10/8-18-93 JSD

LBL-32837
UC-602

Lawrence Berkeley Laboratory

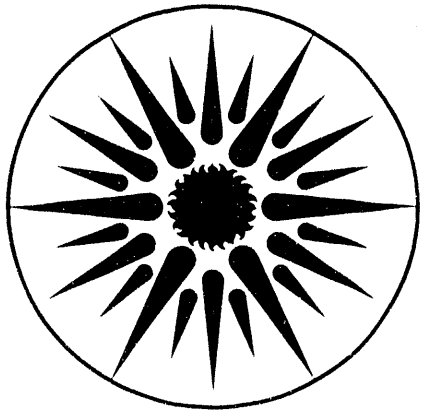
UNIVERSITY OF CALIFORNIA

ENERGY & ENVIRONMENT DIVISION

Spatial and Temporal Variations in Toxicity in a Marsh Receiving Urban Runoff

R. Katznelson, W.T. Jewell, and S.L. Anderson

June 1993



ENERGY & ENVIRONMENT DIVISION

Prepared for the U.S. Department of Energy under Contract Number DE-AC03-76SF00098

LBL-32837

SPATIAL AND TEMPORAL VARIATIONS IN TOXICITY IN A MARSH RECEIVING URBAN RUNOFF

Revital Katznelson, William T. Jewell and Susan L. Anderson

Energy and Environment Division
Lawrence Berkeley Laboratory
University of California
Berkeley CA 94720

June 1993

This work was supported by the California State Water Resources Control Board through the U.S. Department of Energy under contract No. DE - AC03 - 76SF00098.

FORWARD

This research was performed under a contract (# 0-137-120-0) between the Lawrence Berkeley Laboratory and the California State Water Resources Control Board under a contract through the U.S. Department of Energy (No. DE - AC03 - 76SF00098). The project was administered by the San Francisco Bay Regional Water Quality Control Board with Mr. Michael Carlin as contract officer. Support services were graciously contributed by Woodward-Clyde Consultants through the Alameda County Urban Runoff Clean Water Program. The contents of this document do not 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.

CONTENTS

EXECUTIVE SUMMARY

This project is composed of two sections. The first section describes dry weather toxicity surveys to evaluate the distribution of toxicity in the waters of San Francisco Bay and adjacent wetland habitat, and the second is a series of wet weather toxicity studies with emphasis on a marsh receiving urban runoff. The dry weather studies are reported in the appendices, while the wet weather work comprises the main report.

The wet weather toxicity study included two types of hydrological systems. These were the Crandall Creek and DUST Marsh (DUST System), in which urban runoff is retained within a small freshwater marsh for some time after the storm, and the Arrowhead Marsh system, in which stormwater is released directly into San Leandro Bay and is flushed twice daily by the tide. Toxicity was detected in creeks providing inputs to both systems after storms, but no toxicity was detected within Arrowhead Marsh. In contrast, in the DUST system ttoxicity was detected in the receiving waters as well. Thus, the DUST System served as an excellent site for the study of the distribution and fate of toxic substances in receiving waters.

The DUST System was used as an experimental system for the characterization of spatial and temporal distribution of toxicity. This characterization allowed us to answer important marsh design questions relating to its performance in containing, diluting, and removing toxicity. Our studies were performed on a total of seven storm events during the winter of 1991-1992 and the autumn of 1992.

The results of toxicity screening tests in the two types of hydrological systems indicated that *Ceriodaphnia dubia* is the preferable test organism for evaluating effects of toxic substances present in stormwater generated in the Oakland and Fremont drainage areas. The incidence of response of *C. dubia* was very high, whereas no toxic response was observed with fathead minnow larvae or with *Selenastrum capricornutum*. The most useful expression of toxicity in the *C. dubia* test was the median time to lethality (LT_{50}). Reproduction in *C. dubia* did not seem to be adversely affected by DUST System samples, even in toxic samples where mortality occurred later in the test.

Electrical conductivity (EC) was a convenient tool to trace the stormwater as it flows through the marsh and mixes with preexisting marsh water. We found very good correlation between EC values and LT_{50} in samples collected within 30 hours of the storm. Spatial characterizations of EC and toxicity toxicity at this time reveal that big storms flush the marsh, while small storms create horizontal gradient. The low-conductivity (and toxic) stormwater tends to remain on the surface of the waterbody, creating vertical gradients in EC and toxicity. The marsh may remain stratified for several days after the storm.

The intensity of toxicity in the Crandall Creek and the DUST Marsh diminished with time, as observed after four storm events. This could be related to three main performance questions:

1. **Does the DUST marsh contain runoff toxicity?** Measurements show that in most storm events, some toxic water flows out of the marsh through the exit culvert. Since the purpose of this system is to reduce toxicity inputs into the San Francisco Bay, it is recommended to design marshes that will contain runoff effectively.
2. **Does the marsh dilute toxicity?** Direct reduction in toxicity occurs as toxic stormwater is mixed with preexisting non-toxic marsh water. This toxicity-reduction process is not as rapid as it could be, due to the stratification of the waterbody which limits the extent of dilution. To protect the marsh biota from the impact of toxic runoff, it is recommended that the mixing processes in the marsh be ameliorated.
3. **Does the marsh treat toxicity?** In samples collected several days after storms, toxicity was reduced to non-detectable levels or to intensities much lower than those predicted according to dilution only. Toxic substances may be removed from the water by sequestration and/or sedimentation and/or degradation. To allow sufficient time for these processes to occur, it is recommended that the stormwater be stored within the marsh for several days after each storm.

Following structural modifications in the DUST Marsh (September 1992) flow patterns in the marsh were slightly altered and a portion of the marsh became isolated soon after being flushed with stormwater. The small pond thus created could be studied like a mesocosm. In this pond very little mixing occurred and the reduction in toxicity was extremely slow. This

observation emphasizes the importance of mixing with marsh water to facilitate removal of toxicity.

Phase I Toxicity Identification Evaluations (TIE) tests were performed by Woodward-Clyde Consultants using a sample collected in Crandall Creek during the storm of October 1992. Toxicity was removed by the C-18 columns and by aeration with air, indicating that oxidizable non-polar organic molecules could be the substances causing toxicity in *Ceriodaphnia dubia*. In this TIE effort and in all seven runoff samples studied at LBL, toxicity seemed to be associated with the soluble fraction of the sample, rather than with particles. If indeed the major toxicants entering the DUST Marsh are organic molecules, there is hope that the system will be able to degrade, rather than accumulate, these pollutants.

The results of toxicity monitoring in the DUST Marsh provide insight into the relationship between engineering design and treatment performance in a way which could not be achieved by monitoring of chemicals and of hydrological factors alone. Furthermore, characterization of runoff pollutants causing toxicity in a given watershed may help determine the suitable design for construction of treatment facilities, and toxicity monitoring may provide useful guidelines for management of these facilities.

TABLES

FIGURES

Chapter 1

INTRODUCTION

Restoration and protection of wetland ecosystems has become an important national environmental goal. Nevertheless, little research has been conducted to determine whether wetlands, created and designed to treat wastewater or polluted urban runoff, also provide beneficial habitat for wildlife. Marshes have been created to treat wastewater and to provide wetland-habitat enhancement, but although many workers study the performance of marshes as treatment facilities, toxicologists are rarely consulted to determine whether the marshes act as a viable habitat (Hammer 1990).

Recently, there has been heightened interest in using marshes as treatment facilities for stormwater. Over the past several years, the toxicity and pollutant concentrations of urban runoff have been characterized at several sites in the United States (WCC 1989, WCC 1991a,c). The load assessments of pollutants in stormwater from urban, industrial, and commercial areas reveal that these non-point sources contribute pollutants in quantities that rival those attributable to traditional point sources (WCC 1991a). However, the emerging body of data still provides little information on toxicity of urban runoff and its impact in the receiving water.

Although short-term toxicity tests have been widely implemented in water quality assessment (e.g. Anderson *et al* 1991), they have not been widely applied to marsh restoration and management. Evaluations of pollutant assimilative capacity for marshes have tended to focus on chemical analysis of selected substances, rather than on toxicity attributable to complex mixtures. Toxicity tests are a powerful approach because they can be used to rapidly evaluate: 1) spatial distribution of toxicity in complex environments, 2) magnitude and temporal variations of toxicity, and 3) toxicity reduction potential and options.

We have recently completed a study of an experimental marsh, the Demonstration Urban Stormwater Treatment (DUST) Marsh in southern Alameda County. Our efforts, which were carefully integrated with projects conducted by the Alameda County Flood Control and Water Conservation District and the San Francisco Bay Regional Water Quality Control Board, demonstrate the efficacy of combining toxicity assessment with engineering design.

The DUST System was established in 1983-1984 as a research facility to determine whether a wetland system can be effective in treating stormwater. Runoff from a residential area was directed, via Crandall Creek, into the DUST Marsh which was constructed on preexisting salt marsh and excavated areas. Studies in 1985-1986 concluded that the marsh was effective in removal of suspended solids, nutrients and some metals (Meiorin, 1986). Analyses of metals (copper, lead and zinc) in stormwater and sediments emphasize the capacity of the earth-lined Crandall Creek to remove metals (WCC 1991b). Whereas a substantial body of data on metals and other pollutants exists, no previous toxicity studies in the DUST System have been performed. In addition, studies evaluating the impact of specific toxic chemicals on wetland habitats have been reported, but these studies lack a toxicity bioassay component (Woodward *et al* 1988, Johnson 1986, Lee *et al* 1982).

The first question addressed in our research was whether toxicity could be detected in the marsh after a storm. When this was confirmed, we proceeded to ask questions relating to the performance of the marsh in 1) containing the toxic runoff, 2) diluting toxicity, and 3) removing toxicity from the water. Bioassays using the water flea *Ceriodaphnia dubia* were performed to detect and quantify toxicity in the system. *C. dubia* assays were also used in toxicity identification evaluations (TIE) to assess the nature of the toxic substances in the DUST System. The different electrical conductivity values recorded in the marsh before, during and after a storm event were used to trace the distribution of stormwater in the marsh and, supplemented with a toxicity dilution experiment, to construct a preliminary toxicity dilution model for the DUST Marsh.

Additional aspects of this report include assessment of toxicity in a second hydrological system (Arrowhead Marsh) during wet weather.

Our dry weather marsh studies included the Fairfield-Suisun survey, in which two major sloughs and their tributaries were sampled, and the San Pablo Bay survey, in which some of the major inputs of freshwater and effluents into San Pablo Bay were sampled. In addition, two Bay Background surveys were conducted as part of the project. The results of our dry weather marsh studies and the Bay Background surveys are arranged as appendices to this report.

Chapter 2

METHODS

2.1 DESCRIPTION OF STUDY AREAS

2.1.1 DUST System: Crandall Creek and DUST Marsh

Runoff from urban drainage area of 4.6 square miles in Fremont (California) is directed into the 55-acre DUST Marsh via the earth-lined Crandall Creek, into which storm drains open at various spots (Figure 1). The marsh has been constructed on preexisting wetland (System C) as well as on excavated areas (Systems A and B) and is now within the Coyote Hills Regional Park.

The Station numbers, SFBRWQCB Station Code designation, and locations, are as follows:
Station 1 (MA01): Crandall Creek at Fremont Blvd., opening of the main culvert.
Station 3 (MA03): Crandall Creek at Ardenwood Blvd., south of the road.
Station 5 (MA05): Debris basin, near the concrete sill.
Station 5.6 (MA16): System A, across the sill from the debris basin.
Station 6 (MA06): Northeast shore of System A.
Station 5.7 (MA17): System B, across the sill from the debris basin.
Station 7 (MA07): North shore at the center of System B, beyond the reed bed.
Station 8 (MA08): Beginning of System C, about 100 m west of the bend.
Station 9 (MA09): Northwest exit of DUST marsh, near culverts leading to North Marsh.
Station 13 (MA13): Southwest exit of the marsh, near culverts leading to Main Marsh.

Runoff generated in about 24% of the drainage area flows through the culvert at Station 1 and comes into contact with soil and vegetation at its opening. Hence, the creek is a wide vegetated channel. The western part of the creek channel runs parallel to the Alameda Flood Control Channel, and was influenced by seawater leaking into the creek from the Channel through floodgates situated 50 m upstream of Station 3, until March 24, 1992

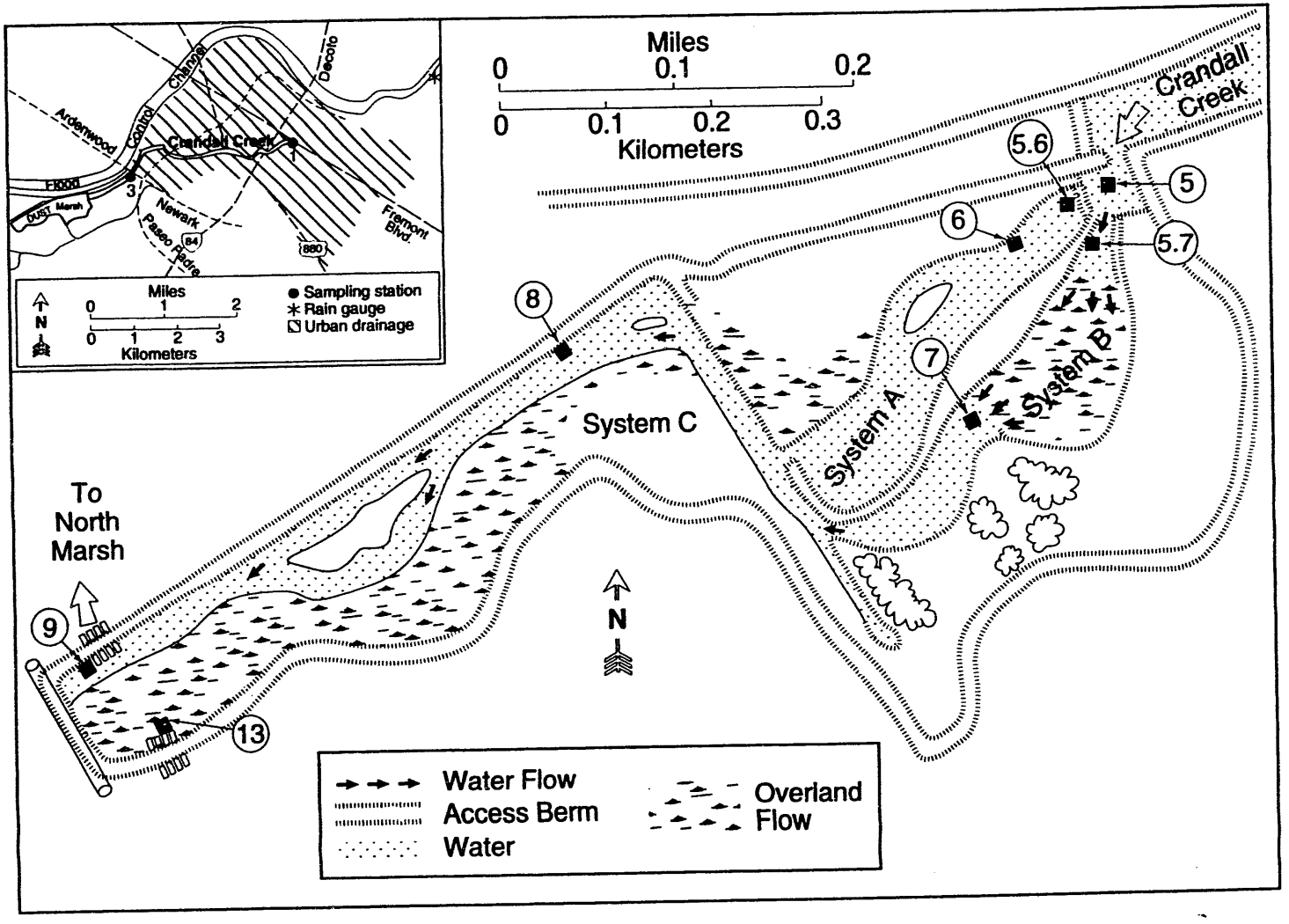


Figure 1: Sampling stations in Crandall Creek and the DUST Marsh, Fremont, CA. Map source: ABAG (Meiorin, 1986).

when flashboards were installed on the floodgates. During low flow after storms, some oily discharges were observed coming from a culvert which drains the western part of the drainage area and opens into Station 3 site. About 1 km west of Ardenwood Boulevard the creek is diverted into a small debris basin. Two concrete sills direct water into Systems A and B. System B was not studied during winter 1991-92, but after the installation of a 20 cm berm on top of sill A (September 1992) System B received the bulk of stormwater flow and was monitored accordingly. During dry weather, some portions of the creek remain wet but there is very little flow of water into the marsh.

2.1.2 Arrowhead Marsh

San Leandro Bay (Oakland, California) was sampled during a storm on February 1, 1992 (Figure 2). The three creeks leading into Arrowhead Marsh were sampled several hundred meters upstream of confluence in San Leandro Bay. Since the sampling was carried out during low tide, the creeks samples consisted mainly of runoff. A marsh sample was collected close to the outfall of the creeks, and a Bay Background sample was collected at the exit of San Leandro Bay.

The Station numbers, SFBRWQCB Station Code designation, and locations are as follows:
Station 1 (MB10): Arrowhead Marsh dock, end of boardwalk leading north upon the marsh.
Station 2 (MB12): San Leandro Creek at Hegenberger Road bridge.
Station 3 (MB13): Elmhurst Creek ("South Coliseum") at Oakport St. bridge.
Station 4 (MB14): Damon Slough ("North Coliseum") at Oakport St. bridge.
Station 5 (MB15): Exit of San Leandro Bay, under Doolittle Drive bridge.

2.2 STUDY DESIGN

Stormwater studies were conducted in three main phases. The first phase involved toxicity screening in the DUST System (Crandall Creek and DUST Marsh) and in Arrowhead Marsh. Fathead minnow (*Pimephales promelas), Ceriodaphnia dubia*, and *Selenastrum capricornutum* tests were conducted with six DUST System samples in November 1991.

