "critical condition" defined by EPA as: *"...the critical condition, that condition where the copper concentration can be expected to be highest relative to the WER…"* (USEPA 2001). A common goal of all WER studies to identify site-specific objectives that will protect water quality under the critical condition.

Prior monitoring of MdRH for metals shows that elevated copper concentrations occur throughout the harbor. Consistent spatial patterns in MdRH copper are weak, due to the diffuse nature of the primary source (i.e., leaching from thousands of boat hulls distributed among multiple locations). Thus, the critical condition for MdRH can also be defined as that combination of factors resulting in the lowest WER, with the assumption that the location of areas with the highest copper concentration is variable.

This report summarizes the results of field studies to characterize the magnitude and variability in water quality characteristics likely to influence the WER in MdRH. Three site characterization sampling events were conducted in 2018 and included measurements of water quality characteristics known to influence copper bioavailability (e.g., pH, dissolved organic carbon, salinity), as well as copper concentration and toxicity. The results are interpreted with the objective of identifying spatial or temporal patterns that are likely to represent the critical condition for a copper WER in MdRH.

STUDY DESIGN

The site characterization studies were designed to evaluate the spatial and temporal variability in water quality factors considered to be important to determining copper bioavailability in MdRH. Spatial variability was assessed by analyzing water samples from 11 stations located throughout the harbor (Figure 1). These stations represented three different hydrologic regions of the harbor (main channel, front basins, back basins), as well as potential discharges from point (shipyard) and nonpoint sources (Ballona Creek, Oxford Basin, storm drains). Sample depth was also considered, by collecting samples from both near surface and near bottom. Temporal variability was investigated by conducting sampling on three events, with each representing different seasonal conditions: winter-wet weather, spring-dry weather, summer-dry weather. Short-term variability in water characteristics, potentially related to tidal exchange, was investigated during the summer sampling event by collecting multiple samples from the same location at different times of the day. A summary of the sampling events is shown in Table 1.

Figure 1. Station locations in Marina del Rey Harbor.

Table 1. Site characterization study design parameters.

Event	Date	Weather	Precipitation (inches)	Sampling depths	Tide Stage		Tide height range relative to MLLW (ft)	Duplicate samples	Stations with repetitive sampling
					Flood	Ebb			
1	3/23/2018	Wet	1.1	B, S	X		0.61 to 2.95	1	0
2	5/21/2018	Dry	0	B, S	X		-0.22 to 3.14	1	0
3	9/10/2018	Dry	0	S	X	X	3.0 to 5.7	2	2

B=bottom depth S=surface depth MLLW=mean lower low water

METHODS

Water samples were collected from each of the stations using a peristaltic pump and Teflon-lined tubing (Table 2). Samples from two-depths (1 m above the sediment and 1 m below the water surface) were collected in the first two events. Event 3 samples were collected from the surface only. The specific coordinates listed (Table 2) are from the third sampling event. The timing of each collection event was planned to collect samples from at least two different tide profiles to evaluate potential effects from tide on the critical condition. For all events, sampling times were related to tide heights based on the Mean Lower Low Water (MLLW), which is the average of the lower low water height of the tidal day. The tidal ranges for each sampling period are presented as a function of time (Figure 2). These data were retrieved from NOAA's Tides and Currents website for the Santa Monica location (https://tidesandcurrents.noaa.gov/waterlevels.html?id=9410840).

Subsamples were immediately filtered through a 0.45 μm polyethersulfone syringe filter for analysis of dissolved metals and dissolved organic carbon (DOC). Additional subsamples were taken for analysis of total metals, toxicity, and chlorophyll. Field measurements of sample pH, dissolved oxygen (DO), temperature, and salinity were made using electrodes.

Dissolved and total concentrations of copper and zinc were measured. Metal analysis was conducted according to USEPA Method 1640 for trace elements in water, using inductively coupled plasma mass spectrometry. In this procedure, trace elements are pre-concentrated based on their reductive precipitation by sodium tetrahydroborate; iron and palladium are added to samples to aid co-precipitation of metal borides and to enhance the precipitation of metals coming out in the elemental form.

Dissolved organic carbon analyses were conducted using USEPA Method 9060a for the analysis of organic carbon by combustion or oxidation.

Toxicity of the water samples was measured with the 48-hour mussel embryo development test using *Mytilus galloprovincialis* (USEPA 1995). The test was conducted under standard conditions specified by USEPA guidance. Toxicity tests were initiated within 48 hours of sample collection. Embryos were preserved for examination at the end of the exposure period. The preserved samples were examined using a microscope to determine the numbers of normal and abnormal surviving embryos. The percent of normal embryos was calculated from the count.

To better evaluate differences in the water quality data collected over time and depth several statistical analyses were applied. One-way analysis of variance (ANOVA) was used to determine

if there were significant differences in DOC, chlorophyll, or dissolved copper concentration by time for all three events. If significant, the Tukey HSD post-hoc test was used to determine the differences. A t-test was also used to determine if there were significant differences by depth for events 1 and 2. Additionally, it was used to determine if the duplicate DOC and chlorophyll data were significantly different from the repeated visit data. Spearman's correlation analysis was also used. Significance was determined if the p value was less than an alpha level of 0.05.

The marine Biotic Ligand Model (BLM) was used to predict the potential for copper toxicity using site water quality data (Arnold et al. 2005). This BLM is currently under review by the USEPA but has not yet been approved for use in water quality criteria development. Water quality data (pH, temperature, DOC, and salinity) from all three sampling events were used in the BLM to calculate a site-specific predicted copper EC50 value for each sample. This value is comparable to an EC50 value for the 48-hour mussel embryo development test using *Mytilus galloprovincialis* (USEPA 1995). This embryo development test was used to evaluate the toxicity of samples collected from each of the three site characterization events.

Table 2. Station location coordinates within Marina del Rey Harbor.

Station ID	Description	Latitude	Longitude
MdRH-MC1	Main Channel, end	N 33° 58.814'	W 118° 26.886'
MdRH-MC2	Main Channel, middle	N 33° 58.330'	W 118° 26.892'
MdRH-MC3	Main Channel, entrance	N 33° 58.880'	W 118° 27.316'
MdRH-A	Front Basin A, middle	N 33° 58.348'	W 118° 27.194'
MdRH-B	Front Basin B, middle	N 33° 58.504'	W 118° 27.189'
MdRH-C	Front Basin C, middle	N 33° 58.665'	W 118° 27.253'
MdRH-D	Back Basin D, middle	N 33° 58.827'	W 118° 27.243'
MdRH-E	Back Basin E, middle	N 33° 58.977'	W 118° 27.191'
MdRH-F	Back Basin F, middle	N 33° 58.919'	W 118° 26.697'
MdRH-G	Front Basin G, middle	N 33° 58.776'	W 118° 26.626'
MdRH-H	Front Basin H, middle	N 33° 58.584'	W 118° 26.676'

RESULTS

Sampling

Winter-wet weather event

During the winter-wet weather event, the previous 48 hours produced 1.1 inches of rainfall at the Ballona Creek rain gauge, which was above the minimum required rainfall of 0.2 inches specified in the draft work plan. Surrounding areas had rainfall values of 1.41 inches (Santa Monica, north of MdRH), and 0.75 inches (83rd Street Yard, south of MdRH). Sampling started at 8:20am and ended at 5:15pm. The first sample (MdRH-H surface) was taken just before the low tide at 0.7 ft, and the final sample (MdRH-MC1 bottom) was taken around the high tide of +2.9 ft. The overall tide change for the day was +2.3 ft (Figure 2).

Spring-dry weather event

Sampling started at 8:40am and ended at 2:37pm. The tidal regime for this event was similar to that for the winter sampling. The first sample (MdRH-MC3 surface) was taken just before the low tide at +0.16 ft, and the final sample (MdRH-H bottom) was taken around the high tide of +3.14 ft. The overall tide change for the day was +3.36 ft.

Summer-dry weather event

Sampling started at 7:44am and ended at 12:25pm. The first sample (MdRH-H-1 surface) was taken approximately 3.5 hours after the low tide (-0.35 ft) at +3.0 ft, and the final sample (MdRH-H-3 surface) was taken approximately two hours after the high tide (+5.75 ft) at +4.63 ft. The overall tide change for the sampling window was +2.75 ft.

Water Quality

All results for water quality, metals concentrations, toxicity, and BLM-based predicted EC50 values for each event are reported in the Appendix. Harbor-wide averages for each sampling event are shown in Table 3.

Salinity measurements showed little change over time and depth. However, there was higher variability in the salinity data during the wet weather event (higher coefficient of variation), which could be due to the freshwater input from rainfall and runoff. Temperature did not vary much by depth but showed a steady increase over time moving from winter through summer. The average pH values were within 0.1 pH units, with similar pH values measured in March and September (7.95) and a slightly reduced pH value (7.84) in May.

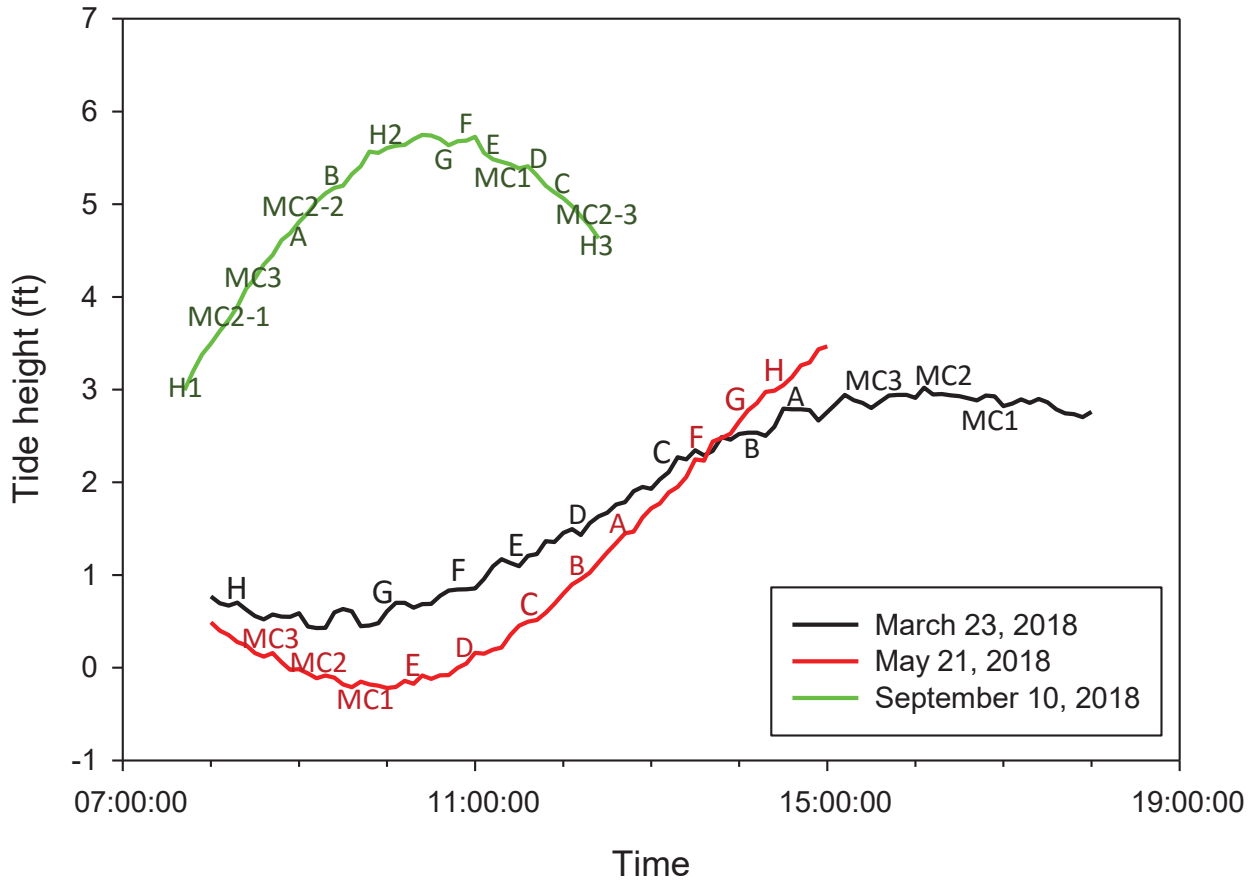

Figure 2. Tide height over the timeframe for each sample collection event: March (black), May (red), and September (green).

DOC and chlorophyll values were the most variable measures by time and depth when compared to temperature, pH, and DO. DOC was higher at most stations in the winter-wet weather event compared to the two dry weather events (Figure 3). The lowest DOC values were frequently in the front basins (A) or in the main channel near the mouth of the harbor (MC3). The location of higher DOC values was more variable, but they frequently occurred in the back basins. This pattern is likely due to water circulation patterns in the Harbor. Chlorophyll showed an increase in concentration from the first event to the second and third events. Chlorophyll concentrations also varied spatially with higher concentrations usually present in the back basins.

Both DOC and chlorophyll were significantly different over time. It was determined that for both DOC and chlorophyll, data from events 2 and 3 (both dry weather events) were different from event 1 (wet weather) but not different from each other. Significant differences by depth were found for DOC and chlorophyll for event 1 (wet weather) but not event 2 (dry weather). Because of the higher variability in time, depth, and space, DOC and chlorophyll may be important determinants of the critical condition.

Harbor average copper concentration was similar among sampling events. Dissolved copper concentrations were not significantly different over time or depth. For all three sampling events, dissolved copper concentrations were similar among most stations except for the main channel

stations in the front basin area (Figure 4). Dissolved copper concentrations at most stations exceeded the current water quality objective of 3.1 µg/L. Stations MdRH-MC2 and -MC3 frequently had lower copper concentrations in surface and/or bottom water samples compared to samples from the rest of the Harbor. Surface concentrations during the winter-wet weather event tended to be higher than bottom concentrations, but these differences were not statistically significant.

Table 3. Harbor-wide average values for MdRH water quality parameters by sampling event and depth. Data are presented as "average (coefficient of variation)".

Sampling Event	Depth	Dissolved Copper (µg/L)	DOC (mg/L)	Chlorophyll (µg/L)	pH*	Salinity (ppt)	Temperature (°C)
1	Surface	6.64 (33)	1.10 (12)	1.72 (41)	7.93	33.22 (1.7)	16.97 (2.7)
	Bottom	4.70 (54)	0.94 (13)	3.69 (61)	7.95	33.90 (3.1)	16.73 (3.3)
	All	5.71 (44)	1.02 (15)	2.66 (71)	7.94	33.54 (2.6)	16.85 (3.0)
2	Surface	7.02 (17)	0.77 (20)	3.14 (42)	7.84	33.28 (0.38)	19.55 (3.4)
	Bottom	5.64 (47)	0.76 (23)	4.25 (63)	7.84	33.18 (0.18)	19.26 (4.4)
	All	6.36 (33)	0.77 (21)	3.67 (57)	7.84	33.23 (0.33)	19.41 (3.9)
3	Surface	5.71 (33)	0.84 (8.6)	3.10 (41)	7.94	34.07 (0.64)	22.45 (2.6)

*Average is based on hydronium ion (H_30^+) concentration and converted back to the log scale. As such, no CV is reported.

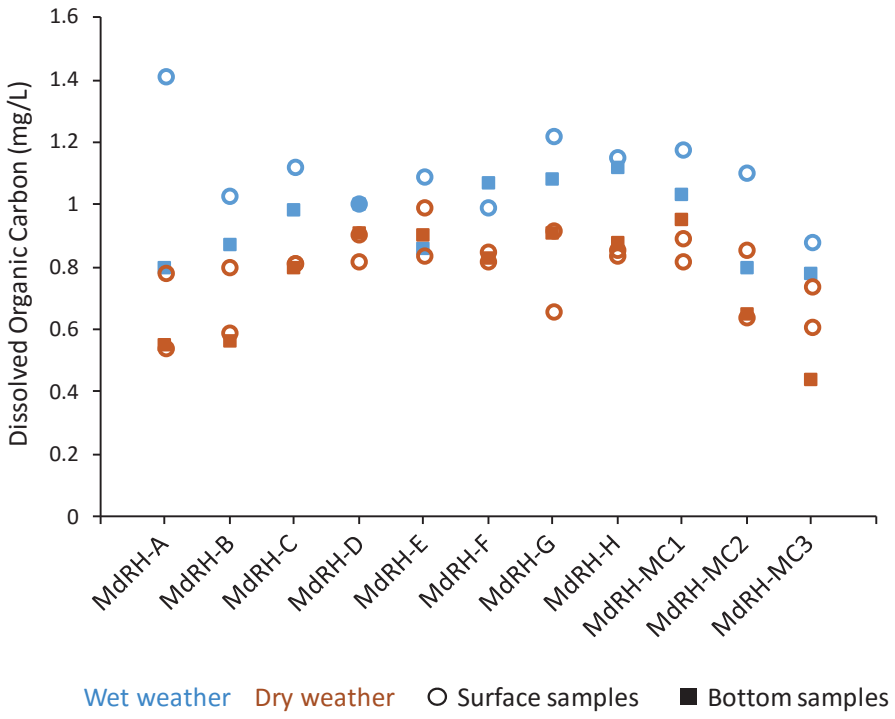

Figure 3. Dissolved organic carbon concentrations (mg/L) at each station by depth (surface=open circles, bottom=closed squares) and weather (wet=blue, dry=orange).

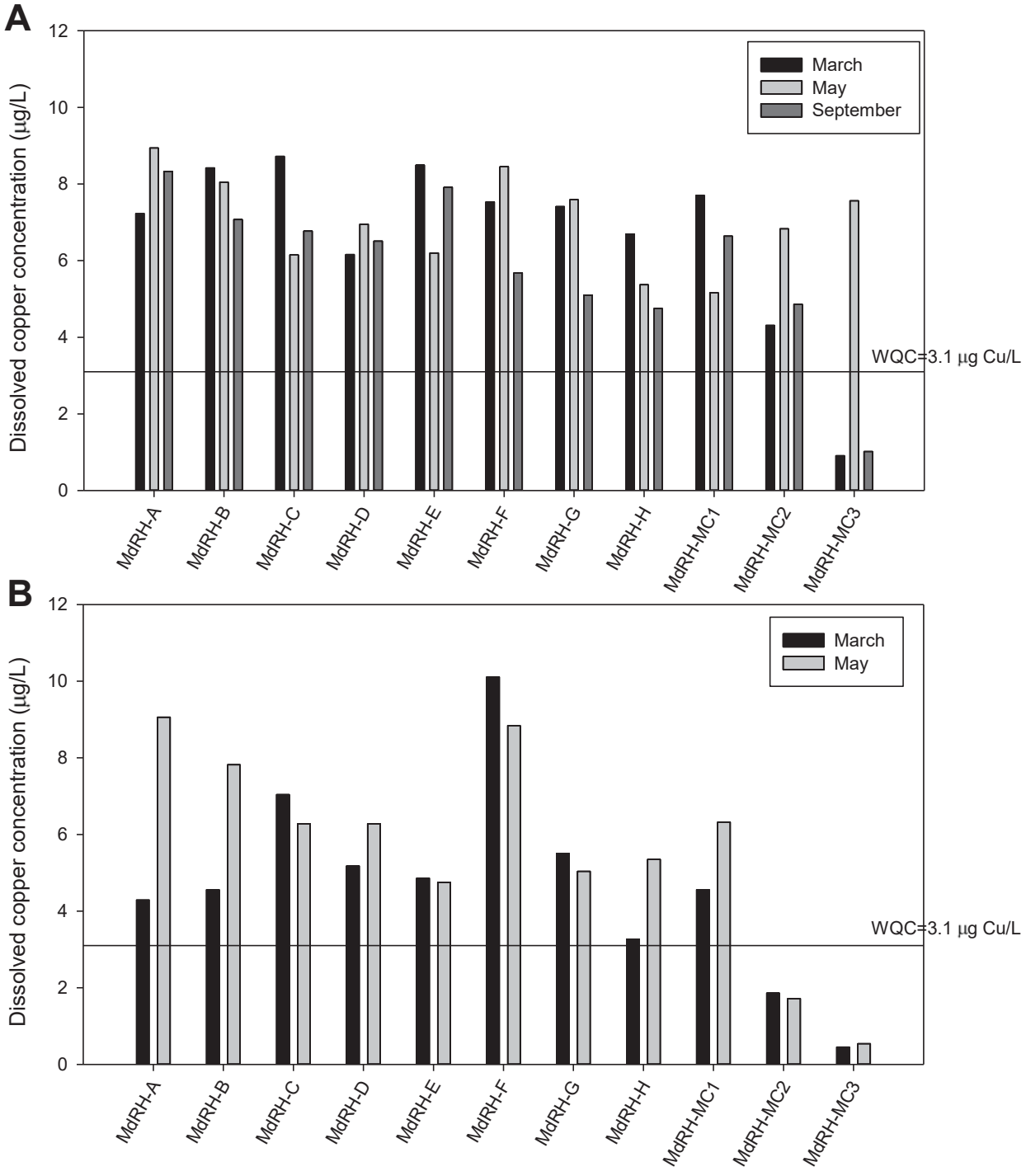

Figure 4. Dissolved copper concentration at each station over time in surface samples (A) and bottom samples (B).

Relationships Among Water Quality Parameters

Correlation analysis was performed for each sampling event to identify relationships between DOC (controlling factor for copper bioavailability) and tide height (as a measure of coastal water influx), chlorophyll, or copper. For the winter-wet weather event, no correlations were found for surface water samples. Bottom samples had a significant correlation (p=0.015) between tide height and DOC concentration. This is an inverse relationship where a higher tide height is related to lower DOC. This correlation is driven by low DOC in two of the main channel stations which were both sampled at high tide. Although this result is statistically significant, this depth-related trend may be an artifact of the station sampling sequence. The main channel stations, expected to have relatively low DOC due to greater mixing with offshore water, were sampled at the end of the day (highest tide height).

