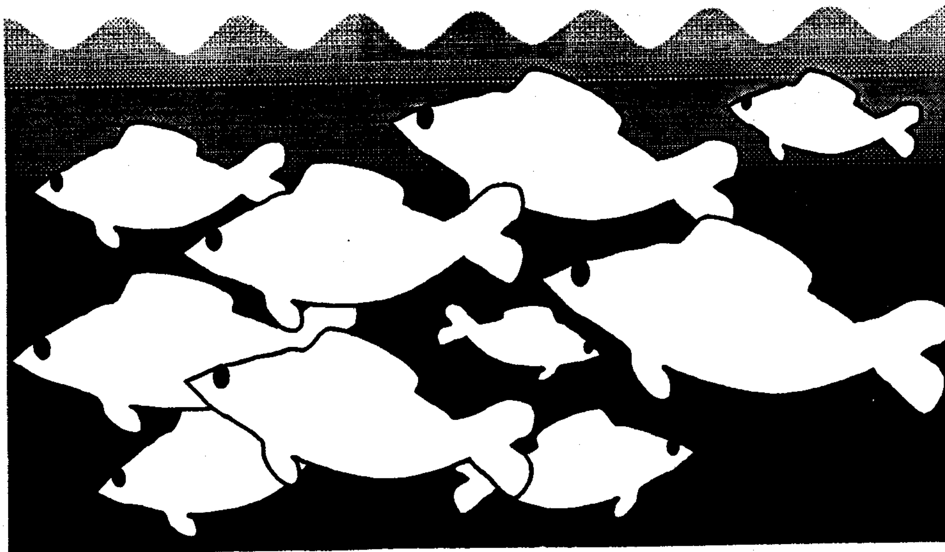
MARINE BIOASSAY PROJECT

NINTH REPORT

Investigations of Receiving Water Toxicity in Coastal Waters



State Water Resources Control Board
California Environmental Protection Agency

December 1998

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CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
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Institute of Marine Sciences, University of California,

California Department of Fish and Game,

STATE WATER RESOURCES CONTROL BOARD
CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY

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Acknowledgments

The authors thank the staff of the Marine Pollution Studies Laboratory for their assistance with all phases of this project: Patricia Nicely, Witold Piekarski, and Michelle White. For their guidance and many helpful suggestions, we are grateful to the members of the Marine Bioassay Project Scientific Review Committee: Steven Bay, Southern California Coastal Water Research Project; Tom Dean, Coastal Resources Associates; and Jo Ellen Hose, Occidental College. We also thank Amy Wagner of the EPA Region IX Laboratory in Richmond, California, for providing laboratory resources in support of field work conducted in San Francisco Bay. This report was prepared through agreement with the California State Water Resources Control Board (Contract No. 2-041-250) and subcontract (FG-5185ES) with the California Department of Fish and Game (Project Managers: Mark D. Stephenson and H. Max Puckett) to the University of California, Santa Cruz (Principal Investigator: Dr. Ron Tjeerdema). This does not signify that the contents necessarily reflect the views and policies of the California State Water Resources Control Board, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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Executive Summary

The goal of the Marine Bioassay Project (MBP), authorized by the State Water Resources Control Board (SWRCB) in 1984, is to protect California's ocean resources by determining the impacts of toxic waste discharges on marine waters. The Project's primary objectives are (1) developing and validating of critical life stage tests to measure the toxicity of these discharges, and (2) providing technical support to SWRCB and Regional Board staff and to outside laboratories performing the toxicity test methods listed in the California Ocean Plan.

In its 15 year history, the MBP has developed four critical life stage protocols using species indigenous to California's coastal waters. These reflect a newer generation of test methods designed to estimate more subtle long term adverse effects of waste discharges that ultimately damage populations of important marine species. The four toxicity test protocols use an alga (giant kelp), a fish (topsmelt), and two invertebrates (red abalone and Pacific mysid crustacean). All of the MBP test methods are on the 1997 Ocean plan approved list of critical life stage protocols for use in NPDES compliance monitoring. The entire Ocean Plan list is published in the Procedures Manual for Conducting Toxicity Tests Developed by the Marine Bioassay Project (96-1WQ).

Two State agencies and one university work cooperatively in operation of the MBP. The Department of Fish and Game's (DFG) Marine Pollution Studies Laboratory (MPSL), serves as the research facility. The University of California at Santa Cruz provides the principal investigators and staff to conduct the research. The SWRCB oversees and provides funding for the Project by contracting with the DFG to develop and conduct tasks specified in the contract's agreement. DFG in turn subcontracts with U.C. Santa Cruz to provide staff and operate the project.

The MBP staff have worked closely with the U.S.EPA in adapting MBP protocols for inclusion into the new U.S.EPA West Coast Methods Manual (EPA/600/R-95/136). This U.S.EPA manual contains seven critical life stage protocols including the four developed by the MBP.

Regulatory Background

Development of toxicity test protocols to estimate long-term effects of waste discharges is consistent with both federal and state requirements. In 1984, the U.S.EPA issued a national "Policy for the Development of Water Quality Based Limitations for Toxic Pollutants" (49 CFR, No. 48, March 9, 1984). This policy outlined a technical approach for controlling discharge of toxic substances through the federal system of discharge permits. In addition to meeting numerical standards for individual chemicals, the policy requires U.S.EPA and the States to use biological testing to complement chemical testing. Biological testing also provides information not available for chemical testing. For example, it incorporates bioavailability and interactions in complicated mixtures of toxic materials.

In 1986, AB 3500 added Section 13170.2 to the California Water Code. In addition to mandating triennial review of the California Ocean Plan, Section 13170.2 requires the SWRCB to develop and adopt toxicity test protocols. Ocean discharges of 100 million gallons per day or more have been required in their permits to use their toxicity test protocols for monitoring complex effluents since January 1, 1991. This requirement extended to smaller dischargers on January 1, 1992. Section 3 of AB 3500 expressed legislative intent that the organisms used in testing be representative marine species.

> "If the State Water Resources Control Board determines through its Marine Bioassay Project that a multispecies toxicity testing program with representative marine species for monitoring complex effluent discharges is appropriate, the

State Board shall use the multispecies toxicity testing program with representative marine species in adopting the toxicity test protocols specified in Section 13170.2 of the Water Code."

On March 19, 1990, the SWRCB adopted a series of amendments to the California Ocean Plan. These amendments included the addition of a chronic toxicity objective for the protection of marine aquatic life. The SWRCB also adopted a list of seven toxicity test protocols deemed sufficiently developed for measuring compliance with the chronic toxicity objective. Included on this list of seven are two protocols developed by the MBP, the giant kelp and the red abalone 48-hour toxicity tests. The MBP has since developed two additional critical life stage tests using the Pacific mysid (*Holmesimysis costata*) and the topsmelt (*Atherinops affinis*). The Pacific mysid and topsmelt toxicity protocols were added to the Ocean Plan list with the adoption of the 1996-1997 Ocean Plan amendments by the SWRCB.

In practice, toxicity requirements in a discharge permit are expressed in toxic units (TU). A TUc is defined as 100 divided by the No Observed Effect Concentration (NOEC):

TUc = 100/NOEC

The NOEC is defined as the maximum percent concentration of effluent, or any water being tested, that does not result in any observed effect on test organisms. Permits would usually require that no sublethal toxicity be observed at a concentration lower than those present within an outfall's designated mixing zone (the "zone of initial dilution"). For example, if a discharger has an outfall design that provides a 99:1 dilution, then no toxicity should be observed in effluent diluted to one percent. The discharge permit would require that the effluent toxicity limit be 100 toxic units or less according to the following effluent limitation equation:

$$Ce = Co + Dm(Co - Cs) \quad (1)$$

$$Ce = 1 + 99(1-0)$$

$$Ce = 100 = TUc$$

where:

Ce = the effluent concentration limit

Co = the concentration (water quality objective) to be met at the completion of initial dilution

Cs = background seawater concentration,

Dm = minimum probable initial dilution expressed as parts seawater per part wastewater

Project Overview and Technical Summary

The Marine Bioassay Project is designed as a multiple phase program to develop and implement short-term tests for toxicity measurement of complex effluents discharged to the ocean. Actual laboratory work is conducted at the California Department of Fish and Game's (DFG) Marine Pollution Studies Laboratory located south of Monterey. To date, eight phases of the Marine Bioassay Project have been completed; chapters of this project describe work performed during the eighth phase. The two primary objectives of the current MBP research were to investigate receiving water toxicity at selected central California sites, and to conduct experiments for assessing receiving water toxicity. A two phase approach was taken. Phase one experiments used laboratory studies of field-collected receiving water or sediment samples to assess toxicity at sites where ambient toxicity was expected. The goal of these surveys was to identify sites which demonstrate consistent toxicity in water column or sediment-water interface exposures. Once identified, these sites were to be used in subsequent Phase Two laboratory and *in situ* investigations designed to assess differences between these two types of exposures and the influence of spatial and temporal variability.

Phase One (November 1984- February 1986)

During the first phase, efforts were made to obtain wide-spread participation in developing the scope of the project. Initially, a draft report was prepared that described a number of potential marine toxicity test species, recommended twelve of these as most suitable, and presented appropriate protocols for each of the twelve. In March 1985, the draft was sent for review to a number of potentially interested agencies (NOAA and DFG), ocean dischargers in southern California, the Southern California Coastal Water Research Project, and a number of individual scientists.

A workshop to discuss report and outline the project's scope was held on April 29, 1985 at the offices of a major ocean discharger, the County Sanitation Districts of Orange County. The purpose was to discuss the proposed toxicity test species and protocols and address questions raised by reviewers of the draft report. Over 50 people attended the workshop and general session and then participated in one of the five sub-committee meetings. The MBP's First Report included a summary of the workshop proceedings in addition to the species descriptions from the pre-workshop draft.

A separate outcome of the workshop was the establishment of a Scientific Review Committee, composed of a small group of outside experts to discuss progress and provide guidance for the Marine Bioassay Project. The first meeting was held in June 1985, and meetings have continued approximately twice a year. The Committee has recommended a number of significant mid-course corrections that have been implemented by the MBP staff. Overall, a major accomplishment in these recommendations has been to refocus daily work on the primary objective: development of short-term protocols for use in performing toxicity tests on complex effluent discharged to the ocean.

Four important laboratory tasks were also completed during Phase One: (1) the Department of Fish and Game laboratory was extensively refurbished for animal culture and rearing of marine species, (2) methods were developed for maintaining and spawning selected marine species, (3) a mobile laboratory was purchased and used to conduct aquatic toxicity tests, and (4) range-finding and definitive tests were developed on two toxicants (pentachlorophenol and endosulfan) of immediate concern to SWRCB. This work is described in the MBP's Second Report (May 1986).