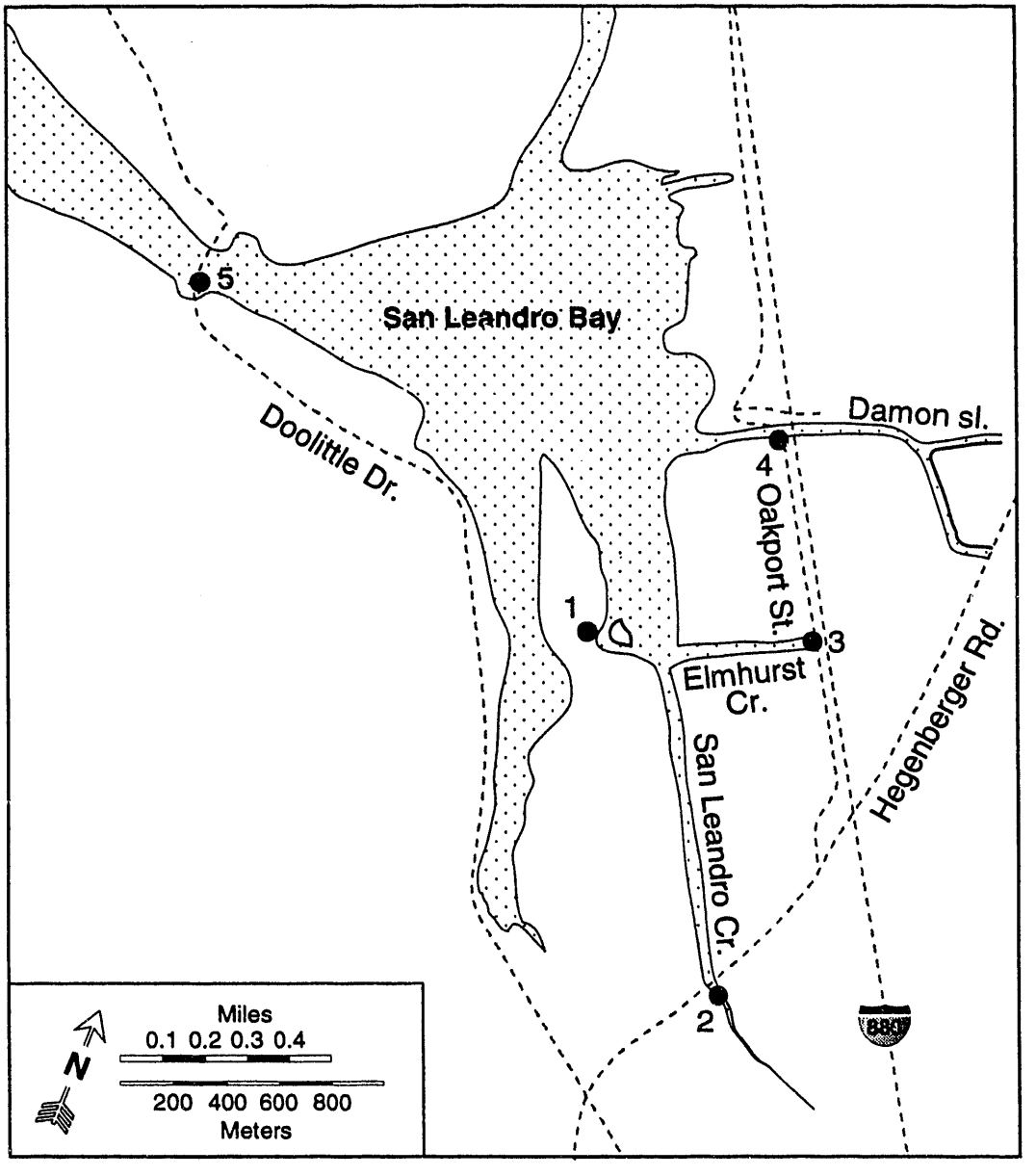


Figure 2: Sampling stations in the Arrowhead Marsh survey, Oakland, CA.

Fathead minnow and mysid tests were also conducted with one DUST sample (Station 3) after the storm of March 5, 1992. In an evaluation of the Arrowhead Marsh (February 1992), six species were tested using samples from 5 stations. The species tested were silverside minnow (*Menidia beryllina), C. dubia, Mysidopsis bahia, Mytilus edulis* and echinoderm (*Strongylocentrotus purpuratus*).

The DUST System was chosen for a comprehensive urban runoff study using *C. dubia* bioassays, and was sampled 13 times throughout the wet weather season of 1991-92. The first phase involved four sampling events. A screening survey was conducted in October, a screening survey with repetitive sampling (2, 4, and 6 days after storm onset) in November, a "dry weather" survey in December, and a survey following a prolonged rainy period in February. During this phase, grab samples were used for acute or chronic toxicity bioassays as specified by the EPA protocols, without modifications.

The second phase of our study, detailed characterization of the spatial and temporal variability of toxicity in the DUST System was conducted during March 1992. This phase involved sampling five field replicates at selected stations and utilized methods which allowed sampling of vertical profiles in the Marsh. The field replication design was applied to two storm events with repetitive sampling (March 5, 1992 and March 14, 1992). It was also applied to sampling of water from the surface and from the bottom (120 cm depth) of the marsh after the storm of March 22, 1992. *C. dubia* bioassays conducted during this phase with field replicates were modified to a 7-day static renewal test with five animals for each field replicate.

The third phase of the DUST Marsh study was directed towards obtaining a dynamic picture of stormwater flow and dilution throughout the marsh before, during and after a storm, and was carried out in October-November 1992. A peristaltic pump was used for sampling, and several (3-5) pump intervals were pooled in each sample bottle. During this phase, the 7-day static renewal toxicity bioassays were performed using four laboratory replicates for each sample, with five animals per replicate.

2.3 SAMPLE COLLECTION

Urban runoff samples for the Arrowhead survey were collected by Woodward Clyde Consultants using a peristaltic pump with silicon tubing. DUST System samples were collected with a diaphragm pump fitted with Bev-A-Line brand tubing during the November 1991 survey, and with a plastic container for the October 1991, December 1991 and February 1992, and for the March 1992 chronic toxicity tests.

For the detailed DUST Marsh study in March 1992 we introduced new sampling methods to allow field replication and to sample vertical profiles. A beaker mounted on a 12-ft pole was used to collect five discreet surface-water samples from defined sub-station locations, spaced a few meters apart from each other, at three sampling stations (creek, marsh entrance and marsh exit). Sampling at several discreet depth along the vertical profile of the water column was performed from the levee, using a manually operated vacuum pump in conjunction with a long Tygon tubing and a 12-ft pole. Field replication for toxicity testing was achieved by moving the pole laterally as five discreet samples were taken at the desired depth.

Sampling before, during and after the storm of October 29, 1992 utilized a peristaltic pump with silicon tubing for most surface and depth samples and was done in collaboration with Woodward Clyde Consultants. A few surface water samples were obtained with a beaker mounted on a 12 ft pole.

All sample containers were presoaked overnight with deionized water or sea water, and all equipment and containers were rinsed with sample water prior to sample collection. All samples were stored in coolers and chilled during transport.

2.4 SAMPLE PREPARATION AND WATER CHEMISTRY

2.4.1. Storage

Samples were stored in refrigerators at 4-7°C. Generally, samples were held 12-48 hours prior to test initiation. Sampling for each stormwater test was conducted only once, and the derived sample was used for test initiation and six renewals, being held for up to 168 h. Waters for the sea urchin and mollusc tests were held 24 hours. Waters for the algal growth test were held for 48 h prior to test initiation. Samples were always shaken vigorously to resuspend particulate matter.

2.4.2 Salt and pH adjustments

Samples were filtered during sampling or upon arrival in the laboratory through a 37 μm Nitex mesh to remove large particulates and predatory organisms. Salinity was adjusted prior to testing to conform with salinity ranges specified in the various protocols. Salinities were reduced for some *C. dubia* bioassays by adding dilution with control water (a mixture of 80% Arrowhead Spring water with 20 % Evian mineral water) to yield conductivities lower than 2000 μmhos/cm. This adjustment was performed only in tests for which reproduction data were collected, since it was found that salt at higher concentrations affects reproduction. Salinities were increased for the sea urchin and mollusc bioassays using fresh brine made by concentrating fresh Bodega Bay seawater. Care was taken during the concentration process to keep the temperature below 40°C and the final salinity below 90 ppt. Salinity adjustments for the silverside minnow and the mysid bioassays were performed with brine made by dissolving Forty Fathoms artificial sea salts in Arrowhead Spring water to 115 ppt. When salinity alteration resulted in a dilution of the ambient sample, the final ambient concentrations are recorded as "Ambient %" in the results.

The pH was routinely adjusted to 8.0 with 0.5N HCl for the sea urchin and mollusc test. In DUST samples with initial pH higher than 8.4, the pH was decreased to 7.5 before exposure of *C. dubia*. This adjustment prevented the development of high pH (>9.0) in the

test chambers during incubation. Other than that, no pH adjustment was necessary with ambient samples.

2.4.3 Water Quality Measurements

Water Quality measurements were made for each toxicity test according to specifications in EPA and ASTM protocols. Dissolved oxygen was measured with a Yellow Spring Instruments model 57 Oxygen Meter. The pH was measured using the Orion model 601 digital Ionalyzer, which was also employed in the determination of ammonia with a specific electrode. Electrical conductivity, salinity and temperature were measured with a Yellow Springs Instruments model 33 S-C-T meter. Alkalinity was determined by acid titration using a Hach kit (Loveland, CO), and hardness was measured by EDTA titration with Calmagite indicator using an Aquarium Pharmaceuticals Inc. kit (Chalfont, PA). Total suspended solids were determined gravimetrically using Glass Fiber filters type A/E supplied by Gelman Sciences (Ann Arbor, MI). Any deviations from the specified ranges are noted in the results.

2.5 TOXICITY TEST PROCEDURES

Toxicity tests were generally conducted according to EPA and ASTM protocols. Additional specifications on test conduct and deviations from protocol are described below for each test.

2.5.1 Silverside Minnow and Fathead Minnow Larval Growth Tests

The larval growth and survival tests using the fathead minnow (*Pimephales promelas*) and the silverside minnow (*Menidia beryllina*) were performed according to the EPA protocols (USEPA, 1988; USEPA, 1989). Fathead minnow, less than 24 hours old, were supplied by Aquatic Resources (Sebastopol, CA) and were used for tests initiated within 20 h of their arrival. Silverside minnow larvae, 6-7 days old upon arrival, were supplied by Aquatic Resources (Sebastopol, CA) at salinity within 4 ppt of the salinity required for each test.

Tests were initiated with 7-9 day old fish that were salinity acclimated. The dilution and control water used for the fathead minnow test was a mixture of 80% Arrowhead Spring water with 20% Evian mineral water. In the silverside minnow test, we used seawater collected at Bodega Bay Marine Laboratory as a natural seawater control and Arrowhead Spring water with 40 Fathoms artificial sea salts added as a salinity-adjustment control. Copper sulfate was used as a reference toxicant in the range of 50-400 μg/l copper.

2.5.2 Echinoderm Fertilization Tests

The echinoderm sperm cell bioassay was conducted using the purple sea urchin (*Strongylocentrotus purpuratus*). We followed the protocol developed in our laboratory (Anderson et al., 1990) after the general approach of Dinnel (1987) with modifications suggested by Cherr et al. (1987). Briefly, spawning was induced by injection of 0.5-1 ml of 0.5M KCl in seawater into the oral cavity of each animal. Sperm was collected as dry spawn from the aboral surface using a syringe, and stored in a container on ice. Females were placed upside down on top of a beaker full of cold seawater, and the eggs were released into the beaker. We routinely conducted a sperm:egg ratio pre-test using a range of ratios to determine the lowest ratio that resulted in 95% fertilization in the seawater control. Bodega Bay seawater was used as the natural seawater control and Arrowhead Spring water, salinity adjusted with freshly prepared natural seawater brine, was used as the brine control. All sample and control waters were salinity-adjusted to 30 ppt and pH-adjusted to 8.0 $\pm$ 0.1 before testing. Sodium azide at concentrations of 400, 300, 200 and 100 mg/l was used as a reference toxicant. Tests were conducted at 14°C in test tubes with 2 ml solution for the duration of 40 minutes (20 minute sperm exposure), and were terminated by addition of formaldehyde (1% final concentration). Adult sea urchins were obtained from the Bodega Bay Marine Laboratory (Bodega Bay, CA).

2.5.3 Mollusc Embryo Development Tests

Mollusc embryo development tests, using the bay mussel (*Mytilus edulis*), were conducted according to ASTM protocol (ASTM, 1987). Adult bay mussels were obtained from Cove

Mussel Company (Marshall, CA) and were held dry at low temperature for several hours. Spawning was induced by incubating the mussels in seawater at 25°C (Anderson et al. 1990). Sodium azide was used as a reference toxicant, at concentrations of 50, 30, 10, and 5 mg/l. Sample and control waters adjustments were identical to those described for the echinoderm fertilization test. The embryos were incubated in 10 ml vials for 48 h at 16°C and the test was terminated by the addition of formaldehyde (0.5% final concentration).

2.5.4 Mysid Survival Tests

The Mysid (*Mysidopsis bahia*) short-term chronic test was performed according to the EPA protocol (USEPA, 1988), with 7-day old animals supplied by Aquatox (Hot Springs, AK) for the DUST Marsh test. We used Bodega Bay seawater diluted with Arrowhead Spring water as a dilution control and Arrowhead Spring water salinity adjusted with 40-Fathoms artificial seawater brine as a brine control. Five mysids per 300 ml chamber with 8 replicate chambers per treatment were used. In the Arrowhead Marsh survey, we transferred samples to Aqua Terra Technologies Aquatic Bioassay Laboratory (Walnut Creek, CA) for mysid bioassays. In this test, 10 mysids per chamber of 500 ml and 4 replicate chambers per treatment were used.

2.5.5 *Ceriodaphnia dubia* Survival and Reproduction Test

The water flea (*Ceriodaphnia dubia*) survival and reproduction test was performed using inhouse cultures and according to the EPA protocol (USEPA, 1989). A mixture of 80% Arrowhead Spring water and 20% Evian mineral water was used as control and dilution water and as a base for culture medium for stock cultures. Sodium chloride at various concentrations in the range of 0.5 - 3 g/l was used as a reference toxicant. Stock cultures and test animals were fed the YCT mixture specified in the protocol (USEPA, 1989) and *Selenastrum* suspension with an average density of 1×10^7 cells/ml. Each day, 0.12 ml of each food suspension was added to the 15-ml cup containing 1-5 animals.

Our inhouse *Ceriodaphnia dubia* cultures were cultivated individually in control water supplemented with 20% (v/v) natural waters, usually DUST Marsh water from samples which enhanced fecundity and were not toxic to the cladoceran. The culture medium used in March 1992 was also supplemented with 2 μg/l selenium, after Winner (1989). Starter culture of *C. dubia*, originally obtained from Chesapeake Cultures (VA), were the source of animals in tests conducted during October 1991-February 1992. Animals obtained from Aquatic Research Organisms (Hampton, NH), were used to start inhouse cultures which supplied animals for tests conducted during March 1992 and thereafter.

Chronic toxicity tests, namely the 7-day static renewal survival and reproduction test employing ten replicate test chambers with one animal in each, were performed in most of our surveys. After the November 1991 storm we performed 4-day acute toxicity tests for some samples, using a sample dilution series with five animals in each of three replicates. A modified 7-day static renewal test design, using 100% sample (without dilution series) in four replicates with five animals in each, was employed during the storm event of October 1992. During some tests conducted in March 1992, we altered the sampling method and took five field replicates at each site; the ensuing 7-day static renewal tests consisted of five animals in one cup with sample water from each field replicate.

2.5.6 *Selenastrum capricornutun* Growth Tests

Algal growth tests were performed on freshwater samples using the *Selenastrum capricornutum* bioassay (USEPA, 1989). All ambient samples were filtered to 0.45 μm prior to testing, and nutrients were added from sterile stock solutions (without EDTA) to concentrations matching those of the control medium. We used our inhouse culture throughout the project. The starter was received from S.R. Hansen and Associates who obtained their culture from Aquatic Research Organisms (Hampton, NH) and was cultivated in the growth medium specified in the EPA protocol. The culture was maintained axenic by periodic streaking on solid media and selection of bacteria-free colonies. Liquid cultures were not allowed to reach high pH to prevent flocculation. Cultures for growth tests were harvested at logarithmic phase, washed aseptically in sterile medium without EDTA, and used for

inoculation. *Selenastrum* cultures grown for feeding *C. dubia* were washed with distilled water and kept in the dark at 4°C for a few days, to minimize photosynthetic activity which might raise the pH in the cladoceran medium.

2.6 REFERENCE TOXICANT TESTS

Reference toxicant tests were appended to each echinoderm and mollusc test that was conducted. *C. dubia* reference toxicant tests were performed periodically during the project, and for each of the chronic toxicity tests with *C. dubia* one or two salt (NaCl) controls in conductivities matching those of the ambient samples were added. Reference toxicant tests were conducted periodically for the fish larvae. The responses of each of the various test organisms were generally consistent throughout the project period.

2.7 STATISTICAL ANALYSES

2.7.1 General Approach

Statistical analyses were carried out according to the guidelines presented in the EPA protocols for each species. Data were entered into the TOXIS database (EcoAnalysis, Ojai, CA) which is equipped with the TOXSTAT package produced by University of Wyoming. Each data-set was tested for normality using either a Chi-squared or Shapiro-Wilks test, and for homogeneity by either a Bartletts or Hartley test. When data passed normality and homogeneity tests, they were analyzed for significance using a Dunnett's test. Datasets which were found non-normal or heterogeneous were analyzed for significance by a nonparametric test (Kruskal-Wallis test for datasets with 3 replicates and Steel's many one rank test for 4 replicates). Non-normal or heterogeneous proportional data (Abnormality in the mollusc bioassay, reduced fertilization in the echinoderm bioassay, and survivorship in the *Mysidopsis*, silverside minnow and fathead minnow bioassay) could be arcsine transformed to achieve normality and/or homogeneity of variance. Nonproportional data (growth in *Selenastrum*, reproduction in *C. dubia*, and larval weights in both the silverside and fathead minnow bioassays) invariably passed the normality and homogeneity tests and were analyzed by the

Dunnett's procedure. Statistical analysis of *C. dubia* reproduction data often required a Bonferroni T-test instead of a Dunnett's Test. This occurred when replicate sizes were unequal due to the presence of males, which are not included in the calculation of average young per female.

Significance was determined by comparison to both brine and dilution-water controls (where available) and p-values less than 0.05. Survival data for the *C. dubia* bioassay was analyzed using either the Fisher's Exact test or various methods for calculation of median time to lethality.

2.7.2 *Ceriodaphnia dubia* LT_{50}'s

The Median lethality time (time-to-death, LT_{50}) was used to compare the relative toxicities of selected samples to *C. dubia*. LT_{50} values were calculated by the graphical method (USEPA 1991), by the Trimmed Spearman-Karber method (Montana State University program), or by the Probit method. These methods, devised to calculate the Median lethal concentration (LC_{50}) or Median effective concentration (EC_{50}), were used for calculation of Median time to lethality by replacing "concentrations" with "hours". Only one method was used for each array of datasets within one comparison.