For the spring-dry weather event, there was no correlation between DOC and tide height. Surface samples had two significant correlations: a correlation (p=0.031) between dissolved copper and DOC concentration, and a correlation (p=0.011) between DOC concentration and chlorophyll concentration. The first is an inverse relationship where a higher DOC concentration is related to lower copper concentration, and the second is a direct correlation where a higher DOC concentration is related to a higher chlorophyll concentration. The bottom samples only had one significant direct correlation between DOC concentration and chlorophyll concentration (p=0.002).

For the summer-dry weather event, none of the parameters were significantly correlated with DOC. The lack of correlation may have been due to the narrow range of DOC values, as there is a minimal spread of data with which to observe a correlation.

Table 4. Summary of parameter correlations with DOC.

		DOC (mg/L)		
Depth	**Parameter**	**March**	**May**	**September**
Surface	Chlorophyll (ug/L)	-0.40 (0.19)	*0.69 (0.011)**	0.20 (0.43)
	Tide height (ft)	-0.011 (0.96)	-0.37 (0.22)	-0.10 (0.69)
	Diss. Copper (µg/L)	0.32 (0.31)	*-0.62 (0.031)**	0.41 (0.15)
Bottom	Chlorophyll (ug/L)	0.036 (0.90)	*0.79 (0.002)**	
	Tide height (ft)	*-0.70 (0.015)**	-0.077 (0.80)	
	Diss. Copper (µg/L)	0.50 (0.11)	0.011 (0.97)	

Data is presented as "correlation coefficient (p Value)". A negative correlation coefficient signifies an inverse relationship. **Denotes significance*.

Short-term variability in water quality associated with tide height was investigated in the summer sampling event. Stations MdRH-H and MdRH-MC2 were sampled three times over the duration of the sampling event, with a duplicate sample taken during the second visit. Variability in DOC and chlorophyll was compared to results for the duplicate samples (i.e., sampling variability). DOC concentration in the repeated samples was more variable than in the duplicates for both stations (Figure 5). The highest DOC value was measured in the last sample collected, which represented an outgoing tide. The magnitude of the DOC change was relatively small, however. Greater variability in the chlorophyll data was observed for the repeated samples (Figure 6).

However, the range of concentrations among the repeated samples was similar to that for the duplicate, indicating little effect of tide height on the results.

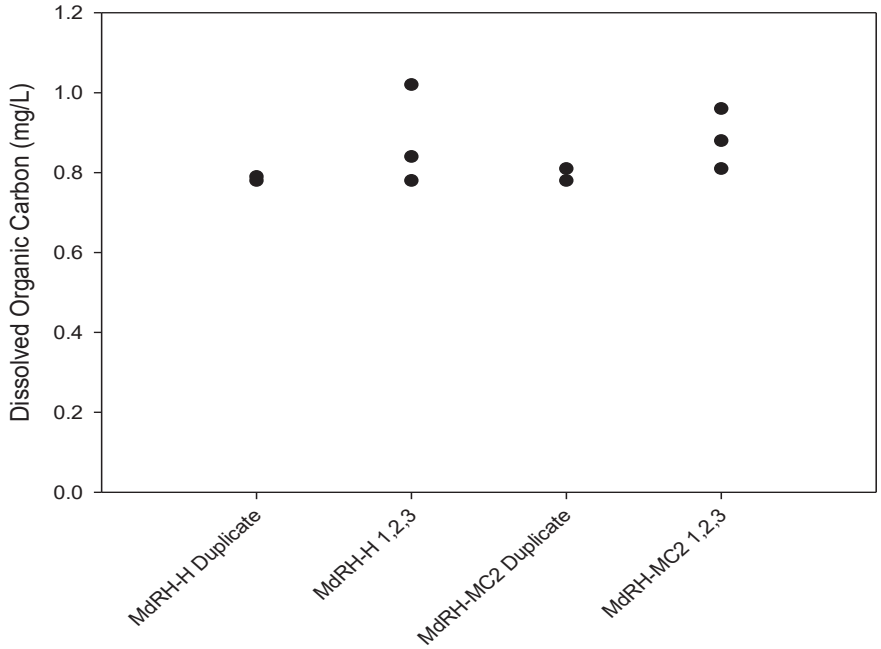

Figure 5. Dissolved organic carbon concentration (mg/L) as a function of duplicate and repeated measurements at stations MdRH-H and -MC2.

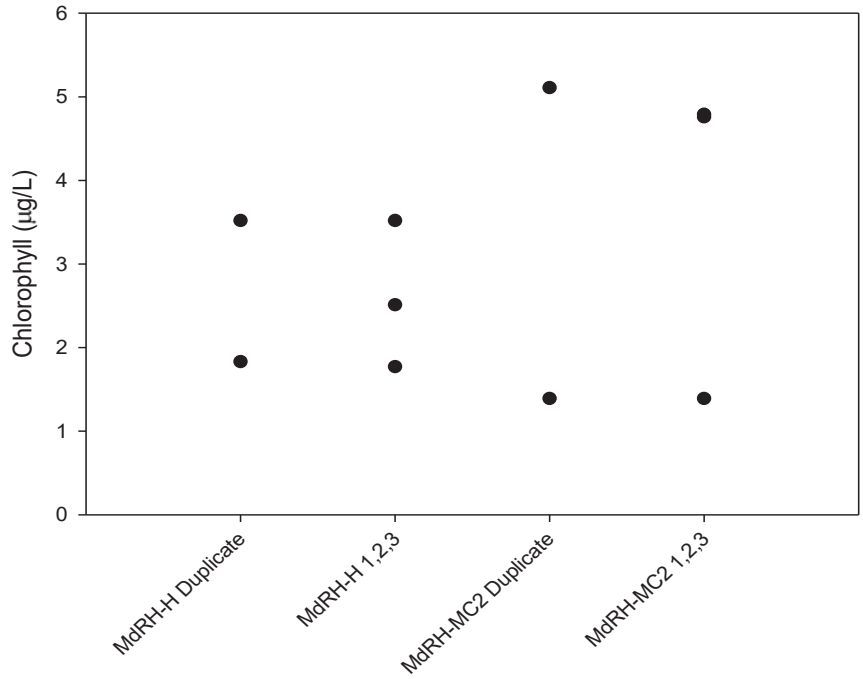

Figure 6. Chlorophyll concentration (mg/L) as a function of duplicate and repeated measurements at stations MdRH-H and -MC2.

Toxicity

Toxicity tests were performed on all samples collected, using the mussel embryo development test. All toxicity results are listed in the Appendix (Tables A1, A2, and A3). These data are used to better characterize MdRH and determine the ambient toxicity in preparation for the WER study, but they are not required to determine the critical condition. The tests for events 1 and 2 met all applicable test performance criteria. The event 3 control (76% normal) fell below the control acceptability threshold of 90% normal. No definitive cause for the low control result was determined but may have been related to general difficulty in obtaining good quality spawning during the warmer summer months. As such, the toxicity results from September are uncertain.

Reference toxicity tests using copper were conducted for each round of testing and all resulting EC50 concentrations fell within two standard deviations of our control chart mean, indicating similar sensitivity of the organisms used in each test. The calculated EC50 value for events 1, 2, and 3 were 8.81 µg Cu/L, 8.23 µg Cu/L, and 7.33 µg Cu/L, respectively. Based on our control chart, these organisms have similar sensitivity to copper as seen in previous tests.

No toxicity was observed for samples from events 1 and 2 (Figure 7). Four stations from event 3 displayed high toxicity: MdRH-B, -E, -F, and -MC1. These results ranged from 12.1-38.7% normal (control-adjusted). However, for all three events, no significant correlation was found between the toxicity test results and the dissolved copper concentrations; there were nontoxic samples from all three events with copper concentrations that were similar to those showing toxicity.

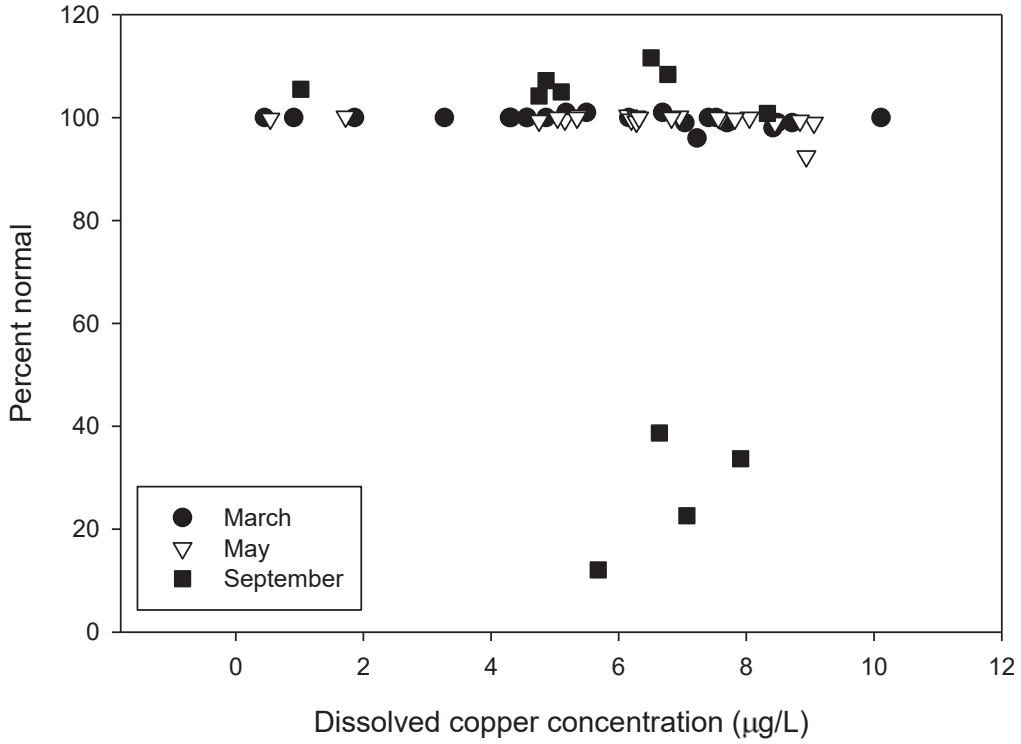

Figure 7. Toxicity as a function of dissolved copper concentration (µg/L) for the three sampling events: March (circles), May (triangles), and September (squares).

Biotic-Ligand Model Predictions

The Biotic Ligand Model developed for the draft EPA marine water quality criteria for copper was applied, utilizing the pH, temperature, salinity, and DOC data for each station for all three events. The BLM results provide a predicted acute EC50 concentration based on dissolved copper for *Mytilus galloprovincialis* (same species used in this study). The EC50 value is the concentration at which 50% of the organisms are affected. Thus, it is possible to see some toxicity in water samples with measured concentrations lower than the predicted EC50. The BLM EC50 provides a prediction of toxicity potential for copper, which can be used as another indicator of water quality conditions corresponding to the critical condition. The BLM predicted acute EC50 values were calculated for each station from all three sampling events. Predicted EC50 values ranged from 5.7 to 10.3 µg/L for event 1, 4.0 to 7.3 µg/L for event 2, and 5.8 to 7.6 µg/L for event 3.

Most of the event 1 samples had dissolved copper concentrations less than or equal to the predicted EC50 value (Figure 8A, B). This suggests that low or no toxicity is expected at those stations, which was consistent with the results of the toxicity test. No toxicity was observed for any sample. The results were more variable for event 2, with approximately half of the measured copper values greater than the predicted EC50 (Figure 8C, D). Based on these predictions, about half of the water samples would be expected to show toxicity. However, no toxicity was observed in any sample from event 2. Event 3 results were also variable with six of the measured values less than the predicted EC50 and seven of the measured values greater than the predicted EC50 (Figure 8E). Of the four samples that displayed toxicity, three had measured copper concentrations greater than the predicted EC50. Of the nine non-toxic samples, three of them had measured dissolved copper concentrations greater than the predicted EC50. Overall, 9 out of 13 toxicity test results matched with expectations based on the BLM predicted EC50 value and measured dissolved copper concentrations.

The lack of correspondence between BLM predictions and toxicity results is greater than expected (B. Santore, pers. Comm.), and no explanation is available at this time. Possible factors influencing the results include variations in the sensitivity of the toxicity test that are not identified from the reference toxicant test, toxicity due to materials other than copper, and presence of unmeasured factors influencing bioavailability. Additionally, the nature of the DOC may vary and influence the bioavailability of copper (De Schamphelaere et al., 2004; Nadella et al., 2009).

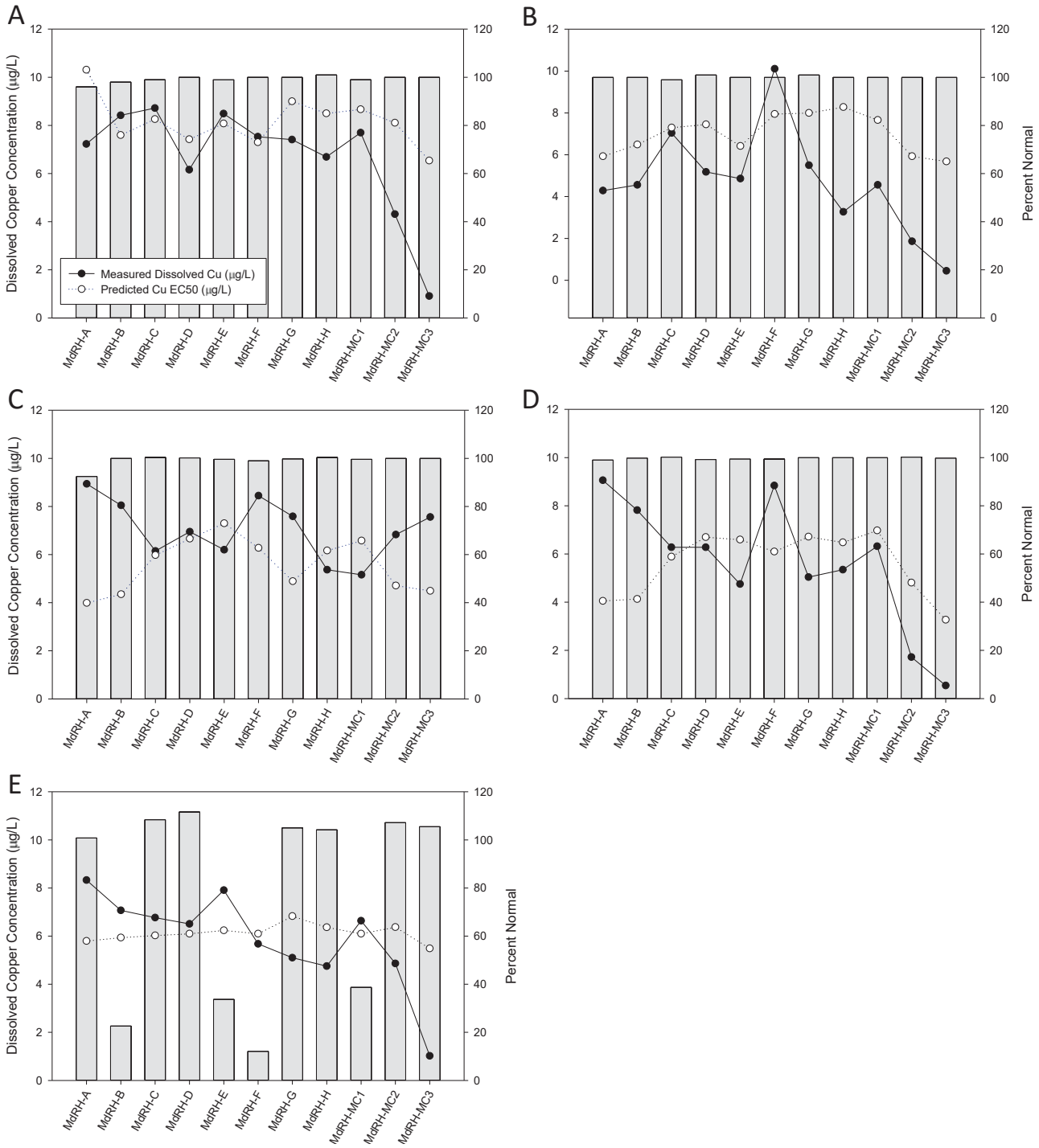

Figure 8. A comparison of the measured (black circles) dissolved copper concentration (µg/L) to the BLM predicted EC50 value (white circles, µg/L) and the toxicity test results (grey bars) for surface and bottom water samples from March (A and B), surface and bottom water samples from May (C and D), and surface water samples from September (E).

SUMMARY

The critical condition is based on when copper bioavailability is expected to be highest, resulting in the greatest toxic potential to resident organisms. This study investigated the influence of nine water quality and other factors on bioavailabity (Table 5). Three factors were identified as having the strongest influence on bioavailability: weather (e.g., presence of stormwater runoff), DOC, and location within the harbor. DOC is established in the scientific literature as having a dominant influence on copper bioavailability in marine waters, which supports the conclusion that DOC is a primary factor influencing the critical condition in MdRH. Wet weather conditions that result in stormwater runoff discharges to the harbor are likely to reduce copper bioavailability through an increase in DOC content. However, the nature of DOC input from stormwater may differ from the natural marina DOC which can alter the type of organic matter present between wet and dry events leading to differences in copper bioavailability.

Station location also had a strong influence on DOC content, and thus predicted bioavailability. Stations located near the front region of the harbor generally had the lowest DOC concentrations, possibly due to greater tidal exchange with offshore water having lower DOC. Station location was the factor with most consistent influence on DOC concentration for all three sampling events.

Tide stage and chlorophyll were shown to influence some water quality parameters and potentially bioavailability, but to a minor degree. Correlations between tide height and DOC or copper were occasionally present. However, the magnitude of variation associated with tide stage was similar to that observed among different station locations. Variation in chlorophyll was occasionally associated with DOC variability, likely due to variations in phytoplankton abundance in the harbor. This result indicates that bioavailability of copper may be reduced when phytoplankton blooms are present.

The copper and toxicity measurements made in this study do not directly influence conclusions about the critical condition, as they do not affect bioavailabity. However, these parameters are important to developing a study design that includes both the critical condition and conditions representative of harbor locations where impacts are likely to occur. Copper concentrations were similar among most harbor stations located in basins, with the greatest variation apparently associated with seasonal factors. Toxicity to mussels was infrequent in this study, with most of the toxic stations located in the harbor back basins. Thus, a study design that includes back basin locations is likely to represent conditions where biological impacts from dissolved copper are greatest.

In summary, the critical condition (greatest copper bioavailability) is most likely to occur at harbor locations with the lowest DOC. DOC is expected to be lowest during dry weather periods throughout the year and at locations nearest the harbor entrance where water exchange is likely greatest (e.g., main channel and front basins). However, variations in water quality is also expected to occur because of other factors that are poorly understood or difficult to predict. Thus, sampling at multiple locations in the harbor, and over multiple time points, is needed to characterize variations in copper bioavailability.

It is recommended that a study design that includes multiple harbor locations and time points be used for water effect ratio determination in MdRH. Sampling at five locations, representing all three main regions of the harbor, should be sufficient to capture the critical condition for copper variability, as well representing the breadth of harbor conditions for evaluation of a site-specific objective. The recommended station locations are shown in Figure 9.

Table 5. Summary of the effects of the various parameters on the critical condition.

Parameter	Effect on the Critical Condition?		
	Yes	No	Minor
Weather	X		
Tide height			X
Sampling depth		X	
Chlorophyll			X
DOC	X		
Temperature		X	
Salinity*		X	
pH*		X	
Station location	X		

*Data did not vary greatly over time or space. With these minor changes, no effect on critical condition was observed.

Figure 9. Proposed stations for WER analysis.

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APPENDIX: SITE CHARACTERIZATION DATA

Table A1. Data collected from the winter wet weather site characterization sampling event on March 23, 2018.