Phase Two (March 1986 - October 1987)

In Phase Two, three new short-term protocols were developed after repeated testing using zinc as a reference toxicant. In addition, longer term reference toxicant tests were used with each species to calibrate the relative sensitivity of the short-term test protocols. All three short-term test protocols developed were static tests; that is, the test solutions are not changed during testing. Each protocol measured a different effect or endpoint.

These protocols, designed to estimate the chronic toxicity of discharges to ocean waters, utilized sensitive life stages of three marine species: the red abalone *Haliotis rufescens*; a mysid shrimp, *Holmesimysis costata*; and the giant kelp, *Macrocystis pyrifera*. After some refinement, preliminary testing with the three protocols was performed on complex effluents from two representative municipal treatment plants.

The short-term larval abalone toxicity test protocol is a 48-hour test in which abnormal shell development is the endpoint as the measured effect of toxicity. The short-term giant kelp toxicity test is a 48-hour test that measures two different endpoints: zoospore germination and growth of the germination tube. The short-term mysid toxicity test is 1 7 day test with endpoints of survival and growth to juvenile mysids.

Phase Three (November 1987-December 1988)

During this phase, the abalone, kelp, and mysid shrimp tests developed during Phase Two and described above were further refined using complex effluent from two large municipal ocean dischargers. In addition, preliminary tests were conducted using a fish species, topsmelt *Atherinops affinis*. The project's fourth report provides detailed descriptions of work completed in Phase Three.

Phase Four (January 1989-December 1989)

During phase four, the giant kelp, red abalone, and topsmelt toxicity tests developed in previous phases were refined by testing with complex effluents, a manual entitled "Procedures Manual for Conducting Toxicity Tests Developed by the Marine Bioassay Project" was prepared for the red abalone, giant kelp, mysid shrimp, and topsmelt protocols. This manual is available from the SWRCB (Report 90-10WQ).

Phase Five (January 1990-December 1990)

In Phase Five, complex effluent tests were conducted and toxicity protocols developed using topsmelt, a fish native to the California coast. Also, a sub-lethal endpoint was established for the mysid shrimp protocol, and additional research was conducted in the areas of complex effluent testing and interlaboratory verification. Finally, technical support was provided by the MBP staff to laboratories using MBP protocols. This support included training of laboratory technicians, providing broodstock organisms, detailing specific requirements for test acceptability of individual protocols and performing interlaboratory tests with discharges exceeding 100 million gallons per day of complex effluent.

Phase Six (January 1991-June 1993)

Work performed for Phase Six included completion of the four toxicity test methods developed by the project and continued implementation of the marine toxicity testing program.

The following tasks were accomplished:

1. Completing the test protocols for the mysid shrimp and the topsmelt,

2. Providing additional technical training and support for dischargers and consulting laboratories,

3. Evaluating the biological significance of the short-term (48 hr) red abalone routine test, by comparing it to both long-term and exposure-recovery tests,

4. Investigating the use of marine organisms for measuring ambient toxicity from non-point sources,

5. Evaluating the applicability of sodium azide as a replacement toxicant for copper when conducting toxicity studies with the giant kelp *Macrocystis pyrifera*.

Phase Seven (July 1993-March 1996)

Phase Seven (eighth report) is organized into six sections. Section 1 examined salinity effects on larval topsmelt *Atherinops affinis*, sensitivity. Section 2 evaluated the sensitivity, precision, and logistical feasibility of the mysid, *Holmesimysis costata*, 7-day growth and survival toxicity test. Section 3 investigated the utility of smaller test

containers for toxicity testing using the red abalone, *Haliotis rufescens*. In Section 4, the giant kelp exposure recovery experiments investigated biological significance of endpoints measured in the 48 hour (short-term) *Macrocystis* germination and growth protocol. These experiments were designed to determine whether vegetative effects (percent germination and kelp germ tube length) observed in short-term exposures are indicative of longer-term reproductive effects, and whether cultures are capable of recovery when transferred to toxicant free media. Section 5 describes the use of Minimum Significant Difference (MSD) criteria to address differences in test sensitivity among MBP protocols. Section 6 summarizes the several remaining tasks the MBP has completed. These tasks include (1) promoting use of the various toxicity test protocols by establishing a network of reliable test organism suppliers, (2) facilitating development of a fully operable toxicity database/statistics package for use statewide, (3) conducting a workshop as part of an ongoing statewide training for Whole Effluent Toxicity (WET) testing for U.S.EPA, SWRCB and Regional Board staff, (4) participating in an EPA-sponsored workshop on bioremediation metabolite toxicity, and (5) participating in several meetings and workshops on statistical issues concerning toxicity test data.

Phase Eight (January 1, 1996- June 30, 1998)

In Phase Eight (ninth report) the MBP investigated receiving water toxicity at selected central California sites and conducted experiments designed to evaluate appropriate exposure designs for assessing receiving water toxicity. Surveys were initially conducted to identify sites which demonstrated consistent toxicity in water column or sediment-water interface exposures. The identified sites were then used in laboratory and *in situ* investigations designed to assess differences between these two types of exposures and the influence of spatial and temporal variability.

Additional tasks completed include: (1) establishing a reliable supply network for all four test organisms (red abalone (*Haliotis rufescens*), giant kelp (*Macrocystis pyrifera*), topsmelt (*Atherinops affinis*), and Pacific mysid (*Holmesimysis costata*)) used

in toxicity tests developed by the MBP and (2) conducting toxicity test methods workshops at U.S.EPA's Region 9 field station in Richmond and at the Society of Environmental Toxicology and Chemistry annual meeting in San Francisco.

Section 1

Ambient Toxicity Monitoring

Previous Use of Toxicity Tests in Ambient Water Monitoring

Whole Effluent Toxicity (WET) test methods are a primary means of determining compliance with NPDES acute and chronic toxicity objectives. Toxicity test protocols, such as those developed by the Marine Bioassay Project (MBP) and the U.S. Environmental Protection Agency are sufficiently developed for this application (EPA 1996). Typical exposures lasting from 20 minutes to seven days have been shown to be adequate to determine the chronic toxicity of point source discharges. As WET test methods are incorporated into state and federal effluent monitoring programs, it is anticipated that receiving water toxicity associated with point source discharges will be significantly reduced. However, receiving waters may continue to be impacted by a variety of non-point source discharges. These include: stormwater runoff (Fisher et al., 1995; Bay et al., 1997); agricultural runoff (Norberg-King et al. 1991; Hunt et al., In Press), and aerial deposition (Carey et al., 1998). In addition, marine and estuarine waters may be impacted by chemicals released from contaminated sediments via flux (Officer and Lynch, 1989; Reidel et al., 1989), or resuspension.

As more regulatory emphasis is placed on reducing non-point pollution sources, it is anticipated that biological toxicity tests developed for WET testing may be used for this application. Current marine WET test protocols may require further refinement in order to be used for receiving water toxicity investigations, particularly for *in situ* exposures. Potential modifications fall into several categories: (1) choice of appropriate species and endpoints, (2) choice of appropriate exposure methods, and (3) development of appropriate experimental designs to assess spatial and temporal variability in receiving water toxicity. The studies reported here emphasized adapting West Coast marine WET protocols for receiving water toxicity assessments in both laboratory and *in situ* exposures. As discussed above, receiving waters may be impacted by a variety of pollution sources including point and non-point sources, as well as contaminated sediments. In the following discussion, the terms "ambient toxicity" and "receiving water toxicity" are used interchangeably. In some cases ambient toxicity was assessed in water column samples, in other cases ambient toxicity was assessed at the sediment-water interface. In the latter cases, sediment overlying water toxicity was assessed and the

toxicity in these samples was assumed to have originated from chemicals fluxed from sediments.

Laboratory testing of receiving water toxicity has been used extensively in freshwater systems (e.g., Grothe et al., 1996; deVlaming 1995), and to a lesser extent in marine sytems (.e.g., Konar and Stephenson, Fairey et al. 1996; Bay et al. 1997, SFEI 1997, Schimmel et al., 1989). In this approach field samples are tested under controlled laboratory conditions to minimize impacts of factors affecting test organism response (eg., salinity, temperature, food, dissolved oxygen). In general these are static or static renewal bioassays. Examples of laboratory toxicity testing of marine receiving waters using current California Ocean Plan protocols include studies using bivalve (*Crassostrea gigas*) embryo-larval development, abalone (*Haliotis rufescens*) embryo-larval development, and sea urchin (*Strongylocentrotus purpuratus*) fertilization. Konar and Stephenson (1995) collected receiving water samples from a variety of enclosed bays throughout California and assessed toxicity using the 48-h embryo development test (*Crassostrea gigas*). These authors observed adverse effects on oyster embryo development at stations which previously showed elevated contaminant bioaccumulation in mussel tissues. Fairey et al. (1996) reported receiving water impacts on development of abalone embryos. In this study, receiving water toxicity was assessed at stations where sediment toxicity and benthic community degradation were investigated. Bay et al. (1997) used laboratory toxicity tests and chemical analyses to assess the quality of receiving waters impacted by storm water runoff. Significant inhibition of sea urchin fertilization was detected in some receiving waters, and toxicity was apparently restricted to surface receiving waters.

Marine Bioassay Project Investigations of Receiving Water Toxicity in Coastal Waters

The two primary objectives of the current MBP research was to investigate receiving water toxicity at selected central California sites, and to conduct experiments designed to evaluate appropriate exposure designs for assessing receiving water toxicity. A two-phase approach was taken. Phase one experiments used laboratory studies of

field-collected receiving water or sediment samples to assess toxicity at sites where ambient toxicity was expected. The goal of these surveys was to identify sites which demonstrate consistent toxicity in water column or sediment-water interface exposures. Once identified, these sites were to be used in subsequent Phase Two laboratory and *in situ* investigations designed to assess differences between these two types of exposures and the influence of spatial and temporal variability. Potential sites were investigated using either embryo-larval development tests with bivalves (*M. galloprovincialis*) or sea urchins (*S. purpuratus*), or juvenile survival tests with mysids (*H. costata*). These species were selected for initial screening tests because they represent 3 distinct phyla having varying sensitivities to contaminants, and all are suited for short-term exposures requiring minimal maintenance. The following is a discussion of each site investigation conducted as part of the Phase One research.

Old Salinas River Channel at Sandholdt Bridge, Moss Landing

Introduction

The Sandholdt Bridge site has been identified as a toxic site through a number of monitoring programs including the State Mussel Watch and the Bay Protection and Toxic Cleanup Program (BPTCP). These studies have indicated this site is contaminated by elevated concentrations of metals, PAHs and pesticides. Laboratory water column toxicity tests conducted by Konar and Stephenson et al. (1995) found significant inhibition of oyster larval development in samples from this site. Previous testing as part of the BPTCP has indicated toxicity to sea urchin and bivalve embryos tested at the sediment-water interface (Downing et al. 1998). For the current Marine Bioassay Project studies, a number of water column and sediment-water interface tests were conducted from June, 1996 through May, 1997 to further investigate ambient toxicity at this site.