2.7.3 Other Statistical and Mathematical Analyses

Regression, correlation, t-tests, box plots and other multiple comparison ANOVAs using confidence intervals were performed by the conventional statistical methods, as supplied with the Minitab or the Quattro-Pro packages. For the calculation of the "predicted toxicity due to dilution" in DUST Marsh samples collected during the Oct 29, 1992 storm event, the curve-fit program supplied by SigmaPlot 5.0 was used to extract the parameters for the polynomial describing the mathematical relationship between LT_{50} values and dilution factors as obtained in a series of bioassays on mixtures of stormwater and preexisting DUST Marsh water.

2.8 SUPPLEMENTARY DATA

Rainfall data supplementing the DUST System drainage area were obtained from Rain Alert Gauge system, Alameda County, as daily totals measured at Station Pt. 1940, ACWD-Niles, located at Mission Blvd. and Alameda Creek. Generally, rainfall daily totals obtained at this station were comparable to those measured at other corners of the drainage area, namely Station Pt. 2102 (Alvarado - Union City) and Station Pt. 2210 (San Francisco Bay Refuge).

Chapter 3

RESULTS

3.1 DETECTION OF TOXICITY IN WET WEATHER MARSH STUDIES

There is ample evidence that runoff from residential and commercial areas in Alameda County contain substances toxic to test organisms (WCC, 1991), but the question whether toxicity can be detected in receiving waters has not been addressed. To answer this question, we chose two types of hydrological systems: The Crandall Creek and DUST Marsh (DUST System), in which urban runoff is retained within a small freshwater marsh for some time after the storm, and the Arrowhead system, where stormwater is released directly into San Leandro Bay which is subject to tidal action. The initial toxicity characterization involved several test organisms in each system.

3.1.1 DUST System: Crandall Creek and DUST Marsh

The results of toxicity tests with DUST System samples collected on Nov 18, 1991, two days after the storm onset, indicate that survival and growth of fathead minnow was not impaired in any sample tested (Table 1). *Ceriodaphnia dubia* were affected in all samples. Most animals did not survive exposure, and the median time to lethality (LT_{50}) increased as the distance from the Crandall Creek increased. Reproduction of the cladoceran was not adversely affected, even in toxic samples in which mortality occurred later in the test. Calculation of the number of offspring per female on reproductive days preceding mortality actually revealed enhanced reproduction in the marsh samples as compared to the control. After the storm event of March 5, 1992, toxicity of a creek (Station 3) sample was also tested with *Mysidopsis bahia* to determine whether crustaceans could be unusually sensitive. No toxicity to mysids was observed.

The growth of the green algae *Selenastrum capricornutum* was slightly inhibited in the sample collected at the upstream station of Crandall Creek, enhanced significantly in the

TABLE 1: SCREENING OF TEST ORGANISMS WITH DUST MARSH SAMPLES COLLECTED AFTER STORMS

SAMPLING DATE	STATION	FATHEAD MINNOW		CERIODAPHNIA			SELENASTRUM	
		Mean Surv (%)	Mean Wt. (mg)	Surv (%)	LT_{50} (hrs)[1] 95% C.I.	Mean Repro[2]	Growth[3]	% of Cont.
11/18/91	1	97	0.58	0[4]	4.9 N.C.	0[4]	0.77	75
	3	97	0.60	0[4]	4.9 N.C.	0[4]	1.62	156
	5	100	0.63	0[4]	100 90 - 111	6.0[4]	2.85	276
	5.6	87	0.66	50[4]	168 N.C.	33.9	0.35[4]	34
	8	97	0.76	0[4]	131 124 - 138	23.3	0.26[4]	25
	9	93	0.85	20[4]	149 135 - 164	28.7	0.33[4]	32
	DILUTION CONTROL	97	0.65	100		21.7[5]	1.03	
3/6/92	3	100	0.58	40	161 154 - 169	34.4		
	DILUTION CONTROL	100	0.63	100		34.4[5]		

[1] Median time to lethality and confidence intervals (Trimmed Spearman-Karber method). Values for Stations 1 and 3 were calculated based on observations at 0.1, 1, 24 and 48 hours. N.C., confidence interval Not Calculable.

[2] Reproduction endpoint: Average number of offsprings per female.

[3] Growth expressed in cells/ml x 10^6. Log transformed data were used for the Dunnett's test.

[4] Significantly lower than the control.

[5] Control solution used for 11/19/91 test was the regular mixture, while control solution for the 3/7/92 test was the mixture supplemented with 20% non-

other creek samples, and inhibited markedly in the marsh samples (Table 1). At the time of sampling, the creek stations (including the debris basin) were separated from the marsh by the sill, and it appears as if the marsh water has a strong inhibitory effect on *Selenastrum* growth. Results obtained in a preliminary test with this algae after the October storm were similar. Creek samples enhanced growth, while marsh samples were inhibitory.

3.1.2 Arrowhead Marsh

The Arrowhead Marsh stations were sampled shortly after the onset of the February 1, 1992 storm in order to obtain samples of peak stormwater flows. Sampling coincided with low tide, so the creek samples consisted primarily of runoff. The receiving water was sampled 1 hour later at Arrowhead Marsh dock.

Table 2 summarizes the results of six-species toxicity tests. Silverside minnows, fathead minnows, and *Mytilus edulis* (Bay Mussel) embryos were not affected by any of the samples tested. Mortality in *Ceriodaphnia dubia* and inhibition of echinoderm fertilization were observed in all three creek samples, while mysid survival was significantly lower in two of the three samples taken at the creeks. Toxicity was not detected in the receiving water sample taken at Arrowhead Marsh dock during the storm.

The results of toxicity screening tests in the two types of hydrological systems indicated that *Ceriodaphnia* is the preferable test-organisms for urban runoff toxicity studies, since it was the most sensitive freshwater species among the species tested. The detection of post-storm toxicity in the receiving waters at the DUST Marsh confirmed that the DUST System is suitable for the study of the distribution and fate of toxic substances in receiving waters, and subsequent studies were focused on this system.

TABLE 2: TOXICITY OBSERVED IN SAMPLES COLLECTED AT ARROWHEAD MARSH DURING THE STORM OF FEBRUARY 1, 1992

STATION		SAL[2]	MENIDIA			FATHEAD MINNOW		MOLLUSC		URCHIN		MYSID		CERIODAPHNIA		
No.[1]	Location	(ppt)	Mean Surv (%)	Mean Wt (mg)	Amb (%)	Mean Surv (%)	Mean Wt (mg)	Abnormal (%)	Amb (%)	Fert (%)	Amb (%)	Mean Surv (%)	Amb (%)	Surv (%)	LT_{50} (hrs) 95% CI	Mean Repr[4]
1 MB10	Arrowhead Marsh Dock	25.5	87	0.86	100			7	92	89	92	88	70			
2 MB12	San Leandro Creek	0	97	0.90	78	80	0.65	3	59	47[5]	59	38[5]	100	0[5]	63 58-69	
3 MB13	Elmhurst Creek	1	90	0.97	79	80	0.64	5	61	10[5]	61	15[5]	100	0[5]	25 14-45	
4 MB14	Damon Slough	2.5	93	0.87	81	80	0.84	6	62	31[5]	62	85	100	10[5]	143 133-154	20.7
5 MB15	Bridge, Doolittle Dr.	25.5	97	0.88	100			6	92	83[7]	92	88	70			
	DILUTION CONT.					87	0.67	4		100[6]				90		16.7[8]
	BRINE CONT.	24	97	0.98	75			7		99	53	93	100			

[1] Lower number represents Station Code designated by the Regional Water Quality Control Board.

[2] Numbers entered for the control indicate test salinity for Menidia. Test salinity for the mollusc and urchin tests was 30 ppt. Salinity adjustments of samples were done by addition of brine, resulting in sample dilution to the concentration entered as % amb (ambient). Salt was added to samples for salinity adjustments in the mysid test. In the Ceriodaphnia test sample 4 was diluted to 70% ambient using dilution control water.

[3] Median time to lethality and 95% confidence interval, as calculated by the Trimmed Spearman-Karber method.

[4] Reproduction endpoint: Average number of offsprings per female.

[5] Significantly lower than brine control (Urchin, mysid) or dilution control (Ceriodaphnia).

[6] This control was significantly different from all stations.

[7] Of the three replicates averaged to yield this number, one was an outlier (64%) which could result from incorrect handling of test-tube. The mean fertilization with the outlier omitted is 92%, and is not significantly different from the brine control.

[8] Animals in control solution (Regular dilution water with 0.7% NaCl) were held through the eighth day of the test.

3.2 SPATIAL DISTRIBUTION OF TOXICITY IN THE DUST SYSTEM

3.2.1 Seasonal Overview

Figure 3 shows the qualitative relationships between storm events and toxicity in the DUST Marsh as monitored during six of the nine major storms of the 1991-1992 winter. Excluding the storm events of December, January and early February (which were not monitored), it is clear that toxic substances in detectable amounts were introduced into the DUST Marsh throughout the season. Toxicity could not be detected after the mid-February storm, probably due to dilution of these substances in the voluminous runoff generated by this storm. It is important to note that the incidence of detectable toxicity in water entering the marsh (Station 5) was much higher than in water at the marsh exit (Station 9).

Electrical conductivity values varied systematically before, during and after storm events. Conductivity values of surface water were very high (3500-4000 μmhos/cm) before the first storm of the season, dropped sharply during storms and increased gradually as time passed after each storm.

Varying patterns of *Ceriodaphnia* survival in surface water samples were observed following each of the six storm events studied during the 1991-92 winter (Figure 4). In these studies, the samples were collected several hours after the stormwater flows into the marsh had subsided. Toxicity was quite intense throughout the DUST System after the first storm of the season (2" storm, October 26, 1991), as can be judged from the relatively short exposure durations leading to mortality. The second storm (0.2" storm, November 17, 1991) brought a limited amount of highly toxic runoff, which dispersed through the system creating a gradient of toxicity along its horizontal axis. It is reasonable to assume that Crandall Creek inputs were the dominant source of toxicity during that storm. The dry weather study performed in December 1991 did not reveal significant toxicity (not shown). In our third study, after a long spell of wet weather in February 1992, toxicity was not detected in the marsh either. After the March 5, 1992 storm (fourth study), an inverse gradient in toxicity was observed, with the marsh stations 8 and 9 more toxic than the creek samples.

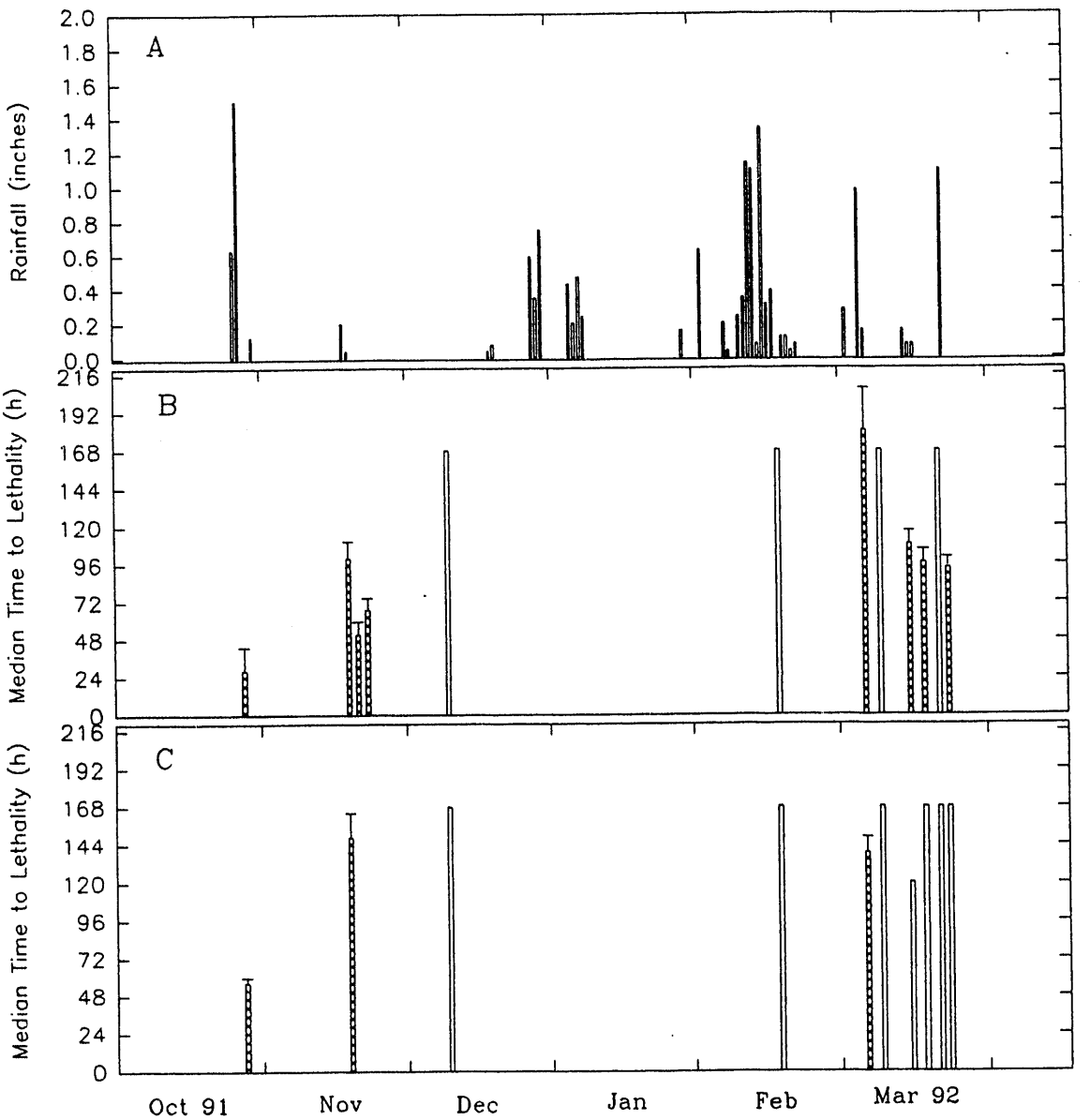


Figure 3: Toxicity observed at DUST Marsh entrance (Station 5) and exit (Station 9) in relation to rainfall.

A. Daily total rainfall at the Fremont watershed during the winter of 1991-1992.
B. Median time to lethality in *Ceriodaphnia dubia* exposed to Station 5 samples. Hollow bar, no detectable effect by the time indicated; checked bar with error indicator, LT_{50} and upper 95% confidence interval as calculated by the Trimmed Spearman-Karber method.
C. Median time to lethality in *C. dubia* exposed to Station 9 samples. Symbols as in B.

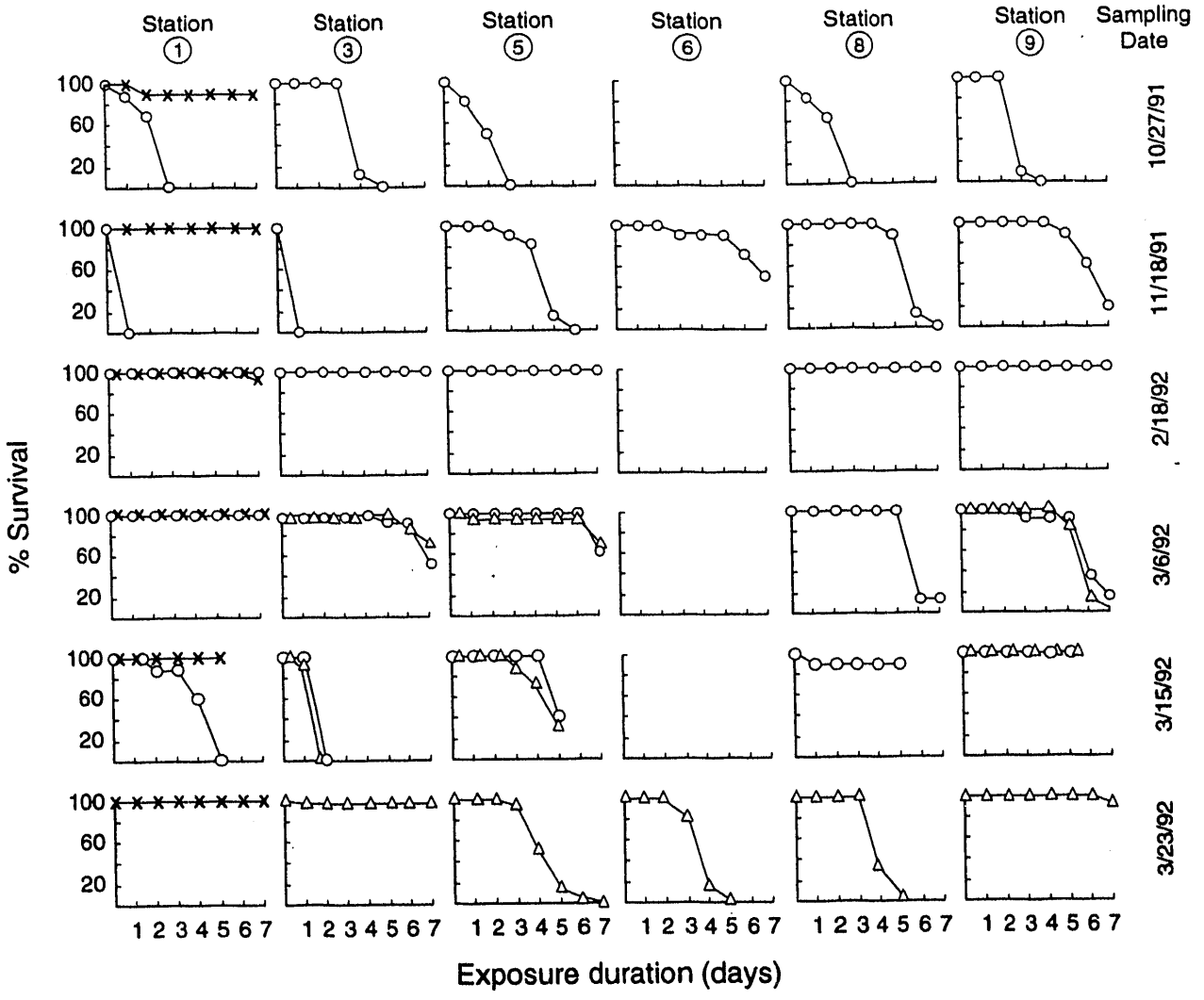


Figure 4: *Ceriodaphnia dubia* survival curves in surface water samples collected at the DUST System one day after each storm event.

x symbols, control; closed symbols, samples; circle, 10x1 design; triangle, 5x5 design. For the 10x1 design, Fisher Exact test was conducted for each bioassay date. Survival of 60% or less is significantly different from the control ($p < 0.05$). For the 5x5 design, Steel's test was conducted for each bioassay date. Survival of 64% or less is significantly different from the control ($p < 0.05$).