Station	Depth	Sample Time	Tide (ft)	pH	Salinity (ppt)	Temp (°C)	DO (mg/L)	DOC (mg/L)	Chlorophyll (µg/L)	Total Cu (µg/L)	Diss. Cu (µg/L)	Total Zn (µg/L)	Diss. Zn (µg/L)	Mussel embryo %Normal (Control Adjusted)	BLM Diss. Cu (µg/L) - Predicted Acute EC_{50}
MdRH-A	Surface	1440	2.789	7.76	33.3	16.3	7.8	1.41	1.50	9.36	7.23	55.1	38.5	96.4	10.31
MdRH-A	Bottom	1440	2.789	7.97	33.4	16.5	7.8	0.80	2.01	4.91	4.29	35.1	24.0	100.5	5.93
MdRH-B	Surface	1408	2.536	7.93	32.9	17.5	7.7	1.03	0.88	9.43	8.42	52.2	42.5	98.0	7.60
MdRH-B	Bottom	1408	2.536	7.93	34.7	16.9	8.8	0.87	7.65	9.44	4.56	56.8	25.2	100.2	6.49
MdRH-C	Surface	1313	2.11	7.92	32.8	17.5	7.7	1.12	1.01	8.51	8.72	56.4	45.8	98.8	8.26
MdRH-C	Bottom	1313	2.11	7.94	34.5	17.4	7.8	0.98	1.80	8.24	7.04	49.6	39.3	99.2	7.30
MdRH-D	Surface	1212	1.43	7.90	33.1	17.2	7.8	1.02	2.53	8.51	6.58	56.4	38.3	100.2	7.53
MdRH-D-Dup	Surface	1212	1.43	7.88	33.4	17.5	7.4	0.99	3.12	7.82	5.74	48.6	34.7	100.6	7.32
MdRH-D	Bottom	1250	1.903	7.95	34.6	17.2	7.6	1.00	7.01	8.24	5.18	49.6	33.5	100.5	7.45
MdRH-E	Surface	1132	1.096	7.93	33.7	17.1	7.6	1.09	2.31	10.28	8.49	64.8	55.6	98.7	8.08
MdRH-E	Bottom	1132	1.096	7.87	35.0	17.1	7.0	0.86	4.42	7.10	4.86	52.4	33.6	100.2	6.42
MdRH-F	Surface	1050	0.843	7.98	32.9	16.9	7.4	0.99	1.84	8.89	7.53	48.3	37.4	99.6	7.30
MdRH-F	Bottom	1050	0.843	7.88	34.3	16.9	6.9	1.07	3.88	7.37	10.11	45.9	30.2	99.8	7.95
MdRH-G	Surface	1000	0.607	7.97	32.9	16.7	7.4	1.22	1.07	8.24	7.41	49.8	35.5	99.8	9.00
MdRH-G	Bottom	1000	0.607	7.98	33.7	16.8	7.9	1.08	2.72	6.41	5.50	39.9	28.7	100.7	8.00
MdRH-H	Surface	820	0.702	7.92	33.2	16.8	7.2	1.15	0.93	7.03	6.69	38.8	28.8	100.6	8.50
MdRH-H	Bottom	820	0.702	7.93	33.2	17.2	7.6	1.12	1.07	6.81	3.27	40.4	23.2	100.2	8.28
MdRH-MC1	Surface	1651	2.936	7.98	32.3	17.0	7.9	1.18	2.10	8.66	7.70	52.1	38.8	98.8	8.67
MdRH-MC1	Bottom	1651	2.936	7.98	34.4	16.4	8.2	1.03	5.69	6.97	4.56	45.4	29.7	99.8	7.66
MdRH-MC2	Surface	1620	2.953	8.05	33.5	17.0	8.2	1.10	1.41	5.32	4.31	37.3	24.8	100.0	8.11
MdRH-MC2	Bottom	1620	2.953	7.99	33.8	15.9	8.1	0.80	1.66	3.81	1.86	30.7	11.4	100.4	5.93

Station	Depth	Sample Time	Tide (ft)	pH	Salinity (ppt)	Temp (°C)	DO (mg/L)	DOC (mg/L)	Chlorophyll (µg/L)	Total Cu (µg/L)	Diss. Cu (µg/L)	Total Zn (µg/L)	Diss. Zn (µg/L)	Mussel embryo %Normal (Control Adjusted)	BLM Diss. Cu (µg/L) - Predicted Acute EC_{50}
MdRH-MC3	Surface	1535	2.871	8.04	34.6	16.1	9.3	0.88	1.88	1.32	0.91	19.8	4.9	100.5	6.54
MdRH-MC3	Bottom	1535	2.871	8.07	31.3	15.7	8.8	0.78	2.71	0.83	0.45	17.3	4.1	99.6	5.68
QA samples															
Field Bk*										<0.005	0.03	17.9	0.878		
Travel Bk										<0.005	NA	<0.005	NA		
Tubing Bk										0.14	0.072	13	12.8		

Diss.-Dissolved DO-Dissolved oxygen DOC-Dissolved organic carbon Zn-Zinc Cu-Copper

*The Field blank includes the use of the sample containers and syringe filter system.

Table A2. Data collected from the spring dry weather site characterization sampling event on May 21, 2018.

Station	Depth	Sample Time	Tide (ft)	pH	Salinity (ppt)	Temp (°C)	DO (mg/L)	DOC (mg/L)	Chlorophyll (µg/L)	Total Cu (µg/L)	Diss. Cu (µg/L)	Total Zn (µg/L)	Diss. Zn (µg/L)	Mussel embryo %Normal (Control Adjusted)	BLM Diss. Cu (µg/L) - Predicted Acute EC_{50}
MdRH-A	Surface	1238	1.34	7.83	33.2	19.7	7.4	0.54	1.57	10.9	8.94	34.2	32.0	92.5	3.99
MdRH-A	Bottom	1246	1.47	7.82	33.1	19.3	7.0	0.55	2.05	12.0	9.06	34.0	31.3	99.0	4.05
MdRH-B	Surface	1211	0.96	7.83	33.1*	20.3	7.3	0.59	1.85	9.94	8.05	31.0	30.8	100.0	4.35*
MdRH-B	Bottom	1219	1.02	7.83*	33.1	19.8	7.2	0.56	2.50	9.45	7.82	30.8	29.1	99.8	4.13*
MdRH-C	Surface	1141	0.52	7.86	33.3	19.0	7.3	0.81	2.40	8.41	6.15	30.4	26.6	100.4	5.98
MdRH-C	Bottom	1152	0.69	7.82	33.2	19.7	6.7	0.80	5.38	8.35	6.28	29.5	26.7	100.2	5.89
MdRH-D	Surface	1053	0.05	7.83	33.2	19.5	7.1	1.00	2.87	13.0	6.71	42.3	34.1	100.0	7.36
MdRH-D-Dup	Surface	1105	0.15	7.83*	33.2*	19.5*	7.1*	0.81	5.55	11.1	7.19	39.7	35.3	100.4	5.97*
MdRH-D	Bottom	1115	0.19	7.83*	33.2	19.7	6.5	0.91	3.85	17.0	6.28	46.2	34.4	99.2	6.90*
MdRH-E	Surface	1019	-0.17	7.84	33.3	19.2	6.4	0.99	5.07	12.7	6.20	49.3	40.0	99.6	7.30
MdRH-E	Bottom	1031	-0.12	7.79	33.2	19.4	5.9	0.90	3.60	16.8	4.75	47.8	34.6	99.4	6.60
MdRH-F	Surface	1333	2.24	7.87	33.2	20.9	7.3	0.85	3.79	10.4	8.45	36.7	36.0	99.0	6.28
MdRH-F	Bottom	1339	2.44	7.81	33.1	20.3	6.8	0.83	5.09	10.3	8.84	39.0	40.4	99.4	6.10
MdRH-G	Surface	1400	2.66	7.93	33.3	20.1	7.1	0.66	2.04	9.68	7.59	31.9	30.9	99.8	4.89
MdRH-G	Bottom	1408	2.78	7.86	33.2	19.6	7.9	0.91	10.39	6.91	5.04	21.2	22.3	100.0	6.72
MdRH-H	Surface	1426	2.99	7.79	33.2	19.8	7.1	0.84	3.44	8.79	5.37	30.5	22.4	100.4	6.17
MdRH-H	Bottom	1437	3.14	7.81	33.3	19.4	7.0	0.88	4.21	7.08	5.35	24.2	22.5	100.0	6.48
MdRH-MC1	Surface	0949	-0.18	7.86	33.4	19.3	6.6	0.89	3.88	9.79	5.16	38.7	24.3	99.6	6.58
MdRH-MC1	Bottom	0959	-0.22	7.81	33.2	19.2	6.5	0.95	6.80	8.32	6.32	32.3	30.2	100.0	6.98
MdRH-MC2	Surface	0917	-0.08	7.79	33.5	18.6	6.8	0.64	3.63	8.47	6.83	33.2	28.6	100.0	4.71
MdRH-MC2	Bottom	0928	-0.18	8.01	33.2	18.3	6.9	0.65	2.15	4.21	1.72	11.8	6.79	100.2	4.81
MdRH-MC3	Surface	0840	0.16	7.80	33.5*	18.7	5.4	0.61	1.62	9.10	7.56	29.8	29.1	100.0	4.49*
MdRH-MC3	Bottom	0854	-0.02	7.92	33.2*	17.2	5.6	0.44	0.76	1.48	0.541	3.63	2.14	99.8	3.27*

Station	Depth	Sample Time	Tide (ft)	pH	Salinity (ppt)	Temp (°C)	DO (mg/L)	DOC (mg/L)	Chlorophyll (µg/L)	Total Cu (µg/L)	Diss. Cu (µg/L)	Total Zn (µg/L)	Diss. Zn (µg/L)	Mussel embryo %Normal (Control Adjusted)	BLM Diss. Cu (µg/L) - Predicted Acute EC_{50}
QA samples															
Field Bk**										<0.005	<0.005	<0.005	0.202		
Travel Bk										<0.005	<0.005	<0.005	<0.005		
Tubing Bk										0.118	<0.005	19.5	2.26		

Diss.-Dissolved DO-Dissolved oxygen DOC-Dissolved organic carbon Zn-Zinc Cu-Copper

*Main salinity meter was giving values which seemed very high. A second, uncalibrated unit that was giving more reasonable values was used at the remaining sites. Missing values were completed using the other field measurement (surface or bottom) as there was little observed difference. These values were used in the calculation of the BLM Predicted FAV.

**The Field blank includes the use of the sample containers and syringe filter system.

Table A3. Data collected from the summer dry weather site characterization sampling event on September 10, 2018.

Station	Depth	Sample Time	Tide (ft)	pH*	Salinity* (ppt)	Temp (°C)	DO (mg/L)	DOC (mg/L)	Chlorophyll (µg/L)	Total Cu (µg/L)	Diss. Cu (µg/L)	Total Zn (µg/L)	Diss. Zn (µg/L)	Mussel embryo %Normal (Control Adjusted)	BLM Diss. Cu (µg/L) - Predicted Acute EC_{50}*
MdRH-A	Surface	0851	4.69	7.90	34.1	22.1	7.1	0.78	1.36	10.1	8.33	39.0	36.9	100.8	5.80
MdRH-B	Surface	0925	5.18	7.90	34.0	22.4	7.8	0.80	4.15	8.45	7.07	32.9	30.8	22.6	5.94
MdRH-C	Surface	1157	5.13	7.90	34.2	23.0	7.1	0.81	3.56	7.71	6.77	32.4	30.1	108.4	6.03
MdRH-D	Surface	1139	5.41	7.89	34.4	23.1	7.1	0.82	4.08	9.37	6.51	39.3	35.5	111.6	6.10
MdRH-E	Surface	1112	5.49	7.88	34.0	23.0	7.0	0.84	2.55	9.80	7.91	51.5	48.9	33.7	6.24
MdRH-F	Surface	1057	5.69	7.92	34.0	23.0	7.0	0.82	2.70	8.90	5.68	30.9	26.9	12.1	6.10
MdRH-G	Surface	1041	5.64	7.91	34.0	22.7	7.7	0.92	4.40	6.67	5.10	25.2	23.2	105.0	6.83
MdRH-H-1	Surface	0744	3.00	7.98	33.9	22.2	6.5	0.84	1.77	NA	NA	NA	NA	NA	6.24
MdRH-H-2	Surface	1001	5.61	7.93	34.1	22.4	7.6	0.78	3.52	6.91	4.87	23.8	21.4	102.6	5.80
MdRH-H-2 Dup	Surface	1010	5.64	8.03	33.8	22.4	7.3	0.79	1.83	6.44	4.63	23.7	22.1	105.8	5.86
MdRH-H-3	Surface	1225	4.63	7.99	34.3	22.6	7.7	1.02	2.51	NA	NA	NA	NA	NA	7.58
MdRH-MC1	Surface	1125	5.43	7.92	34.1	22.9	7.4	0.82	1.68	8.41	6.64	34.7	31.0	38.7	6.10
MdRH-MC2-1	Surface	0812	3.75	7.98	34.6	22.2	7.7	0.88	4.76	NA	NA	NA	NA	NA	6.56
MdRH-MC2-2	Surface	0903	4.91	7.95	34.0	22.1	7.8	0.81	1.39	6.14	4.96	23.4	23.6	104.7	6.02
MdRH-MC2-2 Dup	Surface	0911	5.03	7.94	33.9	22.2	7.7	0.78	5.11	5.85	4.76	24.1	20.9	109.7	5.80
MdRH-MC2-3	Surface	1213	4.87	8.03	34.1	22.7	8.3	0.96	4.79	NA	NA	NA	NA	NA	7.12
MdRH-MC3	Surface	0828	4.20	7.96	33.7	20.6	7.9	0.74	2.53	1.42	1.02	5.68	4.09	105.5	5.49
QA samples															
Field Bk**										<0.005	<0.005	1.34	2.44		
Travel Bk										<0.005	<0.005	<0.0025	<0.0025		
Tubing Bk										0.755	0.376	10.9	18.5		

Diss.-Dissolved DO-Dissolved oxygen DOC-Dissolved organic carbon Zn-Zinc Cu-Copper NA-Not Analyzed

*Salinity and pH meters were not functional in the field. Data were recorded in the laboratory on September 11, 2018 using water samples collected from the field.

**The Field blank includes the use of the sample containers and syringe filter system.

APPENDIX E
COMMENT RESPONSE TABLE

Commenter No.	Author
1.	Technical Advisory Committee (TAC Summary Recommendations Memo)
2.	Los Angeles Regional Water Quality Control Board (communicated by Shana Rapoport)
3.	Los Angeles Waterkeeper and Heal the Bay (communicated by Arthur S. Pugsley)
4.	Stakeholder (Johntommy Rosas; comments provided in four emails)
5.	Stakeholder (Douglas P. Fay)

The comments tabulated in the following pages are numbered according to comment letter.

No.	Comment	Response	Change
1.1	Charge Question 1: "As with all questions about study design, there is a trade-off between sampling effort and cost. The TAC discussed this question mainly considering roughly the same effort as in the proposed sampling design, that is, about 30 samples, rather than thinking about how additional samples could be added to improve the design. With this in mind, the TAC considered how well the sampling design would provide representative samples in terms of space and time. We propose composite sampling as a general way to increase representativeness without increasing analytical costs."	The TAC has proposed both spatial and temporal compositing to provide a more representative sampling scheme that takes into account spatial variability and variability associated with time (i.e., weather and tidal changes). We agree that this sample compositing will provide a better representation of the condition in the Harbor.	Composite sampling has been added to Sampling Design (Section 2.2.3) and Appendix A (Water Sampling Methods).
1.2	Charge Question 2a: "Zinc is a difficult metal to study, due to the ease with which samples can be inadvertently contaminated. It is essential that trace-metal-clean protocols be used for sample collection and that the Quality Assurance/Quality Control protocols include the appropriate use of certified reference materials (CRMs). The Shelter Island study is a good model for the proper use of CRMs both for copper and DOC."	We agree that trace metal clean protocols will be used. Additionally, we will add a DOC and dissolved copper CRM to the sample analysis and QA/QC methods.	This has been added to 2.2.5 Sample Collection and Processing, 2.4.2 Chemistry Quality Assurance/Quality Control, and Appendix A.
1.3	Charge Question 2b: "The choice of filters here is critical, as inadvertent contamination of the samples with metals or dissolved organic carbon must be avoided. Note that choice of filters may well be different for samples destined for metal analyses and for those that will be analyzed for DOC."	We agree that background contamination by filters should be avoided as well as taken into account through appropriate field blanks for all sample types.	A DOC field blank has been added to Table 9 and Appendix A (Water Sampling Methods).
1.4	Charge Question 2c: "Table 7: Containers for water samples. The glass vials used to collect and store the water samples destined for DOC analysis should be pre-combusted."	We agree. We use pre-combusted amber glass vials for DOC sample collection.	Clarification made in Table 7.
1.5	Charge Question 2d: "The TAC also suggested that the samples collected for DOC analysis could also be subjected to simple spectrophotometric and spectrofluorometric characterization [...] This additional information about the nature of the DOC will be particularly useful in the planned comparisons between the observed toxicity and the BLM-predicted toxicity."	We agree that better characterization of the DOC may be useful for comparisons of observed toxicity and predicted toxicity test results.	Selected samples will be characterized, and the results compared to the

No.	Comment	Response	Change
			toxicity results (Table 6).
1.6	Charge Question 2e: "Among the advantages of using this test organism, the TAC considered its recognized sensitivity to copper, the existence of a wealth of published data on copper – *Mytilus* early life stage interactions and the precedent that the development of Site-Specific Objectives (SSO) for copper in other coastal environments in California had used this organism and this test. The TAC also suggested that if it proved difficult to induce spawning in laboratory cultures on *M. galloprovincialis*, other sensitive test organisms could be considered. These include embryos/larvae from *Mytilus californianus* (California mussel) and *Crassostrea gigas* (Pacific oyster). "	We agree that the chosen test species (*Mytilus galloprovincialis*) is sensitive to copper and appropriate for the WER testing. We also agree that similarly sensitive organisms could be substituted if needed.	Clarification made in Toxicity Test Species and Method Selection.
1.7	Charge Question 3: "The TAC is satisfied that the proposed use of the BLM in the work plan, i.e., as a tool to compare measured with predicted toxicities, is the correct manner to proceed. In the view of the TAC, it would be premature to use the marine copper BLM to calculate the SSO for Marina del Rey, i.e., as substitute for the toxicity tests and their use in a WER procedure. However, the use of the BLM to compare predicted and observed toxicities in the unspiked and copper-spiked samples will help answer one of the questions raised by stakeholder participants in the TAC December meeting, namely is copper the only stressor to which *M. galloprovincialis* is responding in the toxicity tests. Consistent agreement between the predicted and observed toxicities would support the argument that copper is the principal chemical stressor in Marina del Rey waters."	We agree. Due to several other comments regarding use of the BLM, the workplan will be revised to reflect the TAC's recommended use of the BLM for comparison purposes only.	Clarifications made in the Introduction.
1.8	Charge Question 4: "The determination of the final WER (fWER) is complicated and more complex than the TAC can fully resolve at this time. […] The TAC believes that the proposed study plan will provide sufficient data, as identified by the USEPA Interim Guidance, to justify using the geometric mean when calculating the WER. This is the fundamental premise of the sampling design; if	We agree. As part of the quarterly TAC meetings and data review process, all stakeholders will be able to interact with the TAC prior to their final data analysis recommendation. This will ensure the final data analysis is	Clarifications made in Study Design and Methods, Water Effect Ratio Investigation, and

No.	Comment	Response	Change
	we didn't believe the proposed sampling design was adequate, we would be proposing a different design. However, we also note that the final decision about statistical methods and the final WER determination doesn't have to be made now. Preliminary analyses of the data after they are collected can inform the final analysis. If it appears the data are not sufficient, then alternate ways of calculating the WER can be determined then."	appropriate and based on sufficient data.	Deliverables and Reporting.
2.1	Will the work plan be completed if the WER appears to be less than 1?	The work plan will be finalized prior to any data collection. If the results of the WER indicate an SSO<1, those results will be presented.	No change made.
2.2	Will the BLM be used to calculate WERs, SSOs or both?	The BLM will only be used as a comparative analysis to the WER results. The SSO will be based on the results of the WER procedure.	No change made.
2.3	Has spiking samples with zinc (as will be done with copper) been considered?	Zinc will not be spiked into the samples as the subject of this SSO study is copper. Since copper is spiked into water from the Harbor, there will be background levels of other metals, including zinc, that will be present in the WER toxicity tests.	No change made.
2.4	The critical condition should be defined based on study results. As there is not yet a U.S. EPA approved version of the saltwater BLM, it is not appropriate to utilize the BLM to define the critical condition in advance of sampling	The BLM does not define the critical condition. We used the BLM during the site characterization events to better characterize the Harbor and highlight certain site-specific parameters that play an important role in copper toxicity.	No change made.
2.5	Please include a minimum of monthly DOC sampling for 1 year in Marina del Rey Harbor to evaluate when during the year DOC is highest and lowest.	Additional DOC monitoring for one year will be done as part of the TMDL monitoring program.	A description of the monthly monitoring plan has been added to

No.	Comment	Response	Change
			the Sampling Design.
2.6	Please state in the Work Plan whether Method 1 or Method 2 from EPA's Interim Guidance are the basis of the study design.	We will be using Method 2.	Clarification made in Study Design and Methods.
2.7	Section 1.2.3: Please discuss previous toxicity testing in Marina del Rey Harbor, including where highest water column toxicity has been observed in the past.	Limited prior toxicity data is available. Some of the data includes the DPR study and recent TMDL monitoring.	Discussion of this topic has been added to the Introduction.
2.8	Section 1.2.3: DPR conducted an investigation of copper levels in California marinas that included BLM application in Marina del Rey Harbor. Please add discussion of this work in the review.	We agree. This is relevant background information.	Discussion of this topic has been added to the Introduction.
2.9	Section 1.2.3: Please remove discussion of studies that did not result in adoption of an SSO by a Regional Board.	The discussion of previous WER studies provides necessary context and background to this study. While not all of these studies resulted in adoption of an SSO, the information regarding their methods and results helps to inform future work in this field.	No change made.
2.10	Please clarify if preliminary sampling for the BLM will be conducted prior to or in conjunction with sampling in Table 5.	This sampling occurred during the site characterization events in 2018. Preliminary sampling was conducted for a critical condition determination.	Revision made to Study Design and Methods to clarify this.
2.11	Please state EC50 values for *M. galloprovincialis* and confirm that the relevant endpoint for toxicity tests in laboratory dilution water is close to but not lower than the CCC and CMC as recommended in the 1994 Interim Guidance (pg. 21).	In the 2003 Draft Update of Ambient Water Quality Criteria for Copper, the species mean acute value (SMAV) for *Mytilus sp.* is 6.19 µg/L, which is the most sensitive species. The CCC (3.1 µg/L) was set to the *Mytilus* SMAV to protect this commercially important species. The EC50 for our test organism	This information has been added to the Toxicity Test Species and Method Selection.