Methods

The following methods were used for all toxicity assessments; these apply to initial studies conducted at Sandholdt Bridge, and subsequent studies conducted in San Francisco Bay and Monterey Harbor. Water column samples (Sandholdt Bridge - Salinas River Channel) were collected approximately 1 foot below the surface mid-channel using

one-liter amber bottles deployed in a PVC sampler (Hunt et al., in press). Two stations were sampled, one immediately adjacent to the Sandholdt Bridge, and one up-stream from the bridge (Figure 1). Samples were tested with 3 (static) laboratory toxicity tests: 48-h bivalve embryo development *(Mytilus galloprovincialis,* EPA 1996), 96-h embryo development (*Strongylocentrotus purpuratus*, EPA 1996), 96-h mortality test (*Holmesimysis costata*, EPA 1993). All responses to site water were compared to responses in Granite Canyon (control) seawater.

In addition to water column samples, toxicity of sediment at the Sandholdt Bridge station was also assessed. Toxicity to sea urchin and bivalve embryos exposed at the sediment-water interface (SWI) followed methods described by Anderson et al. (1996). The SWI exposure system is designed to assess toxicity of chemicals fluxed out of the sediment into overlying water. Intact (un-homogenized) sediment cores were collected by skin divers and transported to MPSL on ice. All cores were tested 2 days after sampling. On the day before the test, 300 mls of 1 um-filtered Granite Canyon seawater was added to the cores, and the cores were allowed to equilibrate overnight. A screen tube was added to each core, and 1000 newly fertilized bivalve embryos were added into each screen tube, so that embryos developed 1 cm above the sediment. All cores were gently aerated and tests were terminated after 48-h. Results of the Sandholdt Bridge SWI exposures were compared to SWI exposures using sediment from Yaquina Bay, Oregon. This sediment has been demonstrated to have low concentrations of contaminants, and serves as a negative control for amphipod bulk-phase toxicity tests. Statistical differences between site samples and controls were compared using t-tests ($p = 0.05$).

Results

Site water from Sandholdt Bridge did not significantly inhibit bivalve (*M. edulis*) embryo development in four tests conducted between June 1996, and March 1997 (Table 1). In addition, Sandholdt Bridge sediment was not toxic to bivalve embryos exposed to intact sediment cores at the SWI in March, 1997. Water samples from Sandholdt Bridge and upstream in the Old Salinas River Channel were not toxic to mysids (*H. costata*) in 96-h exposures conducted in May, 1997 (Table 1). These results demonstrate that although this site has been consistently toxic to amphipods in sediment exposures, and intermittently toxic to bivalve (*M. galloprovincialis*; *C. gigas*) larvae in sediment and water exposures, ambient toxicity was not observed during the current project.

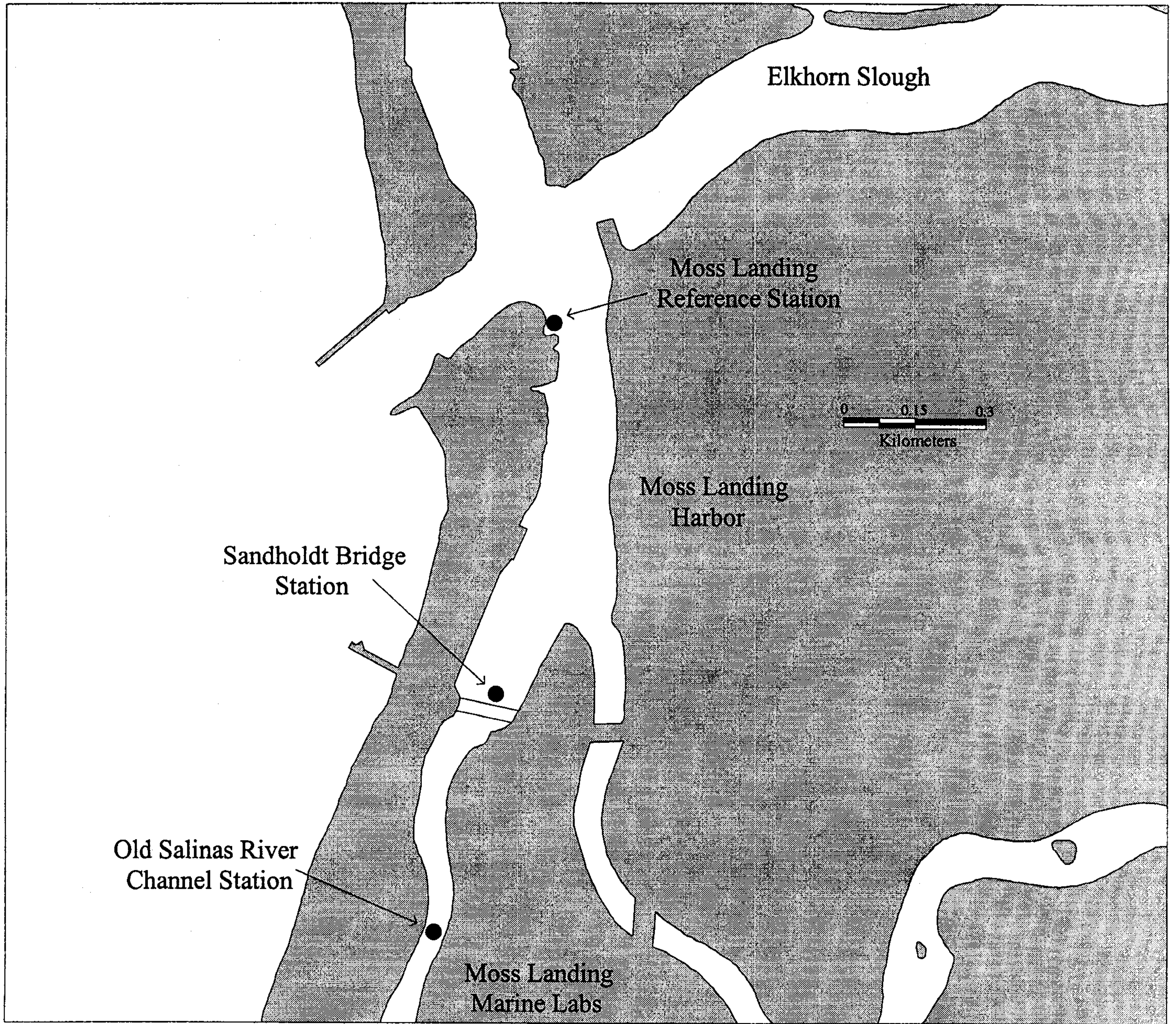

Figure 1. Location of ambient sampling stations in Moss Landing study area.

Table 1. Summary of Tests Conducted for Marine Bioassay Project Ambient Toxicity Studies.

Date	Location	Species – Sample Type	Survival or Normal Dev. Mean (SD)
6/11/96	Sandholdt Bridge-Moss Landing	*M. galloprovincialis*-Water	75.0 (10.0)
12/16/96	Sandholdt Bridge-Moss Landing	*M. galloprovincialis*-Water	89.6 (7.0)
1/8/97	Sandholdt Bridge-Moss Landing	*M. galloprovincialis*-Water	79.0 (5.0)
3/5/97	Sandholdt Bridge-Moss Landing	*M. galloprovincialis*-Water	98.6 (10.9)
3/5/97	Sandholdt Bridge-Moss Landing	*M. galloprovincialis*-SWI	81.3 (10.8)
5/13/97	Sandholdt Bridge-Moss Landing	*H. costata*- Water	100.0 (0.0)
5/13/97	Old Salinas River Channel-M Landg.	*H. costata*- Water	100.0 (0.0)
8/28/97	Monterey Harbor	*S. purpuratus*- SWI	84.0 (10.2)
10/10/97	Islais Creek – San Francisco		
	Upper Channel – midwater	*S. purpuratus*- Water	90.0 (4.0)
	Upper Channel – bottom	*S. purpuratus*- Water	90.0 (3.0)
	Middle Channel – midwater	*S. purpuratus*- Water	89.0 (4.0)
	Middle Channel – bottom	*S. purpuratus*- Water	92.0 (3.0)
	Lower Channel – midwater	*S. purpuratus*- Water	90.0 (2.0)
	Lower Channel – bottom	*S. purpuratus*- Water	91.0 (5.0)
10/10/97	Mission Creek – San Francisco		
	Upper Channel – midwater	*S. purpuratus*- Water	84.0 (11.0)
	Upper Channel – bottom	*S. purpuratus*- Water	94.0 (3.0)
	Middle Channel – midwater	*S. purpuratus*- Water	93.0 (6.0)
	Middle Channel – bottom	*S. purpuratus*- Water	90.0 (2.0)
	Lower Channel – midwater	*S. purpuratus*- Water	92.0 (1.0)
	Lower Channel – bottom	*S. purpuratus*- Water	93.0 (1.0)
10/20/97	Islais Creek – San Francisco		
	Upper Channel – midwater	*H. costata*- Water	90.0 (4.0)
	Upper Channel – bottom	*H. costata*- Water	90.0 (3.0)
	Middle Channel – midwater	*H. costata*- Water	89.0 (4.0)
	Middle Channel – bottom	*H. costata*- Water	92.0 (3.0)

Table 1 (cont.). Summary of Tests Conducted for Marine Bioassay Project Receiving Water Toxicity Studies.

	Lower Channel - midwater	*H. costata*- Water	90.0 (2.0)
	Lower Channel - bottom	*H. costata*- Water	91.0 (5.0)
10/20/97	Mission Creek - San Francisco		
	Upper Channel - midwater	*H. costata*- Water	84.0 (11.0)
	Upper Channel - bottom	*H. costata*- Water	94.0 (3.0)
	Middle Channel - midwater	*H. costata*- Water	93.0 (6.0)
	Middle Channel - bottom	*H. costata*- Water	90.0 (2.0)
	Lower Channel - midwater	*H. costata*- Water	92.0 (1.0)
	Lower Channel - bottom	*H. costata*- Water	93.0 (1.0)

Monterey Harbor

Introduction

Sediment collected from one station in Monterey Harbor was shown to inhibit development of sea urchin embryos exposed at the SWI in studies conducted as part of the Bay Protection Program. This station was located near a former lead slag deposit from an adjacent rail line, and is also adjacent to a boatyard and small craft harbor (Figure 2). Sediment collected from this station was moderately contaminated by several trace metals (Downing et al. 1998). This station was revisited as part of the current MBP in an effort to identify a toxic site to be used for subsequent laboratory and *in situ* comparisons.