The source of toxic runoff during that storm could have been the P-Line, which drains runoff from an industrial area south of Coyote Hill Regional Park into the DUST Marsh at the southwest corner. Sampling Station # 11 (MA11) is located on the P-Line at the road leading to the visitor center of Coyote Hill Regional Park. Toxicity was not monitored regularly in the P-line, but a sample tested in October 1991 was toxic. In tests performed during our fifth study, after the storm of March 14-15, toxicity was most intense at Station 3 and was not detected in System C at all. Finally, in the runoff brought by the storm of March 22, 1992 (sixth study), toxicity was detected in the surface water collected close to the center of the DUST System (Stations 5, 6 and 8). This could have been due to agricultural drain pumped into system B near Station 5.7. In the second year a seventh storm event was monitored in the DUST System during the storm of October 29, 1992, details of which are presented in Section 3.3 below.

3.2.2 Horizontal Distribution of Toxicity

The first storm of the winter in October 1991 brought nearly 2 inches of rain. The DUST Marsh was "flushed" with a huge volume of stormwater, some of which was not retained in the marsh. Samples collected in the Crandall Creek and DUST Marsh stations after the storm were toxic to *Ceriodaphnia*, while conductivity values were generally low. After the 0.2-inch storm of November 1991, we observed a horizontal gradient of electrical conductivity in the system. Toxicity and conductivity data along the creek and the marsh are presented in Figure 5 for the two storms. Toxicity is expressed in time units indicating the duration of exposure which caused mortality in 50% of the test animals (Median time to lethality, LT_{50}). Linear regression of LT_{50} vs. sampling site (dotted line) yielded a slope which was not significantly different from zero ($p=0.778$) for the October (2") storm, and a slope different from zero ($p=0.026$) for the November (0.2") storm. There was a good correlation ($r=0.88$) between toxicity and conductivity after the October storm and a high correlation ($r=0.94$) between these parameters after the November storm. Since the conductivity value reflects the fraction of stormwater in the sample, indicating the degree of dilution of the toxic stormwater in preexisting marsh water, this correlation is to be expected.

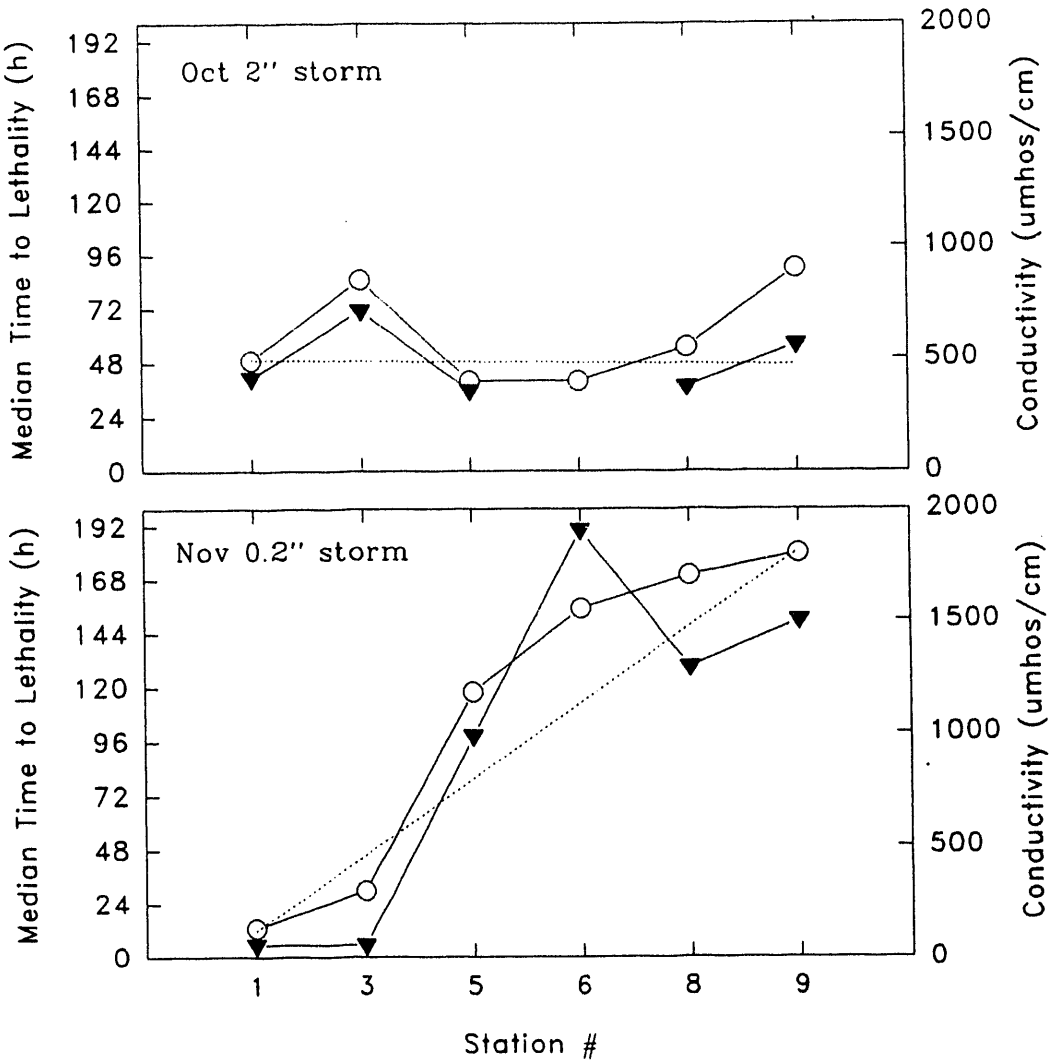


Figure 5: Spatial distribution of toxicity and conductivity in the DUST System following two storm events.

Hollow circle, conductivity; full inverted triangle, LT_{50} as calculated by the Probit method; dotted line, linear regression of LT_{50} versus sampling site. Resulting slopes of -1.4 with std. error of 4.54 for the October 1991 (2") storm, and a slope of 31.7 with std. error of 9.2 for the November 1991 (0.2") storm.

3.2.3 Vertical Distribution of Toxicity in the DUST Marsh

The increase in conductivity of surface-water observed during our post-storm sampling trips in March 1992 was too sharp to be explained by evaporation, suggesting that other hydrological factors may be involved. Previous hydrological studies in the marsh (Meiorin, 1986) led to the conclusion that "the influent stormwater may be subjected to a combination of plug flow and mixing, complicated by stratification and short-circuiting". The variations in salinity, as observed by conductivity measurements, may reflect differences in specific density. If low-density (low conductivity) stormwater floods the marsh surface and does not mix with preexisting, higher density marsh water, the marsh may remain vertically stratified for some time after a storm. Subsequent mixing would bring preexisting marsh water to the upper layer and explain the increase in conductivity measured in the surface water samples. In a stratified marsh, shortly after a storm, toxicity is more likely to be detected in the stormwater than in the preexisting marsh water, so there will be a vertical gradient in toxicity.

To test the latter hypothesis, we measured conductivity at various depths in the marsh before and after the March 22, 1992 storm, and exposed *C. dubia* to surface-water and depth-water samples collected after the storm. We observed very mild temperature and conductivity vertical gradients before the storm and very sharp gradients one day after the storm. Toxicity was detected in surface waters and was not detected in samples drawn from 120 cm depth. A detailed study was performed during the storm event of October 29, 1992. Clear vertical gradients in toxicity and conductivity could be seen 30 hours after the onset of the storm (Figure 6).

3.2.4 Variability in Toxicity Within Sampling Sites

To characterize the variability in toxicity within sampling sites, an altered sampling design was introduced to provide field replication. During the storm events of March 1992, we collected five field replicates, taken several meters apart from each other, at station 3, 5 and 9 and tested them separately. For logistical reasons it was not possible to use this design

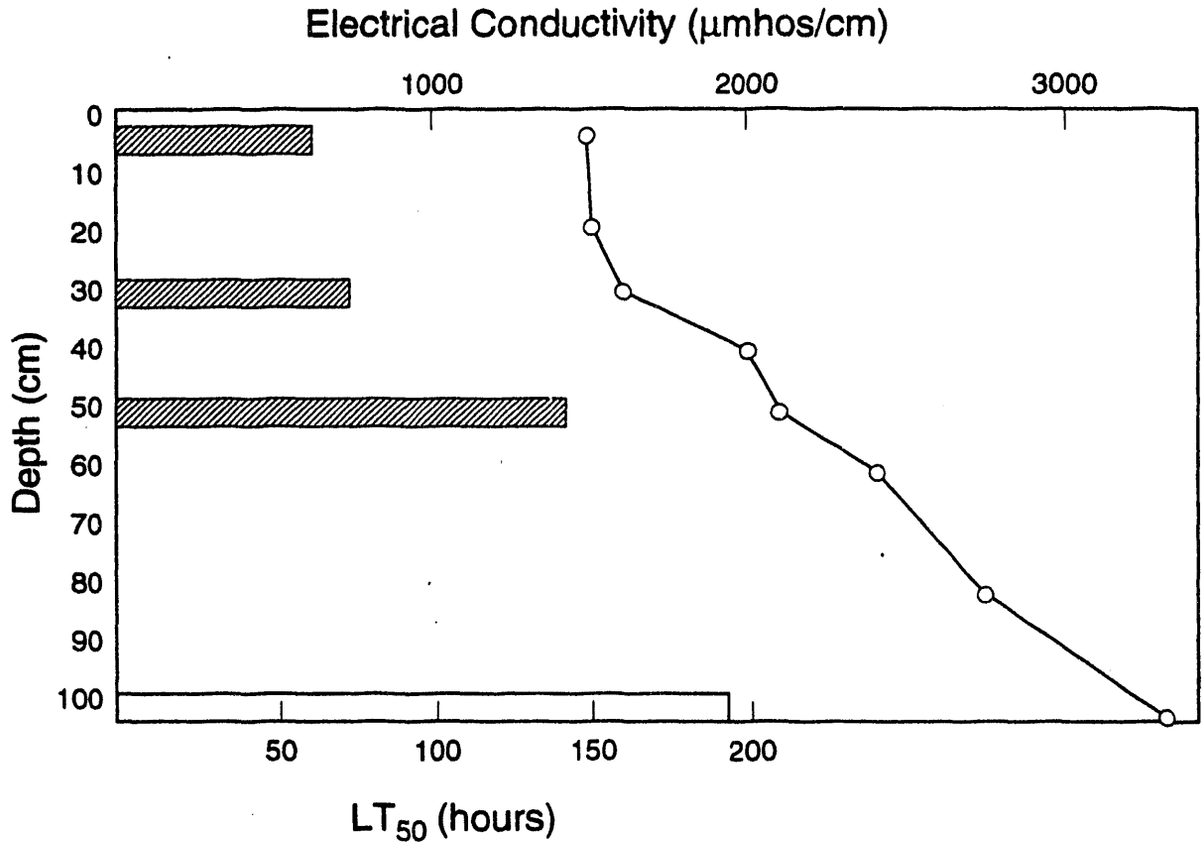


Figure 6: Vertical conductivity and toxicity profiles observed in the DUST Marsh 32 h after the storm onset.

Hollow bar, no mortality by the time indicated; checked bar, LT_{50} (Probit method); circle, electrical conductivity (μmhos/cm).

during the storm event of October 29, 1992, and the selected alternative was to pool several pump-intervals into one sample container during sampling and test these samples using four laboratory replicates (4x5 design). The LT_{50} values obtained for the separate replicates in four samples of each design are shown in Table 3. The mean of the coefficients of variation (CV), which express the variability among replicates, was not significantly different for the two designs, indicating that the predominant source of variability is the toxic response of the test animals rather than the sampling spot in the marsh.

3.3 TEMPORAL DISTRIBUTION OF TOXICITY IN THE DUST SYSTEM

To measure the intensity of toxicity as a function of time, repetitive sampling and testing were performed after storm events. Figure 7 shows *C. dubia* survival curves in various dilutions of Station 3 samples, taken 2,4,6, and 22 days after onset of the November 1991 storm. There was little flow in the creek at that period. Diminution of toxicity in the creek is obvious after six days. In the debris basin (Station 5), toxicity actually increased after 3 days and decreased after six days (see LT_{50}'s in Figure 3). This could be due to toxic water moving downstream from Station 3 to Station 5. There was no detectable toxicity in the entire system after 22 days. Table 4 presents similar results obtained after the storm of March 5, 1992. Toxicity was detected in the creek and the marsh two days after the storm onset; however, toxicity was not detected within five days in both parts of the system. There was a constant flow of water through the system during that time. The same question was addressed again after the March 14, 1992 storm and the results are presented in Figure 8. This time, flow through the system ceased 3 days after the storm, while the water was still toxic, and that water was retained in the creek and the debris basin. Four days later, no toxicity was detected in the debris basin (Station 5) nor was any detected in the creek (Station 3). The latter data indicate that dissipation of toxicity could be related to toxicity-removal processes which may take place in the creek and debris basin.

The studies conducted during the winter of 1991-92 suggested that three major factors may be responsible for the diminution of toxicity observed after storms:

TABLE 3: VARIABILITY OF LT_{50} VALUES IN FIELD REPLICATION AND LABORATORY REPLICATION DESIGNS

DESIGN		FIELD REPLICATION (5X5)				LAB REPLICATION (4X5)			
SAMPLE		9Mar6	5Mar18	5Mar23	6Mar23	D7D1	D9P1	D5.7P2	D8P2
Parameter	Rep								
LT_{50}	A	124	100	106	82	77	75	85	82
	B	133	106	91	75	82	84	88	106
	C	126	78	98	85	81	103	82	87
	D	136	78	88	82	74	104	85	128
	E	129	125	88	80				
Average		129.6	97.4	94.2	80.8	78.5	91.5	85.0	100.8
Std. Deviation		4.93	19.97	7.76	3.70	3.70	14.34	2.45	20.90
Coefficient of Variation (%)[1]		3.80	20.50	8.24	4.58	4.71	15.67	2.88	20.75

[1] Comparison of the Coefficient of Variation values obtained for the two designs by t-Test yielded t= 0.337, p=0.758. The means are not different at the 0.05 level.

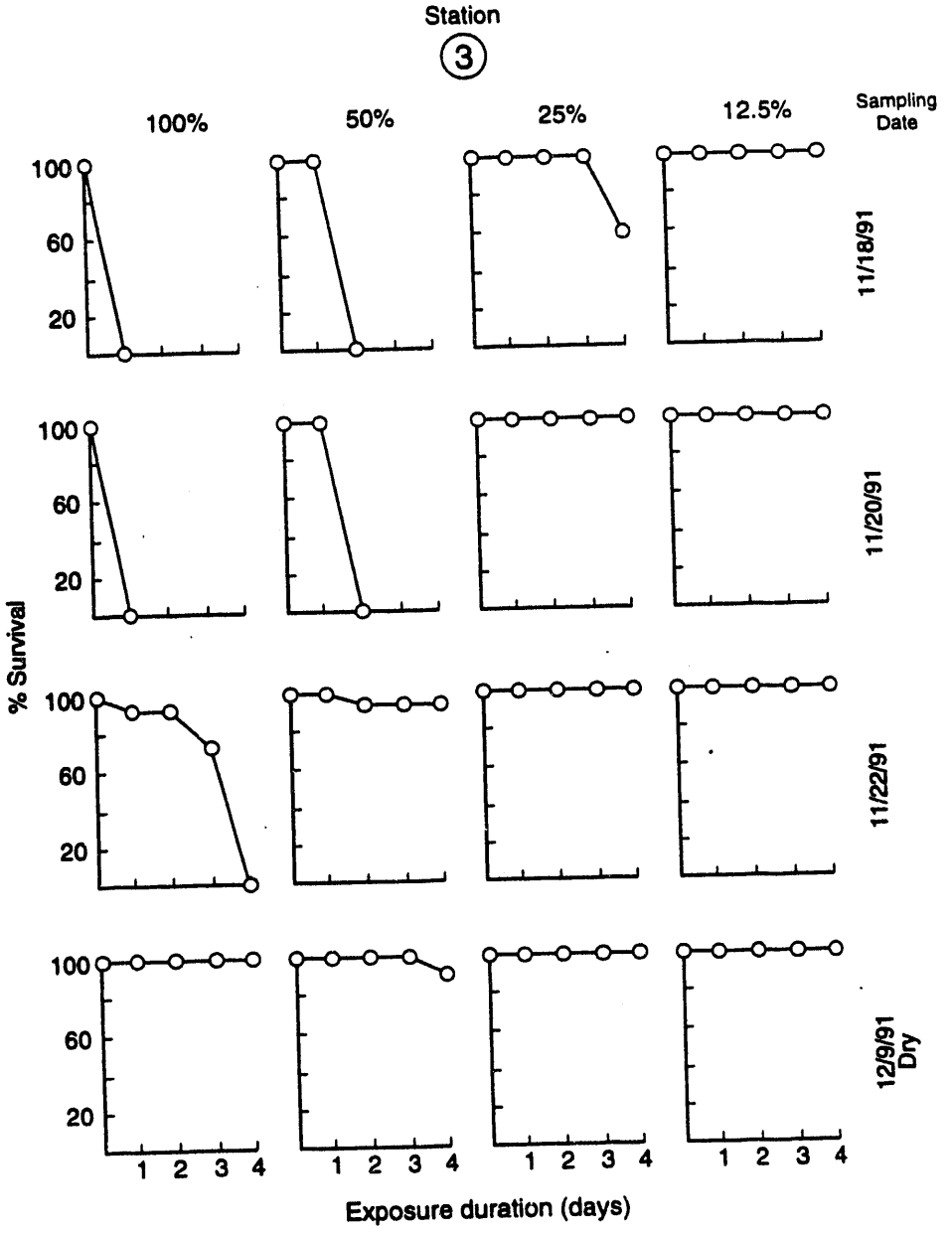


Figure 7: Survival of *Ceriodaphnia dubia* in various dilutions of samples collected at Station 3 (DUST System) after the November 1991 storm.