No.	Comment	Response	Change
		(*Mytilus galloprovincialis*) in SCCWRP tests is 8.12 µg/L.	
2.12	Please identify the first and second most sensitive species to copper in the national copper database and why *M. galloprovincialis* is preferred.	The most sensitive species is *Mytilus sp.* with a SMAV of 6.19 µg/L. The second most sensitive species is *Mytilus edulis* with a SMAV of 21.50 µg/L. This results in a genus mean average (GMAV) of 11.53 for *Mytilus*. The second most sensitive genus is *Crassostrea* with a GMAV of 12.60. *Mytilus galloprovincialis* is the preferred species as it is part of the most sensitive species (*Mytilus sp.*), as well as the most sensitive genus (*Mytilus*). Using results based on this species will provide protection for this commercially important species, as intended by the EPA water quality criteria.	This information has been added to the Toxicity Test Species and Method Selection.
2.13	Please clarify that all individual WER exposures will be initiated within 36 hours of sample collection. This clarification is requested to confirm that tests will not be initiated after 36 hours.	Currently the workplan states "WER exposures will be initiated within 36 hours of sample collection." (Section 2.1.1 Test Method)	No change made.
2.14	Table 1 – Test conditions in the EPA method indicate salinity should be 30°° +/- 2°° and the test chamber should be 30mL. Please discuss the rationale for utilizing different test conditions in the study.	Although the EPA test method indicates a salinity of 30 ± 2 ‰, we use 32 ± 2 ‰ to better reflect the salinity in local waters. If we adjusted the salinity down to the EPA range it would require dilution of the sample. Our test chamber volume is 30 mL. The 10 mL listed in the Table reflects the sample volume, which is consistent with the EPA method.	This change has been clarified in the Test Method description.

No.	Comment	Response	Change
2.15	Tables 3 and 4 are inconsistent regarding whether samples near the bottom were collected during event 2 or 3.	This is a typo. The first column, row 6 should say “2”, not “3”.	Table 4: Correction made for event number in row 6.
2.16	Table 5 – Please add details in the text regarding the decision process for sample event 6 and how stakeholders will participate in this process.	The decision process for Event 6 will be done in consultation with the TAC during one of the quarterly update meetings. There will also be an opportunity for stakeholder input during those meetings.	A footnote has been added to Table 5 indicating decision process and stakeholder involvement.
2.17	Table 5 – In order to characterize the variability of Marina del Rey Harbor water quality, the Regional Board would like to see three years of sampling.	The TAC has reviewed the proposed workplan and concluded that the current study design is adequate to address temporal and seasonal variations over time (Appendix F, TAC response to charge question 1 and Regional Board question 7)..	No change to study duration made. Text clarified to indicate that the TAC will review preliminary WER results and make a determination as to the need for additional analyses (2.2.3).
2.18	Please add storm drain outlets to Figure 3 as discussed in text.	We agree with this addition.	Figure 4 has been added to the work plan to show storm drain outlets.
2.19	“Some of the water quality parameters needed for BLM analysis will be measured in the field…” Please clarify when and how other parameters needed for the BLM analysis will be obtained.	DOC is the only other required parameter. It will be collected as a grab sample and analyzed in the laboratory.	Clarified text in Parameters to be Analyzed.
2.20	Please add TSS and turbidity to the parameters that will be analyzed.	These parameters are not relevant to the study as they are not needed for the	No change made.

No.	Comment	Response	Change
		WER study nor are they used in the BLM.	
2.21	Please clarify whether or not sampling near the bottom of the water column is included in the work plan. Please add sample collection at 1 meter depth into the work plan to capture area in the water column most impacted by discharge from boat hulls.	Samples will only be collected 1 m below the water surface.	Clarified in Sampling Design.
2.22	Please clarify whether filtering of samples collected in the field for use in toxicity tests will occur in the field or in the laboratory.	Filtration of DOC and dissolved metals samples will be performed in the field. Per recommendation by the TAC, toxicity test samples will be filtered in the laboratory prior to spiking with copper to remove any organisms that may interfere with the test.	Text added for clarification in Sample Collection and Processing.
2.23	Please clarify the toxicity testing procedures. This paragraph indicates there will be no dilution or spiking; however, the draft work plan includes spiking of samples with copper.	We agree. The toxicity test methods were based on the standard test but were inaccurate for the WER study design. The revisions to this text will reflect the accurate methods described in more detail in Section 2.1.1 Test Method.	Text revised in Analysis Methods.
2.24	Please utilize the version of the BLM and reference material included in U.S. EPA's 2016 Draft Aquatic Life Ambient Estuarine/Marine Water Criteria for Copper. The 2012 document sited in the Work Plan does not appear to be included in the 2016 draft criteria.	The reference will be updated.	Reference revised in the "Biotic Ligand Model Analyses" and "References" sections.
2.25	Please increase field QA samples to two field blanks and two field duplicates per sampling event, collected at dispersed times during the sample event.	The number of blanks used for this study (one of each type per sample batch) is consistent with standard practice.	No change made.
2.26	Please clarify the procedure for spiking samples with copper. How will samples with no effect be obtained for sites where toxicity is exhibited in the site water?	We agree this needs clarification. The laboratory control water will range from no effect to complete inhibition of	Revision made to "Water-effect

No.	Comment	Response	Change
		normal development. The field samples will be spiked with the same range of copper concentrations and the results will be recorded. It is not possible to know the level of toxicity in field samples prior to testing. As such, the resulting toxicity test data will be reviewed to determine if the level of ambient toxicity in any given field sample is too high to provide a useable WER value for that sample.	Ratio Testing" section.
2.27	The Regional Board would like verification of dissolved copper in all toxicity treatments and total copper in at least some of the toxicity treatments. This change should be made to Pg. 11, paragraph 5 as well.	We agree. One replicate of all treatments will be analyzed for total and dissolved copper.	Revisions made to clarify this in both Water-effect Ratio Testing and Toxicity test species and method selection.
2.28	For what analyses will recovery surrogates be utilized for QC rather than reference materials?	Per the TAC's recommendation, reference materials for DOC and copper will be analyzed.	Clarification made to Chemistry Quality Assurance/Quality Control.
2.29	Please include the criteria and statistical methods that will be used to evaluate sWER sample size precision in the Final Work Plan.	The TAC will provide a recommendation on this data analysis during the data review process. Additionally, we will calculate the coefficient of variation and 95% confidence intervals for the sWER results.	Clarification made to text in the Water-Effect Ratio Investigation.
2.30	Please include submittal of data to CEDEN as part of data management.	Data will be provided in CEDEN format and forwarded to LA County for submission to the CEDEN system.	This has been clarified in Deliverables and Reporting.

No.	Comment	Response	Change
2.31	Please include randomization sheets in study documentation and toxicity data quality review	These are included in the umbrella term "laboratory bench sheets"; however, we have included text for clarity.	Toxicity test randomization sheets have been included in the study documentation and toxicity data quality review.
2.32	Public Participation: Please clarify the recipients of each of the reports. Do the draft and final reports discussed in paragraph 3 apply to all task and study reports?	The draft and final report discussed in paragraph 3 refers to the Site-specific Objective Study Report only. Draft results will be provided to the TAC and available to stakeholders as part of the periodic consultation meetings.	Clarification provided in Deliverables and Reporting.
2.33	Public Participation: Please detail how potential decisions and/or changes to the work plan will be communicated (i.e. TBD information for sample event #6, whether or not data indicates the need for additional samples).	Discussion of data, potential changes to the workplan/methods, and any potential decisions will be made during the quarterly meetings with TAC. These meetings will be open to stakeholders.	Clarification provided in Deliverables and Reporting.
2.34	Please change public comment periods from "30 days" to "a minimum of 30 days" to allow for potential requests for additional time to review documents.	We will make the suggested text revision.	Revision made to "Public Participation Plan" section.
2.35	Table 11 – Please clarify whether or not all TAC meetings/calls listed will be open to the public.	Yes, the TAC meetings/calls listed in Table 10 under Project Schedule will be open to stakeholders.	Clarification provided in a footnote for Table 10.
2.36	Please include in the work plan a discussion of whether or not a translator may be used in the study to convert dissolved to total copper. If a translator will be used, please describe how the translator will be selected.	Translators will not be needed or used as we will be measuring dissolved copper in the collected samples.	No change made.
2.37	ELAP Certificate #2769 for Physis Environmental Laboratories appears to be an interim accreditation that expired on 8/31/18.	Their ELAP certification has been renewed and expires on 8/31/2019. The	No change made.

No.	Comment	Response	Change
	Please confirm whether or not Physis Environmental Laboratories is currently accredited by ELAP for analyses they will be performing for this study.	lab will maintain current ELAP certification throughout the study period.	
2.38	As recommended in the Interim Guidance document, please compare at least some toxicity test results obtained with results obtained in at least one other laboratory. Guidance pg. 8, paragraph 2 – "…it is important to compare at least some results obtained in the laboratory dilution water with results obtained in at least one other laboratory."	We will include analysis of a split dilution water sample by another laboratory as part of this study.	A description of this comparison has been added to Toxicity Quality Assurance/Quality Control.
2.39	Please include full details of anticipated WER and BLM calculations/modeling in the Work Plan.	Discussion of the BLM-based calculations are provided in the Biotic Ligand Model Analyses section of the workplan. We can provide additional references regarding the model. WER calculations are described in Sections 1.2.1 Water-Effect Ratio and 2.4 Water-Effect Ratio Analysis and Interpretation.	Additional BLM references and description of data analysis and use has been added to Biotic Ligand Model Analyses.
2.40	QAPP – Table B-1 - Please provide a reference for an EPA method referencing data quality objectives for each parameter.	These measurements (pH, DO, temperature, and salinity) are taken in the field using meters with the appropriate probes. They do not have an EPA method; however, we agree the data quality objectives and calibration details should be added to clarify the data quality of these measurements.	Table B-1 has been revised to include data quality objectives and calibration procedures for these measurements.
2.41	QAPP – Pg. B-5, paragraph 1: Modifications in analytical methods must be approved by the TAC.	We agree. Any changes in methods must be approved by the TAC.	Text has been added to the QAPP to reflect this addition.
2.42	QAPP – Please add QA/QC procedures used to evaluate quality/acceptability of seawater from Granite Canyon. Are hold times implemented for the seawater?	The acceptability of seawater from Granite Canyon is based on low metals content, low DOC content, no ambient toxicity, and previous acceptance by	A description of these procedures has been added to Laboratory Quality

No.	Comment	Response	Change
		EPA for use in water quality criteria development. These parameters will all be analyzed in the unspiked control. No specific hold time will be implemented; however, we will minimize and document holding times. We will use recently collected water for each test.	Control in the QAPP.
2.43	QAPP – Please add a description of personnel training to the QAPP.	We agree. A description of personnel training will be added for the field and laboratory components of this project.	A section on Personnel Training has been added to the QAPP (Appendix B).
2.44	QAPP – Please include a page for signatures of those certifying the adequacy of the QAPP.	We agree a signature page is needed.	A signature page has been added to the QAPP.
3.1	III. General Comments, Section A, Page 3: "The Regional Board should ensure that the approval of the MDR Draft Work Plan does not foreclose analysis of reasonable alternatives to a Copper WER."	This is a comment directed to the Regional Board.	No change made.
3.2	III. General Comments, Section B, Page 4, Paragraph 1: "Other SSO and WER studies can be illuminating but should not be considered to create binding precedent on the methodology of the MDR Harbor SSO study."	We agree. These prior SSO studies provide examples of this process but are not directing the methodology proposed in this workplan.	No change made.
3.3	III. General Comments, Section B, Page 4, Paragraph 2: "We strongly urge the MDR Harbor SSO study authors as well as the Regional Board to make source control, particularly control of copper anti-fouling paint, a much higher priority for the MDR Harbor SSO study."	These issues will be addressed in the implementation report.	No change made.
3.4	III. General Comments, Section B, Page 4, Paragraph 3: "A clear explanation (at a minimum) of the reasonably foreseeable regulatory effects (or lack thereof) of any MDR Harbor SSOs should be included with the MDR Harbor SSO study itself, given the importance of the anti-degradation policies to the ultimate success of the MDR Harbor SSO study."	We agree. These issues will be addressed in the implementation report.	No change made.

No.	Comment	Response	Change
3.5	III. General Comments, Section B, Page 4, Paragraph 4: "The San Francisco Bay 2007 Proposed BPA and Staff Report relied on dissolved organic carbon (DOC) as a surrogate for toxicity in follow-up monitoring. This might be a defensible or even preferred approach for MDR Harbor, but study authors need to carefully justify any proposed use of that approach in MDR Harbor, rather than relying on the approved methodology of past studies."	We agree. For this project we will be using toxicity tests and will not use DOC as a surrogate for toxicity.	No change made.
3.6	III. General Comments, Section B, Page 4, Paragraph 5: "Similarly, the San Francisco Bay 2007 Proposed BPA and Staff Report, as well as the WER study itself, relied on translators, since conversions from total to dissolved copper were necessary in San Francisco. It is unclear to what extent, if any, such translators would be needed or even appropriate in the MDR Harbor SSO study."	Translators will not be used in this study as dissolved copper will be measured.	No change made.
3.7	III. General Comments, Section B, Page 6, Paragraph 1: "LAW and HTB therefore request that if specific methodologies, findings, etc. from previous SSO studies are being imported into the MDR Harbor SSO study, that the study authors include an explanation of the appropriateness in the current context of MDR Harbor, and how the methodology proposed could affect the margin of safety and the protectiveness of the site specific objectives and/or site specific TMDL modifications in MDR Harbor."	We agree. No specific methodologies, findings, etc. will be used from prior SSO studies. These studies are presented in the workplan to provide background and context for the use of SSOs in the state of California.	No change made.
3.8	IV. Comments on the MDR Draft Work Plan, Page 6, Paragraph 3: "We suggest revising the text of the first full paragraph to reflect that site-specific parameters can increase, as well as decrease, toxicity to aquatic organisms (as would be reflected whenever WER < 1.0). In addition, we request a comparison of the margin of safety in the unadjusted WQOs, versus the margin of safety in any WER-adjusted standards."	We agree. We will clarify that the WER can identify an increase or decrease in toxicity based on site-specific parameters. The margin of safety will remain the same as intended by EPA's process for establishing aquatic life criteria (protective of 95% of aquatic life).	Revised to clarify text in the Introduction.
3.9	IV. Comments on the MDR Draft Work Plan, Page 6, Paragraph 4: "We request clarification on why the authors believe use of the BLM is appropriate despite the lack of approval for use of the model in marine waters. Also, we recommend considering revision	We recognize that the BLM is not yet approved for use in marine systems. We will only use the BLM as a comparative	Clarified text in the Introduction regarding use of the BLM.

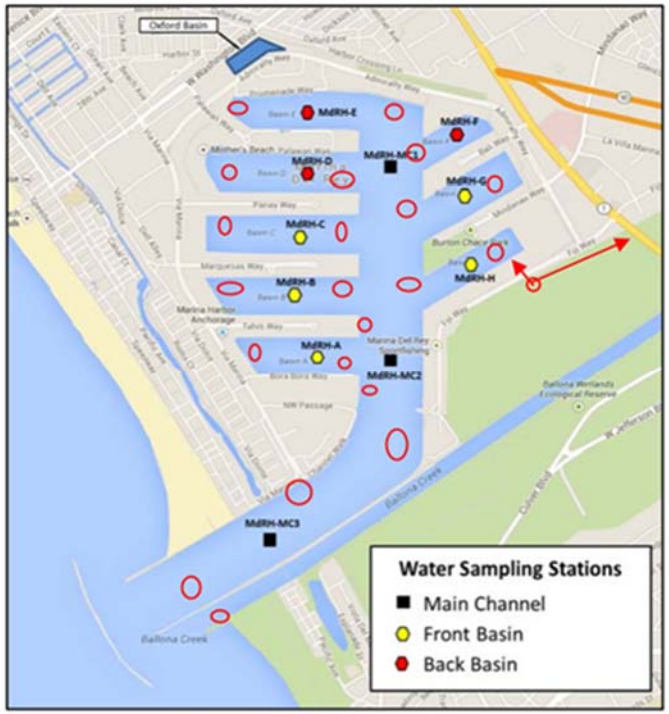
No.	Comment	Response	Change
	of the MDR Draft Work Plan to include an alternative study design, if reliance on the BLM turns out to be inappropriate or scientifically less than robust (or if use of the BLM remains unapproved for marine waters as sample WERs are derived)."	analysis to the WER results. The SSO will be based only on the WER results.	
3.10	IV. Comments on the MDR Draft Work Plan, Page 6, Paragraph 5: "LAW and HTB are also concerned that use of the BLM to target the conditions likely to result in the lowest WER (i.e., the critical condition), may not be appropriate if the BLM is not approved for use in marine waters. In any event, it is of the utmost importance that the MDR Harbor SSO study demonstrate that the critical condition has been fully evaluated as part of the study, regardless of whether the BLM is used or not."	The BLM was used to gain more insight during the site characterization study and highlighted DOC as an important site-specific parameter related to predicted copper toxicity. The WER study will still entail six sampling events over a full-year during both wet and dry weather.	Clarified text regarding use of the BLM in the Introduction.
3.11	IV. Comments on the MDR Draft Work Plan, Page 7, Paragraph 1: "The dispute over the critical condition was one of the single most contentious items in the Los Angeles River Copper WER Study in 2015, so we urge the study authors to carefully justify any claims about the timing of the critical condition, especially if the MDR Harbor SSO study relies of the critical condition relies on a model not approved for use in marine waters."	We agree. The results of the site characterization study suggest the critical condition is when DOC is low. This information helped in the development of the workplan and study design; however, the proposed WER study design includes six sampling events over the course of a year which allows for inclusion of many conditions. Additionally, monthly DOC samples will be analyzed as part of the TMDL monitoring program which will help track any potential fluctuations in the condition of the Harbor.	Clarified text regarding critical condition in the Introduction and Study Design and Methods.
3.12	IV. Comments on the MDR Draft Work Plan, Page 7, Paragraph 2: "Assuming successful resolution of the methodological concerns expressed elsewhere, we agree that the fWERs can be used to adjust both the CCC (chronic) and CMC (acute) WQOs, as well as TMDL adjustments associated with the same location."	We agree. Thank you.	No change made.
3.13	IV. Comments on the MDR Draft Work Plan, Page 7, Paragraph 3-4: "The summary of various other marine WER/SSO studies raises the	We did not rely on methods from prior WER/SSO studies. These studies were	No change made.

No.	Comment	Response	Change
	question of to what extent the MDR Draft Work Plan relies on methodologies in prior studies" and "…the San Diego Regional Board had serious concerns with the design of the Shelter Island Yacht Club WER study (ironically, the WER study that produced the lowest WER values of any of the studies summarized). The MDR Harbor SSO study authors may thus wish to consult with San Diego Regional Board staff for additional information on these apparent concerns."	provided as background and context for WER/SSO studies previously performed in CA.	
3.14	IV. Comments on the MDR Draft Work Plan, Page 7, Paragraph 5: "…we urge the authors, Regional Board staff, SCCWRP staff, and the Technical Advisory Committee members to consider basing fWERs on the lowest value produced by methodologically correct testing, rather than relying on a geometric mean (or any type of averaging) of several values. This is especially important if the sample WERs (sWERs) show high variability."	Thank you for your suggestion. We have requested the TAC to provide a recommendation regarding this.	The TAC will be consulted to provide a recommendation regarding fWER calculation method (Section 2.5).
3.15	IV. Comments on the MDR Draft Work Plan, Page 8, Paragraph 1: "We strongly suggest that the study design for the MDR Harbor SSO study include more than three sampling events, and that the number of sampling events in the study (as well as exclusion of data from any sampling event from fWER calculations) be fully justified."	We agree. We will have six sampling events (Table 5).	No change made.
3.16	IV. Comments on the MDR Draft Work Plan, Page 8, Paragraph 2: "We request citations to support the statement that DOC concentration is the primary variable controlling bioavailability of copper in marine systems. Additionally, we request additional evidentiary support that DOC concentration is closely correlated with BLM modeling results, and an explanation whether this DOC/BLM correlation is being used to justify reliance on BLM results despite lack of EPA approval for use of the BLM in marine waters."	We agree that citations are needed. Additionally, the BLM uses DOC, salinity, temperature, and pH as the input variables in the model. Based on our site characterization study, DOC was the most important parameter with regard to the predicted toxicity from the model results. As previously mentioned, the BLM will only be used as a comparison to the WER results.	Citations and clarification provided in Site Characterization.

