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Ambient Toxicity Characterization of San Francisco Bay and Adjacent Wetland Ecosystems

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and J. Harte

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AMBIENT TOXICITY CHARACTERIZATION OF SAN FRANCISCO BAY AND ADJACENT WETLAND ECOSYSTEMS

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FORWARD

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CONTENTS

	Page
FORWARD	i
EXECUTIVE SUMMARY	iv
LIST OF TABLES	vi
LIST OF FIGURES	vii
1. INTRODUCTION	1
1.1 Background	1
1.2 Purpose and Approach	2
1.3 Scope and Limitations	5
2. METHODS	6
2.1 Toxicity Test Procedures	6
2.2 Sample Collection	11
2.3 Sample Preparation and Water Chemistry	12
2.4 Water Quality Measurements	13
2.5 Reference Toxicant Tests	13
2.6 Statistics	13
3. RESULTS OF BAY BACKGROUND SURVEYS	15
3.1 Introduction	15
3.2 First Survey--Survey Design and Field Conditions	17
3.3 First Survey--Results	17
3.4 Second Survey--Survey Design and Field Conditions	17
3.5 Second Survey--Results	19
3.6 Third Survey--Survey Design and Field Conditions	19
3.7 Third Survey--Results	19
3.8 Fourth Survey--Survey Design and Field Conditions	22
3.9 Fourth Survey--Results	22
3.10 Preliminary TIE Study--Results	22
3.11 Metals Survey--Results	24
3.12 Summary of Bay Background Survey Findings	24
4. RESULTS OF BAY MARSH SURVEYS	27
4.1 Introduction	27
4.2 San Francisco Bay National Wildlife Refuge--Survey Design and Field Conditions	27
4.3 San Francisco Bay National Wildlife Refuge--Results	29

Contents Continued

	Page
4.4 Hayward Marsh Reclamation Project--Survey Design and Field Conditions	31
4.5 Hayward Marsh Reclamation Project--Toxicity Characterization Results	34
4.6 Hayward Marsh Reclamation Project--Results of Toxicity Correlations	37
4.7 Hayward Marsh Reclamation Project--Results of Toxicity Identification Evaluations	44
4.8 Mountain View Sanitary District Marsh Reclamation Project--Survey Design and Field Conditions	47
4.9 Mountain View Sanitary District Marsh Reclamation Project--Results	47
4.10 Sunnyvale Marsh and Wastewater Treatment Plant--Survey Design and Field Conditions	50
4.11 Sunnyvale Marsh and Wastewater Treatment Plant--Results	53
4.12 San Jose/Santa Clara Marsh and Wastewater Treatment Plant--Survey Design and Field Conditions	55
4.13 San Jose/Santa Clara Marsh and Wastewater Treatment Plant--Results	58
4.14 Summary of Marsh Survey Results	58
5. RESULTS OF ANCILLARY SURVEYS	61
5.1 Introduction	61
5.2 Oakland Harbor and Dredge Survey--Survey Design and Field Conditions	61
5.3 Oakland Harbor and Dredge Survey--Results	63
5.4 USS Posco Steel Refinery--Survey Design and Field Conditions	63
5.5 USS Posco Steel Refinery--Results	66
6. METHODOLOGICAL FINDINGS	70
6.1 Introduction	70
6.2 Intralaboratory Precision	70
6.3 Salinity Adjustment	72
6.4 Sperm:Egg Ratio in the Echinoderm Test	72
7. DISCUSSION AND CONCLUSIONS	78
7.1 Potential Significance of Toxicity Observed in Bay Background Surveys	78
7.2 Potential Significance of Toxicity Observed in Bay Marsh Surveys	79
ACKNOWLEDGEMENTS	82
REFERENCES	83

EXECUTIVE SUMMARY

The goal of this project was to evaluate the spatial and temporal distribution of toxicity in the waters of San Francisco Bay, with special emphasis on nearshore marsh habitats. Short-term chronic toxicity tests were used to characterize toxicity in ambient samples. The program was comprised of three components.