TABLE 4: SURVIVAL OF *CERIODAPHNIA DUBIA* AFTER SEVEN-DAY EXPOSURE TO SAMPLES TAKEN AT THE DUST SYSTEM AFTER THE MARCH 5, 1992 STORM

SAMPLING DATE	CONTROL		STATION 3		STATION 5		STATION 9	
	Lab Reps	Surv (%)	Sub Station	Surv (%)	Sub Station	Surv (%)[1]	Sub Station	Surv (%)[1]
3/6/92	A	100	3A	80	5A	80	9A	0
	B	100	3B	40	5B	60	9B	0
	C	100	3C	100	5C	60	9C	0
	D	100	3D	80	5D	60	9D	20
	E	100	3E	80	5E	60	9E	0
3/9/92	A	100	3A	100	5A	100	9A	100
	B	100	3B	80	5B	100	9B	100
	C	100	3C	100	5C	100	9C	100
	D	100	3D	100	5D	100	9D	100
	E	100	3E	100	5E	100	9E	100

[1] Survival in samples from Station 5 and Station 9 was significantly different from the control (Steel's test, $p<0.05$) for March 6, 1992. Survival in all samples collected on March 9, 1992 was not significantly different from the control.

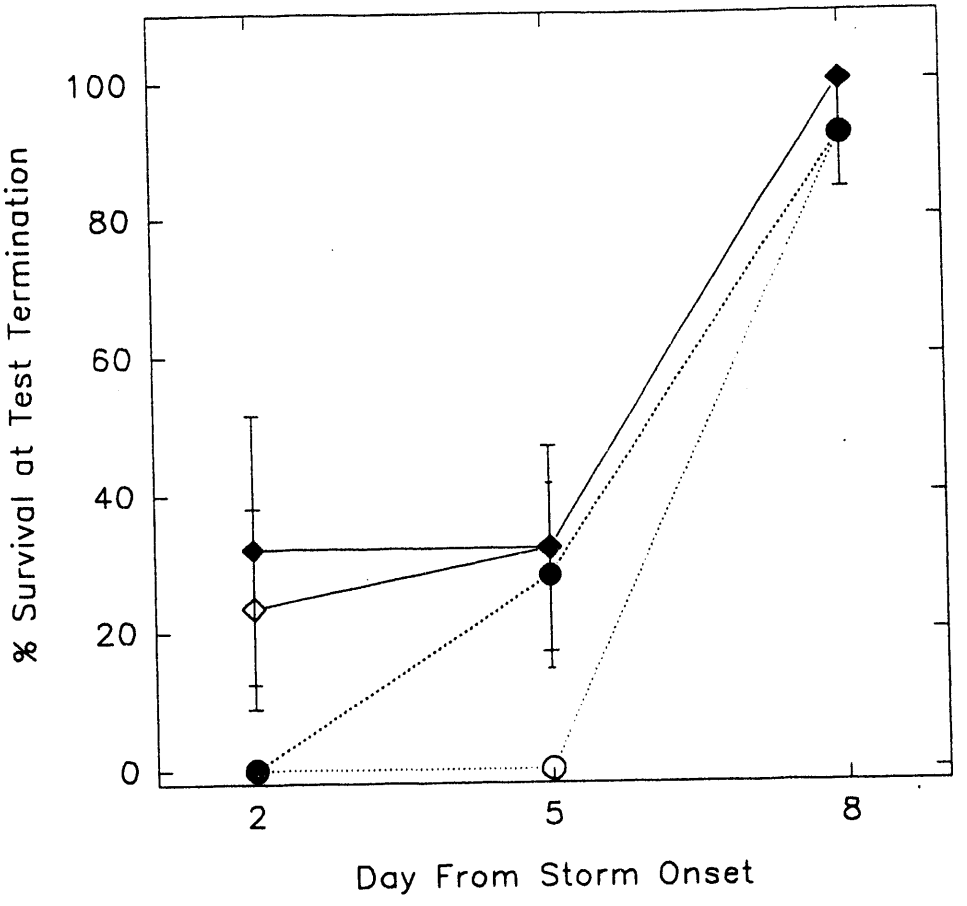


Figure 8: Survival of *Ceriodaphnia dubia* in DUST System samples taken after the storm of March 14, 1992.

Survival in all control chambers was 100% at test termination, which was after 7 days except for the test with March 15 samples. Full diamond, survival (mean and std. error) after 5-day exposure to Station 5 samples; hollow diamond, same, males excluded; full circle, survival (mean and std. error) after 7-day exposure to Station 3 samples; hollow circle, same, males excluded.

1) Flushing: Some toxic water flow out of the marsh.
2) Dilution: Toxicity diminishes when stormwater is mixed with preexisting marsh water.
3) Removal: Toxic substances are removed from the water by sequestration/sedimentation or by degradation.

To evaluate the contribution of each factor to diminution of toxicity, we monitored the flows, the water levels, the conductivity values and the toxicity intensities along the marsh before, during and after the storm of October 29, 1992 (Figure 9). A series of vertical profiles show the effects of mixing in the System C channel (Figure 10). After the onset of the storm (Time 0 in Figure 9), stormwater accumulated in the creek for several hours and the water level rose by 50 cm before the flow into the debris basin was evident. Once the level in the debris basin rose above the concrete sills, most of the stormwater went into System B (The survey was performed after the addition of a 20 cm berm over sill A to divert the flow to System B). Low-conductivity stormwater could be detected at Station 7 across the reed bed several hours later, and the peak concentration of stormwater was observed in that station 33 hours after the rain began. In Station 9, the peak water level was recorded at 32 hours, but the lowest conductivity values were measured 80 hours after the storm onset. This may indicate plug flow of preexisting marsh water towards the marsh exit. In Station 13, at the southwest corner of the DUST Marsh, peak levels and outflow velocities were recorded at 30 hours, concomitant with the lowest conductivities observed in that station. Some time between 30 and 80 hours the direction of flow at Station 13 had reversed, and water was now flowing from the Main Marsh back into the DUST Marsh.

As time passed after the storm, water levels in the marsh decreased while conductivity increased. Both of these changes could be due to loss of stormwater from the marsh, since a substantial amount of low-conductivity water was flowing out of the marsh at Station 9. However, the gradual dissipation of the vertical conductivity gradient (Figure 10) implied that mixing processes were occurring as well. The temperature profiles were quite uniform, indicating that thermal inversions, caused by the cooling of surface water at night and the "sinking" of this water to deeper layers, could contribute to mixing. Wind mixing action is not ruled out either, as the fetch in System C may be sufficient.

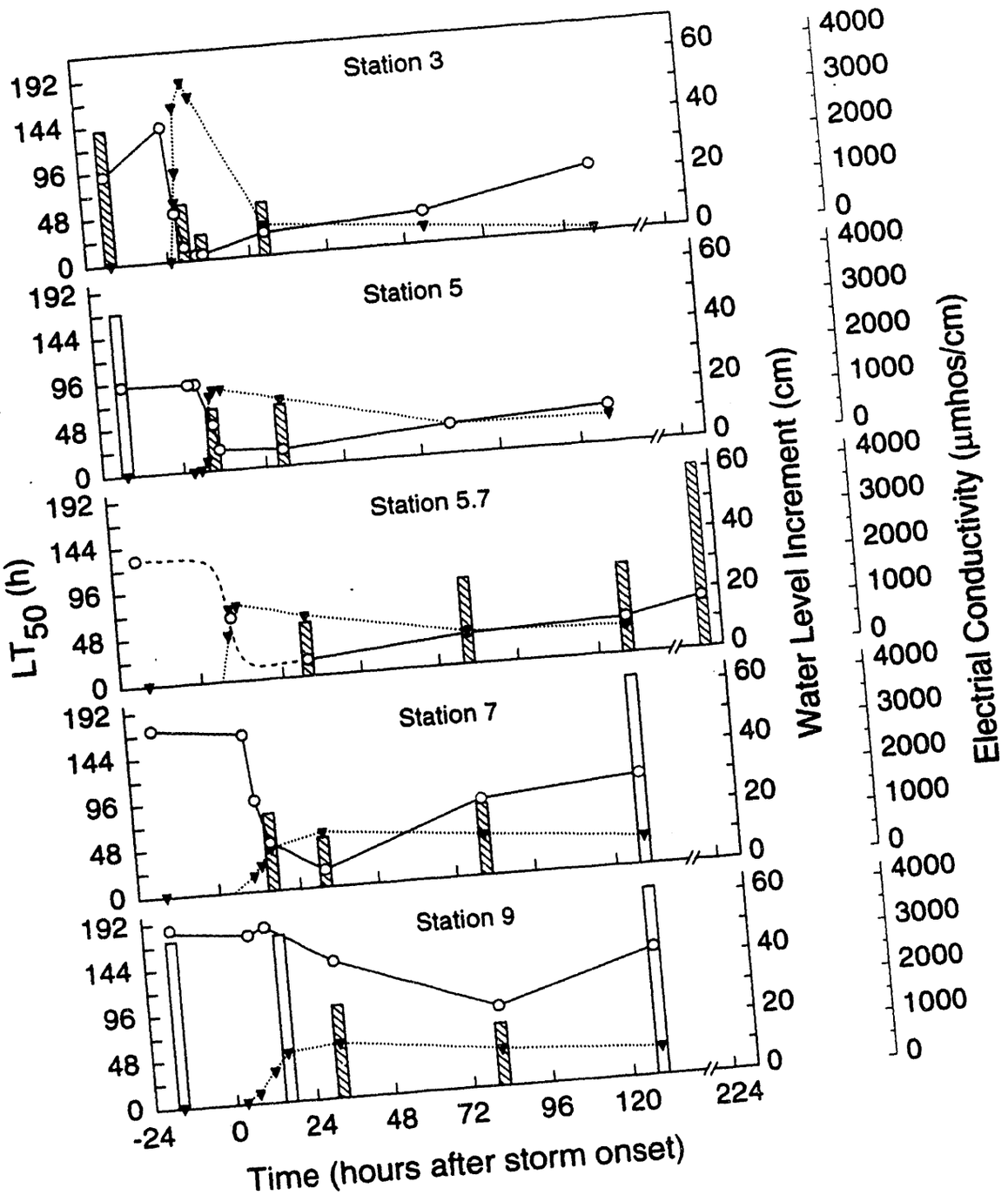


Figure 9: Spatial and temporal distribution of toxicity, conductivity and water levels during the storm of October 29, 1992.

Hollow bar, no mortality by the time indicated; checked bar, LT_{50} (Probit method); circle, electrical conductivity (µmhos/cm), inverted triangle, water level increment.

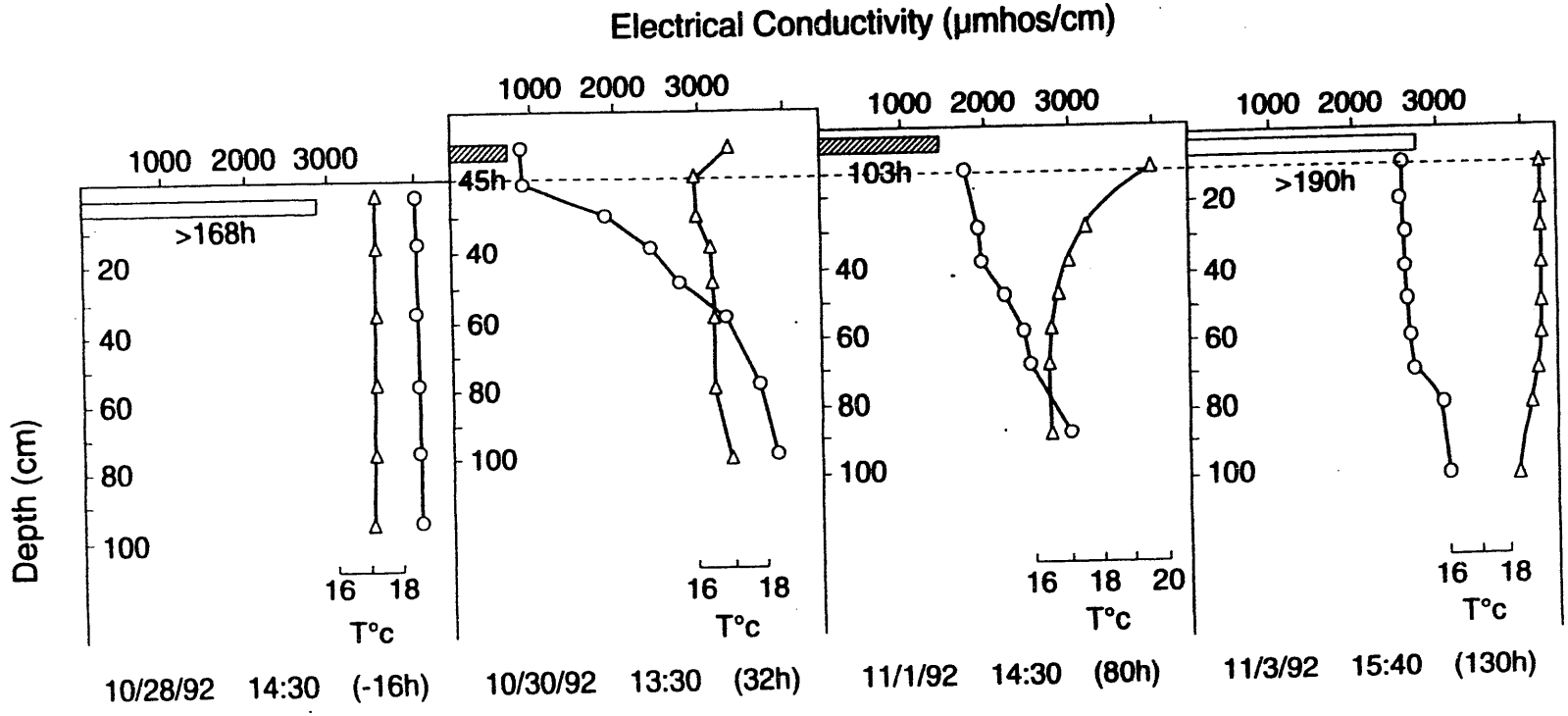


Figure 10: Vertical profiles in the DUST Marsh before, during and after a storm event.

Hollow bar, no mortality by the time indicated; checked bar, LT_{50} (Probit method); circle, electrical conductivity (µmhos/cm); triangle, temperature (°C).

Toxicity was detected throughout the marsh and was associated with low conductivity. In the main marsh waterbody, which was subject to mixing, toxicity diminished to non-detectable levels after five days. On the other hand, the reduction of toxicity in Station 5.7 was extremely slow. This station is located in the front end of System B, which became isolated as soon as the water level decreased to sill level, and the water in the resulting pond (the "Ag Pond") was not mixed.

Our interpretation of the cumulative data obtained in the DUST System over the project period is summarized schematically in Figure 11. As low-conductivity, toxic stormwater flows into the marsh, horizontal and vertical gradients in conductivity and toxicity are created. The marsh contains most of the stormwater. The mixing which occurs after the storm effectively dilutes the stormwater, rendering toxicity undetectable within several days.

3.4 TOXICITY DILUTION MODEL FOR THE DUST MARSH

To determine the extent of toxicity reduction which is due solely to dilution, a dilution experiment was conducted in the laboratory. A toxic stormwater sample collected at Station 3 was mixed, in various proportions, with non-toxic sample which was collected at Station 9 before the storm. The resulting LT_{50} values were plotted against the dilution factor, and the mathematical relationship between these variables was obtained by a curve-fit program (Figure 12). The derived formula was then used to compute the predicted LT_{50} values in samples collected in the marsh during and after the storm, based on the dilution factor of each sample as calculated from conductivity measurements (Table 5).

This preliminary model was based on the assumptions that the creek sample taken at peak flow represents the bulk of the stormwater entering the marsh, and there are no inputs of salts or non-toxic freshwater into the system. Conductivity measurements in the DUST System throughout the project period indicate that, during low flow, there are some salt inputs in the creek which influence the conductivity at Station 5 and that there are salt inputs at the western end of System C which may raise the conductivity values recorded at Station 13 and Station 9. On the other hand, with our present knowledge of the DUST

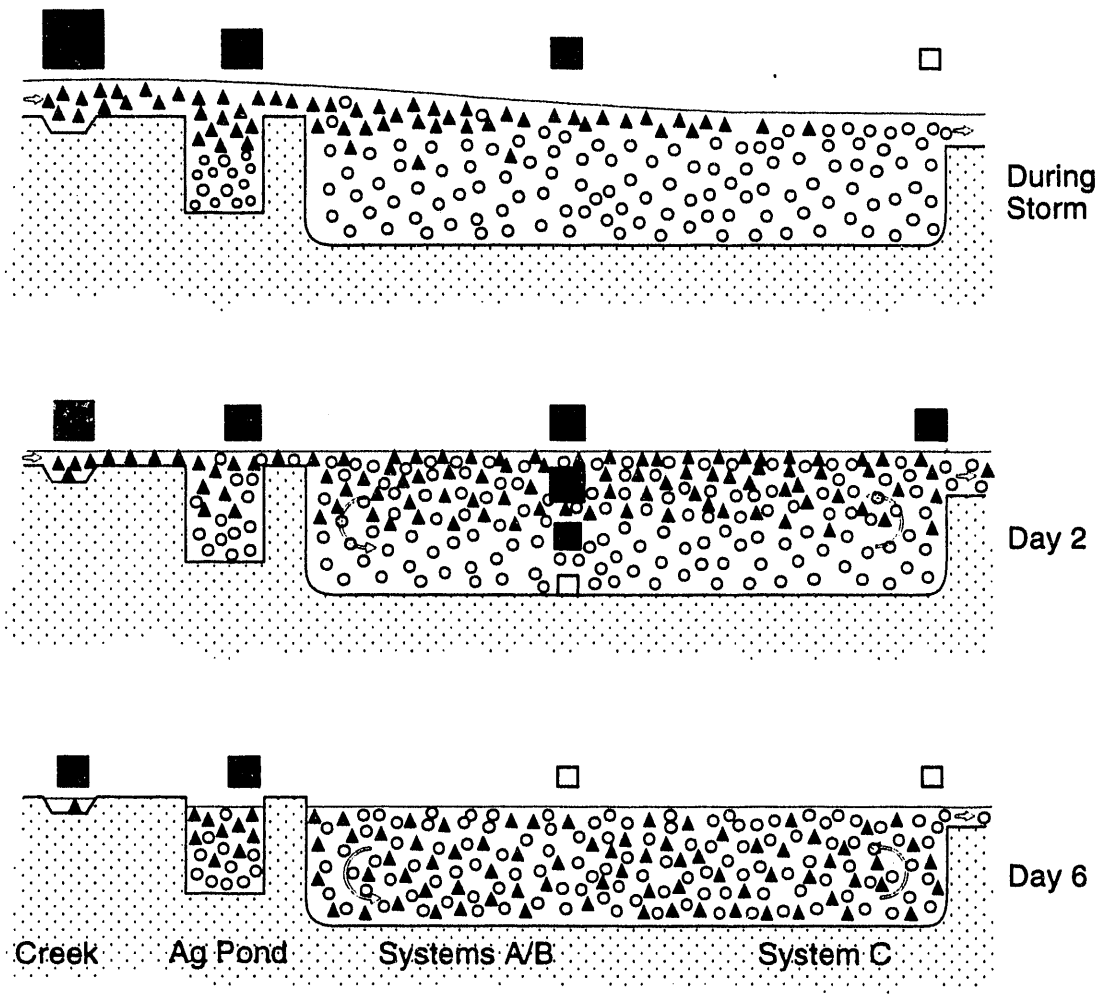


Figure 11: Schematic representation of the spatial and temporal distribution of toxicity in the DUST System.