No.	Comment	Response	Change
3.17	IV. Comments on the MDR Draft Work Plan, Page 8, Paragraph 3: "…we recommend that additional DOC monitoring be undertaken prior to WER sampling to confirm the DOC concentration distribution pattern in the study area."	Monthly DOC samples will be collected and analyzed as part of the County's ongoing TMDL monitoring program.	A description of the monthly monitoring plan has been added to the Sampling Design.
3.18	IV. Comments on the MDR Draft Work Plan, Page 8, Paragraph 4: "Similarly, we recommend additional confirmation backed by rigorous data and robust modeling that the critical condition is likely to occur during dry weather in winter or spring."	Based on our site characterization study, the critical condition is likely to occur in lower DOC conditions.	This discussion has been clarified in Site Characterization.
3.19	IV. Comments on the MDR Draft Work Plan, Page 9, Paragraph 1: "Given the probable lowest DOC concentrations in the main channel, we recommend considering adding a second water sampling station in the main channel, in the area of the channel to the east of the terminus of Bora Bora Way, to improve the robustness of the study results."	The TAC has recommended a revised sampling design and station locations.	Study and sampling design revised in accordance with TAC recommendations.
3.20	IV. Comments on the MDR Draft Work Plan, Page 9, Paragraph 2: "We recommend that the study authors consider adding at least a second sampling event in wet conditions."	We've asked the TAC to advise on this matter. The sixth WER sampling event can be used as an additional wet-weather event if recommended.	Sampling design clarified to indicate TAC will provide recommendation regarding additional wet weather sampling (Table 5).
3.21	IV. Comments on the MDR Draft Work Plan, Page 9, Paragraph 3: "We support the collection of grab samples, and support analyzing zinc concentrations in those samples. However, we ask for greater clarification of the claim that inclusion of zinc in the study will facilitate understanding copper toxicity." Comment continued through page 10, "In any event, we urge study authors to more fully explain how the MDR Draft Work Plan accounts for synergism,	We agree that this text is unclear. By including zinc in the metals analysis, we hope to gain some insight as to whether zinc is a likely contributor to toxicity. We will not be able to determine a specific relationship between copper and zinc (i.e., synergism, additivity, etc.).	Text clarified in Parameters to be Analyzed.

No.	Comment	Response	Change
	additivity, or reduced efficacy of detoxifying metal-organic complexes when multiple metals are present."		
3.22	IV. Comments on the MDR Draft Work Plan, Page 10, Paragraph 1: "The question of whether the WER sample size and precision allows for a defensible fWER is extremely important. We again urge the study authors to use the lowest WER obtained plus a margin of safety, rather than an average of sWERs, to ensure that the critical condition has been captured, and that the SSOs do not suffer from a potentially serious anti-degradation policy consistency problem as a result."	The TAC will advise on the final data analysis. An additional margin of safety will not be needed as the water quality criteria is designed to protect 95% of aquatic life. An WER-based SSO is an adjustment of the objective so that the same level of aquatic life protection is attained.	The TAC will be consulted to provide a recommendation regarding fWER calculation method (Section 2.5).
3.23	IV. Comments on the MDR Draft Work Plan, Page 10, Paragraph 2: "We thus strongly encourage revision to this section of the MDR Draft Work Plan, to include a firm commitment to either collect such data until the data set is robust enough to support calculated fWERs, or else to discontinue the process of seeking approval of SSOs if data is insufficient to support calculation of defensible fWERs."	We agree that sufficient data is necessary to determine an SSO. This workplan includes six sampling events and exceeds the minimum recommended by EPA guidance. The data will be reviewed by the TAC prior to calculation of the fWER.	Text added to clarify this in the Final Water-Effect Ratio Calculation (Section 2.4.3).
3.24	IV. Comments on the MDR Draft Work Plan, Page 10, Paragraph 3: "We strongly recommend formal written responses to comments be prepared, even if the Regional Board believes it does not have a strict legal obligation to prepare such a document as part of its Work Plan review."	We agree. We have provided written responses to the comments received on the draft workplan via this document, which is an appendix in the final workplan.	Formal comment responses added to the workplan as an appendix.
3.25	IV. Comments on the MDR Draft Work Plan, Page 10, Paragraph 4: "We also request that data collected to support the MDR Harbor SSO study be made available in as close to real time as possible, to further enhance transparency, and to provide for potential feedback to allow identification (and presumably correction) of any problems as early in the process as possible."	Preliminary data will be made available to stakeholders when it is sent to the TAC for review.	Text added to Deliverables and Reporting to clarify this.
3.26	IV. Comments on the MDR Draft Work Plan, Page 11: Concerns with the June 1994 Environmental Protection Agency Interim Guidance on the Determination and Use of Water Effect Ratios for Metals	The comments provided here are related to the Interim EPA guidance document and do not have any specific suggestions regarding the workplan.	No change made.

No.	Comment	Response	Change
		These concerns are largely addressed in prior comments.	
4.1	Email 1: Several links to a video presentation and resources and an attached Word document with more resource links.	Thank you for the provided resources.	No change made.
4.2	Email 2: "I hope to see sufficient testing and additional testing areas per this citation [USEPA 1994]". This citation recommends sampling multiple stations over a minimum of three sampling events that include different seasons and locations.	We agree. The draft workplan currently adheres to this guidance with a plan to collect samples at five stations with six sampling events over the course of one year.	No change made.
4.3	Email 2: "It's also important to test appx 2 years to get a secondary year to compare with"	The TAC has provided recommendations on the number of sampling events and sampling timeframe. The TAC agrees that the six sampling events distributed over 12 months is an adequate sampling design. The workplan includes 2 years of sampling, 1 year for site characterization and 1 year of WER sampling.	TAC recommendations have been incorporated into the Study Design and Methods section of the workplan.
4.4	Email 2: "The suggested testing areas are not sufficient. There should be an additional 22 stations and 22 locations used for metals analysis."	The TAC has provided recommendations on the number of stations and sampling strategy.	TAC recommendations have been incorporated into the Study Design and Methods section of the workplan.
4.5	Email 2: "Some testing should be near the vessels as well, where the actual discharging occurs."	The goal of this study is to determine the condition of the entire Harbor and its impact on copper bioavailability and toxicity. The TAC has indicated that a representative sample is more important than sampling hot spots.	No change made.

No.	Comment	Response	Change
4.6	Email 2: "There should be habitat testing additionally to have the negative impacts on sea life/habitat etc. documented."	This is outside the scope of this project.	No change made.
4.7	Email 3: Suggested station locations  **Figure 3. Candidate sampling stations in Marina del Rey Harbor.**	The TAC has provided recommendations on the number of stations and sampling strategy	TAC recommendations have been incorporated into the Study Design and Methods section of the workplan.
4.8	Email 4: "I have reviewed your draft document and I approve of it"	Thank you for your review and approval.	No change made.
5.1	Page 1, Paragraph 4: It is suggested that the County remove the contaminated sediment from the Harbor to improve the water quality.	This is outside the scope of this project. The focus of this work is on toxicity due to copper in the Harbor water, not the sediment.	No change made.
5.2	Page 2, Paragraph 7: "In the Development of Site-Specific Objectives, it states protection of aquatic life is uncertain. It further states that this study does not account for physical constituents for example, particulate and dissolved organic matter."	These statements from the Draft Workplan refer to the USEPA's Interim Guidance on Determination and Use of Water-Effect Ratios for Metals (USEPA-	No change made.

No.	Comment	Response	Change
		823-B-94-001, 1994). The current water quality criteria were based on data that did not consider site-specific water quality parameters such as dissolved organic matter. The purpose of this project is to determine what threshold is appropriate and specific to the Harbor to protect aquatic life by considering those site-specific water quality parameters.	
5.3	Page 2, Paragraph 8: "It does not mention providing the required level of protection for human health."	This is outside the scope of this project. This water quality criterion is only used for protection of aquatic life.	No change made.
5.4	Page 2, Paragraph 11: "The Water-Effect Ratio Study Design – Station Locations excludes Oxford Basin. Why?"	This is outside the scope of this project. The study is evaluating a site-specific objective for the Harbor only. The TAC has reviewed the workplan and provided recommendations regarding sampling station location and number.	No change made.
5.5	Page 2, Paragraph 12: "Absent from the Draft Work Plan is public comment and Q&A at the TAC review meetings and the first public workshop."	The TAC meeting to review the Draft Workplan was public and allowed time for public comments and questions. Additionally, future TAC meetings and workshops will be open to public participation.	Clarification made in the Public Participation Plan.
5.6	Page 2, Paragraph 13: "Under section VII Implementation Report is California Water Code Section 13241 anti-degradation review (as appropriate). If the Draft Work Plan proceeds as proposed degradation of the Harbor will continue and reviewing now is appropriate."	This review will be performed as part of the implementation report.	No change made.

APPENDIX F
TAC AND PUBLIC COMMENTS

Technical Advisory Committee Comment Letter

TAC Review of Marina del Rey Harbor Site-Specific Objective Study Workplan
February 15, 2019

Prepared by: **Richard F. Ambrose (University of California, Los Angeles)**
Peter G. C. Campbell (University of Quebec)
Gary N. Cherr (University of California, Davis)

This document presents the consensus comments and recommendations by the Technical Advisory Committee (TAC) regarding the draft workplan for the Marina del Rey Site-Specific Objective Study (October 2018 version). Responses are organized in two sections. The first section addresses charge questions that were provided in conjunction with the December 18 work plan review meeting, held in Marina del Rey on December 18, 2018. The second section includes responses to questions regarding the work plan conveyed by staff of the Los Angeles Regional Water Quality Control Board.

TAC responses to Charge Questions

1. Is the study sampling design (e.g., number of stations, frequency, duration) sufficient to represent the variations in MDRH water quality necessary to determine representative and protective water effect ratios?

As with all questions about study design, there is a trade-off between sampling effort and cost. The TAC discussed this question mainly considering roughly the same effort as in the proposed sampling design, that is, about 30 samples, rather than thinking about how additional samples could be added to improve the design. With this in mind, the TAC considered how well the sampling design would provide representative samples in terms of space and time. We propose composite sampling as a general way to increase representativeness without increasing analytical costs.

<u>Spatial representativeness</u>

The main consideration for spatial representativeness is how conditions might vary across the harbor. Given the limited amount of spatial variability reported in the pilot study, we find the rationale for proposing fewer stations than were sampled in the pilot study is reasonable. Thus, the proposed distribution of sampling stations, with two in basins near the back of the Marina, two in basins near the front of the Marina, and one in the main channel (Figure 1), is appropriate for the purposes of this study.

Figure 1. Proposed stations for WER analysis, from the SSO Draft Final Work Plan.

However, a single location near a particular basin will not capture variability in conditions among different locations in the basin (such as the blind end and middle). Therefore, we recommend that each basin sample consist of samples taken from the end, middle and mouth of the basin that are combined to form a single composite sample to represent the water in that basin.

We suggest that each of these samples be taken 1 m below the surface of the water. Although conditions at the very surface and near the bottom might be different, a sample taken 1 m below the surface of the water will be representative of the majority of the water in the Marina. We do not recommend taking samples close to the docks because these are also microhabitats that do not represent the majority of the water in the Marina.

<u>Temporal representativeness</u>

There are two kinds of temporal changes that seem most likely to influence the results of this sampling program: (1) wet vs. dry weather, and (2) tidal stage.

To capture the temporal variability in WER, the Draft Work Plan proposes six sampling events distributed over 12 months (Table 1). Four of those events were to occur during dry weather, when the critical condition is expected to be present. Two dry weather samples would occur during summer and two would occur during dry weather in winter. One sample would be taken during wet weather in winter. The timing for the sixth sample is not specified ahead of time, but would be "adaptive," that is, determined based on initial sampling results. In terms of tidal stage, dry weather samples would be split between flood tides and ebb tides.

Table 1. Proposed WER sampling events, from the SSO Draft Final Work Plan.

Event	Tide Stage		Summer	Winter	
			Dry Weather	Dry Weather	Wet Weather
	Flood	Ebb	April – October	November – March	November – March
1	X		X		
2	X			X	
3		X	X		
4		X		X	
5	NA	NA			X
6	TBD	TBD	TBD	TBD	TBD

Notes:
Wet weather sampling is not dependent on tide stage. Sampling event characteristics to be determined based on results of previous events.
NA = not applicable
TBD = to be determined

The TAC agrees with the strategy of distributing samples between dry and wet weather. The Draft Work Plan states that one wet weather sampling event is proposed "to confirm preliminary findings that indicate relatively low copper bioavailability during this time." It is also our understanding that the sixth sample (indicated as 'TBD' in Table 1) is designed to offer the possibility to adjust the sampling plan once WER data have been obtained for both dry and wet sampling periods.

The TAC has recommendations about the wet weather sampling. The goal is to sample after a representative storm, and to sample at a time that represents conditions after storms. Of course, predicting storm timing and intensity is challenging, and for this study it is not necessary to sample during a storm. We recommend that sampling occur one day after a storm. Representative sampling will only occur if the storm is large enough to discharge substantial stormwater into the Marina del Rey. Thus, bigger storms are better than smaller, but we don't want to miss sampling opportunities by waiting for a large storm that might not occur. Therefore, we recommend sampling after a 0.5" storm. The current storm threshold is 0.2", but the TAC is concerned that such a small storm may not discharge sufficient stormwater into the Marina, hence the recommendation for a larger storm.

For tidal differences, the proposed sampling plan has samples taken on flood and ebb tides, but makes no mention of spring versus neap tides. The TAC thinks that the magnitude of tidal variation could affect WER, so samples should be taken on spring and neap tides, when tidal changes are maximum and minimum, respectively. It is not necessary to pick the most extreme spring tides since the goal is to acquire representative samples. Rather than having one sample taken on a flood tide and a different sample taken on an ebb tide, if feasible samples should be taken throughout the flood and ebb tide periods of one tidal cycle. To minimize analytical costs, and because we don't actually care about

differences between flood and ebb tides but rather just want representative samples, these samples can be combined to create a single composite sample for each sampling event. There will be some logistical challenges from occupying a sampling site over a full tidal cycle (through both flood and ebb tides), but if possible to accomplish, this will provide the most representative sample.

2. Are the proposed methods for toxicity and chemistry analyses appropriate and based on sound scientific practice?

The TAC initially had some questions about collection and filtration of the water samples, and about the details of the analysis of dissolved organic carbon (DOC). These questions were submitted to the Southern California Coastal Water Research Project (SCCWRP) scientists before the TAC meeting in December 2017 and were discussed at the meeting. At that time, we understood that SCCWRP was going to take our comments into account in the design of the final work plan. For clarity, the comments and questions that were submitted before the TAC meeting and then discussed are summarized below (the page references are to the work plan, 30 October 2018 version).

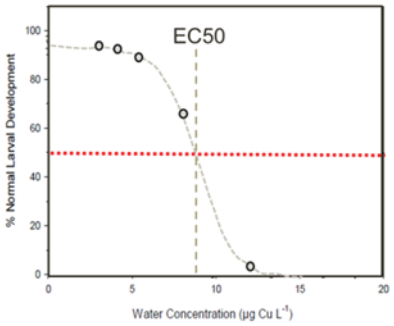
- p.20: "Concentrations of both copper and zinc will be measured, as both of these metals may be elevated in harbors and contribute to ambient toxicity." Zinc is a difficult metal to study, due to the ease with which samples can be inadvertently contaminated. It is essential that trace-metal-clean protocols be used for sample collection[1, 2] and that the Quality Assurance/Quality Control protocols include the appropriate use of certified reference materials (CRMs). The Shelter Island study is a good model for the proper use of CRMs both for copper and DOC.[3]
- p.21: "Samples for measurement of DOC and dissolved metals will be filtered on site within 15 minutes of collection using plastic syringes fitted with 0.45-micron filters." The choice of filters here is critical[4, 5], as inadvertent contamination of the samples with metals or dissolved organic carbon must be avoided. Note that choice of filters may well be different for samples destined for metal analyses and for those that will be analyzed for DOC.
- p.22, Table 7: Containers for water samples. The glass vials used to collect and store the water samples destined for DOC analysis should be pre-combusted.

The TAC also suggested that the samples collected for DOC analysis could also be subjected to simple spectrophotometric and spectrofluorometric characterization. Such analyses require only small sample volumes and it should not be difficult to access the necessary equipment (i.e., a UV-visible spectro-photometer and an excitation-emission fluorimeter).[6-8] This additional information about the <u>nature</u> of the DOC will be particularly useful in the planned comparisons between the observed toxicity and the BLM-predicted toxicity.

With respect to the planned <u>toxicity</u> tests, the work plan refers to the SCCWRP Toxicology Laboratory, Standard Operating Procedure for Mussel Embryo Development Test and states, "This test is based on methods in the EPA's Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms (EPA/600/R-95/136)." The TAC discussed this test and the choice of text organism (*Mytilus galloprovincialis*). The toxicity test assesses mortality of embryos/larvae and at low concentrations, focuses on subtle larval shell morphology. It is well known that mollusc embryos/larvae are exquisitely sensitive to metals. Among the advantages of using this test organism, the TAC considered its recognized sensitivity to copper, the existence of a wealth of published data on copper – *Mytilus* early life stage interactions and the precedent that the development

of Site Specific Objectives (SSO) for copper in other coastal environments in California had used this organism and this test. The TAC also suggested that if it proved difficult to induce spawning in laboratory cultures on *M. galloprovincialis*, other sensitive test organisms could be considered. These include embryos/larvae from *Mytilus californianus* (California mussel) and *Crassostrea gigas* (Pacific oyster). The California mussel is located along the open coast and possibly at the entrance of MDRH, while the Pacific oyster is found on hard substrates throughout the MDRH. The embryos/larvae of both species have been used in toxicity tests with metals, including copper, and have been shown to be as sensitive as *Mytilus galloprovincialis*.

The TAC did raise some questions about the WER approach as it would be applied to the Marina del Rey, where there will be an appreciable background level of copper in the test waters collected from the Marina. In the documentation supplied to the TAC before the December meeting, it was unclear whether the EC_{50} for the <u>site</u> water would include the background copper concentration or not. In other words, does the EC_{50} site value include the background copper, or just the added copper?



With reference to the adjacent figure, taken from the work plan, for the unspiked site water the X-axis will start not at 0 but rather at a value of close to 4 or 5 µg copper/L.

The SCCWRP scientists confirmed that the EC_{50} value would indeed include both the background copper and the added copper.

3. To what degree should the Biotic Ligand Model be used for data analysis and WER development?

The TAC is satisfied that the proposed use of the BLM in the work plan, i.e., as a tool to compare measured with predicted toxicities, is the correct manner to proceed. In the view of the TAC, it would be premature to use the marine copper BLM to calculate the SSO for Marina del Rey, i.e., as substitute for the toxicity tests and their use in a WER procedure. However, the use of the BLM to compare predicted and observed toxicities in the unspiked and copper-spiked samples will help answer one of the questions raised by stakeholder participants in the TAC December meeting, namely is copper the only stressor to which *M. galloprovincialis* is responding in the toxicity tests. Consistent agreement between the predicted and observed toxicities would support the argument that copper is the principal chemical stressor in Marina del Rey waters.

4. Are the proposed statistical methods for data analysis and final WER determination appropriate?

Questions about the statistical methods proposed for this work have focused on the calculation of the final WER. The Draft Final Work Plan states that "the fWER will be calculated as the geometric mean of the group of single WERs (sWERs) selected for analysis, as recommended in the USEPA (1994) Interim Guidance."

There are other possible ways to calculate the final WER (fWER). The LA Waterkeeper suggests that using the lowest sWER value might be most protective, acknowledging that the USEPA Interim Guidance defines fWERs as geomeans but stating that p. 135 argues for using the lowest value.

The determination of the final WER (fWER) is complicated and more complex than the TAC can fully resolve at this time. We note that the USEPA Interim Guidance has an entire section on "How should an fWER be derived?", and there are many considerations. Most of the EPA's discussion revolves around the need to have an adequate number of representative samples, and the guidance also explains a number of different approaches to calculating the fWER under different scenarios. Although the scenarios often don't relate to the situation at MDRH, the conclusions from the Interim Guidance's discussion of this issue are relevant. In discussing the calculation of the fWER as an adjusted geometric mean, the Guidance notes that the level of protection *would* be greater if the lowest WER, rather than an adjusted geometric mean, were used as the fWER. However, the Guidance goes on to state that the intended level of protection is provided when the criterion is derived according to the national guidelines, and there is no reason to add a substantial margin of safety and thereby change the intended level of protection if sufficient data are available and it is clear how the data should be used.

The TAC believes that the proposed study plan will provide sufficient data, as identified by the USEPA Interim Guidance, to justify using the geometric mean when calculating the WER. This is the fundamental premise of the sampling design; if we didn't believe the proposed sampling design was adequate, we would be proposing a different design. However, we also note that the final decision about statistical methods and the final WER determination doesn't have to be made now. Preliminary analyses of the data after they are collected can inform the final analysis. If it appears the data are not sufficient, then alternate ways of calculating the WER can be determined then.

TAC responses to Water Board Questions

1. Are either the Resident Species or Recalculation procedures potentially appropriate for deriving a site-specific objective for Marina del Rey Harbor?
    - For resident species: there are no threatened or endangered species to be focused on. For recalculation: not that much new data.

2. Selection of Test Species
    - Is *Mytilus galloprovincialis* the most sensitive species? Yes. Bivalve larvae are known to be the most sensitive marine species/life stage for copper, and *Mytilus galloprovincialis* is established as one of the most sensitive of all marine species at the early life stages.
    - Should testing be conducted with multiple species during the study? For determining the WER, use of the most sensitive species that is relevant is all that is necessary.
3. Is the toxicity test proposed appropriate for use in developing a single WER for use in calculating both acute and chronic SSOs in Marina del Rey Harbor?
    - Yes, and it provides good links to earlier SSO work in coastal marine waters in California (San Francisco Bay; Shelter Island Yacht Basin).

4. Are the locations of the sites appropriate for the study? Do the sites capture the variability in Marina del Rey Harbor?
    - With the proposed modifications to the sampling plan, yes. Recall that the TAC suggested that the study design should integrate across the Marina, to be representative, rather than looking for hotspots.

5. Will all potential sources of variability be sufficiently investigated? Episodic events are mentioned in the background section of the work plan but not currently included in the study design. Would sampling during a planktonic bloom, if one occurs during the study period, enhance study results?
    - While it is impossible to design a short-term study to determine all sources of variability in copper levels or in bioassay responses, the current design focuses on the WER for copper and takes into account seasonal differences in copper bioavailability in wet and dry seasons, salinity, pH, etc.