The first component was a background survey to determine the potential for toxic effects within the Bay, but remote from discharge locations. Sampling was conducted at 12 fixed locations on four occasions. Three species were studied in all but the fourth survey. The key finding of the Bay Background surveys was that, with few exceptions, toxicity was only observable using the echinoderm fertilization assay. The most widespread effects were observed during the spring of 1989. The cause of the toxicity observed was not identified; but it was determined that, in at least one incidence, the toxic effects were not persistent.

The second component of the program was designed to evaluate toxicity in five Bay marshes. This component was considered significant because no surveys have been performed to evaluate short-term toxic effects in Bay marshes, despite the fact that wastewater is discharged into much of the remaining wetland habitat or is used to create such habitat. Little is known about the potential toxicological effects of such practices nationwide.

Toxic effects were observed in samples from four of the five marshes surveyed. The Hayward Marsh Reclamation Project was the most toxic of all marshes studied, with acute and chronic effects detected at several stations using a variety of test species. Toxicity correlation and toxicity identification studies revealed that the toxic effects were largely attributable to unionized ammonia. Nevertheless, unionized ammonia does not explain all of the toxicity observed in all of the samples. Toxic effects were also observed in the marsh area immediately adjacent to the Sunnyvale Wastewater Treatment Plant. Significant effects were observed with three of the four species tested, but only the responses of the water flea, *Ceriodaphnia,* were clearly related to the concentration of effluent in the receiving water. At the Mountain View Sanitary District Marsh Reclamation Project and

at the San Francisco Bay National Wildlife Refuge, significant effects were observed; but these were relatively subtle in nature and not widespread in occurrence. The only site at which no toxic effects were documented with the short-term chronic tests was the marsh area adjacent to the San Jose/Santa Clara Wastewater Treatment Plant.

The third program component involved the conduct of two ancillary surveys. These surveys included an evaluation of toxicity associated with dredging activities in the Oakland Outer Harbor and an evaluation of toxicity in the New York Slough area adjacent to the USS Posco Steel Refinery. The most intriguing finding obtained in this program component was the observation that acute toxic effects occurred in the Contra Costa Canal, a drinking water conveyance system.

Selected methodological findings are also presented. These include the intralaboratory precision of selected toxicity tests, the efficacy of various salinity adjustment techniques, and the effects of varying sperm to egg ratios in the echinoderm fertilization assays.

The major implications of these findings are twofold. First, it is concluded that the occurrence of widespread toxicity using the echinoderm fertilization assay is perplexing but not unprecedented, and that further investigation is warranted to determine whether periods of elevated toxicity reoccur. Second, it is concluded that improved characterization and control of toxicity in wetland habitats is vital, and that ecotoxicological investigation can be constructively coupled with engineering design evaluations. The complex interrelationships between the need for further ecotoxicological characterization in marshes and the attainability of related, but potentially conflicting, policy objectives is discussed.

FIGURES

		Page
Figure 1.	Ambient Toxicity Survey Sites	3
Figure 2.	Echinoderm Fertilization Success in Four Bay Background Surveys	25
Figure 3.	San Francisco Bay National Wildlife Refuge Ambient Toxicity Survey	28
Figure 4.	Hayward Marsh Ambient Toxicity Survey I	32
Figure 5.	Hayward Marsh Ambient Toxicity Survey II	33
Figure 6.	Ambient Toxicity Survey at Mountain View Sanitary District Marsh	48
Figure 7.	Sunnyvale Ambient Toxicity Survey	52
Figure 8.	Ambient Toxicity Survey at San Jose Marsh	56
Figure 9.	Summary of Findings at Five Marsh Sites	60
Figure 10.	Dredge and Oakland Harbor Ambient Toxicity Survey	62
Figure 11.	USS Posco Ambient Toxicity Survey	65