Full triangle, stormwater; hollow circles, preexisting marsh water; hollow squares, toxicity not detected; full squares, toxicity detected (decrease in square size indicates decrease in toxicity).

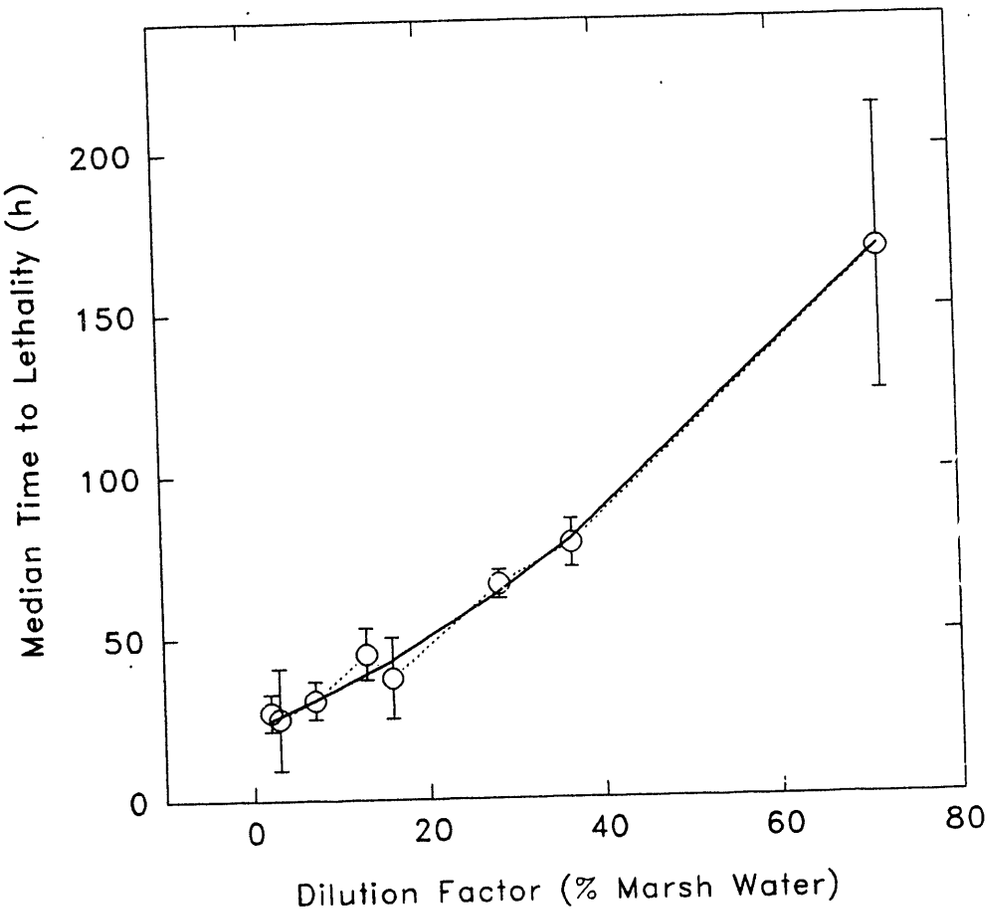


Figure 12: Dilution experiment of a toxic Crandall Creek Sample in preexisting DUST Marsh water.

Circles, LT_{50} (mean and std. error of four values, as calculated by the Probit method for each replicate). Solid line, empirical curve $y=0.01356x^2+1.044x+23.52$ fitted to data.

TABLE 5: TOXICITY DILUTION MODEL: PRELIMINARY COMPARISONS OF PREDICTED AND OBSERVED TOXICITIES IN DUST MARSH SAMPLES

STATION	TIME[1] (h)	EC (µmhos/cm)	DILUTION FACTOR[2] (% preexist. DUST water)	PREDICTED LT_{50}[3] (h)	OBSERVED LT_{50} (h)	EUGLENOIDS (cells/ml)[4]
5.7 surf	30	330	6.8	31.3	53.3	
	82	690	20.4	50.5	86.7	
	129	760	23.0	54.7	84.5	
	224	1090	35.5	77.7	184.5	4.4 ± 0.4
6 surf	32	1550	43.8	95.1	60.5 *	862 ± 228
6 30 cm		1700	48.4	105.8	72.2 *	183 ± 40
6 50 cm		2100	59.1	132.6	142.8	21 ± 5
7 surf	14	1080	26.2	60.2	78.8	
	33	390	6.8	31.2	47.6	5.4 ± 1.0
	80	1700	43.7	95.0	70.0 *	171 ± 49
	128	2050	53.5	118.2	>190	3.8 ± 1.8
8 surf	32	950	21.3	51.9	44.2 *	179 ± 52
	80	1780	43.5	94.6	101.3	19 ± 3
	130	2500	62.7	142.3	>190	5.8 ± 0.3

[1] Sample collected at the specified time after the storm onset (Beginning of rain, Fremont)

[2] The dilution factor (i.e. fraction of DUST water in each sample) was calculated using conductivity values recorded in that station before the storm (Figure 9) and in the sample as collected after the storm.

[3] Predicted LT_{50} was calculated for each sample according to its dilution factor, using the mathematical relationship obtained in Figure 12.

[4] Numbers indicate density of euglenoids in the sample used for the bioassay.

Marsh it is reasonable to believe that for the other stations the conductivity values recorded reflect the actual dilution. In most of the DUST samples from Stations 5.7, 6, 7, and 8 the predicted LT_{50} values fell below the observed values, meaning that the samples are less toxic than would be anticipated due to dilution only (Table 5). The difference is wider in samples collected 80 and 130 hours after the storm onset and may indicate that toxicity attenuation factors are indeed taking place in the DUST Marsh. However, surprisingly small LT_{50} values were observed with some samples. Microscopic observations revealed that unicellular algae of the Euglena taxon were abundant in these samples. Cell counts indicate that these samples contain 170 cells/ml or more, while the other samples counted contained less then 25 euglenoid cells/ml (Table 5). It is conceivable that the presence of algae in high densities created unfavorable conditions in the test chambers, and the stress upon the cladocerans acted synergistically with the toxic substances, reducing their survival period. It is advisable that biotic factors, such as algal blooms in the marsh, be incorporated into the model.

3.4 PHYSICAL AND CHEMICAL PROPERTIES OF DUST MARSH WATER

Electrical conductivity values measures in the DUST system were variable, responding to stormwater inputs and to mixing action. The alkalinity and hardness values correlated with conductivity ($r = 0.81$ and $r = 0.97$, respectively). For 1800-2000 μmhos/cm samples, alkalinity values approximated 250 mg/l and hardness ranged from 450 to 550 mg/l (as $CaCO_3$). Dissolved oxygen values, as measured during the early afternoon hours, ranged between 8-11 mg/l at the surface and 4-6 mg/l at 100 cm depth. Super-saturation concentrations of oxygen, as well as high pH values, are probably due to photosynthetic activity. The pH values of most DUST Marsh samples ranged between 7.8 and 8.2, with a slight decrease observed as holding time proceeded, but during algal blooms (Fall 1992) pH values of up to 9.3 were recorded in some samples. The buffer capacity of DUST samples, however, was very low, as was evident after incubation with *C. dubia* fed with photosynthetically-active *Selenastrum* cultures. pH values of 9.0 were measured in some test chambers, and this was a cause for concern when ammonia was present. Therefore, in samples with initial pH values

above 8.5, the pH was adjusted to 7.5 prior to test solution renewal in the bioassay. Total ammonia measured in fresh DUST samples during March 1992 and October 1992 ranged between 0-0.5 mg/l, but after incubation with animals and food ammonia concentration could rise to 1 mg/l due to ammonification and excretion. Total suspended solids (TSS) were in the range of 27-34 mg/l in DUST samples collected one day after the March 5, 1992 storm, and dropped to 11-13 mg/l in samples collected 3 days later. TSS as high as 60 mg/l were found at Station 5 after the March 22, 1992 storm. Metal concentrations in the DUST Marsh did not reveal significant differences between wet weather and dry weather samples. Copper and lead concentrations ranged between 1-15 μg/l, mostly in the particulate fraction, and zinc concentrations were in the range of 5-60 μg/l (WCC 1991c, WCC 1993).

The nature of the toxic substances in a DUST sample representing the peak stormwater flow during the storm of October 29, 1992 can be inferred from TIE efforts conducted by Woodward Clyde Consultants (WCC 1993). Starting with baseline toxicity of LT_{50} of 25 hours, passage through the C-18 columns or aeration with air rendered the sample nontoxic inasmuch as no *C. dubia* mortalities were observed after exposure for 96 hours. Filtration did not remove any toxicity. After aeration with nitrogen gas, toxicity was somewhat reduced (to LT_{50} of 71 hours). Following pH adjustment manipulations, the LT_{50} value was shifted to 73 hours for pH 11 and 83 hours for pH 3. EDTA at non-toxic concentrations shifted the median time to lethality to 30 hrs, indicating that metals were not an important cause of toxicity in the sample. Rather, the toxic substance(s) were oxidizable, non-polar organic molecules.

Ancillary tests conducted in our laboratory with stormwater samples yielded two major findings. First, toxicity was very persistent in low-TSS samples held refrigerated in the laboratory. For instance, the sample collected on March 15, 1992 at Station 3 was used in tests started 1,2,6, and 25 days after collection, and respective LT_{50}'s of 36, 48, 48 and 84 hours were obtained. A sample collected in the Crandall Creek after the October 1991 storm was still toxic after 167 days. In contrast, toxicity was rapidly lost in the high-TSS

sample of March 23, 1992. Second, removal of particulate matter by filtration to 0.45 μm or 1.2 μm did not diminish toxicity in the four DUST samples, one Arrowhead sample, and additional rooftop and street samples which were tested. There was no toxicity associated with particulate matter which was removed from the toxic Station 3 sample of March 15, 1992 by centrifugation and resuspended in control water.

Chapter 4

DISCUSSION

In our preliminary attempts to detect toxicity in waters receiving urban runoff we studied two types of hydrological systems. The first system is the Crandall Creek and DUST Marsh (DUST System), in which urban runoff is retained within a small freshwater marsh for some time after the storm. The second is the Arrowhead system, where stormwater is released directly into San Leandro Bay which is flushed twice daily by the tide. Toxicity was detected in creeks of both systems after storms. However, no toxicity was detected in Arrowhead marsh itself, whereas in the DUST system toxicity could be clearly demonstrated in marsh waters. Thus, the DUST System was an excellent site for the study of the distribution and fate of toxic substances in receiving waters.

The results of our toxicity screening tests in the two types of hydrological systems indicated that *Ceriodaphnia dubia* is the preferable test organism for evaluating the effects of stormwater generated in the watersheds which we have sampled. This conclusion is supported by numerous stormwater toxicity tests conducted in residential, commercial and industrial areas during 1989-1993 in Santa Clara County and Alameda County (WCC 1989, 1991a, 1991c, 1993), in which the incidence of response of *C. dubia* was much higher than that of fathead minnows *(Pimephales promelas)* or *Selenastrum capricornutum*. In our study, the same pattern was observed. In fact, the response of the algae was inverse to that of *C. dubia*: growth was enhanced in toxic creek stations and inhibited in marsh stations. The algae may have been affected by "antibiotics" secreted by marsh biota, or growth-limited as a result of trace-element deficiency due to chelating agents which were probably not carried in with stormwater. Interpretation of algal bioassays requires information on nutrients and element concentration and availability (Miller *et al* 1978), which was beyond the scope of the present study. Nevertheless, the conclusion that stormwater collected at Crandall Creek was not toxic to *S. capricornutum* is valid. As for marine test species (Arrowhead survey),

we saw significant toxic effects on *Mysidopsis bahia* juveniles and on echinoderm *(Strongylocentrotus purpuratus)* gametes in the creek samples (which were acutely toxic to *C. dubia*), while *Menidia beryllina* larvae and mollusc *(Mytilus edulis)* embryos were not affected.

Local cladocerans and copepods, collected in the DUST Marsh and in the Main Marsh of the park, were not affected by DUST System samples which caused mortality in *C. dubia.* In this respect, the laboratory test organism may reflect the fate of sensitive organisms no longer existing in the DUST System. For the purpose of measuring and comparing toxicity levels in one system, the use of a single, responsive test organism seemed justified (Doherty 1983). Consequently, *Ceriodaphnia dubia* bioassays became the major research tool in the DUST Marsh.

The most useful unit to describe the intensity of toxicity was the median time to lethality (LT_{50}). This variable could be calculated from mortality data derived by any test design and by any length of test up to 168 hours. It enabled us to compare results of tests over a wide range of toxicity "intensities". Attempts at running dilution series and calculating 48h LC_{50} for very toxic samples produced results which could not be compared with most other tests. Subsequently, effort was put into frequent observations of animals in ambient samples without dilution, especially during the first 48 h of exposure; this increased the resolution power of LT_{50}'s in very toxic samples.

Reproduction in *C. dubia* did not seem to be adversely affected by DUST System samples, even in toxic samples where mortality occurred later in the test. In some samples, reproduction was actually enhanced in comparison to controls. Moreover, we found a multigeneration drift in the numbers of healthy offsprings in the controls. For all these reasons we do not believe that reproduction analysis can detect chronic toxicity in the DUST System.

Stormwater toxicity was introduced into the DUST System throughout the wet weather season of 1991-1992 and during the first storm of the following winter. Correlation with

conductivity indicates that Crandall Creek inputs were the dominant source of toxicity as detected in the first storms of each season. However, Crandall Creek may not be the only source of toxic runoff in the DUST System. In subsequent storms we saw patterns in which toxicity was "centered" in other stations along the marsh axis. Assuming that this does not reflect a moving peak of toxicity (plug flow), it is conceivable that the p-Line runoff, entering the marsh through the southwest culverts, could have introduced toxicity at Station 9 in the March 5, 1992 event, and that agricultural drainage, pumped into System B, could be the source of toxicity found in March 23, 1992.

Electrical conductivity (EC) was a convenient tool to trace the stormwater as it flows through the marsh, and provided a measure of the extent of mixing that has occurred. Measurements of conductivity and temperature along vertical profiles of the DUST marsh revealed that the marsh was stratified after storms; this stratification was also evident in the intensity of toxicity. Furthermore, we found very good correlation between EC values and LT_{50} in samples collected within 30 hours of the storm. This led to the construction of a preliminary toxicity dilution model which allowed the calculation of predicted LT_{50} in marsh stations based on the observed conductivity. In most of the samples, the predicted LT_{50} values were less than the observed values; this means that these marsh samples were less toxic than predicted according to dilution only, and could imply that toxicity removal processes were taking place. However, in some samples we observed toxicity which was more intense than the predicted toxicity, and microscopic observations suggested that some biotic factors, namely populations of euglenoids and/or flagellates, may be correlated with enhanced toxicity.

The intensity of toxicity in the Crandall Creek and the DUST Marsh diminished as time passed after the storms, as observed after four storm events. This could be related to three main performance questions:

1. **Does the DUST marsh contain runoff toxicity?** Measurements show that in most storm events some toxic water flows out of the marsh through the exit culvert. The marsh may remain stratified for several days after the storm, and it is mainly surface (low conductivity, toxic) water that flows out. The proportion of toxic stormwater

contained in the marsh depends on the volume of stormwater inputs, the water level in the marsh (which determines the outflow rate), and the wind conditions during the days following the storm.

2. Does the marsh dilute toxicity? Direct reduction in toxicity occurs as toxic stormwater is mixed with preexisting non-toxic marsh water. This toxicity-reduction mechanism /process is not as rapid as it could be, due to the stratification of the waterbody which limits the extent of dilution. Subsequent mixing and dissipation of vertical gradients contribute to dilution of toxicity.

3. Does the marsh treat toxicity? In samples collected several days after storms, toxicity was reduced to non-detectable levels or to levels much lower than those predicted according to dilution only. Toxic substances may be inactivated by factors present in the marsh. They may also be removed from the water by volatilization, sequestration and/or sedimentation, or by degradation.

Since the purpose of the DUST system is to reduce toxicity inputs into the San Francisco Bay, efforts to contain as much runoff as possible within the marsh are recommended. To protect the marsh biota from the impact of toxic runoff, enhancing the mixing processes in the marsh is also recommended. In addition, mixing with marsh water seems to be an important factor in facilitation of processes which remove toxicity: The reduction of toxicity within the isolated portion of System B (Ag Pond), as observed after the storm event of October 29, 1992, was much slower than in the other marsh stations, and conductivity values indicated that very little mixing occurred in this pond. To allow sufficient time for the mixing and toxicity removal processes to occur, containment of stormwater within the marsh for several days after each storm is recommended.

Phase I Toxicity Identification Evaluations (TIE) tests performed with a sample collected in Crandall Creek during the October 29, 1992 storm showed that toxicity was removed by the C-18 columns and by aeration with air, indicating that oxidizable non-polar organic molecules could be the substances causing toxicity in *C. dubia*. Toxicity was reduced very slightly by chelation with EDTA, indicating that only a small portion of the toxicity observed may be attributed to metals. In this TIE effort and in all seven runoff samples studied at

LBL, toxicity seemed to be associated with the soluble fraction of the sample, rather than with particles. If indeed the major toxicants entering the DUST Marsh are organic molecules, there is hope that the system will be able to degrade, rather than accumulate, these pollutants.

The extensive body of toxicity data collected in the DUST system has been used to evaluate the performance of this system as a stormwater treatment facility. The general approach developed in this project can be easily applied to evaluating the potential performance of urban runoff treatment marshes. The DUST Marsh study also highlights the importance of integrating engineering design with toxicity monitoring. For example, engineering guidelines for construction of treatment marshes emphasize sedimentation processes and advocate structures which will minimize mixing. Whereas this approach provides optimal removal of particulates, our toxicity study indicates that addition of a mixing phase may facilitate the removal or attenuation of soluble pollutants. In conclusion, marsh design and management practices may benefit from toxicity studies in ways that could not have been accomplished by chemical testing and hydrological studies alone.