6. Does the study include a sufficient number of sample sites and samples at each site?
    - The TAC believes that for establishing a WER for MDRH an integrative approach is much more relevant than attempting to determine hotspots of copper in the basins. Hot spot determinations would be appropriate for a very different study design of copper sources. The SSO WER study is focused on DOC and copper bioavailability throughout the Harbor in an integrative manner.

7. Will study design capture a sufficient range of conditions to encompass drought and wet year conditions?
    - If the question is if a one-year study is sufficient, the TAC believes it is. The variation in the revised study design covers dry weather and wet weather, capturing this range within a year should cover dry and wet year conditions. Monitoring of physical and chemical parameters (DOC, copper, pH, salinity, etc.) can be continued after the WER sampling via the long-term monitoring program within in MDRH, but toxicity testing should not be necessary beyond the one year of the proposed study.

8. What is the potential effect of discharge from Oxford Basin on study results? Will study design allow for this evaluation? Would an additional sample site near Oxford Basin and/or a storm drain outlet enhance the study results?
    - This study is not designed to address the Oxford basin directly, however site sampling at MDRH-F which is where Oxford Basin drains during tidal flux will provide data on possible contributions of Oxford outflow to MDRH. If high levels of copper are present at MDRH-F, follow-up sampling at the discharge point of Oxford Basin would be warranted.

9. What potential synergistic effects may affect copper toxicity in Marina del Rey Harbor? Are these sufficiently evaluated in the study design?
    - In the data interpretation phase of the project, the planned use of the marine copper BLM will help identify if factors other than copper are affecting the test species. For example, it the measured toxicity proves to be consistently close to that predicted by the copper-BLM, then the effects of other stressors or toxicants in Marina del Rey waters can probably be discounted. On the other hand, if the measured toxicity is consistently greater than that predicted by the copper-BLM, this would suggest that some factors other than copper may be affecting the test species. Note, however, that the performance of the BLM may also be sensitive to other factors, such as the molecular nature of the dissolved organic carbon present in Marina del Rey waters (see the TAC's response to the original charge question #2). In other words, the BLM results will help reply to this question from the Water Board, but they may not provide an unequivocal answer.

10. Is calculation of a final WER in Marina del Rey Harbor through use of a geomean likely to provide a final WER that provides a level of protection equivalent to that of the California Toxics Rule?
    - Based on WER calculations at other sites (San Francisco and San Diego Bays), the TAC presumes that the geomean approach will provide the level of protection expected. However, this will not be determined until sufficient data are available from the proposed study.

11. How will implementation of sediment TMDLs affect the study and results?
    - There is a sediment toxics TMDL already, but no specific plan. The TAC does not know how to answer this directly, but we presume it would have a very minor effect because the water column copper is more influenced by watershed/hydrology factors and by leaching from antifouling paints than by what is in the sediment. Furthermore, copper associated with sediments would be tightly bound due to sulfide binding, as was indicated in previous studies (S. Bay, personal communication), so suspension of MDRH sediments is unlikely to contribute to water column ionic copper levels.

12. What is the likelihood a WER calculated during the proposed study period will be reflective of conditions in Marina del Rey Harbor upon during and after the implementation of the sediment TMDLs for Marina del Rey Harbor?
    - For questions 11 and 12, it is worth mentioning first of all that no remedial measures are planned for the period during which the work plan will be carried out. If dredging <u>is</u> carried out to remove copper-contaminated sediment, then based on the comprehensive studies performed by the US Army Corps of Engineers in their Dredged

Material Research Program, there should be negligible release of additional dissolved copper to the water column during the dredging operations. Dredging necessarily leads to increases in the concentration of fine particles in the water column and it also promotes the formation of hydrous iron oxides when the ferrous iron present in the sediment pore water in introduced into the oxic water column[9]. In both cases, the copper in the water column will tend to adsorb onto these particles and sink to the bottom of the marina, thus leading to a decrease in the dissolved copper concentration.

13. How might potential electrical discharges into the marina affect copper toxicity and a potential WER?
    - The TAC is not clear what this question is asking. If this is related to electrolysis of metals in seawater and release of copper ions, or if the question deals with high voltage from shore-based electrical sources. In either case, the TAC believes that this source of copper in seawater in MDRH would be relatively trivial.

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6. Tait, T. N.; Cooper, C. A.; McGeer, J. C.; Wood, C. M.; Smith, D. S., Influence of dissolved organic matter (DOM) source on copper speciation and toxicity to *Brachionus plicatilis*. *Environ. Chem.* **2016,** *13*, (3), 496-506.

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Los Angeles Regional Water Quality Control Board Comment Letter

Regional Board Feedback Regarding Draft Work Plan: Marina del Rey Harbor SSO Study
Provided by Los Angeles Water Board on December 4, 2018

Questions for TAC:

- Are either the Resident Species or Recalculation procedures potentially appropriate for deriving a site-specific objective for Marina del Rey Harbor?
- Selection of Test Species
    - Is *Mytilus galloprovincialis* the most sensitive species?
    - Should testing be conducted with multiple species during the study?
- Is the toxicity test proposed appropriate for use in developing a single WER for use in calculating both acute and chronic SSOs in Marina del Rey Harbor?
- Are the locations of the sites appropriate for the study? Do the sites capture the variability in Marina del Rey Harbor?
- Will all potential sources of variability be sufficiently investigated? Episodic events are mentioned in the background section of the work plan but not currently included in the study design. Would sampling during a planktonic bloom, if one occurs during the study period, enhance study results?
- Does the study include a sufficient number of sample sites and samples at each site?
    - The Regional Board would like to see additional sample sites, potentially near areas where organisms aggregate (e.g. dock pilings) and where copper concentrations are highest (e.g. near boats). Consider multiple sample sites within a basin.
- Will study design capture a sufficient range of conditions to encompass drought and wet year conditions?
- What is the potential effect of discharge from Oxford Basin on study results? Will study design allow for this evaluation? Would an additional sample site near Oxford Basin and/or a storm drain outlet enhance the study results?
- What potential synergistic effects may affect copper toxicity in Marina del Rey Harbor? Are these sufficiently evaluated in the study design?
- Is calculation of a final WER in Marina del Rey Harbor through use of a geomean likely to provide a final WER that provides a level of protection equivalent to that of the California Toxics Rule?
- How will implementation of sediment TMDLs affect the study and results?
- What is the likelihood a WER calculated during the proposed study period will be reflective of conditions in Marina del Rey Harbor upon during and after the implementation of the sediment TMDLs for Marina del Rey Harbor?
- How might potential electrical discharges into the marina affect copper toxicity and a potential WER?

Questions regarding Draft Work Plan:

- Will the work plan be completed if the WER appears to be less than 1?
- Pg. 3, paragraph 2 – Will the BLM be used to calculate WERs, SSOs or both?
- Pg. 20, paragraph 4 – Has spiking samples with zinc (as will be done with copper) been considered?

Requested edits to Work Plan:

- The critical condition should be defined based on study results. As there is not yet a U.S. EPA approved version of the saltwater BLM, it is not appropriate to utilize the BLM to define the critical condition in advance of sampling.
- Please include a minimum of monthly DOC sampling for 1 year in Marina del Rey Harbor to evaluate when during the year DOC is highest and lowest.
- Please state in the Work Plan whether Method 1 or Method 2 from EPA's Interim Guidance are the basis of the study design.
- Section 1.2.3
    - Please discuss previous toxicity testing in Marina del Rey Harbor, including where highest water column toxicity has been observed in the past.
    - DPR conducted an investigation of copper levels in California marinas that included BLM application in Marina del Rey Harbor. Please add discussion of this work in the review.
    - Please remove discussion of studies that did not result in adoption of an SSO by a Regional Board.
- Pg. 10, paragraph 1 – Please clarify if preliminary sampling for the BLM will be conducted prior to or in conjunction with sampling in Table 5.
    - Pg. 10, paragraph 4 – Please state EC50 values for *M. galloprovincialis* and confirm that the relevant endpoint for toxicity tests in laboratory dilution water is close to but not lower than the CCC and CMC as recommended in the 1994 Interim Guidance (pg. 21).
- Pg. 11, paragraph 1 – Please identify the first and second most sensitive species to copper in the national copper databse and why *M. galloprovincialis* is preferred.
- Pg. 11, paragraph 5 – Please clarify that all individual WER exposures will be initiated within 36 hours of sample collection. This clarification is requested to confirm that tests will not be initiated after 36 hours.
- Pg. 13. Table 1 – Test conditions in the EPA method indicate salinity should be 30°° +/- 2°° and the test chamber should be 30mL. Please discuss the rationale for utilizing different test conditions in the study.
- Pg. 17, Table 4 – Tables 3 and 4 are inconsistent regarding whether samples near the bottom were collected during event 2 or 3.
- Pg. 19, Table 5
    - Please add details in the text regarding the decision process for sample event 6 and how stakeholders will participate in this process.
    - In order to characterize the variability of Marina del Rey Harbor water quality, the Regional Board would like to see three years of sampling.
- Pg. 20, paragraph 2 – Please add storm drain outlets to Figure 3 as discussed in text.
- Pg. 20, paragraph 3 – "Some of the water quality parameters needed for BLM analysis will be measured in the field…" Please clarify when and how other parameters needed for the BLM analysis will be obtained.
- Pg. 20, paragraph 3 – Please add TSS and turbidity to the parameters that will be analyzed.
- Pg. 20, paragraph 4 – Please clarify whether or not sampling near the bottom of the water column is included in the work plan. Please add sample collection at 1 meter depth into the work plan to capture area in the water column most impacted by discharge from boat hulls.
- Pg. 21, paragraph 1 – Please clarify whether filtering of samples collected in the field for use in toxicity tests will occur in the field or in the laboratory.
- Pg. 23, paragraph 2 – Please clarify the toxicity testing procedures. This paragraph indicates there will be no dilution or spiking; however, the draft work plan includes spiking of samples with copper.

- Pg. 23, paragraph 3- Please utilize the version of the BLM and reference material included in U.S. EPA's 2016 Draft Aquatic Life Ambient Estuarine/Marine Water Criteria for Copper. The 2012 document sited in the Work Plan does not appear to be included in the 2016 draft criteria.
- Pg. 24, paragraph 2 – Please increase field QA samples to two field blanks and two field duplicates per sampling event, collected at dispersed times during the sample event.
- Pg. 24, paragraph 3 – Please clarify the procedure for spiking samples with copper. How will samples with no effect be obtained for sites where toxicity is exhibited in the site water?
- Pg. 25, paragraph 2 – The Regional Board would like verification of dissolved copper in all toxicity treatments and total copper in at least some of the toxicity treatments. This change should be made to Pg. 11, paragraph 5 as well.
- Pg. 26, paragraph 3 – For what analyses will recovery surrogates be utilized for QC rather than reference materials?
- Pg. 27, paragraph 4 – Please include the criteria and statistical methods that will be used to evaluate sWER sample size precision in the Final Work Plan.
- Pg. 29 – Please include submittal of data to CEDEN as part of data management.
- Pg. 29, paragraph 3 – Please include randomization sheets in study documentation and toxicity data quality review
- Pg. 30, Public Participation
    - Please clarify the recipients of each of the reports. Do the draft and final reports discussed in paragraph 3 apply to all task and study reports?
    - Please detail how potential decisions and/or changes to the work plan will be communicated (i.e. TBD information for sample event #6, whether or not data indicates the need for additional samples).
- Pg. 31, paragraph 1 – Please change public comment periods from "30 days" to "a minimum of 30 days" to allow for potential requests for additional time to review documents.
- Pg. 33, Table 11 – Please clarify whether or not all TAC meetings/calls listed will be open to the public.
- Please include in the work plan a discussion of whether or not a translator may be used in the study to convert dissolved to total copper. If a translator will be used, please describe how the translator will be selected.
- ELAP Certificate #2769 for Physis Environmental Laboratories appears to be an interim accreditation that expired on 8/31/18. Please confirm whether or not Physis Environmental Laboratories is currently accredited by ELAP for analyses they will be performing for this study.
- As recommended in the Interim Guidance document, please compare at least some toxicity test results obtained with results obtained in at least one other laboratory.
    - Guidance pg. 8, paragraph 2 – "…it is important to compare at least some results obtained in the laboratory dilution water with results obtained in at least one other laboratory."
- Please include full details of anticipated WER and BLM calculations/modeling in the Work Plan.
- QAPP
    - Table B-1 - Please provide a reference for an EPA method referencing data quality objectives for each parameter.
    - Pg. B-5, paragraph 1: Modifications in analytical methods must be approved by the TAC.
    - Please add QA/QC procedures used to evaluate quality/acceptability of seawater from Granite Canyon. Are hold times implemented for the seawater?
    - Please add a description of personnel training to the QAPP.
    - Please include a page for signatures of those certifying the adequacy of the QAPP.

Los Angeles Waterkeeper and Heal the Bay Comment Letter

December 3, 2018

Via electronic mail to ashleyp@sccwrp.org , steveb@sccwrp.org , and shana.rapoport@waterboards.ca.gov

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RE: Draft Work Plan for Marina del Rey Harbor Site-Specific Objective Study for Toxics (Dissolved Copper): Joint Comments of Los Angeles Waterkeeper and Heal the Bay

Dear Dr. Parks, Mr. Bay, and Ms. Rapoport:

Thank you for the opportunity to review and comment on the Draft Work Plan for the Marina del Rey Harbor Site-Specific Objective Study for Toxics (Dissolved Copper) (hereafter "MDR Draft Work Plan"). Los Angeles Waterkeeper (LAW) and Heal the Bay (HTB) jointly submit the following comments on the MDR Draft Work Plan.

I. Our organizations advocate on behalf of water quality in Marina del Rey Harbor and throughout Southern California.

LAW is a nonprofit environmental organization with over 25 years of experience and 3,000 members dedicated to protecting and restoring the inland and coastal surface and ground waters throughout Los Angeles County. LAW docks its boat (coated with Hullspeed copper free anti-fouling paint) in MDR Harbor, and has been a visible and active part of the MDR Harbor community for more than 20 years. Heal the Bay is a nonprofit environmental organization with over 30 years of experience and 15,000 members dedicated to making the coastal waters and watersheds of greater Los Angeles safe, healthy, and clean.

Together, our organizations have used a mix of advocacy, education, outreach, engagement, and (where necessary) litigation to ensure that Water Quality Objectives (WQO) and Total Maximum Daily Loads (TMDL) are promulgated as required; and that WQOs and

TMDLs are properly protective of the environment and of all beneficial uses at all times. We also prioritize the compliance of all local water bodies with all applicable WQO and TMDL limits.

II. LAW and HTB have actively participated in the review of previously proposed SSOs and have generally been critical of SSOs as a weakening of existing environmental protections.

LAW and HTB have historically been quite skeptical of the use of Site Specific Objectives (SSOs) and/or site specific modifications to TMDLs when the effect is to raise regulatory numeric standards.[1] We have been particularly concerned with the use of Water Effect Ratios (WER) in setting SSOs, even more so than the use of Recalculation Procedures or other methods of deriving SSOs. Nonetheless, we recognize that SSOs (including WERs) are allowed (although not mandated) by regulation. (See for example 40 C.F.R. §131.11 subd. (b).) LAW and HTB have consistently stressed the absolute necessity that ***any SSOs and site specific TMDL adjustments be as protective as the standards or limits they replace or modify***. Additionally, any promulgated SSOs and site specific TMDL adjustments must avoid potential degradation of water quality, and demonstrate consistency with all other applicable Anti-degradation Policies. (See State Water Resources Control Board Resolution 68-16; 40 C.F.R. § 131.12 subd. (a)(1), (a)(2)(ii).)

Despite our continued misgivings on the use of SSOs, our organizations see grounds for cautious optimism at this point in the process for SSO development in MDR Harbor. The role of SCCWRP has been beneficial, providing a layer of oversight and editorial independence that, from our perspective anyway, might not have always been present in the often-contentious Los Angeles River Copper and Lead SSO review process. We are also encouraged by the willingness of Regional Board staff to engage environmental groups such as LAW and HTB, and to address crucial issues transparently and early in the review process. While we anticipate occasional strong differences of professional opinion as the MDR Harbor SSO review process plays out, we will strive to make sure other participants in the process clearly understand the basis for our opinions. In other words, we recognize that engagement with study authors and regulators is a two way street.

We have reviewed the MDR Draft Work Plan, the interim and streamlined[2] WER guidance from the federal Environmental Protection Agency (EPA), and a previous WER study in San Francisco Bay that lead to the promulgation of Water Effect Ratios. We have also briefly reviewed several other WER studies in California that did not result in promulgation of Water Effect Ratios. We have divided the remainder of our comments into general comments, specific comments on the MDR Draft Work Plan, and comments on the interim EPA guidance.

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[1] We are not aware of any SSO that had the effect of lowering regulatory numeric standards.
[2] We agree with the study authors (MDR Draft Work Plan, Page 3, fn 1) that the interim EPA guidance applies to the MDR Work Plan, but that the streamlined EPA guidance does not. We therefore offer general comments on only the interim EPA guidance, to the extent of any divergence between the two.

III. General Comments:

A. The Regional Board should ensure that the approval of the MDR Draft Work Plan does not foreclose analysis of reasonable alternatives to a Copper WER.

It is important to remember that the Regional Board must review any proposed final WERs (fWERs) pursuant to the California Environmental Quality Act (CEQA) as a proposed Basin Plan Amendment (BPA).[3] Because significant resources are being invested now in studies that will presumably produce proposed fWERs, LAW and HTB wish to sound a note of caution that a range of reasonable alternatives to proposed WERs will need to be evaluated as part of the Basin Plan Amendment process. We wish to go on record now with our position that the BPA CEQA documents should evaluate the no-build alternative (i.e., keeping all current standards in place); as well as a range of alternatives to a copper WER including but not necessarily limited to a ban on copper anti-fouling paint in MDR Harbor implemented by California state agencies[4]; a partial ban designed to lower copper levels by the amount necessary to meet TMDL requirements; other source control measures implemented by the state and/or County of Los Angeles (such as an incentive program to convert to copper free paints); use of Resident Species Procedures and/or Recalculation Procedures instead of WER-based SSOs; and other reasonable alternatives that may emerge as the MDR Harbor SSO study process continues.

We also urge the Regional Board to ensure that planned compliance with anti-degradation policies (and to the extent applicable, anti-backsliding requirements) is integrated into the review process for any MDR Harbor SSO as early as possible. More broadly, we urge the Regional Board to integrate its reviews under the Porter-Cologne Water Quality Act of any SSOs with its CEQA review of the same. This approach is arguably required of the Regional Board anyway (see *Banning Ranch Conservancy v. City of Newport Beach* (2017) 2 Cal. 5th 919), but we believe that the anti-degradation analysis in particular would benefit greatly from a more integrated and holistic review approach. Required findings and any eventual Regional Board approval resolution would have more of a substantial evidentiary basis as well if the environmental reviews are better integrated. Our broad concern with integrated environmental review also extends to SCCWRP and the TAC review processes to some extent, even if *Banning Ranch,* strictly speaking, does not bind SCCWRP or the TAC. For example, integrating concerns with the anti-degradation analysis required as part of any eventual BPA into the MDR Harbor SSO study design now would tend to result in heightened attention to the critical condition. We believe such focus could only improve the final results of the MDR Harbor SSO study.

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[3] The BPA would also require approval by the California State Water Resources Control Board, the California Office of Administrative Law, and the federal Environmental Protection Agency.
[4] LAW has already shared a memorandum with the Regional Board outlining its position that the County of Los Angeles is likely preempted by California law from directly regulating copper paint as a nuisance in MDR Harbor, but that the Water Boards are not so preempted, if a demonstration is made that the proposed Water Boards' regulatory action supports attainment of water quality objectives and/or TMDL waste load and load allocations.

B. Previous WER studies should be considered illustrative, but should not be treated as creating binding precedent for the MDR Harbor SSO study.

Other SSO and WER studies can be illuminating, but should not be considered to create binding precedent on the methodology of the MDR Harbor SSO study. This is especially true given the overall lack of specific methodological guidance in the interim EPA guidance. This overall lack of guidance has led to considerable variation in WER study designs around California.

For example, the San Francisco Bay 2007 Proposed BPA and Staff Report for copper SSOs in San Francisco Bay was based on a copper WER study. However, that SSO process included only a perfunctory discussion of potential source control measures, devoting just one page of discussion in the Staff Report to control of copper anti-fouling paint. The Regional Board in San Francisco further declined to commit to any specific source control measures as part of the approval process. (See San Francisco Bay 2007 Proposed BPA and Staff Report, Page 5-6). We strongly urge the MDR Harbor SSO study authors as well as the Regional Board to make source control, particularly control of copper anti-fouling paint, a much higher priority for the MDR Harbor SSO study. We see source control as both a potentially viable alternative to a copper SSO, but also as a threshold inquiry to help determine whether a copper SSO is appropriate in MDR Harbor in the first place. It is thus conceptually difficult to separate source control from the MDR Harbor SSO study itself.

The San Francisco Bay 2007 Proposed BPA and Staff Report contained a fairly detailed anti-degradation analysis (an analysis we generally find much more informative than the conclusory analysis prepared in conjunction with the Los Angeles River SSOs). But one of the apparent conclusions in the anti-degradation analysis was that Numeric Effluent Limitations would likely not change in any existing permits if the proposed fWERs were approved, because of the effect of preexisting regulatory and permitting requirements (including anti-backsliding requirements). Since SSOs are widely perceived by both proponents and opponents alike as a form of regulatory relief, to us this conclusion begs the question of why any discharger with an existing permit would bother with a lengthy and expensive SSO study in the first place. (See 2007 Proposed BPA and Staff Report, p. 6-7.) A clear explanation (at a minimum) of the reasonably foreseeable regulatory effects (or lack thereof) of any MDR Harbor SSOs should be included with the MDR Harbor SSO study itself, given the importance of the anti-degradation policies to the ultimate success of the MDR Harbor SSO study.