Methods and Results

Because it was considered unlikely that water column samples from Monterey Harbor would demonstrate toxicity due to the absence of significant inputs, toxicity of sediments from Monterey Harbor were assessed using sediment-water interface exposures. As discussed above, this exposure system assesses toxicity of chemicals fluxed into sediment overlying waters by exposing animals to field-collected sediment cores. Intact sediment cores were collected at Station 1 (Figure 2) in Monterey Harbor by divers in August, 1997 and brought back to the laboratory. The sediment cores were

treated as described above for sediment-water interface studies conducted at Sandholdt Bridge. Granite Canyon seawater was added to the top of the cores the day before the test and allowed to incubate for 24-h. Sea urchin embryos were exposed to the sediment cores in screen tubes at the SWI for 96-h. The sediment from this station was not significantly toxic to sea urchin embryos (Table 1).

San Francisco Bay Sites – Islais Creek and Mission Creek

Introduction

Using monitoring data from the Bay Protection program, two candidate toxic hot spots were identified in San Francisco Bay (Islais Creek and Mission Creek). Both sites are impacted by combined sewage overflow (CSO) outfalls operated by the City of San Francisco, and receive urban storm runoff. Both sites have been characterized as contamination gradients with heavy sediment pollution near the outfalls, and less sediment pollution near the channel mouths. Sediment pollutants include heavy metals, PAHs, PCBs and pesticides (Hunt et al. 1998).

Methods and Results

MBP ambient monitoring at Mission and Islais Creeks was designed to assess receiving water toxicity. Receiving water samples were collected from a boat-deployed sampler. Samples were collected at two depths and 3 stations in both channels (Figure 3). Water samples were collected within approximately 0.5 m of the bottom and at midwater at three stations along the sediment contamination gradient from the back of each channel to the front (i.e., three stations at each of two channels). Toxicity of all water samples was assessed with two toxicity tests: 96-h embryo development using the purple sea urchin (*S. purpuratus*), and 96-h survival using neonates of the mysid (*H. costata*). No toxicity to sea urchins or mysids was observed at any of the stations in either channel (Table 1).

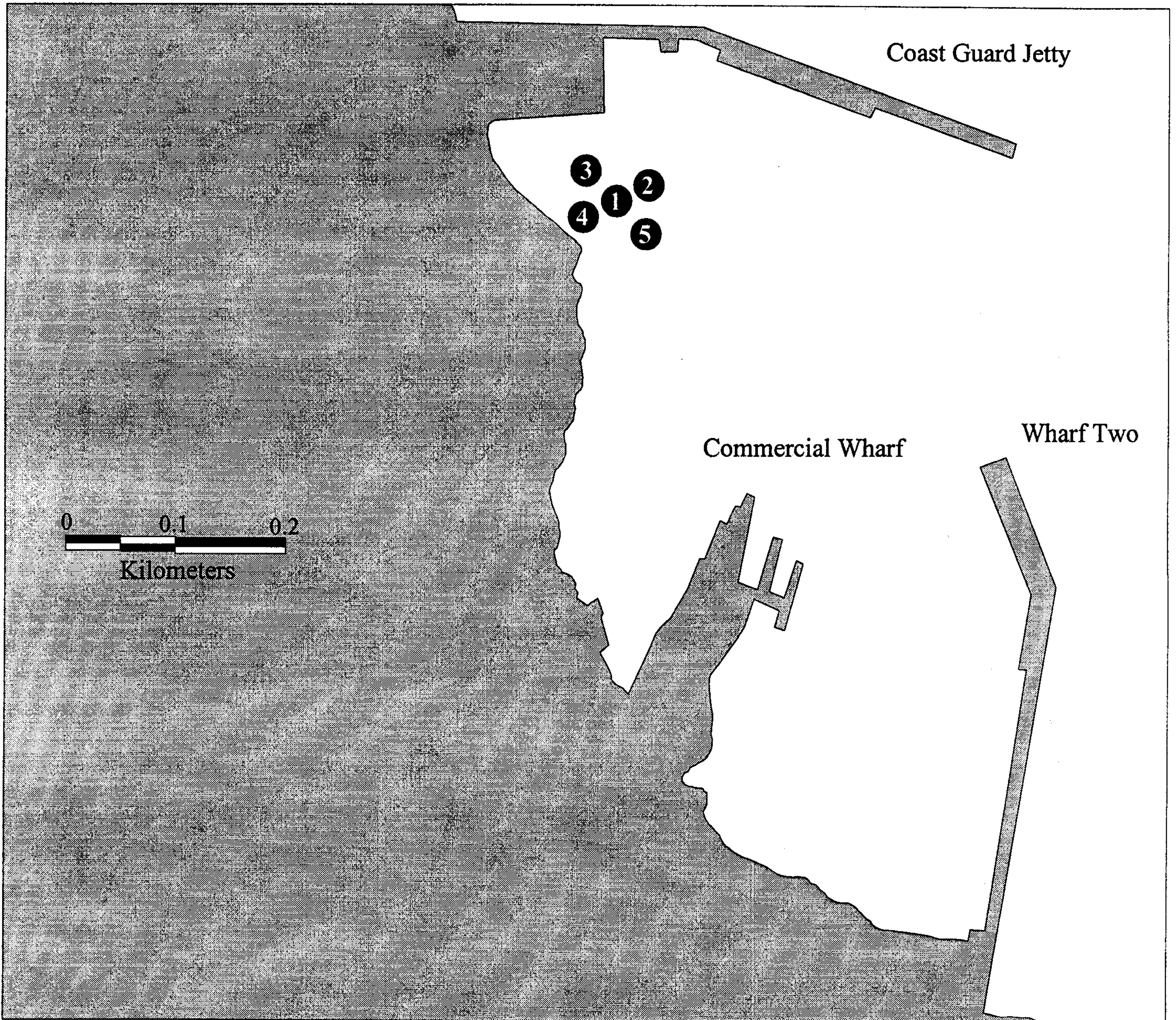

Figure 2. Locations of Monterey Harbor stations. The initial laboratory experiment to asssess sediment toxicity was conducted using sediment collected at station 1. Subsequent comparisons of laboratory and field exposures were conducted at stations 1-5.

The results of these studies indicate that all of the protocols used were amenable to laboratory assessments of receiving water toxicity. None of the samples required salinity adjustments, which is one of the major impediments to using marine species to assess estuarine receiving waters. The results also indicate that despite the fact that all of the stations tested have demonstrated sediment toxicity in past monitoring studies, water from these stations was not toxic during the current project. It should be noted that except for Sandholdt Bridge, these tests were conducted on single grab samples and therefore did not account for temporal variability in toxicity at these sites.

Toxicity at these sites was assessed, in part, to investigate the appropriateness of using WET protocols for identifying coastal pollution sources, and to identify a toxic site for use in subsequent experiments designed to assess differences between laboratory and *in situ* exposures using West Coast WET protocols. After reviewing results of the experiments discussed above with the MBP Scientific Review Committee (SRC), it was decided that additional experiments should be conducted which assessed toxicity to organisms exposed at the sediment-water interface. This decision was based on the observation that sediments are a major source of non-point source contamination. The MBP SRC felt that by emphasizing assessments of sediment overlying water toxicity, project staff would have a better chance of detecting significant toxicity at polluted sites. The SRC suggested that *in situ* experiments designed to assess sediment overlying water toxicity be conducted at the Sandholdt Bridge station in Moss Landing in order to determine whether temporal variability of toxicity events at this site were not accounted for in the original assessments. The results of these and other experiments are discussed in the following section.

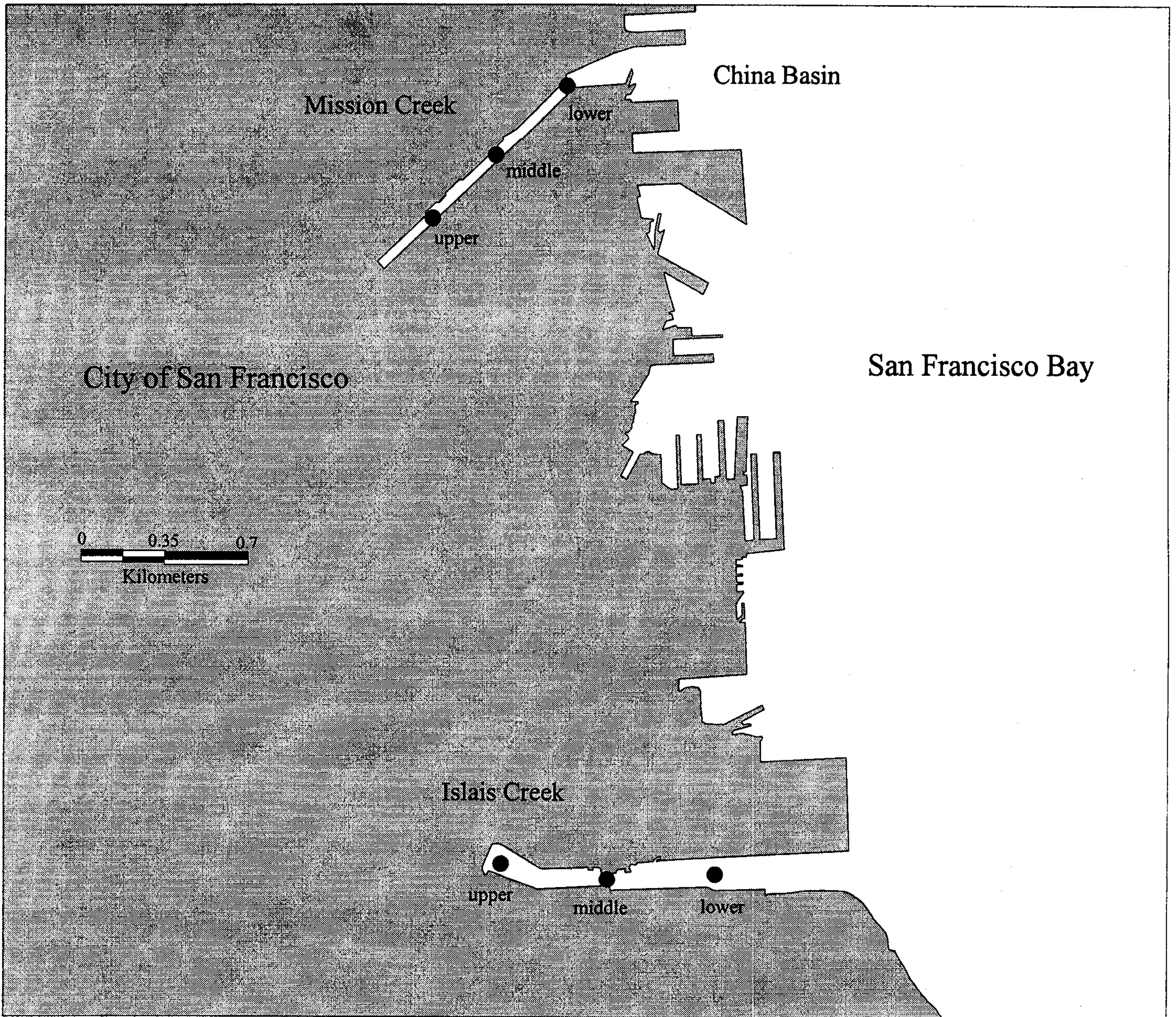

Figure 3. Mission Creek and Islais Creek ambient sampling stations.