ACKNOWLEDGEMENTS

The project was conducted as a joint effort between the Lawrence Berkeley Laboratory (LBL), the San Francisco Bay Regional Water Quality Control Board (SFBRWQCB), and Woodward-Clyde Consultants (WCC). We would like to thank our colleagues at LBL, Gillian Wild, Walter Sadinski and Pamela Reynolds, for their help and support. The coordination efforts and sampling assistance, which were provided by Michael Carlin, Karen Taberski and Tom Mumley of the San Francisco Bay Regional Water Quality Control Board, are greatly appreciated. We are grateful to Richard Wetzig of the Alameda County Public Works Agency for his cooperation and help with the field work. The contribution of Chow Lee, Terry Cooke, Mike Paquet, Bill Martin and Francesca Demgen of Woodward-Clyde Consultants, who provided valuable information and data on the DUST System and participated in the sampling efforts is greatly appreciated.

REFERENCES

American Society for Testing and Materials. 1987. Guide for conducting static acute toxicity tests with embryos of four species of saltwater bivalve molluscs. In *Committee E-47, American Society for Testing and Materials Standards.* Document E 724-80. Philadelphia, PA.

Anderson, S.L., E. Hoffmann, D. Stewart and **J. Harte.** 1990. Ambient toxicity characterization of San Francisco Bay and adjacent wetland ecosystems. Lawrence Berkeley Laboratory, Report # 29579.

Anderson, S.L., M.P. Carlin and **A.L. Suer.** 1991. Effluent and ambient toxicity programs in the San Francisco Bay region. *Can. Tech. Rept. Fish. Aquat. Sci.* **1**:152-164.

Brennan, K.M. 1985. Effects of wastewater on wetland animal communities. In P.J Godfrey, E.R. Kaynor, S. Pelczarski and J. Benforado, eds. *Ecological Considerations in Wetlands Treatment of Municipal Wastewaters.* Van Nostrand Reinhold Co., New York, NY.

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

Dinnel, P.A., J.M. Link and **Q.J. Stober.** 1987. Improved methodology for a sea urchin sperm cell bioassay for marine water. *Arch. Environ. Contam. Toxicol.* **16**:23-32.

Doherty, F.G. 1983. Interspecies correlations of acute aquatic median lethal concentration for four standard testing species. *Environ. Sci. Technol.* **17**:661-665.

Hammer, D.A. 1990. *Constructed Wetlands for Wastewater Treatment. Municipal, Industrial and Agricultural.* Lewis Publishers Inc., Chelsea, MI.

Johnson, B.T. 1986. Potential impact of selected agricultural chemical contaminants on a northern prairie wetland: A microcosm evaluation. *Environ. Toxicol. Chem.* 5:473-485.

Lee, C.R., B.L. Folsom and **R.M. Engler.** 1982. Availability and plant uptake of heavy metals from contaminated dredged material placed in flooded and upland disposal environments. *Environment International* 7:65-71.

Meiorin, E.C. 1986. Demonstration of urban stormwater treatment at Coyote Hills Marsh. Report by Association of Bay Area Goverments (ABAG), Oakland CA.

Miller, W.E., J.C. Green and **T. Shiroyama.** 1978. The *Selenastrum capricornutum* Prinz algal assay bottle test. EPA 600/9-78-018. Corvallis, OR.

U.S. Environmental Protection Agency (USEPA). 1985. Methods for measuring the acute toxicity of effluents to freshwater and marine organisms, 3rd ed. EPA-600/4-85-013. Cincinnati, OH.

U.S. Environmental Protection Agency (USEPA). 1988. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms. EPA-600/4-87-028. Cincinnati, OH.

U.S. Environmental Protection Agency (USEPA). 1988b. Methods for aquatic toxicity identification evaluations: Phase I toxicity characterization procedures. EPA-600/3-88-034. Duluth, MN.

U.S. Environmental Protection Agency (USEPA). 1989. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms, 2nd ed. EPA-600/4-89-001. Cincinnati, OH.

Winner, R.W. 1989. Multigeneration life-span tests of the nutritional adequacy of several diets and culture waters for *Ceriodaphnia dubia*. *Environ. Toxicol. Chem.* **8**:513-520.

Woodward, D.F., E. Snyder-Conn, R.G. Riley and **T.R. Garland.** 1988. Drilling fluids and the arctic tundra of Alaska: Assessing contamination of wetlands habitat and the toxicity to aquatic invertebrates and fish. *Arch. Environ. Contam. Toxicol.* **17**:683-697.

Woodward-Clyde Consultants (WCC). 1989. Santa Clara Valley Nonpoint Source Study, Volume 1: Loads Assessment Report. Report prepared for the Santa Clara Water District. Submitted to the San Francisco Bay Regional Water Quality Control Board, Oakland CA.

Woodward-Clyde Consultants (WCC). 1991a. Santa Clara Valley Nonpoint Source Study. Loads Assessment Report. Report prepared for the Santa Clara Water District. Submitted to the San Francisco Bay Regional Water Quality Control Board, Oakland CA.

Woodward-Clyde Consultants (WCC). 1991b. Sediment and storm runoff concentrations of copper, zinc and lead in the Crandall Creek-DUST Marsh system. Report prepared for County of Alameda Public Works Agency, Hayward CA. Submitted to the San Francisco Bay Regional Water Quality Control Board, Oakland CA.

Woodward-Clyde Consultants (WCC). 1991c. Loads Assessment Summary Report. Report prepared for Alameda County Flood Control and Water Conservation District, Hayward CA. Submitted to the San Francisco Bay Regional Water Quality Control Board, Oakland CA.

Woodward-Clyde Consultants (WCC). 1993. Annual Monitoring Report. Report prepared for Alameda County Urban Runoff Clean Water Program, Hayward CA. Submitted to the San Francisco Bay Regional Water Quality Control Board, Oakland CA. (in preparation).

APPENDICES

APPENDIX A: DRY WEATHER MARSH STUDIES

A.1 FAIRFIELD-SUISUN SURVEY

A.1.1 Survey Design and Field Conditions

The goal of this survey was to evaluate the potential for ambient toxicity in Suisun Marsh adjacent to areas of human influence. The survey included two of the sloughs which flow into Suisun Marsh. The first, Boynton Slough, receives effluent from the Fairfield-Suisun Sanitary District (FSSD), either directly via a discharge pipe opening into it, or indirectly after land application. The second, Hill Slough, receives sporadic runoff from Travis Air Force Base (TAFB). Sites were selected to encompass freshwater inputs from both sloughs into Suisun Slough, and the sampling stations are depicted in Figure A-1. The two major creeks leading to Hill Slough were sampled at Station 1 (Union Creek at Hwy 12) and Station 3 (McCoy Creek at Hwy 12). There was no visible flow from TAFB, upstream Union Creek during the survey. Station 2 was in Hill Slough at Grizzly Island Road, and Station 4 at the mouth of Hill Slough at Suisun Slough. Stations 5, 6, 7, and 8 were located along Boynton Slough, from the railroad tracks (5) to the mouth at Suisun Slough. Station 9 samples were the pre-chlorinated effluent of the FSSD treatment plant.

The methodology for sampling and toxicity testing follows that of the attached report, with specific details as added below. The bioassay organisms used were minnows, echinoderms and water fleas (*Menidia Beryllina, Strongylocentrotus purpuratus,* and *Ceriodaphnia dubia*). Sampling was carried out on September 23, September 25, and September 27, 1991, about 2 hours before low tide. Samples were drawn with a diaphragm pump fitted with Bev-A-Line brand tubing and transferred through a 37 um net into cubitainers. Stations 1,2 and 3 were sampled by foot and the others by boat; the latter were taken from the leeward side of the boat to avoid contamination from the engine. For the seven-day toxicity tests, each sample was used for two or three renewals, thus being held no more than 72 h. Adult sea urchins were obtained from Marinus (Los Angeles, CA) for the Fairfield-Suisun survey, and samples collected on September 25, 1991 were used for the fertilization test 24 hours later.

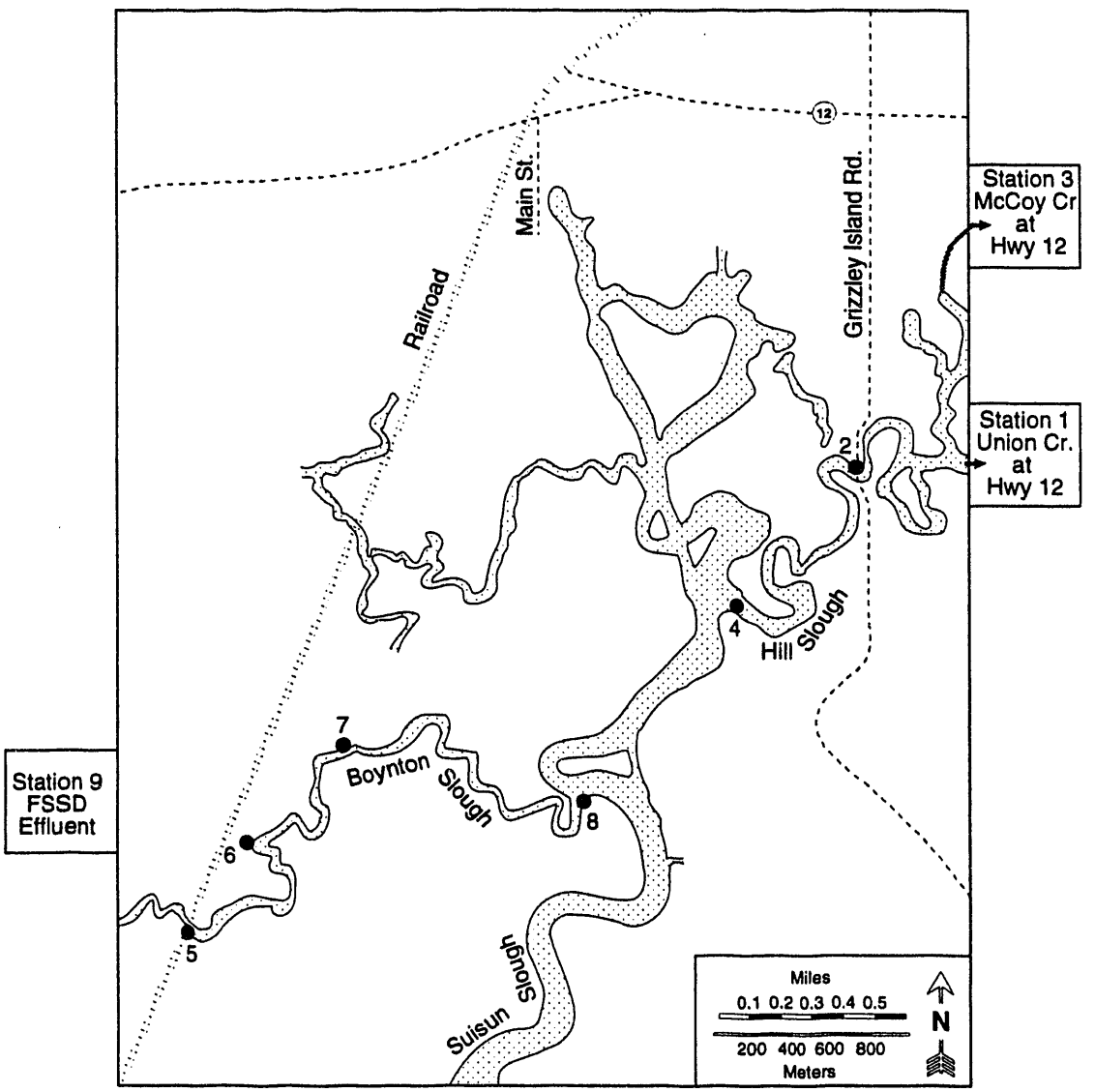


Figure A-1: Sampling Sites for the Fairfield-Suisun Survey.

A.1.2 Results and Discussion

A variety of responses was observed in the bioassays with the three species tested (Table A-1). Silverside minnow larvae survived in all samples but their growth seemed to be slightly but significantly impaired in samples 2,3, and 5. Echinoderm fertilization was not inhibited in samples derived from Hill Slough. Within the Boynton Slough system, on the other hand, all samples inhibited fertilization to some degree or another. There was one biotic factor, a planktonic diatom with long extensions which was present in the 37-um-filtered samples 5-8, and was seen to entangle sperm cells and interfere with their free movement under the microscope. This diatom was not detected in the Hill Slough samples. The water flea *Ceriodaphnia dubia* was not significantly affected by any of the freshwater samples tested. In the FSSD effluent, only echinoderm fertilization was significantly inhibited. In previous monitoring of FSSD effluents over 6 sampling events during July 1989 to March 1990 (ref), no mortality of minnows or water fleas was observed. *C. dubia* reproduction was not affected either, but *Menidia beryllina* growth was significantly impaired in one event.

The results of our Fairfield-Suisun survey indicate that during dry weather there is no acute toxicity in the sites tested, but there could be chronic effects associated with effluent inputs. Some biotic factors, which may be related to human activity (e.g. algal blooms due to nutrient inputs), could have interfered with our toxicity bioassays.

A.2 SAN PABLO BAY SURVEY

A.2.1 Survey Design and Field Conditions

This survey was designed to monitor the potential for toxicity in some of the major inputs of freshwater and effluent into San Pablo Bay. Significantly, almost nothing has been done to characterize potential toxicity inputs to the Bay in this region. The survey was carried out in January 1992 after a dry spell of 2 weeks, during a period of considerable flow of "non-runoff" freshwater. This was also a period in which most sewage treatment plants discharge

TABLE A-1: TOXICITY OBSERVED IN SAMPLES COLLECTED DURING THE FAIRFIELD-SUISUN SURVEY (SEPTEMBER 23-27, 1991)

STATION		SAL[2]	MENIDIA			SEA URCHIN		CERIODAPHNIA		
No.[1]	Location	(ppt)	Mean Surv (%)	Mean Wt (mg)	Amb (%)	Fert (%)	Amb (%)	Survival (%)	Mean Repro[3]	Amb (%)
1 MF27	Union Creek	0.4	100	1.09	93	85	74	100	30.7	100
2 MF26	Hill Slough bridge	8	100	1.05[6]	100	96	78			
3 MF25	McCoy Creek	9	100	1.02[6]	100	93	78			
4 MF24	Hill Slough mouth	8	100	1.15	100	85	78			
5 MF14	Boynton Slough railroad (C-2)	2.5	100	1.00[6]	95	59[5]	75	100	27.3	50
6 MF10	Boynton Slough discharge(C-1)	4	100	1.28	96	68[4]	75			
7 MF11	Boynton Slough (C-3)	6	100	1.20	97	46[5]	77			
8 MF12	Boynton Slough mouth (C-4)	8	95	1.17	100	72[4]	78			
9	FSSD Effluent	0.5	100	1.16	93	56[5]	74	100	18.3	100
	DILUTION CONTROL					96	100	100	22.7	100
	BRINE CONTROL	9	100	1.25	93	81	78			

[1] Lower number represents Station Code designated by the Regional Water Quality Control Board.
[2] Numbers entered for the control indicate test salinity for Menidia. Urchin test was run at 30 ppt. Salinity adjustment with brine resulted in sample dilution to the ambient concentration specified under Amb (%). In the Ceriodaphnia test sample 5 was diluted to 50% ambient in control water.
[3] Reproduction endpoint: Average number of offsprings per female.
[4] Significantly lower than Dilution Control only.
[5] Significantly lower than both Dilution and Brine Controls.
[6] Significantly lower than Brine Control.

treated effluent directly into rivers, rather than diverting effluents for land application. The sampling sites around San Pablo Bay were selected to allow characterization of the Las Gallinas SD, the Petaluma River area and the Napa River area (Figure A-2). Station 1 was located in Miller Creek, upstream, at Las Gallinas Ave. corner of Roundtree Blvd. There was a considerable flow of low-conductivity (280-370 umhos) water. Station 2: Miller Creek at bridge, 50 meters upstream of Las Gallinas Valley Sanitary District (LGVSD) discharge point (Freshwater to brackish, tidally influenced). Station 3: Discharge point of LGVSD effluent in Miller Creek. Station 4: McInnis Park, 100 meters east of entrance gate, dock into Gallinas Creek (Brackish, tidally influenced slough). Station 5 was at Pacheco Pond, on Bel Marin Keys Blvd. about 150 meters east of Galli Drive corner. (Freshwater, possibly runoff). Station 6 was at the mouth of Petaluma River, from boat ramp on south bank under Hwy 37 bridge (brackish to seawater, tidally influenced). Station 7: Petaluma River, upstream, dock at Papa's Taverna on Lakeville Road 6 miles north of Hwy 37 junction (Brackish, tidally influenced). This station is about 2 miles downstream of the winter-discharge point of the City of Petaluma Water Pollution Control Plant, in which treated effluent, after prolonged detention in maturation ponds, flows into the river. Station 8 was on Napa River, upstream, at boat ramp at the end of Cuttings Wharf Road (Brackish, tidally influenced). This station is about 1.5 miles downstream of Napa Sanitary District WWTP effluent discharge point. Station 9: Napa River, mouth, boat ramp off Curtola Pkwy 0.2 miles west of Sonoma Blvd. in Vallejo (Brackish to seawater).

Sampling and toxicity testing were conducted as described in the attached report, with additional specifications supplied below. Toxicity test species included *Menidia beryllina, Pimephales promelas, Strongylocentrotus purpuratus, Mysidopsis bahia* and *Ceriodaphnia dubia.* Samples were collected from docks or bridges on January 21, January 23, and January 25, 1992, within 2 hours of low tide. Grab sampling, using a plastic container, was combined with filtration through a 37 μm net. Seven-days old Mysids were supplied by Aquatic Indicators (St. Augustine, FL). With a few exceptions (see Results) each sample was used for two or three renewals, thus being held no more than 72 h. Adult sea urchins were obtained from the Bodega Marine Laboratory (Bodega Bay, CA), and samples collected on January 23, 1992 were used for the fertilization and development tests 24 hours later.