The San Francisco Bay 2007 Proposed BPA and Staff Report relied on dissolved organic carbon (DOC) as a surrogate for toxicity in follow-up monitoring. This might be a defensible or even preferred approach for MDR Harbor, but study authors need to carefully justify any proposed use of that approach in MDR Harbor, rather than relying on the approved methodology of past studies.

Similarly, the San Francisco Bay 2007 Proposed BPA and Staff Report, as well as the WER study itself, relied on translators, since conversions from total to dissolved copper were necessary in San Francisco. It is unclear to what extent, if any, such translators would be needed

or even appropriate in the MDR Harbor SSO study. The excessive copper discharges in MDR Harbor are almost wholly attributable to copper paint on boats instead of municipal effluent. The water quality objectives and TMDL limits most at issue in MDR Harbor already are tied to concentrations of dissolved copper instead of total copper. We do not see any evidence that discharges by regulated municipal treatment plants have a significant direct effect on bioavailability or toxicity of copper in MDR Harbor, so those effluent limitations expressed in terms of total copper concentration in Hyperion's discharge permits are apparently not an issue.[5] Moreover, other site specific factors such as depth of the water column in MDR Harbor argue against the appropriateness of using translators.

Nonetheless, we request additional clarification on whether translators will be used in the MDR Harbor SSO study. We make this request because use of translators could reduce or even eliminate the margin of safety in the SSOs compared to the underlying standards, and thus effect the protectiveness of the SSOs and the potential for degradation of water quality and the related question of compliance with anti-degradation policies.[6] For example, the 2007 SSO study in San Francisco Bay applied a geometrical mean-calculated translator of 0.70 to the Central and Lower Bay, and a geometrical mean-calculated translator of 0.37 to Suisun and San Pablo Bays. However, the observed highest- and most conservative observed- translator was 1.0 in the Central and Lower Bays,[7] and 0.94 in Suisun and San Pablo Bays. The use of translators, in and of itself, therefore had the effect of significantly relaxing standards. (See 2007 Proposed BPA and Staff Report, Page 4-16.) For LAW and HTB, this feature of translators raises many of the same concerns that use of geometrical mean-based fWERs raised in the Los Angeles River.

The issues raised above are meant to be examples of questions that can arise when trying to apply the methodology of one SSO study to subsequent studies. It is not intended as an exhaustive list of concerns with using old SSO studies such as the 2007 San Francisco Bay study

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[5] Since this issue is not clearly explained in the MDR Draft Work Plan, we request clarification on whether discharges from Hyperion might have a significant direct effect on toxicity of copper in MDR Harbor.

[6] The most conservative copper translator is 1.0, which implies that all copper present in the water column is in dissolved form. The California Toxics Rule contains an approved default translator of 0.83, whose use in SSO studies we would find problematic because 1) there is nothing site-specific about a default translator, so the appropriateness of including this default term in a study meant to support *site specific objectives* is questionable; and 2) translators are typically derived using geometrical means of field samples, so by definition use of the calculated geometrical mean translator provides a less conservative standard than reliance on the highest observed translator would provide. Whether the use of a mean translator also equates to a less protective standard depends on the margin of safety in the original standard, but it seems likely that use of a very low translator would raise many of the same issues as use of a very high WER. Since the two terms could be combined in any SSOs, we have a number of unresolved questions regarding translators. Use of translators is seemingly analogous to promulgating a WER-within-a-WER. Translators were not an issue in the Los Angeles River SSOs, so possible use of translators present an issue of first impression for us here.

[7] The measured 1.0 translator strongly suggests that use of any other translator was inappropriate.

as definitive guides to the current SSO study in MDR Harbor. We also recognize that some methodological issues (perhaps such as translators) might prove irrelevant for the MDR Harbor SSO study.

The broader point we are trying to illuminate with this discussion of the 2007 San Francisco Bay copper SSO is the lack of specific recommended practices in the interim EPA guidance on the proper design of SSO studies. The lack of guidance in the interim EPA guidance creates difficulty in making apples-to apples comparisons of methodology across SSO studies. Unfortunately, reinventing the wheel may be a cost of this lack of specific guidance. LAW and HTB therefore request that if specific methodologies, findings, etc. from previous SSO studies are being imported into the MDR Harbor SSO study, that the study authors include an explanation of the appropriateness in the current context of MDR Harbor, and how the methodology proposed could affect the margin of safety and the protectiveness of the site specific objectives and/or site specific TMDL modifications in MDR Harbor. Our hope is to avoid or resolve any methodological issues early on in the study process, to make the MDR Harbor SSO study as robust and transparent as possible.

IV. Comments on the MDR Draft Work Plan:

To facilitate consideration of our comments on the MDR Draft Work Plan by SCCWRP and the TAC, our comments are presented essentially in the order in which the topics appear in the MDR Draft Work Plan itself. The order of discussion is therefore not necessarily reflective of our organizations' biggest concerns. We have highlighted major concerns as needed for clarity.

Page 2: We suggest revising the text of the first full paragraph to reflect that site specific parameters can increase, as well as decrease, toxicity to aquatic organisms (as would be reflected whenever WER < 1.0). In addition, we request a comparison of the margin of safety in the unadjusted WQOs, versus the margin of safety in any WER-adjusted standards. The use of *M. galloprovincialis* as a toxicity test species (see MDR Draft Work Plan, p.10), appears to increase the margin of safety of associated with any WER-adjusted SSOs, but it is unclear whether the overall effect of the SSO would be to lessen the margin of safety compared to the underlying standards.

Page 2: The Biotic Ligand Model (BLM) is not approved for use in marine waters, but the MDR Draft Work Plan relies heavily on the use of the BLM. This raises a potentially major concern, although we are open to persuasion on use of the BLM in marine waters. We request clarification on why the authors believe use of the BLM is appropriate despite the lack of approval for use of the model in marine waters. Also, we recommend considering revision of the MDR Draft Work Plan to include an alternative study design, if reliance on the BLM turns out to be inappropriate or scientifically less than robust (or if use of the BLM remains unapproved for marine waters as sample WERs are derived).

Page 3: LAW and HTB are also concerned that use of the BLM to target the conditions likely to result in the lowest WER (i.e., the critical condition), may not be appropriate if the

BLM is not approved for use in marine waters. In any event, ***it is of the utmost importance that the MDR Harbor SSO study demonstrate that the critical condition has been fully evaluated*** as part of the study, regardless of whether the BLM is used or not.

Page 3: (see also Page 8): Similarly, use of the BLM to generate "comparative" WERs is also of concern for the same reasons. The dispute over the critical condition was one of the single most contentious items in the Los Angeles River Copper WER Study in 2015, so we urge the study authors to carefully justify any claims about the timing of the critical condition, especially if the MDR Harbor SSO study relies of the critical condition relies on a model not approved for use in marine waters.

Page 4: Assuming successful resolution of the methodological concerns expressed elsewhere, we agree that the fWERs can be used to adjust both the CCC (chronic) and CMC (acute) WQOs, as well as TMDL adjustments associated with the same location.

Pages 5-6 and Page 8: The summary of various other marine WER/SSO studies raises the question of to what extent the MDR Draft Work Plan relies on methodologies in prior studies. The authors intend to "us[e] methods shown to be effective in recent successful California WER studies." (MDR Draft Work Plan, Page 8.) "Success" is not defined, however, and only one of the summarized studies resulted in promulgated WERs (which to us seems like a necessary if insufficient condition for a determination of "success").

LAW's Senior Attorney spoke to his counterpart at San Diego Coastkeeper about the SSO studies there, and he recalled that the San Diego Regional Board had serious concerns with the design of the Shelter Island Yacht Club WER study (ironically, the WER study that produced the lowest WER values of any of the studies summarized). The MDR Harbor SSO study authors may thus wish to consult with San Diego Regional Board staff for additional information on these apparent concerns. (San Diego Coastkeeper unfortunately could not locate its comment letter on this study in its files, so we are passing on all the additional information we have regarding this review.)

Page 10: The authors discuss the significant sources of potential variability in calculation of WERs. With a multi-year dispute over the critical condition in the Los Angeles River fresh in our minds, along with a keen desire to avoid such a dispute this time around, we urge the authors, Regional Board staff, SCCWRP staff, and the Technical Advisory Committee members to consider basing fWERs on the lowest value produced by methodologically correct testing, rather than relying on a geometric mean (or any type of averaging) of several values. This is especially important if the sample WERs (sWERs) show high variability.

Again, the Los Angeles River Copper WER study provides a relevant negative example for us. There, the geometrical mean of five sWERs (6.196, 8.161, 8.689, 9.215, and 17.15) for the Rio Hondo tributary was calculated, yielding an fWER of 9.69. Because of the high variability, and the inclusion of the outlying sWER of 17.15 in the fWER calculation, the fWER was considerably higher than the critical condition, which by definition had to occur with a WER

of no higher than 6.196.[8] In fact, the fWER was higher than 80% of the sWERs. This suggests that the WER- adjusted SSO for Rio Hondo could be considerably less protective than the underlying standard it replaced, and could often allow for increased toxicity. Other tributaries and the LA River mainstem had the same methodological issue. In addition, we believe a strong case exists that WER-based SSO should generally use the lowest sWER value obtained plus a margin of safety, rather than an average of values.

Page 14: We believe the interim EPA guidance is generally poorly suited to Southern California's flashy and highly variable hydrologic cycle, with one of the major shortcomings being that the guidance allows fWERs to be promulgated on as few as three field samples. We strongly suggest that the study design for the MDR Harbor SSO study include more than three sampling events, and that the number of sampling events in the study (as well as exclusion of data from any sampling event from fWER calculations) be fully justified.

Pages 16-17: We request citations to support the statement that DOC concentration is the primary variable controlling bioavailability of copper in marine systems. Additionally, we request additional evidentiary support that DOC concentration is closely correlated with BLM modeling results, and an explanation whether this DOC/BLM correlation is being used to justify reliance on BLM results despite lack of EPA approval for use of the BLM in marine waters. Again, we are open to persuasion on this issue, but we believe additional evidence in support of the study author's position is warranted.

Page 17: Available DOC data suggests that the lowest DOC levels tend to occur in the front basins and main channel of MDR Harbor, with back basins trending towards higher concentrations. While this distribution of concentrations makes intuitive sense, given how crucial distribution of DOC appears to be for the defensibility of the study results (including the calculation of fWERs), we recommend that additional DOC monitoring be undertaken prior to WER sampling to confirm the DOC concentration distribution pattern in the study area.

Page 18: Similarly, we recommend additional confirmation backed by rigorous data and robust modeling that the critical condition is likely to occur during dry weather in winter or spring.[9] Since this period is quite different from the apparent occurrence of the critical condition in the freshwater system of the Los Angeles River (where the Regional Board found the critical condition to occur during the peak of the dry season), we request additional discussion of why this large temporal difference in occurrence of the critical condition might result between the freshwater Los Angeles River and the marine waters of MDR Harbor.

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[8] It seems likely that the true critical condition at Rio Hondo would be even lower, since only 5 sample WERs were calculated and the sWERs exhibited high variability.

[9] Ironically, during the review of the Copper WER study for the Los Angeles River and its tributaries, our organizations suggested that dry periods within the rainy season were very plausible candidates for the occurrence of the critical condition, a position that was hotly disputed by both the study authors and the Regional Board at the time. We suggest, at a minimum, that study authors consider a more precise definition for wet and dry weather conditions than that provided in the interim EPA guidance.

Page 18: Given the probable lowest DOC concentrations in the main channel, we recommend considering adding a second water sampling station in the main channel, in the area of the channel to the east of the terminus of Bora Bora Way, to improve the robustness of the study results.

Page 20: We have concerns that a single sampling event to confirm that wet weather copper bioavailability is lower than other periods is insufficient, especially since the study authors believe that winter or spring contains the critical condition. We recommend that the study authors consider adding at least a second sampling event in wet conditions.

Page 20: We support the collection of grab samples, and support analyzing zinc concentrations in those samples. However, we ask for greater clarification of the claim that inclusion of zinc in the study will facilitate understanding copper toxicity.

In other words, we request a greater discussion of how the study design will account for possible synergistic effects of multiple toxins (zinc and lead come to mind in particular). It is unclear how the study design can account for synergistic effects when the test methodology isolates copper for addition to test vessels, but the extent of any variation in copper discharges is unclear,[10] and zinc/lead loading probably tend to occur in unison in real world conditions (along with loading of other pollutants, some of which might exacerbate the toxicity of the metals, and some of which might mitigate the toxicity). This issue proved contentious in the Los Angeles River proceedings, and being familiar with the LA River WER study, we have the impression that environmental advocates and regulators spent much time talking past each other on the issue of synergistic effects, so we would like as much clarification as possible early in the process.

One possible solution would be to essentially sidestep the issue, by demonstrating that the MDR Draft Work Plan builds in a very significant margin of safety, so that artifacts of test methodology do not lead to an underestimate of copper bioavailability. We reiterate how important it is to the success of the study to obtain an accurate estimate of copper bioavailability. If bioavailability is underestimated, proposed fWERs are likely unrealistically high, with all of the attendant problems for protectiveness of the standards, possible degradation of water quality, etc., that such a situation would entail.

The other overlapping possibility is to identify the lowest possible WER and set the SSOs accordingly.[11] In other words, use the critical condition, and not a geometric mean, as the basis for the SSOs. One of the few areas where, in our view, the interim EPA guidance actually provides useful methodological recommendations concerns the potential for synergistic effects, and the interim EPA guidance suggests that it may be most cost-effective to base SSOs on the

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[10] We request a fuller discussion of whether copper discharges are in fact approximately constant, or whether they tend to increase during events when discharges of other pollution tends to increase.

[11] Logically, we would see this as the lowest sWER, plus some defensible margin of safety, or even a non-arbitrary "fudge factor" that can garner consensus support.

lowest WER. (See interim EPA guidance at p. 135.) In any event, we urge study authors to more fully explain how the MDR Draft Work Plan accounts for synergism, additivity, or reduced efficacy of detoxifying metal-organic complexes when multiple metals are present.

Page 27: *The question of whether the WER sample size and precision allows for a defensible fWER is extremely important.* We again urge the study authors to use the lowest WER obtained plus a margin of safety, rather than an average of sWERs, to ensure that the critical condition has been captured, and that the SSOs do not suffer from a potentially serious anti-degradation policy consistency problem as a result.

Page 28: In a similar vein, *LAW and HTB believe that if insufficient data exists to support "the desired level of precision and seasonal specificity" of fWERs after completion of the study, then Regional Board approval of a Basin Plan Amendment based on those fWERs almost by definition would violate both CEQA and Porter-Cologne*, as any findings would lack substantial evidentiary support and/or would not proceed in manner required by law. (See California Code of Civil Procedure §1085 and §1094.5.) We thus strongly encourage revision to this section of the MDR Draft Work Plan, to include a firm commitment to either collect such data until the data set is robust enough to support calculated fWERs, or else to discontinue the process of seeking approval of SSOs if data is insufficient to support calculation of defensible fWERs.

Page 31: We support having public comment periods at key points during the MDR Harbor SSO study process. We strongly recommend formal written responses to comments be prepared, even if the Regional Board believes it does not have a strict legal obligation to prepare such a document as part of its Work Plan review. We recognize that preparing written responses at multiple points in the MDR Harbor SSO study process will be very time consuming and can seem a thankless task. We can assure you that the same is often true of writing multiple comments in the first place. However, the process of preparing formal written responses ensures that 1) all public comments are examined and considered; 2) the author of the comments knows how the comments have been considered and has a sense of the disposition of the issue raised; and 3) issues that might otherwise linger and fester are potentially resolved.[12] It also offers all parties the benefit of a written record, and demonstrates that the Regional Board takes engagement with various interests and perspectives seriously at every step of the process.

We also request that data collected to support the MDR Harbor SSO study be made available in as close to real time as possible, to further enhance transparency, and to provide for potential feedback to allow identification (and presumably correction) of any problems as early in the process as possible.

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[12] As a practical matter, issues that fester would likely reappear as written comments during the BPA review, at which point written comment responses from the Regional Board would be required anyway. (See Pub. Res. Code §21080.5 subd. (2)(D).)

V. Concerns with the Interim EPA Guidance:

Many of the following comments on the "*June 1994 Environmental Protection Agency Interim Guidance on the Determination and Use of Water Effect Ratios for Metals*" (i.e. the interim EPA guidance) are reflected above as applied to specific issues associated with the MDR Draft Work Plan. Nonetheless, our organizations have sufficient concern with the interim EPA guidance generally that we wish to go on record here with those concerns.

First, we were greatly encouraged and relieved during the course of the initial TAC call when Mr. Bay of SCCWRP referred to the interim EPA guidance as an "aspirational" document not to dictate the exact conduct of the MDR Harbor SSO study. Bringing to mind Voltaire's quip about the Holy Roman Empire, we do not consider the interim EPA guidance as "interim" in any meaningful sense of the word (it will celebrate its 25th birthday during the course of the MDR Harbor SSO study), nor do we believe it provides much in the way of useful guidance. However, we will assume, arguendo, that the guidance was actually produced by EPA, or at least under its direction.

One fundamental problem with the document is that it assumes the hydrologic cycle of Southern California is essentially the same as anywhere else. As even a casual observer would recognize, this is far from true. Our organizations have long advocated that the California State Water Resources Control Board promulgate California-specific WER guidance, but apart from production of a draft WER policy over a decade ago we have had no success on this front. So, from our perspective we are essentially stuck with very old WER guidance that does not speak to the extreme variability of California's hydrologic cycle; allows potentially wholesale changes to WQOs and TMDL limits based on as little as three field samples; and provides only vague guidance as to the timing of when those samples should even occur. (See for example, Page 48 of the interim EPA guidance, which cautions that samples should not be "unduly affected by recent runoff events.")

We are also concerned that the document allows extrapolation of assumptions about toxicity over a wide range of organisms. This is less of a concern in this particular instance, since the study design is using the most sensitive species in the national data set for marine organisms, and is using the same organism upon which California Toxics Rule copper criteria are based. (MDR Draft Work Plan, Pages 10-11.) Nonetheless, this breezy acceptance of extrapolation to very divergent organisms raises concerns generally.

The interim EPA guidance is silent on maintaining a margin of safety to account for possible errors in measurements. Because setting a WER too high could (and in many cases, likely would) result in increased toxicity and/or water quality degradation, this is a major omission.

Even where the interim EPA guidance uncharacteristically offers specific help to study authors- most notably by suggesting identification of the lowest possible WER and setting the SSOs accordingly to account for potential synergistic effects that are difficult to account for in study design- the guidance thereby creates an internal inconsistency with the definition of the

fWER, which is defined as a geometrical mean.[13] (See interim EPA guidance at Page 36; cf Page 135.)

VI. Conclusion:

LAW and HTB approach the MDR SSO study with a dose of healthy skepticism, but we are committed to keeping open minds and open channels of communication. The process for the MDR Harbor SSO study so far represents a major improvement over the contentious review of the Los Angeles River Copper and Lead SSOs, although obviously the review process for MDR Harbor is still quite early. We hope to work with SCCWRP, TAC members, the Regional Board, and other stakeholders moving forward to resolve issues as they arise, and to ensure that if site specific water quality objectives and TMDL adjustments are approved for MDR Harbor, that the results are scientifically and legally sound.

Thank you for this opportunity to comment on the MDR Draft Work Plan. We look forward to participating in the in-person TAC meeting in December. Please contact Arthur Pugsley at LAW (arthur@lawaterkeeper.org or 310-394-6162 x 102) or Annelisa Moe at HTB (amoe@healthebay.org or 310-451-1500 x 139) if you have any follow-up questions regarding the above. (Signatures appear on the following page.)

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[13] Unless all data points are exactly equal, of course. Such a situation seems extremely unlikely to occur, and we believe it could be problematic for other reasons if it did.