Section 2

Assessing Ambient Toxicity With Estuarine Invertebrates Using Laboratory and *In Situ* Approaches

Introduction

As discussed above, the primary objective of the current Marine Bioassay Project research was to assess ambient toxicity in coastal waters using WET protocols. However, because the current West Coast WET protocols were designed for use in static or static-renewal laboratory toxicity tests, it was not clear whether these protocols were appropriate for ambient assessments without some modification. For example, there have been numerous studies which have demonstrated that receiving water toxicity may be temporally and spatially variable. It was not clear whether current protocols conducted under laboratory conditions would account for this variability. In addition, ambient toxicity studies conducted primarily in freshwater systems have demonstrated differences between laboratory and *in situ* exposures due to the interaction of spatial and temporal variability, and the influence of physical factors (e.g., temperature, sunlight, dissolved oxygen, pH, etc.). Because of these concerns, the current MBP research was designed to proceed in two phases. In Phase One, toxicity was assessed at a variety of sites expected to be toxic in order to identify a site to be used for future experiments. Once a site demonstrating consistent toxicity was identified, Phase Two research was designed to include a series of laboratory and *in situ* experiments intended to assess spatial and temporal variability of ambient toxicity, and differences between laboratory and *in situ* exposures. The objective of the second phase experiments was to investigate the appropriateness of using static laboratory exposures to assess coastal receiving water toxicity by comparing laboratory and *in situ* exposures. Before proceeding with the Phase Two experiments, a literature review of the use of *in situ* toxicity exposures in freshwater and marine systems was completed. These studies are listed in the references section and are discussed in the following section as they apply to Phase Two research. The next step was to design and test an *in situ* exposure chamber for use with West Coast WET protocols. The following section discusses this part of the research.

Chamber Design

The *in situ* chamber was intended to be adaptable for exposing all of the species and lifestages included in the California Ocean Plan list of acceptable marine toxicity test protocols, with the possible exception of kelp spores (*M. pyrifera*). Because kelp spores

are considerably smaller than most of the other lifestages on the Ocean Plan list, and require a settling substrate, the chamber, as designed, would not work for this protocol. To accommodate the other species, the chamber was designed with sufficient volume for short-term (48-96 hour) survival of invertebrate and vertebrate larvae and other early life stages. In addition, the chamber required a mesh size small enough to retain the smallest lifestages possible while allowing for sufficient water exchange with the environment. Initial development emphasized a chamber amenable to field testing sea urchin (*S. pupuratus*) and bivalve (*M. galloprovincialis, C. gigas*) embryos. These species were chosen for the initial design because they have proven to be relatively sensitive to a wide variety of toxicants (Hunt and Anderson 1993, Bay et al. 1993), require no feeding during the exposure, and two of the three tolerate a fairly wide range of salinities and temperatures (*M. galloprovincialis, C. gigas*). A 25 um (Nitex) mesh screen was used to retain developing embryos of these species. Development of the *in situ* exposure chamber was based on chamber designs reported for freshwater invertebrate species, particularly those reported by Sasson-Brickson and Burton (1991), and the Sediment-Water Interface chamber developed by Anderson et al. (1996).

Polycarbonate tubing (5 cm diameter) was cut into cylinders and glued into a "drum" design that is 44% screened surface (Figure 4). The initial design (Chamber 1) did not have glued interior seams. The final design (Chamber 2) incorporated glued interior seams to prevent the microscopic embryos from being trapped. Dual ports allowed the container to be thoroughly rinsed for cleaning and for complete recovery of test animals. The ports were tapped to accommodate nylon screws that could be removed for test organism inoculation and retrieval.

Throughout the development of the chamber, laboratory and field tests were conducted to determine if the design was adequate. Ten tests were conducted in the laboratory and two in the field (Table 2). Initial tests with Chamber 1 were conducted with sea urchin embryos, and indicated toxicity and some loss of embryos from the chambers. After modifying the chambers so that all interior and exterior seams were glued, recovery improved. Subsequent experiments using Granite Canyon filtered seawater indicated the chamber was toxic to developing sea urchin embryos. It was determined that the acrylic glue used required a long curing time (at least 1 week at 40

°C), and once dried, extensive leaching of the chambers was required (4 days flow-through sea water).

Table 2. Results of initial experiments with *in situ* chambers, tested in clean seawater.

Date	Drum Version	Endpoint	Mean	SD	Notes
6/25/96	1	% Urchin Develop.	53	38	Lab Exp. Low recov.
7/12/96	1	% Urchin Develop.	60	22	Lab Exp. Low recov
7/25/96	1	% Urchin Develop.	76	19	Lab Exp. Low recov
8/12/96	1	% Bivalve Develop.	70	27	Lab Exp. Low recov
9/10/96	1	% Urchin Develop.	54	6	Field Exp. Low recov
10/14/96	1	% Bivalve Develop.	60	21	Lab Exp. Low recov
10/24/96	1	% Urchin Develop.	56	22	Chamber Comparison
	2	% Urchin Develop.	73	31	
11/5/96	1	Bivalve Survival	80	26	Chamber Comparison
	2	% Bivalve Develop.	92	7	
3/5/97*	2	% Bivalve Develop.	0	0	Channel Exposure - High Surge/Waves

*Two additional channel exposures were conducted but not recovered in time to terminate the test due to high surf.

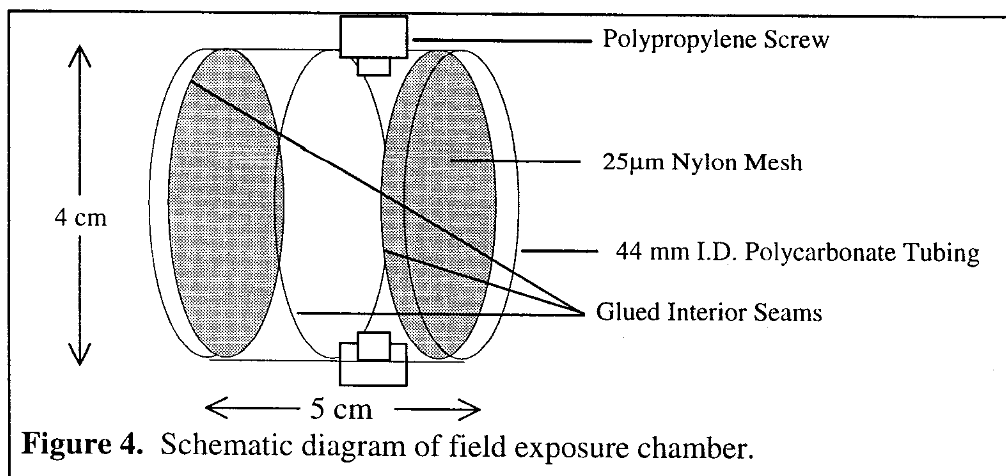

Figure 4. Schematic diagram of field exposure chamber.

It was necessary to experiment with alternative cleaning procedures because of the small mesh and closed nature of the chambers. As experiments proceeded, each chamber was leached inside and out for a minimum of two days using flow-through seawater after cleaning. Initial field experiments in the channel in front of the Granite Canyon marine lab showed arrested bivalve embryo development apparently due to extreme surge and high waves.

Field Experiments- Carlson Creek, Sandholdt Bridge, Monterey Harbor

Introduction

The preceding experiments demonstrated that the *in situ* exposure chamber was sufficiently developed for use in field studies. The next objective was to use the *in situ* chamber in experiments designed to determine whether there were differences in toxicity test results between laboratory and *in situ* exposures, and to consider the results of these comparisons in future studies designed to assess ambient toxicity of non-point source pollution. An initial experiment was conducted at Carlson Creek in San Francisco Bay. Subsequent experiments were conducted at Sandholdt Bridge, and Monterey Harbor in the Monterey Bay area. Depending on the site, the experimental designs were similar. Toxicity of water column or sediments from these sites was assessed using laboratory and *in situ* exposures. In two cases, spatial variability of toxicity was assessed by testing samples along a suspected contamination gradient or by collecting samples arrayed around a suspected pollution hot spot. The results of these experiments were intended to be used in the design of future coastal ambient toxicity monitoring programs conducted as part of the Marine Bioassay Project.

Laboratory and Field Experiments at Carlson Creek

Carlson Creek is located in the Central San Francisco Bay adjacent to the Richmond Field Station. This wetland site receives some urban runoff from the city of Richmond and drains parts of Stege Marsh, which has been designated as a candidate toxic hot spot due to sediment

contamination (eg., PAHs, PCBs, pesticides and heavy metals) and toxicity identified through the Bay Protection Program (Hunt et al., 1998). No chemical analyses have been conducted at the specific part of the creek chosen for the MBP field experiments, but the proximity of this site to Stege Marsh suggested it could be impacted from contaminants migrating off-site. This station is tidally influenced, but the center channel of the creek remains submerged throughout the tidal cycle. Because of the proximity of this site to Stege Marsh, it was assumed that stations closer to Stege Marsh would be more contaminated than those farther along the Creek. This presumed contamination gradient provided an opportunity to use laboratory and *in situ* exposures to assess spatial variability in toxicity.

Laboratory and field comparisons using mussel (*M. galloprovincialis*) embryos were conducted at Carlson Creek in July 1998. On the day the test was initiated (Day 0) mussels were spawned at the Environmental Protection Agency Region IX Laboratory in Richmond, California. Fertilized mussel embryos were loaded into the *in situ* test chambers for field and laboratory testing. Ten chambers were placed in polypropylene mesh bags for field deployment. Additional chambers were kept in a bucket at 15°C to be used in laboratory experiments. Bags were transported to the test site and deployed at three stations along a suspected contamination gradient. Bags were attached to PVC stakes so that embryos were exposed mid-channel at the sediment-water interface. Water samples were collected at each station for laboratory comparisons. Water samples were collected near the bottom of the channel at each of the three stations in 1 liter amber bottles. Salinity was measured at the initiation and termination of the test, and temperature was monitored *in situ* using an Optic Stowaway Temperature Recorder (Onset Computer Corp.). Field-collected water samples were brought back to the lab and distributed into one-liter beakers. A single chamber was placed into each beaker. A duplicate set of chambers that served as travel control chambers was transported to the Carlson Creek station then back to the laboratory to quantify effects of handling on the test embryos. The test was terminated after 48 hours.