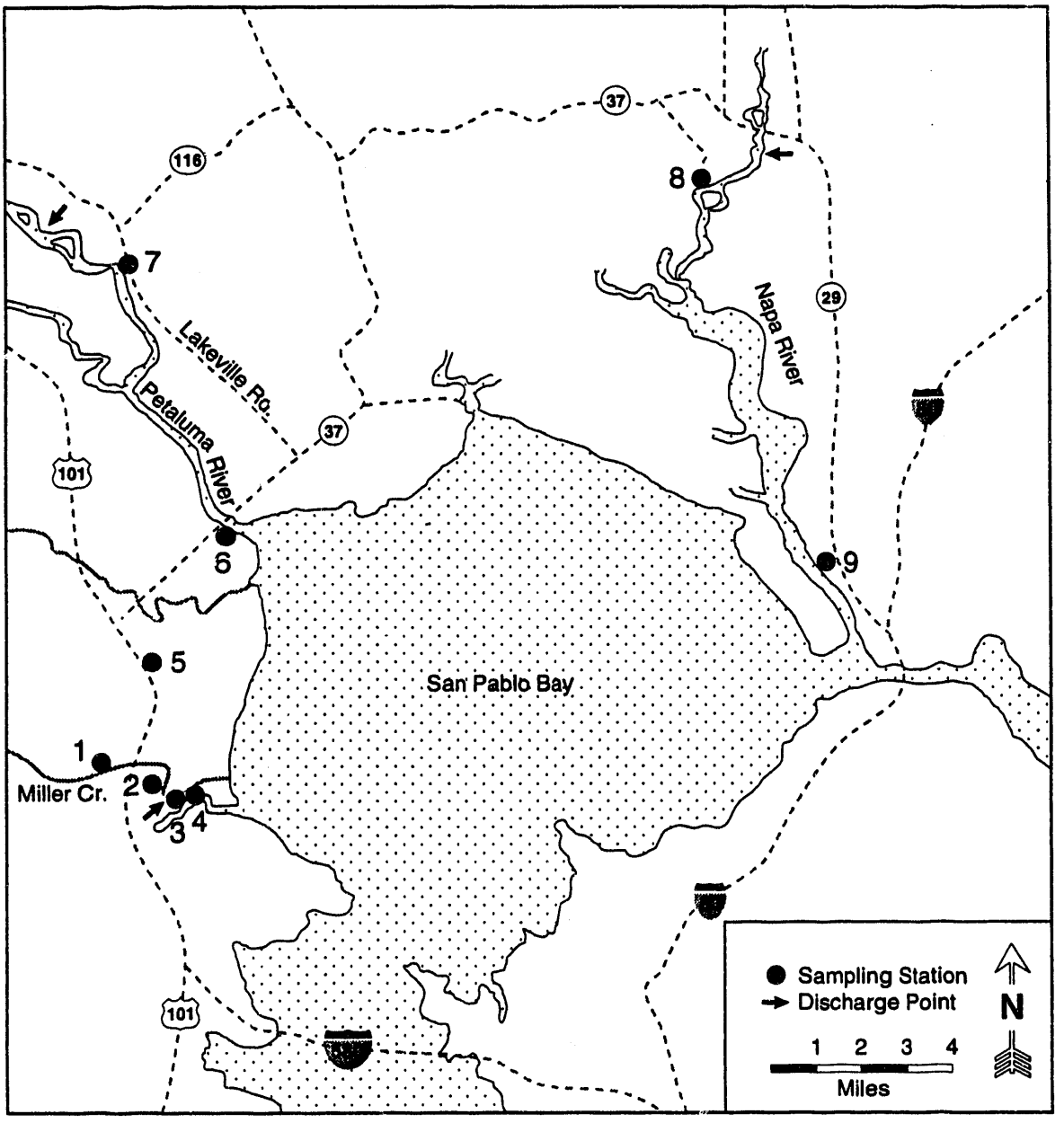


Figure A-2: Sampling Sites for the San Pablo Bay Survey.

A.2.2 Results and Discussion

Ceriodaphnia dubia survived and reproduced in all freshwater samples derived from Miller Creek and LGSD effluent (Table A-2). Two freshwater samples, 2 and 3, had significant effect on fathead minnows survival but not on growth. On the third sampling trip (Saturday January 25, 1992), however, high chlorine concentrations were detected in the LGSD effluent. The free chlorine, as was determined on diluted sample with an aquarium kit two days later, was 20-40 mg/l; on January 29, 1992 the same sample gave a reading of 9.5 mg/l with a chlorine electrode. This sample was not used for renewals; it was tested separately for acute toxicity. The 24 h LC_{50} for *C. dubia* was between 0.1% and 1% sample, and for the fathead minnows it was between 1% and 10% sample.

Echinoderm fertilization was totally inhibited in an LGSD effluent sample that did not contain elevated chlorine, but the same sample did not prevent full gastrulation in echinoderm embryos, nor did any other sample tested. Fertilization was significantly inhibited in the McInnis Park sample as well. Mysids survived in all brackish samples. *Menidia beryllina* larvae survived and developed well in all brackish samples until day 7 of the test, when mortalities occurred in one replicate (out of 3) of sample 5, in two replicates of sample 7, and in all 3 replicates of sample 8. The pH in the affected test chambers was approximately 8.1, and the total ammonia concentrations were 0.1, 0.3, and 1.1 mg/l in samples 5, 7, and 8 respectively, so it does not seem likely that unionized ammonia was the cause of this enigmatic mortality. These samples (January 25, 1992) were used for all renewals of a repeat test with a fresh batch of 7-day old larvae, in which no ill effect was observed. The *M. beryllina* data remain unexplained and laboratory error cannot be ruled out as a potential cause of the mortality observed.

These findings are valuable additions to our knowledge of San Pablo Bay. Our data suggest that ambient monitoring of the region could result in improved water quality management. Emphasis should be placed on the marshes, the rivers adjacent to point source effluents, and on the entire system during wet weather. So far, toxicity has not been characterized under wet weather conditions in the San Pablo Bay region.

TABLE A-2: TOXICITY OBSERVED IN SAMPLES COLLECTED AROUND SAN PABLO BAY (JANUARY 21-25, 1992)

STATION		SAL[2] (ppt)	MENIDIA		MENIDIA[3]		FATHEAD MINNOW		SEA URCHIN			MYSID		CERIODAPHNIA		
No.[1]	Location		Mean Surv (%)	Mean Wt (mg)	Mean Surv (%)	Mean Wt (mg)	Mean Surv (%)	Mean Wt (mg)	Fert (%)	Dev[4] (%)	Amb (%)	Mean Surv (%)	Amb (%)	Surv (%)	Mean Repro[5]	Amb (%)
1 MD12	Miller Cr. upstream	0					100	0.33	97.3		75			100	19.6	100
2 MD11	Miller Cr. downstream	0.5 1 3.2					83[6]	0.27	71.7[6]	98	76			90	23.3	100 80 33
3 MD10	Discharge LGVSD	0.5					70[6]	0.28	0.0[7]	97	75			100	13.3	100
4 MD20	McInnis Park	18	93	0.60					54.0[7]	99	93	87	93			
5 MD22	Pacheco Pond	3	70	0.58	96	0.50			99.3	92	77	91	80			
6 RD10	Petaluma Riv. mouth	19	90	0.72					78.7[6]		89	89	83			
7 RD11	Petaluma Riv. upstr.	16	47	0.86	93	0.47			99.3	98	85	89	80			
8 RD60	Napa River upstream	13	23[8]	0.65	93	0.51			67.1[6]	97	84	85	78			
9 RD61	Napa River mouth	17	100	0.68					97.7		85	88	82			
	DILUT. CONT.						100	0.28	99.7	98	100	96		100	16.0	
	BRINE CONT.	18	100	0.60	97	0.47			69.0	98	78	95	75			

1 Lower number represents Station Code designated by the Reginonal Water Quality Control Board.
2 Numbers entered for the control indicate test salinity for Menidia and mysid. Urchin test salinity was 30 ppt. Salinity adjustments with brine resulted in ambient sample dilution as specified under Amb (%). In the Ceriodaphnia test, samples collected at Station 2 during the 2nd and 3rd trips were diluted with control water for the renewals.
3 Test was repeated, starting January 29, 1992, with remaining portions of samples taken on January 25, 1992.
4 Development endpoint, indicating % embryos which completed gastrulation within 48 h.
5 Reproduction endpoint: Average number of offspring per female.
6 Significantly lower than Dilution Control only.
7 Significantly lower than both Dilution and Brine Controls.
8 In a box plot of this test, the 95% C.I. of Station 8 did not overlap The 95% C.I. of the Control, as was the case for Stations 5 and 7.

APPENDIX B: BAY BACKGROUND SURVEYS

The two Bay Background surveys represent a continuation of an ongoing effort to characterize reference toxicity in the San Francisco Bay system. This effort is important for three reasons. First, there are few data available to determine whether toxicity is widespread in Bay waters. Second, if toxicity is detected in the immediate vicinity of specific discharges, there is little information available to aid in determining whether the observed toxicity contributes to a greater regional problem or whether it is a local concern. Third, there are no data that correlate ambient toxicity in estuarine ecosystems with exceedences of specific chemical criteria. Both surveys were coordinated with other San Francisco Bay Regional Water Quality Control Board (SFBRWQCB) contractors from UC Santa Cruz who were evaluating concentrations of trace metals and organic contaminants in synoptic samples.

B.1 Surveys Design and Field Conditions

The first Background survey of the present project was conducted on June 11-13, 1991. Samples were collected by SFBRWQCB personnel in established monitoring stations (Figure B-1). Silverside minnow bioassay was performed by Aqua Terra Technologies Aquatic Bioassay Laboratory (Walnut Creek, CA), and echinoderm fertilization test was carried out by MEC Analytical Systems, Bioassay Division (Tiburon, CA).

The second Bay Background survey was conducted in the second week of April 1992, during a dry weather period, and included established monitoring stations (Figure B-1). Samples were collected from a boat by SFBRWQCB personnel, using a teflon impeller pump system. The toxicity tests, using silverside minnows and sea urchins, were run in our laboratory in two batches. Samples collected at Port Chicago (BF30) and Grizzly Bay (BF20) on April 7 and samples collected at Pacheco Creek (BF10), Napa River (BD50) and Davis Point (BD40) on April 8 were tested in the first batch. The second batch was run with samples collected on April 9 at Yerba Buena Island (BC10), Oyster Point (BB30), Redwood Creek (BA40), Dumbarton Bridge (BA30) and Extreme South Bay (BA20). Sampling at each site

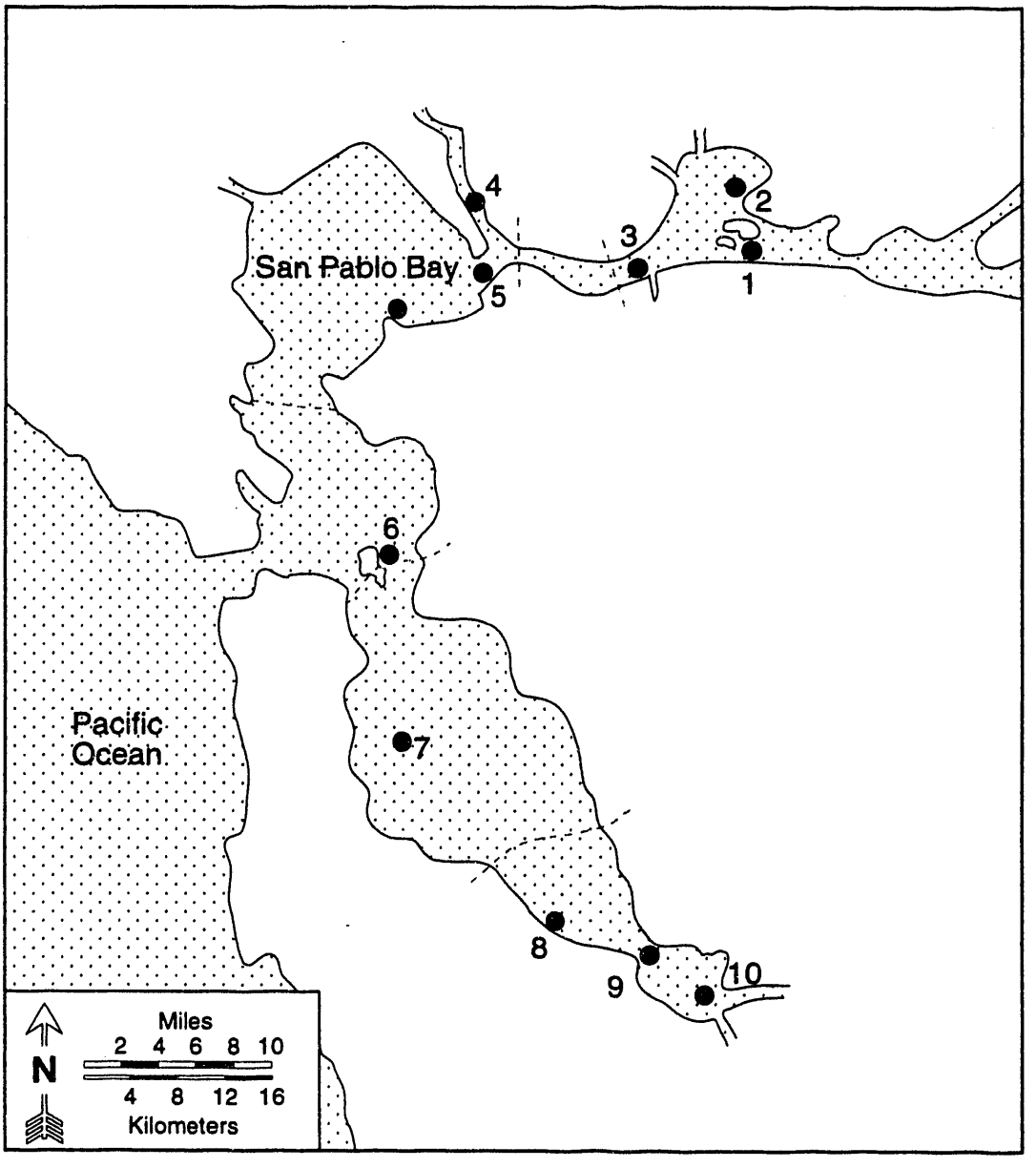


Figure B-1: Sampling Sites for the San Francisco Bay Background Surveys.

was conducted only once, and the derived sample was used for initiation and six renewals of the fish larvae test, being held for up to 168 h. Adult sea urchins were obtained from the Bodega Marine Laboratory (Bodega Bay, CA). Waters for the sea urchin fertilization tests were held 24-48 hours.

B.2 Results and Discussion

In the first Bay Background survey, *Menidia beryllina* survival was significantly affected only in one of the ambient samples (Table B-1). Echinoderm fertilization was inhibited in only one, but a different, sample.

For the second Bay Background survey, results are shown in Table B-2. Larvae of *M. beryllina* survived and gained normal weights in all samples. Fertilization success of *Strongylocentrotus purpuratus* gametes was in the range of 96-100% in all samples. These data, indicating **no significant toxicity in the sea urchin test**, are in contrast with previous findings of Bay Background surveys (Table B-1 above, and the four surveys reported by Anderson *et al*, 1990) in which some samples had inhibitory effect on echinoderm fertilization.

TABLE B1: TOXICITY OBSERVED IN SAMPLES COLLECTED DURING THE FIRST BAY BACKGROUND SURVEY (JUNE11-13, 1991)

STATION				SALINITY (ppt)[2]	MENIDIA		SEA URCHIN
No.[1]	Location	Latitude	Longitude		Mean Surv (%)	Mean Wt (mg)	Fert (%)
BF30	Port Chicago	38.04	122.00		57[3]	1.21	84.3
BF20	Grizzly Bay	38.07	122.01		87	1.25	82.5
BF10	Pacheco Creek	38.03	122.06		90	1.47	75.7
	DILUTION CONTROL			15	90	1.30	88.3
	BRINE CONTROL			15	77	1.49	75.2
BD50	Napa River	38.06	122.16		77	1.42	77.2
BD40	Davis Point	38.04	122.16		67	1.49	83.9
BD30	Pinole Point				83	1.54	88.1
	DILUTION CONTROL			24	90	1.52	88.3
	BRINE CONTROL			24	80	1.50	75.2
BC10	Yerba Buena Island	37.49	122.21		97	1.48	72.0
BB30	Oyster Point	37.40	122.20		87	2.05	68.7
BA40	Redwood Creek	37.33	122.12		80	2.03	58.7[4]
BA30	Dumbarton Bridge	37.30	122.07		93	1.65	78.0
BA20	Extreme South Bay	37.29	122.05		93	1.60	73.2
	DILUTION CONTROL			24	80	1.58	83.7
	BRINE CONTROL			24	87	1.60	78.8

[1] Number represents Station Code designated by the Regional Water Quality Control Board.

[2] Numbers entered for the controls indicate test salinities for Menidia. Urchin tests were run at 30 ppt.

[3] Significantly lower than dilution control only (Fisher-Exact test, $p<0.05$).

[4] Significantly lower than controls (Dunnett, $p<0.05$).

TABLE B2: TOXICITY OBSERVED IN SAMPLES COLLECTED DURING THE SECOND BAY BACKGROUND SURVEY (APRIL 4-9, 1992)

STATION				SALINITY (ppt)[2]	MENIDIA			SEA URCHIN	
No.[1]	Location	Latitude	Longitude		Mean Surv (%)	Mean Wt (mg)	Amb (%)	Fert (%)	Amb (%)
	NORTH BAY								
1 BF30	Port Chicago	38.04	122.00	4.9	100	1.02	97	99	71
2 BF20	Grizzly Bay	38.07	122.01	3.9	97	1.07	95	96	70
3 BF10	Pacheco Creek	38.03	122.06	9.0	100	0.92	100	100	74
4 BD50	Napa River	38.06	122.16	11.0	100	0.92	100	100	77
5 BD40	Davis Point	38.04	122.16	10.5	97	1.05	100	100	77
	DILUTION CONTROL							99	
	BRINE CONTROL			9.5	97	0.84	90	99	67
	CENTRAL & SOUTH BAY								
6 BC10	Yerba Buena Island	37.49	122.21	24.2	97	0.97	100	99	95
7 BB30	Oyster Point	37.40	122.20	22.5	97	1.03	100	100	94
8 BA40	Redwood Creek	37.33	122.12	21.5	93	0.98	100	100	92
9 BA30	Dumbarton Bridge	37.30	122.07	20.5	100	1.21	100	100	91
10 BA20	Extreme South Bay	37.29	122.05	20.5	100	1.12	100	100	91
	DILUTION CONTROL			22.0	97	1.09		100	
	BRINE CONTROL							100	68

[1] Lower number represents Station Code designated by the Regional Water Quality Control Board.

[2] Numbers entered for the controls indicate test salinities for Menidia. Urchin tests were run at 30 ppt. Salinity adjustments with brine resulted in ambient sample dilution to values specified under Amb (%). Samples 6-10 did not require salt adjustment with brine for the Menidia test, therefore Bodega Bay Water diluted with Arrowhead Spring water to 22 ppt was used as control.

DATE
FILMED
10/5/93