Sincerely,

Arthur Pugsley, M.S., LL.M.
Senior Attorney
Los Angeles Waterkeeper

Melissa von Mayrhauser, M.Sc.
Watershed Programs Manager
Los Angeles Waterkeeper

Katherine Pease, Ph.D.
Director of Science & Policy
Heal the Bay

Annelisa Moe, M.S.
Water Quality Scientist
Heal the Bay

cc (via electronic mail): Ms. Jenny Newman (Jenny.Newman@waterboards.ca.gov)
Mr. Michael Tripp (mtripp@bh.lacounty.gov)

Stakeholder (Johntommy Rosas) Comment Letter

Ashley Parks

From: Johntommy Rosas <tattnlaw@gmail.com>
Sent: Wednesday, October 31, 2018 1:00 PM
To: Ashley Parks; Hanks, Michael@Waterboards; Rapoport, Shana@Waterboards; JOHNTOMMY ROSAS
Subject: RE:DRAFT WORK PLAN MARINA DEL REY HARBOR SITE-SPECIFIC OBJECTIVE STUDY
Attachments: Citations for OSHA, etc - Rosas - MIACC-AFS mtg 10-16-18 (1).docx

Hi Dr. Ashley Parks/Shana/Michael,
I made this video presentation for the Fall 2018 Marina Interagency Coordinating Committee and Anti-fouling Strategies Workgroup-
I hope you folks can implement this information for your study-
thanks ,jt
https://www.youtube.com/watch?v=mvTgmKY8pSY

1. https://www.nace.org/Corrosion-Central/Corrosion-101/Galvanic-Corrosion/
2. http://www.cruisersforum.com/forums/attachment.php?attachmentid=172105&d=1529467584
3. https://www.cdpr.ca.gov/docs/emon/pubs/ehapreps/eh0805.pdf
4. https://newcontent.westmarine.com/wm-img/westadvisor/articles/Shore-Power-13.jpg
5. http://assets.bluesea.com/files/resources/newsletter/images/Ground_Fault.png
6. http://www.forestriverforums.com/attachments/photobucket/img_1146680_0_ebf4cd5eca16c8ae1d3bef0d73c8bef4.gif
7. https://darchive.mblwhoilibrary.org/bitstream/handle/1912/191/chapter%2022.pdf?sequence=31
8. https://www.ecmweb.com/content/case-hot-marina
9. https://www.electricshockdrowning.org/esd--faq.html
10. http://www.boatus.com/seaworthy/assets/pdf/electric-shock-drowning-explained.pdf
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14. https://www.waterboards.ca.gov/about_us/performance_report_1617/plan_assess/docs/fy1314/11112_r4_marinadelrey_toxics.pdf
15. https://www.waterboards.ca.gov/losangeles/board_decisions/basin_plan_amendments/technical_documents/96_New/Final%20MdR%20Sediment%20Characterization%20Report_processed.pdf
16. https://www.cdpr.ca.gov/docs/dept/prec/2017/012017_af_paints.pdf
17. https://www.paint.org/article/marine-coatings-making-sense-u-s-state-local-mandates-copper-based-antifouling-regulations/
18. https://www.epa.gov/sites/production/files/2015-10/documents/2001_10_30_nps_mmsp_section4.pdf
19. https://www.dtsc.ca.gov/PollutionPrevention/upload/Scappingand-SandingHullFinal.pdf
20. https://www.usace.army.mil/Portals/2/docs/civilworks/recreation/Water_Safety/tools/guidelines_safe_operation_maint_marinas.pdf?ver=2016-07-25-130937-857
21. https://www.osha.gov/Publications/osha2268.pdf
22. https://www.osha.gov/laws-regs/regulations/standardnumber/1915/1915SubpartC
23. https://www.osha.gov/OshDoc/data_MaritimeFacts/shipbreaking-factsheet.pdf
24. https://www.osha.gov/dts/maritime/standards/guidance/shipyard_guidance.html
25. https://www.osha.gov/dts/maritime/sltc/ships/surfaceprep/surfaceprep_all-in-one.pdf

26. https://www.osha.gov/laws-regs/regulations/standardnumber/1910/1910.16
27. https://www.osha.gov/pls/imis/sic_manual.display?id=921&tab=description
28. https://www.dtsc.ca.gov/PollutionPrevention/upload/MediaBlastingFinal.pdf
29. https://www.cdpr.ca.gov/docs/dept/prec/2017/012017_af_paints.pdf
30. http://www.dfo-mpo.gc.ca/Library/52635.pdf
31. ftp://ftp.sccwrp.org/pub/download/DOCUMENTS/AnnualReports/2003_04AnnualReport/ar04-schiff_pg41-49.pdf
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36. https://www.equipcoservices.com/sales/ysi/pro30.html?gclid=EAIaIQobChMIzvfrqefP3QIVyQoqCh3f3QUKEAAYASAAEgJoA_D_BwE
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38. http://www.electrochemsci.org/papers/vol12/120201232.pdf
39. https://www.paint.org/article/use-copper-based-antifouling-paint-u-s-regulatory-update/
40. https://www.wartsila.com/docs/default-source/marine-documents/encyclopedia/wartsila-o-marine-encyclopedia.pdf?utm_source=web&utm_medium=web&utm_term=marine&utm_content=encyclopedia&utm_campaign=encyclopedia
41. http://www.imo.org/en/About/Conventions/ListOfConventions/Pages/International-Convention-on-the-Control-of-Harmful-Anti-fouling-Systems-on-Ships-(AFS).aspx
42. http://www.imo.org/en/OurWork/Environment/Anti-foulingSystems/Pages/Default.aspx
43. https://www.google.com/search?q=boat+hull+cleaning+divers&num=20&newwindow=1&rlz=1C1CHBD_enUS807US807&source=lnms&tbm=isch&sa=X&ved=0ahUKEwjj_7u968_dAhULKXwKHfgsB4EQ_AUIDygC&biw=1816&bih=974&dpr=2
44. http://events.nace.org/conferences/IMCS2008/papers/15.pdf

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JOHN TOMMY ROSAS
TRIBAL ADMINISTRATOR
TRIBAL LITIGATOR -TATTN JUDICIAL # 0001
TONGVA ANCESTRAL TERRITORIAL TRIBAL NATION
A TRIBAL SOVEREIGN NATION UNDER THE UNDRIP AND AS A TREATY [s] SIGNATORIES RECOGNIZED TRIBE, INCLUDING BY THE STATE OF CALIFORNIA WITH HISTORICAL & DNA AUTHENTICATION ON CHANNEL ISLANDS AND COASTAL VILLAGES - AND AS A CALIFORNIA NATIVE AMERICAN TRIBE / SB18-AB 52-AJR 42-ACHP/NHPA - CALIFORNIA INDIANS JURISDICTIONAL ACT U S CONGRESS APPROVED MAY 18, 1928 45 STAT. L 602

TRUTH IS OUR VICTORY AND HONOR IS OUR PRIZE >TATTN ©

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Ashley Parks

From: Johntommy Rosas <tattnlaw@gmail.com>
Sent: Wednesday, October 31, 2018 3:45 PM
To: Ashley Parks; Hanks, Michael@Waterboards; Rapoport, Shana@Waterboards; JOHNTOMMY ROSAS
Subject: Re: DRAFT WORK PLAN MARINA DEL REY HARBOR SITE-SPECIFIC OBJECTIVE STUDY

I hope to see sufficient testing and additional testing areas per this citation -
" The USEPA (1994) Interim Guidance recommends that WER analyses be conducted over a range of conditions so that the results are representative of the variations in water quality at the site.
The guidance also states that the study should include multiple stations distributed over a minimum of three separate sampling events that include different seasons and locations. "
Its also important to test appx 2 years to get a secondary year to compare with -
in the various seasonal events and effects that occur from the seawater
and the actual vessels different discharges from stray electrical currents
-and those affects that cause additional antifouling paint /copper other noble metals in the coatings to discharge from all vessels -
the suggested testing areas are not sufficent -there should be an additional 22 stations and 22 locations used for metals analysis
"The station locations for the study are a subset of 11 candidate stations used in previous monitoring surveys (Figure 3). These stations include nine locations used for metals analysis in the MdR Harbor TMDL Coordinated Integrated Monitoring Program "
the some testing areas should be near the vessels as well- where the actual discharging occurs- there should be habitat testing additionally to have the negative impacts on sea life / habitat etc- documented-
thanks jt

On Wed, Oct 31, 2018 at 12:59 PM Johntommy Rosas <tattnlaw@gmail.com> wrote:

> Hi Dr. Ashley Parks/Shana/Michael,
> I made this video presentation for the Fall 2018 Marina Interagency Coordinating Committee and Anti-fouling Strategies Workgroup-
> I hope you folks can implement this information for your study-
> thanks ,jt
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> 1. https://www.nace.org/Corrosion-Central/Corrosion-101/Galvanic-Corrosion/
> 2. http://www.cruisersforum.com/forums/attachment.php?attachmentid=172105&d=1529467584
> 3. https://www.cdpr.ca.gov/docs/emon/pubs/ehapreps/eh0805.pdf
> 4. https://newcontent.westmarine.com/wm-img/westadvisor/articles/Shore-Power-13.jpg
> 5. http://assets.bluesea.com/files/resources/newsletter/images/Ground_Fault.png
> 6. http://www.forestriverforums.com/attachments/photobucket/img_1146680_0_ebf4cd5eca16c8ae1d3bef0d73c8bef4.gif
> 7. https://darchive.mblwhoilibrary.org/bitstream/handle/1912/191/chapter%2022.pdf?sequence=31
> 8. https://www.ecmweb.com/content/case-hot-marina
> 9. https://www.electricshockdrowning.org/esd--faq.html
> 10. http://www.boatus.com/seaworthy/assets/pdf/electric-shock-drowning-explained.pdf

Ashley Parks

From: Johntommy Rosas <tattnlaw@gmail.com>
Sent: Wednesday, October 31, 2018 4:04 PM
To: Ashley Parks; Hanks, Michael@Waterboards; Rapoport, Shana@Waterboards; JOHNTOMMY ROSAS
Subject: Re: DRAFT WORK PLAN MARINA DEL REY HARBOR SITE-SPECIFIC OBJECTIVE STUDY

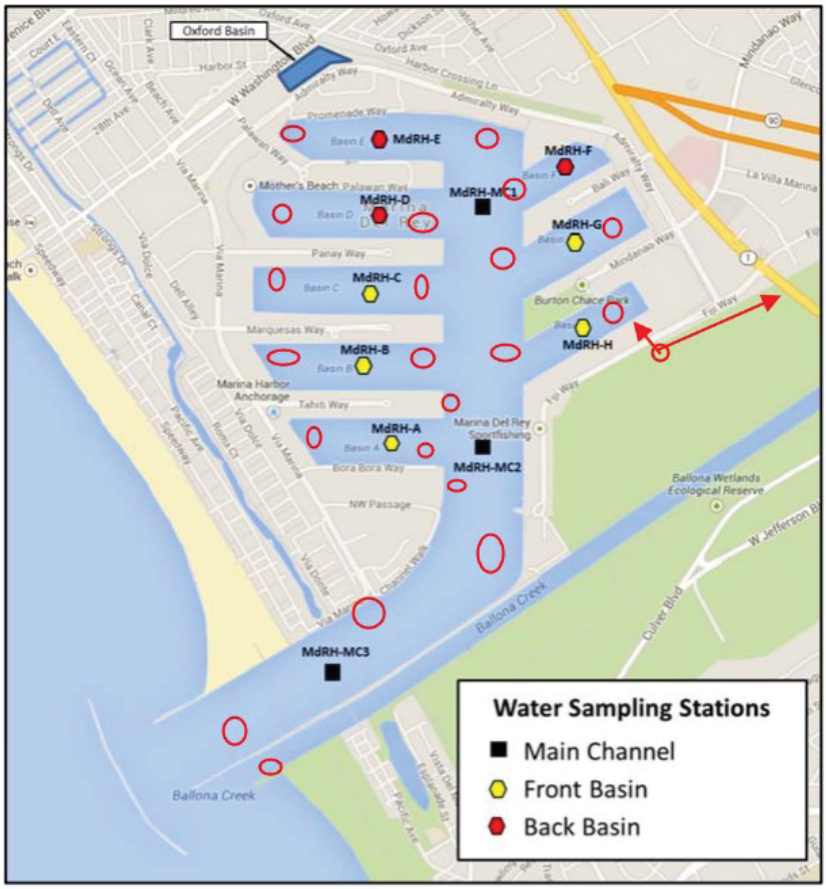

Figure 3. Candidate sampling stations in Marina del Rey Harbor.

please my suggested test stations - please include the fiji channel/ditch [has the areas with arrows]
and ballona[bayona] creek channel that goes into the state BWER -as well
thanks jt

On Wed, Oct 31, 2018 at 3:44 PM Johntommy Rosas <tattnlaw@gmail.com> wrote:

> I hope to see sufficient testing and additional testing areas per this citation -
> " The USEPA (1994) Interim Guidance recommends that WER analyses be conducted over a range of conditions so that the results are representative of the variations in water quality at the site.
> The guidance also states that the study should include multiple stations distributed over a minimum of three separate sampling events that include different seasons and locations. "
> Its also important to test appx 2 years to get a secondary year to compare with -
> in the various seasonal events and effects that occur from the seawater
> and the actual vessels different discharges from stray electrical currents

Ashley Parks

From: Johntommy Rosas <tattnlaw@gmail.com>
Sent: Tuesday, December 11, 2018 4:40 PM
To: Ashley Parks; JOHNTOMMY ROSAS; Michael Tripp; Kang, Jim@Waterboards; Rapoport, Shana@Waterboards
Subject: RE DRAFT WORK PLAN MARINA DEL REY HARBOR SITE-SPECIFIC OBJECTIVE STUDY

I have reviewed your draft document and I approve of it -
The TONGVA NATION continues to fully support of the clean up and remediations for the MDR HARBOR and the SANTA MONICA BAY as approved by the US EPA and CA STATE WATER BOARD-
which is our territorial waters historically and now as recognized by UN UNDRIP which the USA has adopted -
and the state of ca has as well under AJR 42-
thank you
/S/ JOHNTOMMY ROSAS

--
JOHN TOMMY ROSAS
TRIBAL ADMINISTRATOR
TRIBAL LITIGATOR -TATTN JUDICIAL # 0001
TONGVA ANCESTRAL TERRITORIAL TRIBAL NATION
A TRIBAL SOVEREIGN NATION UNDER THE UNDRIP AND AS A TREATY [s] SIGNATORIES RECOGNIZED TRIBE, INCLUDING BY THE STATE OF CALIFORNIA WITH HISTORICAL & DNA AUTHENTICATION ON CHANNEL ISLANDS AND COASTAL VILLAGES - AND AS A CALIFORNIA NATIVE AMERICAN TRIBE / SB18-AB 52-AJR 42-ACHP/NHPA - CALIFORNIA INDIANS JURISDICTIONAL ACT U S CONGRESS APPROVED MAY 18, 1928 45 STAT. L 602

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Stakeholder (Douglas P. Fay) Comment Letter

December 3, 2018

To: USEPA, LARWQCB, LA County BOS, SCCWRP, TAC Members, and other Interested Parties

Re: Draft Work Plan Marina del Rey Site-Specific Objective Study review, comments and concerns by Douglas Fay

Thank you for the opportunity to comment on this controversial Work Plan. I represent the third generation of documented Fay family involvement with the Marina del Rey (MDR) Harbor and Santa Monica Bay (SMB). My children represent the fourth. In 1949, my fisherman grandfather Rimmon Loraine Fay was a member of the pioneering Ocean Fish Protective Association, which formed when our local fishery showed signs of collapsing, which it did. My ocean lifeguard and marine scientist father Dr. Rimmon C. Fay, with PhDs in Chemistry and Oceanography was considered by many as the leading SMB expert for decades. In 1962, the year I was born, my parents bought a home on Howard Street adjacent to the MDR Harbor, where I lived for 3 decades. I have been swimming and diving in the SMB my entire life. I am a certified professional scuba instructor with hundreds of dives in the SMB. I have attended several meetings and commented on MDR water quality concerns in the past.

I have read the Draft Working Plan and am alarmed that this is in fact not a working plan that protects aquatic life in the MdR Harbor as stated. The Introduction of the Draft Working Plan clearly outlines that this is a “reconsideration” of TMDLs for Toxins based on the rationale that there is a need to quantify and/or justify creating a legally and “scientifically defensible” water quality criterion to protect aquatic life in MDR Harbor. It is deception. Without creating a working plan that removes over 50 years of accumulated toxins and organic matter this is impossible. The intent of the Draft Working Plan is to reduce environmental protection by paying SCCWRP and others $4 million to create studies that say everything is okay when in fact it is not. Funding and creation of this study is exempt from CEQA. This move is also an environmental injustice.

I have stated that, “For the recreational users, boaters, divers, tourists, residents and marine life that live and play in Marina del Rey, the Harbor is our aquarium of the Pacific. Aquariums must be maintained. The County of Los Angeles (County) is responsible for maintaining the Harbor. Unfortunately, since the Harbor was built, they have failed to maintain the water quality at an acceptable level.

The Working Plan solution to the 303(d) list impairment that I proposed years ago was for the County to purchase or custom build a commercial boat that can remove, in an environmentally friendly way, vacuum not dredge, an average of 1 ton of sediment per day, transfer the material to a commercial truck, and have it taken to a land based facility for recycling and reuse. Within 2 years, approximately 600 tons of sediment, generated primarily from in water hull cleaning, would be removed and water quality will significantly improve. Ongoing maintenance would be required. The man-made marina does not have adequate circulation. It is a design flaw that requires maintenance as mitigation.

Unfortunately, in 2014, rather than implement a working plan to remove the toxins and organic matter, the County BOS approved another study. There have been several over many years. This one at a cost of $4 million.

The question that needs to be asked is: Do the decision makers and stakeholders clearly understand the objective and reconsideration of this proposed Draft Work Plan? I do not believe the stakeholders do. I know further delays that prevent implementing a solution impose a significant impact on aquatic life.

Absent from Background description is 1954 US Public Law, House Document No.389, the Federal document that outlines the creation and purpose of the Harbor. In 1994, the US House of Representatives approved a Resolution - Docket 2455 that states the Secretary of the Army is required to review a report by the Chief of Engineers on House Document No.389. A determination was to be made prior to environmental restoration projects. Unfortunately, there is no documentation that shows a determination has been made and controversial restoration projects have proceeded.

The November 15, 2018 publication of The Argonaut newspaper Page 10, A Piece of Marina History Goes up for Auction shows an early-1960s watercolor depicting the original design study for dredging Marina del Rey Harbor painted by an architecture coordinator for Los Angeles County. It shows a landscape consistent with what is described in House Document No. 389.

In 1998 the MDR Harbor was added to the 303(d) list by the LARWQCB for high copper levels.

In 2014 the toxin TMDLs were revised and adopted by the LARWQCB and SWRCB.

The SWRCB Resolution 2014-0049 lead to the SSO study in 2017.

In the Development of Site-Specific Objectives, it states protection of aquatic life is uncertain. It further states that this study does not account for physical constituents for example, particulate and dissolved organic matter.

The proposed study is not inclusive and will not produce a comprehensive solution that will protect aquatic life. It does not mention providing the required level of protection for human health. Commercial divers and recreational swimmers enter the MdR Harbor waters daily.

WER is not applicable to MdR.

The Toxicity Test Species and Method Selection is limited to one species, M. galloprovincialis embryos.

The Water-Effect Ratio Study Design – Station Locations excludes Oxford Basin. Why?

The Public Participation Plan states public participation will be actively sought. Absent from the Draft Work Plan is public comment and Q&A at the TAC review meetings and the first public workshop. Why? Limiting public participation to only being able to comment at the final second public workshop is insufficient.

Under section VII Implementation Report is California Water Code Section 13241 anti-degradation review (as appropriate). If the Draft Work Plan proceeds as proposed degradation of the Harbor will continue and reviewing now is appropriate.

Under section VIII Project Schedule is the Work Plan. April 19, 2015, the County BOS approved SCCWRP to initiate the SSO Study.

The Quality Assurance Project Plan will be conducted by SCCWRP. For me, given the history of failed scientific research integrity at SCCWRP, this is problematic. It is well documented in Dirty Water by Bill Sharpsteen that Willard Bascom altered SMB pollution data submitted to him by one of his scientists Dr.

David Brown when he ran SCCWRP. Eventually, the City of Los Angeles lost the 301(h) waiver battle in court at a cost of over $3.5 billion dollars. The scientific evidence that forced them to upgrade sewer infrastructure throughout LA County and at Hyperion came from my marine scientist father Dr. Rimmon C. Fay. With Dr. Fay out of the picture, he passed away January 1, 2008, there are no credible independent scientists to ensure Bascom's behavior isn't continuing at SCCWRP and influencing this process in other ways.

When I read that Richard Ambrose was 1 of 3 TAC Members for this Draft Work Plan and study the alarm bells went off. Like Bascom, Ambrose is a person that is not trusted by SMB environmental activists. The controversial Malibu Lagoon restoration project he supported was not comprehensive in relation to the watershed and SMB. This past summer, after project completion, a massive fish die-off occurred in the Malibu Lagoon. The upstream barrier Rindge Dam that significantly impedes the endangered Southern Steelhead trout from historical migration territory, has not been removed. Upstream pollution and water quality concerns legalized through the TMDL process do not provide adequate protection for the endangered Southern Steelhead. Ambrose also supports the controversial Ballona Wetlands Ecological Reserve (BWER) Alternative 1 proposed alternative, that will transform what was historically a positively charged fresh water estuary into a full tidal habitat. Again, approved through the TMDL process, untreated urban runoff would be introduced into the fragile BWER under the assumption that wildlife areas can assimilate the synthetic toxins without harming species. I have never read a valid scientific report that states unlike in humans, synthetic toxins do not harm wildlife. Maintaining the adjacent MdR Harbor that already is a full tidal habitat for aquatic species would negate the need to transform the BWER. Through his involvement with the Santa Monica Bay Restoration Commission (SMBRC), as a scientist Ambrose has done nothing to right the wrongs of this commission's actions. Currently this commission operates under the SWQCB and is funded in part by the USEPA's National Estuaries Program (NEP) which states the SMB is a 312 square mile estuary, which it is not. It is a bay. His scientific integrity appears to be compromised by politicians. Richard Ambrose does not belong on the TAC. He should be replaced.

The intent by US Representative Mel Levine and California Assemblyman Tom Hayden decades ago, with NEP funding, was to study SMB pollution for 5 years and draft a plan that included the creation of the Santa Monica Bay National Marine Sanctuary. If the sanctuary designation had happened, which it didn't, the MdR Harbor would have been required to be maintained. There is sufficient revenue generated in unincorporated MdR to fund my recommended work plan for the harbor. Unfortunately, the BOS have taken this process as far away from a sustainable outcome as humanly possible and at a significant financial burden to taxpayers.

Respectfully submitted,

Douglas Fay