Results of the field and laboratory exposures were inconclusive. Although embryo development in the seawater control was acceptable, there were no surviving mussel larvae in the laboratory or field exposures. Final salinity measurements were 22‰ in the laboratory exposures and 15 to 17‰ in the field exposures. *M.*

galloprovincialis embryos do not tolerate salinities less than approximately 20 ‰ (ASTM 1993). Because initial field measurements of salinity were approximately 22 ‰, it was felt this was within the range tolerable to bivalve larval development. However, this did not account for variability in salinity over the two tidal cycles that occurred during this study. At the time this experiment was conducted, there was appreciable freshwater runoff into San Francisco Bay from the Delta, and although this site had proven to be primarily marine in previous BPTCP sampling, it was apparently influenced by Delta runoff during this study. In addition, temperature readings in the field ranged from 12.4° to 30.2° C. The upper temperature extremes and the high variation were probably beyond that tolerable by *M. galloprovincialis* embryos.

Laboratory Experiments at Sandholdt Bridge

Previous experiments conducted as part of the current MBP research indicated that the Sandholdt Bridge station was not toxic to juvenile mysids, or mussel or sea urchin embryos exposed to water column samples or at the SWI. However, the MBP Scientific Review Committee (SRC) suggested that *in situ* experiments be conducted at this site because a number of previous studies indicated it was a candidate toxic hot spot (Downing et al. 1998). The SRC felt that the lack of toxicity observed in the initial MBP laboratory experiments may have been due to temporal variability, and that *in situ* experiments might better measure episodic toxicity. With this in mind, the site was monitored twice a day for a week to determine whether salinities were within the range tolerable to mussel embryos. Although no significant rainfall had occurred in the Salinas River watershed during this period, salinities at the site ranged from 8 to 15 ppt, during this survey (August 1998). This was below the range tolerable to bivalve or sea urchin embryos, so the original study design had to be modified. As an alternative, it was decided that sediment samples would be tested in the laboratory using two different exposure regimes, static and flow-through. The rationale for these experiments was that contaminated sediments were assumed to be a primary source of toxicity at this site, and because low salinity prevented *in situ* exposures for most species, sediment-water interface exposures could be designed to approximate *in situ* conditions in the laboratory by using flow-through overlying water in the exposure systems. Previous experiments with Stege Marsh sediments had indicated

greater toxicity to bivalve embryos exposed at the sediment-water interface under flow-through conditions, than those exposed under static conditions (MPSL unpublished data). The objective of these experiments was to compare different exposure regimes for sediment toxicity testing, and to consider the results of these comparisons in the design future monitoring programs which use WET protocols to investigate sediments as a source of ambient toxicity.

Intact sediment cores were collected by skin divers from the Sandholdt Bridge station and a reference site located near the mouth of Moss Landing Harbor (Figure 1). Embryo development has not been inhibited by sediment from this reference station in previous experiments (Anderson et al., 1996; Anderson et al., *In Review*). The sediment cores were transported to MPSL and 300 ml of Granite Canyon seawater was added above the sediment as in the experiments described above. Twelve cores were collected from each site; these were divided into two groups of six cores designated for either static or flow-through overlying water. Both static and flow-through containers were filled with overlying water and allowed to equilibrate overnight. The next morning, a slow flow of filtered (~ 20 um) seawater was introduced to the flow-through containers. Flow rates were adjusted to deliver approximately 5 liters per hour, a sufficient rate to renew the overlying water in the containers approximately 10 times per hour without disturbing the sediment surface.

Approximately 225 sea urchin (*S. purpuratus*) embryos were inoculated into the *in situ* chambers and these were placed in the sediment cores so that the screen was 1 cm above the sediment surface. The test was terminated after 96 hours and animals were preserved for microscopic analysis. Water quality was measured inside the static and flow-through drum containers.

Results of these experiments indicated no significant inhibition of sea urchin larval development in either static or flow-through treatments at either station; recovery of animals was excellent in all treatments (Table 3). There were no differences between water quality parameters measured inside the static and flow-through exposure chambers; all measures were within the range tolerated by this species.

Table 3. Development of sea urchin embryos in Sandholdt Bridge and Moss Landing reference site sediment using static and flow through exposures.

Sample	Static Mean (%)	SD (%)	Flow-Through Mean (%)	SD (%)
Sea Water Control	99	2	99	1
Yaquina Bay Sediment	99	1	98	1
Sandholdt Bridge	99	1	98	1
Moss Landing Ref	95	4	92	10

Table 4. Water quality measurements in static and flow-through test containers.

Station	pH	Dis. O_2 (mg/L)	Salinity ppt	Tot. NH_3 mg/L	Tot. S_2 mg/L
Initial WQ					
Sand. Bridge T_0	7.89	7.81	34	ND	NA
Moss Lan. Ref T_0	7.89	7.64	34	ND	NA
Yaq. Bay Ref. T_0	7.91	7.69	34	ND	NA
GC seawater T_0	7.89	7.79	34	ND	NA
Static Exposures					
Sand. Bridge T_{96}	7.59	5.94	34	1.0	NA
Moss Lan. Ref T_{96}	7.81	7.13	34	1.2	NA
Yaq. Bay Ref. T_{96}	7.95	7.56	34	ND	NA
GC seawater T_{96}	8.04	8.02	34	ND	NA
Flowing Exposures					
Sand. Bridge T_{96}	7.68	6.41	34	0.87	NA
Moss Lan. Ref T_{96}	7.70	6.20	34	0.77	NA
Yaq. Bay Ref. T_{96}	7.90	7.95	34	ND	NA
GC seawater T_{96}	7.91	8.00	34	ND	NA

T_0= Time 0 hours; T_{96}= Time 96 hours; ND = Not Detected; NA = Not Analyzed

Laboratory and Field Experiments in Monterey Harbor

In order to avoid salinity and temperature extremes encountered at the Carlson Creek and Sandholdt Bridge study sites, a deeper water marine site was selected to compare lab and field exposures. Previous data from the Bay Protection Project had indicated that sediments from Monterey Harbor exhibited intermittent toxicity. Although preliminary experiments conducted as part of the current MBP studies showed no toxicity, this site was selected because it offered the most promising opportunity for lab-field comparisons at a potentially toxic marine site which was relatively close to MPSL. This allowed us to spawn animals, inoculate embryos in the test containers and transport them quickly to the study site in order to minimize travel effects. Depth at this site ranged from two to four meters and the salinity stayed within the range of 32-34 ‰.

For these experiments, mussels (*M. galloprovincialis*) were spawned at MPSL and inoculated into the *in situ* chambers. Six chambers were placed in each of 5 mesh bags, and these were transported to the site in a 5 liter plastic bucket. One bag also contained the Onset *in situ* thermometer. Using SCUBA, embryos in mesh bags were placed at five field replicates distributed throughout the inner harbor area (Figure 2). The mesh bags were attached with cable ties to PVC tubes driven into the bottom so that the test chambers were in contact with the bottom at the sediment-water interface (Figure 5). At each field station, six intact sediment cores were collected and transported to MPSL as described above. Laboratory exposures were conducted by placing one chamber in each core as described above. The laboratory exposures were conducted static, using site water collected with the cores as the sediment overlying water. After a 48-h exposure, the chambers were collected from the field and returned to the lab where all test containers were emptied and the contents fixed for microscopic analysis. Representative water samples were collected from both the laboratory and *in situ* chambers to determine any differences in chamber water quality between the lab and field.

Results indicated significant toxicity to bivalve embryo development at Station 5 for both the laboratory and field exposed animals (Figure 6). Significant toxicity was defined as a significant difference between treatment and control (t-test), and sample response being less than 80% of the control value (Phillips et al., 1998). Laboratory exposures were considerably more variable than the *in situ* exposures at stations 3 and 5, but the *in situ* exposures were more variable at Stations 1, 2, and 4. It is not possible to account for between-replicate variability in this study, although we might expect greater

variability in the laboratory exposures. There was a greater likelihood for differences among cores as opposed to among bagged chambers. This is because the sediment samples used in the lab exposures were intact (un-homogenized) cores. Although they were collected at approximately the same stations as the *in situ* exposures, they were collected over a wider bottom area to minimize disturbance of the surficial sediment layers at the five stations by the diver's swim fins. The *in situ* containers were contained in a mesh bag in a much smaller surface area at the site, and therefore were less subject to any heterogeneity in contaminant distributions at this site.

It is not clear from this study what contaminant(s) were responsible for inhibition of bivalve development. Previous chemical analyses of sediments at Station #2 indicated elevated concentrations of lead and to a lessor extent copper, based on comparisons to published TEL (Threshold Effects Level) Sediment Quality Guideline (SQG) values (MacDonald et al., 1995). In addition, sediments at this site contained certain PAH compounds above the ERL (Effects Range Low) SQG. Water quality parameters were all within quality assurance limits with the exception of dissolved oxygen in the laboratory exposure of station 4, which was less than 60% saturation (Table 5). There were considerable differences in pH values in the final water quality measurements taken at 48-h (T_{48}); lab pH values were lower in all samples relative to field samples, sometimes by as much as half a pH unit. Temperatures in the field ranged from 14.5° to 16.6° C, slightly higher than quality assurance limits. Toxicity in these samples could not be attributed to ammonia, which was below known effect thresholds for bivalve embryos (MPSL unpublished data, Knezovich et al. 1995). Although hydrogen sulfide was not measured in these experiments, previous measurements of H_2S at this site were below detection limits of the method.

There are some possible explanations why greater toxicity was observed in the laboratory exposures, particularly at Station 5. Other than water quality parameters, chemistry was not measured in the water contained in the chambers because this was beyond the scope of this study. By placing the chambers on the bottom at the sediment-water interface in both the laboratory and field exposures, we assume that toxicity was largely driven by chemicals fluxed from the sediment. There would presumably be greater dilution of fluxed chemicals in the *in situ* exposures relative to the static

laboratory exposures, and this might explain the differences. In addition, lower pH in the laboratory exposures may have increased the bioavailability of sediment contaminants, particularly metals. Although extremes in pH may affect invertebrate embryo development (Phillips et al., 1997), we do not think that explains the toxicity observed in these samples because the pH of these samples at the start of the exposures were well within the range tolerated by bivalve larvae, and pH apparently elicits a stronger affect on initial developmental stages of this specis (Phillips et al., 1997).

One problem encountered in this experiment was that there were considerably fewer animals in the chamber containers than estimated from the initial density counts. These are counts of the mean number of embryos inoculated into the chambers at the initiation of the experiment, and are estimated by pipeting the same volume of water from the embryo stock beaker into 10 separate scintillation vials, fixing it, and counting the embryos. The mean number of embryos estimated in this way was (242±15), while the mean number of embryos in the control chambers containing Granite Canyon seawater (control) was (133±14). There were few abnormal animals in the control containers, but the density was approximately half that of the initial density counts. We cannot account for this disparity. Other experiments have indicated that chamber densities remain constant. It is possible there was error involved in inoculation of the drums due to insufficient mixing of the embryo stock. The final density of animals exposed to sea water in beakers (ie., not in chambers) was 197 ± 17, which was also somewhat lower than the initial density scintillation vials, but greater than the control drum densities.

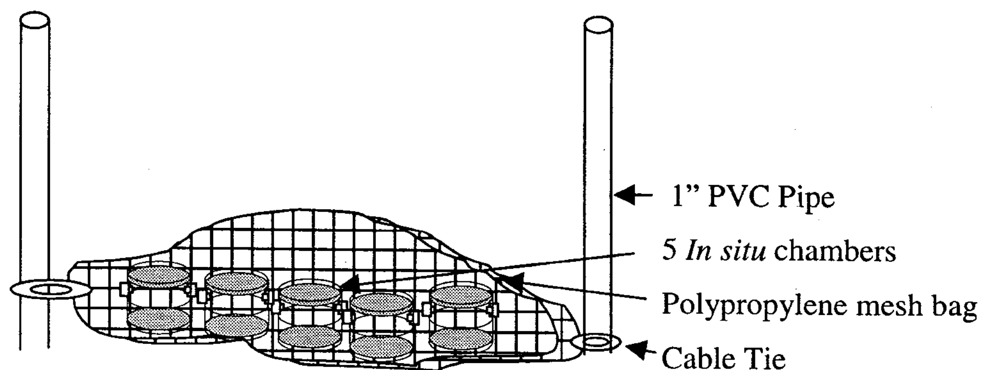

Figure 5. *In situ* chambers in exposure array used in Monterey Harbor *in situ* experiment. The mesh bag was attached to the PVC stakes at the sediment surface.

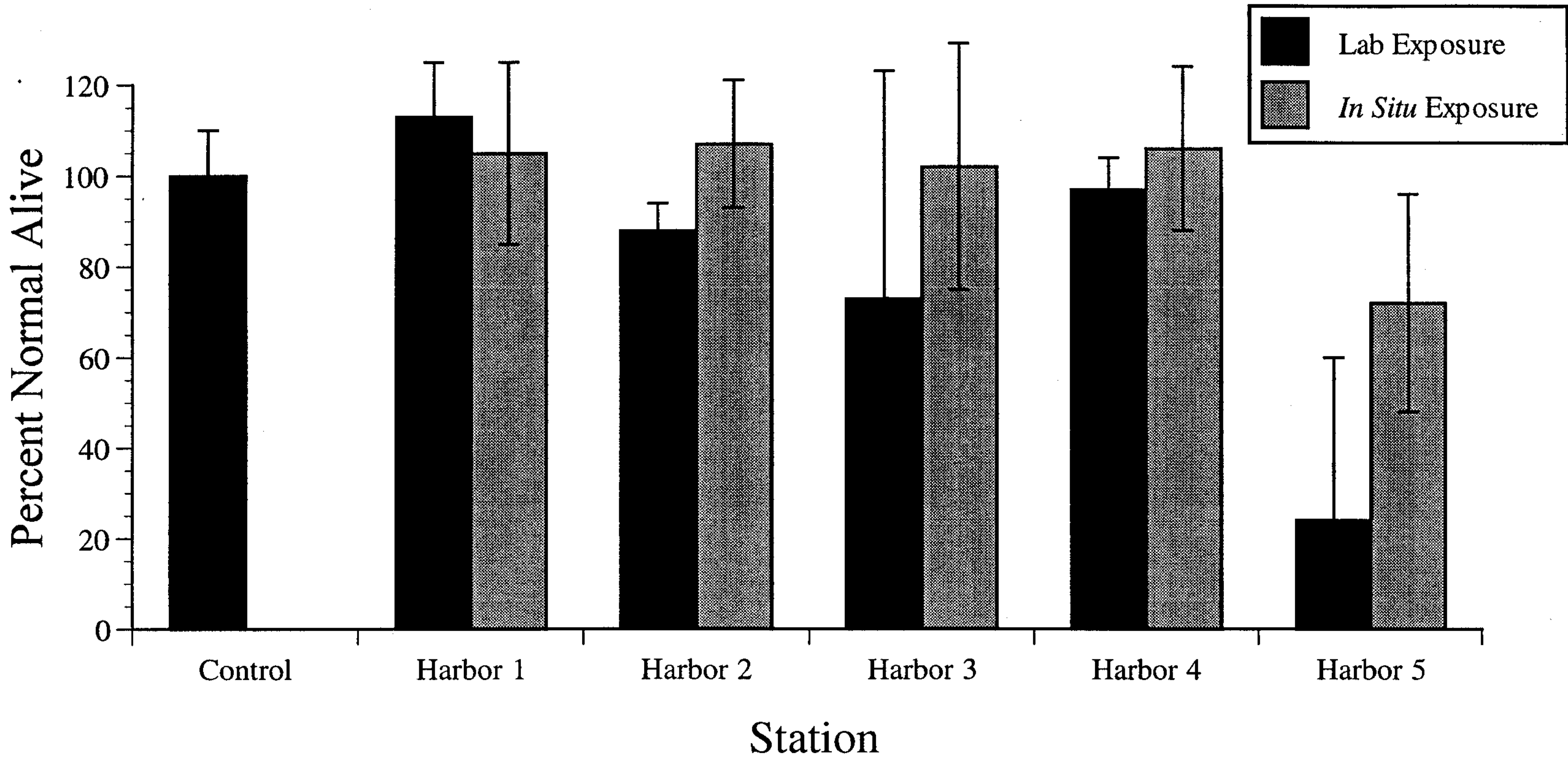

Figure 6. Comparison of Monterey Harbor laboratory and *in situ* exposures using bivalve embryos. Error bars are +/- one standard deviation. Refer to Figure 2 for station locations

Table 5. Water quality measurements in lab and field test containers.

Station	pH	Dis. O_2 (mg/L)	Salinity ppt	Tot. NH_3 mg/L	Tot. S_2 mg/L
1 – lab T_0	7.98	7.00	35	ND	NA
2 – lab T_0	7.96	6.41	35	ND	NA
3 – lab T_0	7.99	6.82	35	ND	NA
4 – lab T_0	8.04	7.33	35	ND	NA
5 – lab T_0	8.00	6.75	35	ND	NA
Reference T_0	7.78	7.60	35	ND	NA
1 – lab T_{48}	7.56	7.22	35	ND	NA
2 – lab T_{48}	7.57	5.10	35	0.56	NA
3 – lab T_{48}	7.84	6.51	35	ND	NA
4 – lab T_{48}	7.67	4.55	35	ND	NA
5 – lab T_{48}	7.84	6.00	35	ND	NA
Reference T_{48}	7.91	7.27	35	ND	NA
GC control T_{48}	7.97	7.17	35	ND	NA
1 – field T_{48}	8.09	7.65	34	ND	NA
2 – field T_{48}	8.04	7.16	35	ND	NA
3 – field T_{48}	8.07	7.65	35	ND	NA
4 – field T_{48}	8.07	7.72	35	ND	NA
5 – field T_{48}	8.05	7.45	35	ND	NA

T_0= Time 0 hours; T_{48}= Time 48 hours; ND = Not Detected; NA = Not Analyzed

These experiments demonstrate that there were differences in the quantification of ambient toxicity depending on whether laboratory or *in situ* exposures were used. Chemical analysis of the water inside the laboratory and *in situ* exposure chambers would have clarified whether the observed differences were due to exposure to chemicals in the water or fluxed from the sediments, or due to other factors such as differences in pH

between laboratory and *in situ* exposures. Regardless, these studies should be regarded as an initial step toward using West Coast marine WET protocols for assessing ambient toxicity of receiving waters and sediments. In order to accurately assess ambient toxicity, it is necessary to have toxicity test methods that are adaptable to different exposure conditions and experimental designs. Although in most cases it would not be practical to conduct *in situ* exposures for routine ambient monitoring, the results from experiments conducted in Monterey Harbor suggest that more research is required to determine why WET tests conducted under laboratory conditions differed from *in situ* exposures. Although previous studies have used marine WET protocols for assessing receiving water toxicity in laboratory exposures, these experiments are the first successful use of a West Coast WET protocol reported in an *in situ* application. This provides an opportunity for further comparisons between laboratory and *in situ* exposures.

Although laboratory bioassays are convenient, cost effective, and provide control over environmental parameters, it is difficult to extrapolate the results of laboratory tests to natural environments because of differences in exposure conditions and organism response (Kimball and Levin, 1985; Crane et al., 1995). Laboratory bioassays are considered to be useful as a first step in testing for chemical effects on ecosystems, but are less adequate for predicting effects on natural populations and on ecosystem-level features (Kimball and Levin 1985). In recognition of the need for more realistic evaluations of exposure-response relationships for biota in contaminated ecosystems, there has been recent emphasis on monitoring ambient toxicity by exposing laboratory bioassay organisms directly in the field (Burton et al., 1996). Field exposures have several advantages compared to laboratory exposures. *In situ* testing avoids excessive sample manipulation that might affect the availability of contaminants. (Sasson-Brickson and Burton, 1991). By combining *in situ* studies with laboratory exposures, multiple stressors can be teased apart and examined individually (Burton et al., 1996), and temporal variations in toxicity can be measured with continuous exposure of organisms in the field (Crane et al., 1995).

Several studies have compared laboratory exposures with either field or microcosm exposures. Clark et al. (1987) compared the effects of fenthion on mysids, grass shrimp, pink shrimp and sheepshead minnows exposed in the laboratory and in the

field. These authors demonstrated that laboratory LC50 values were good predictors of fenthion toxicity in the field for exposures greater than 24 hours, but overestimated toxicity for exposures less than 24 hours. Salazar (1989) exposed mussels to TBT in the laboratory and the field. Mussel survival in the field was much higher than measured or predicted in the laboratory.

Hansen and Garton (1982) assessed the ability of freshwater toxicity tests to predict the effects of diflubenzuron on species diversity and biomass in laboratory stream communities. Streams were equilibrated for three months and treated with diflubenzuron for five months. Single toxicity tests were accurate in predicting lethality to single species in laboratory streams but less successful in predicting community effects. This study is significant because it accounted for spatial and temporal variability of pesticide toxicity. Sherman et al. (1987) compared cadmium toxicity to fathead minnows in laboratory water and experimental ponds with and without sediment. They demonstrated cadmium was less toxic in laboratory water due to variable water quality conditions. Sasson-Brickson and Burton (1991) monitored sediment effects on caged *Ceriodaphnia dubia* in a stream that was impacted by effluents, sewage overflow and a creosote treatment operation. Field exposures were compared to laboratory exposures with sediment, elutriate and interstitial water. Average *in situ* survival in this study was greater than the laboratory exposures. Van Wijngaarden et al. (1996) compared single species tests with chlorpyrifos with short term direct effects in outdoor experimental ditches. Effects were investigated by conducting *in situ* bioassays with caged organisms and surveying zooplankton and macroinvertebrates. This study demonstrated that acute laboratory single-species toxicity tests could be used to estimate short-tem effects in the field for populations of the same species. It is clear from the studies discussed above that the combined use of WET test protocols in laboratory and *in situ* applications increases our ability to detect and interpret ambient toxicity test results. By adapting West Coast WET protocols for different exposure conditions we have a greater capacity to use these tests in a weight-of-evidence approach to assessing impacts of point and non-point source pollution in coastal waters.

Because receiving water toxicity is spatially and temporally variable, future toxicity studies conducted as part of the Marine Bioassay Project should emphasize more intensive sampling to account for sources of variability. This is particularly important when using laboratory exposure of field-collected samples to assess receiving water toxicity. For example, Bay et al. (1997) used horizontal stratification of samples to account for spatial variability of

receiving water toxicity and found toxicity due to storm water inputs was restricted to the surface waters. Stemmer et al. (1990) showed extreme vertical and horizontal variability in toxicity of sediments to cladocerans, sometimes on a scale of centimeters. In addition to spatial variability, future studies should incorporate sampling designs that more rigorously account for temporal variability. Hunt et al. (In press) found that toxicity of agricultural receiving waters to mysids (*N. mercedis*) varied monthly. In some sites, variability of toxicity was correlated with rainfall events, in other sites variability was apparently related to dry season inputs from agriculture and urban sources. Finlayson et al. (1993) found that seasonal variability of toxicity of Colusa Basin Drain water to juvenile mysids (*N. mercedis*) was related to the presence of methyl parathion, which is used seasonally in rice agriculture.

To account for inter-species differences in sensitivity to chemicals in marine receiving waters, future studies should also use a greater number of species than were used in the experiments reported here. It is possible the toxicity tests used in the experiments discussed above lacked adequate sensitivity to the particular contaminants present due to inappropriate species or endpoints or inadequate exposure times. For example, the sea urchin and bivalve embryo larval development tests are particularly sensitive to trace metal toxicity, but may be less sensitive to trace organic chemicals. Bay et al. (1997) found that while the sea urchin fertilization (*S. purpuratus*) test detected significant toxicity of storm waters in 20 minute exposures, the 48-h development test using red abalone (*H. rufescens*) did not. Assessments of water column toxicity in San Francisco Bay as part of the Regional Monitoring Program have shown significant toxicity of receiving waters to mysids (*M. bahia*) where no toxicity to bivalve development (*M. galloprovincialis*) was detected (SFEI, 1997). These studies demonstrate the need for multiple species and endpoints in ambient toxicity investigations. Of particular importance may be the use of more chronic endpoints such as juvenile mysid or larval fish growth.

These experiments also demonstrate the difficulty involved in conducting field experiments with early life stages of marine organisms in estuarine systems. Because of their relatively narrow tolerance of salinity and temperature extremes, these species may only be used for *in situ* experiments in field settings where these factors are consistently within a narrow range. Because non-point source toxicity events in estuarine and coastal systems are often associated with freshwater run-off, assessments of this source of toxicity may rely more on controlled laboratory assessments. Alternatively, *in situ* exposures could be restricted to times of

lower runoff, or to seasons or periods in the tidal cycle when favorable conditions prevail. In this case, it may be possible to conduct abbreviated field exposures during critical periods of development, and return animals to the laboratory to allow final developmental stages to proceed under controlled conditions (eg. Raimondi et al, 1996). Exceptions to this might include use of protocols and species with a wider tolerance for environmental variability. Because topsmelt (*Atherinops affinis*) embryos and larvae tolerate a wide range of salinities and temperatures, this species is probably more amenable to *in situ* exposure. This species may be particularly useful in experiments designed to assess contaminated sediments. For example, Jelinski et al. (1996) have conducted preliminary laboratory experiments with field-collected sediments and found that topsmelt embryos are suitable for sediment-water interface exposures. Embryonic development was not inhibited by reference sediments in this study, but was inhibited by contaminated sediments. Kocan and Landolt (1990) compared laboratory tests with *in situ* exposures using herring embryos, which are developmentally similar to topsmelt. Embryo survival and development in water samples and water extracts of sediments (elutriates) was compared to field exposures. Embryos exposed in the field had lower hatching success than those exposed in laboratory tests. Toxicity was detected with field deployed embryos because the exposure period was longer. Another species which may be particular useful for *in situ* sediment assessments is the free-burrowing estuarine amphipod *Eohaustorius estuarius*. This species also has wide salinity and temperature tolerances and has been demonstrated to be sensitive to a variety of contaminants (EPA 1994). Because sediments are often the source and repository of mixtures of non-point source pollutants, experiments could be designed to assess the toxicity of sediment pollutants using *in situ* exposures. These could be compared to laboratory exposures to assess differences between the two, and would also allow investigation of the relative importance of spatial and temporal variability.

SECTION 3

Organism Supply and Technical Workshops

Test Organism Supply

One of the primary goals of the MBP is to promote use of the various toxicity test protocols by establishing a network of reliable test organism suppliers. Supply has been established for all MBP protocols. Abalone (*Haliotis rufescens*) supply has been accommodated by the various commercial operations who culture this species for the seafood industry. Kelp (*Macrocystis pyrifera*) reproductive blades and mysids (*Holmesimysis costata*) are available year-round from various suppliers who collect these species from the field. Topsmelt (*Atherinops affinis*) embryos and larvae are now available year-round from Aquatic Indicators in Fort Collins, Colorado. Bivalves are also available for year-round use from several suppliers. We have found it possible to culture purple sea urchins in the laboratory for year-round testing and have been supplying sea urchin adults to number of testing laboratories as part of Marine Bioassay Project activities. Sea urchins were shipped to various laboratories approximately 15 times a year during the current contract period. This service has been provided at no charge, but only in situations where commercial supply was not available.

Marine Bioassay Project Workshop

A toxicity testing methods workshop was conducted March 11, 1997 at EPA's Region IX field station in Richmond, in conjunction with the Northern California Toxicity Assessment Group's annual Meeting on March 12. The workshop covered background and methods for the four Marine Bioassay Project protocols using red abalone, giant kelp, topsmelt, and mysids. The workshop format allowed participants to conduct every aspect of each protocol to facilitate a technical exchange between the researchers who developed the protocols, regulators who are using the toxicity test data for NPDES compliance monitoring, and biologists who are conducting the tests on a routine basis as implementation proceeds.

Each section of the workshop began with a slide presentation of a protocol, giving participants an overview on the general principles and underlying assumptions of the test procedure. A question and answer period followed. Participants were then led through hands-on workstations where they could conduct abbreviated segments of the toxicity

tests. Workstations included appropriate equipment and glassware for conducting the test, as well as live test organisms for handling. Table 6 outlines tasks that were presented at each workstation.

Table 6. Summary of workstation tasks presented at methods workshop conducted March 11, 1997 at EPA's Region IX field station in Richmond.

Test Protocol	Workstation 1	Workstation 2
Red Abalone	• Handling and inspecting broodstock • Spawning induction • Fertilization • Washing and concentrating embryos • Density estimation • Delivery into test containers	• Preservation and Analysis of Larvae • Microscopic analysis • Endpoint interpretation • Water Quality considerations
Giant Kelp	• Sporophyll collection and washing • Storage and transport of sporophylls • Zoospore release • Determination of spore viability • Estimating spore density • Inoculation of test containers	• Removal of slides from containers • Microscopic analysis of spores • Germination endpoint determination • Growth endpoint determination
Topsmelt	• Larval handling and feeding • Inoculation of test containers • Test solution renewals	• Quantification of mortality endpoint • Preparation of larvae for weighing • Quantification of growth endpoint
Mysid	• Isolation of gravid females • Isolation of juveniles for testing • Loading of test containers • Feeding and maintenance • Test solution renewals	• Quantification of mortality endpoint • Quantification of length endpoint • Quantification of weight endpoint

SETAC WET Workshop

MBP staff member John Hunt participated as an instructor in a second workshop on November 15-16, 1997 in conjunction with the Society of Environmental Toxicology and Chemistry (SETAC) annual meeting held in San Francisco. This workshop was a two-day instructional short course entitled "Whole Effluent Toxicity Testing from Standards to Enforcement". This course covered technical and policy aspects of implementing WET tests, and incorporated information on WET test requirements from EPA documents. The course was designed for participants from both the regulating and regulated community. John Hunt gave a detailed overview of WET protocols developed with East and West Coast marine species, and discussed results of research conducted as part of the Marine Bioassay Project. Efforts to validate the ecological significance of the MBP protocol endpoints was emphasized, as well as WET QA/QC, and statistical considerations. MBP staff prepared extensive written materials that were included in the EPA/SETAC workshop manual.

State Water Resources Control Board Statistical Workshop

A statistical workshop was presented by Dr. Tom Dean of Coastal Resources and Associates in March, 1996 at the SWRCB office in Sacramento. The workshop was devoted to staff training on statistical analyses of aquatic toxicity data. An outline of the topics discussed is presented below.

Statistical Analysis of Aquatic Toxicity Test Data

Course Outline

1.0 Introduction

A. What will be covered

B. Specific problems from audience

C. Schedule

II. Point Source Testing

A. Statement of Objectives

B. Example of Test Procedure

C. Why do Statistics

III. Point Source Testing - Hypothesis Tests of Differences in Means

A. Null Hypothesis

B. Means and Variance

C. Alpha, Beta, sample size, and power

D. Type 1 and Type 2 errors

E. 2 sample tests and assumptions of t-test

F. Multiple sample tests

IV. Point Source Testing - Regression Approaches (Point Estimation)

A. Dose response data

B. Regression principals, determining effect concentrations

C. Alternative models

D. Confidence Intervals and Hypothesis Tests

E. Allocation of effort *vs* Hypothesis Tests of Differences in Means

V. Comparisons of Means Tests and Regression Approaches

A. Pros and Cons of each method

VI. Concerns about statistical power and possible solutions

A. QA measures - assuring sufficient power

B. Problems with "excessive power"

C. Adjustable α and β

D. Testing an alternative Hypothesis - Bioequivalence

VII. Sample *vs* Discharge Toxicity

A. Statistical Inference and "Sample" *vs* "Discharge" toxicity

B. Psuedoreplication

C. "Multiple Testing" and its effect on alpha

D. Statistical tests of discharge toxicity

VIII. Problems with temporal variability in sensitivity of test organisms - test x dose interactions

A. Example of test x dose interaction

B. Potential solutions

IX. Final Regulatory Decisions - Weighing the costs of Type 1 and Type 2 errors

A. The not so magical level of 0.05

B. Different means to the same end

X. Ambient Toxicity Testing

A. Statement of the Problem

B. Methods of evaluation, Point Estimate *vs* comparison of mean response

C. Pattern recognition, ANOVA with multiple comparisons

D. Testing mean response *vs* internal standard, historical standard, or reference sample

XI. Risk Assessment - Setting chemical standards based on Toxicity Data

A. Use of functional "dose response" relationships

B. Exposure and Response

C. Models combining exposure and response functions

XII. Class Exercise

A. The M&M test of short-term chronic toxicity

XIII. Addressing specific case histories

A. Real world problems and solutions

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