TABLES

		Page
Table 1.	Location of Sampling Stations for Bay Background Surveys	16
Table 2.	Toxicity Observed in Samples Collected During the First Bay Background Survey	18
Table 3.	Toxicity Observed in Samples Collected During the Second Bay Background Survey	20
Table 4.	Toxicity Observed in Samples Collected During the Third Bay Background Survey	21
Table 5.	Toxicity Observed Using the Sea Urchin Bioassay in Samples Collected During the Fourth Bay Background Survey	23
Table 6.	Toxicity Observed in Samples Collected from the San Francisco Bay National Wildlife Refuge	30
Table 7.	Toxicity Observed in the First Survey Conducted at the Hayward Marsh	35
Table 8.	Toxicity Observed in the Second Survey Conducted at the Hayward Marsh	36
Table 9.	Daily Cumulative Survivorship and Free Ammonia Concentration for the Fathead Minnow Bioassay in the Second Hayward Marsh Survey	39
Table 10.	Seven-Day Average Calculations of Free Ammonia from the Second Hayward Marsh Survey	40
Table 11.	Daily Cumulative Survivorship and Adjusted Free Ammonia Concentrations for the Silverside Minnow in the Second Hayward Marsh Survey	41
Table 12.	Free Ammonia in the Sea Urchin Bioassay for the Second Hayward Marsh Survey	43
Table 13.	Hayward Marsh TIE -- Results of pH Adjustment and Ammonia Removal Tests Using Fathead Minnow	45
Table 14.	Toxicity Observed in Samples Collected from Mountain View Sanitary District Marsh	49

Tables continued

		Page
Table 15.	Toxicity Observed in a Survey Conducted in Marshes Adjacent to the Sunnyvale Wastewater Treatment Plant	54
Table 16.	Toxicity Observed in a Survey Conducted in Marshes Adjacent to the San Jose/Santa Clara Wastewater Treatment Plant	57
Table 17.	Toxicity Observed in Samples Collected in Conjunction with Dredging Activities Conducted in the Oakland Outer Harbor	64
Table 18.	Toxicity Observed in Waterways Adjacent to the USS Posco Site	67
Table 19.	Intralaboratory Precision for the Echinoderm, Silverside Minnow, and Mollusc Tests	71
Table 20.	Comparison Between Seawater and Artificial Seasalts Using the Silverside Growth and Survival Test	73
Table 21.	Comparison Between Seawater and Natural Brine Using the Echinoderm Test	74
Table 22.	Comparison Between Seawater and Natural Brine Using the Mollusc Embryo Development Test	75
Table 23.	Example of Sea Urchin Sperm:Egg Ratio Test	77

Chapter 1

INTRODUCTION

1.1 BACKGROUND

For over a decade, the quality of effluents discharged into San Francisco Bay has been assessed using acute toxicity tests and chemical monitoring. However, no systematic efforts have been supported to evaluate the potential for toxicity in the Bay itself. Effectively, there have been few comprehensive attempts to determine whether toxicity monitoring and control strategies have been adequate to protect aquatic communities in San Francisco Bay. This report provides the results of the first surveys to determine the potential for ambient toxicity in Bay waters using short-term chronic toxicity tests. This research effort was directly coordinated with a larger program at the San Francisco Bay Regional Water Quality Control Board to evaluate effluent toxicity. Information obtained from the effluent program was used to identify sites of greatest concern, to select species to be studied at selected sites, and to determine the potential for variation in effluent toxicity.

The toxicity test methods assess short-term toxic responses in a range of standard test species. The tests have been developed and standardized by the US Environmental Protection Agency (EPA) and the American Society for Testing and Materials (ASTM). Effects evaluated include growth and survival in fish larvae, fertilization success in sea urchins, developmental success in bivalve molluscs, and reproduction of water fleas. These tests are widely used in effluent toxicity characterization and control programs across the nation. Laboratory comparisons (using single chemicals) of these tests to longer-term chronic tests indicate that predictions based on the short-term tests are within an order of magnitude of the predictions based on the longer-term tests (McKim, 1977). However, one recent report, involving complex effluents, indicates that the short-term tests may provide estimates of chronic effects that do not differ significantly from predictions made using longer-term embryo-larval assays (Glickman et al., 1988).

Although few studies of the potential biological effects of toxic contaminants have been conducted in Bay waters, the approach used differs from previous studies in the Bay. Past

studies have primarily evaluated physiological indicators of stress in fish species such as striped bass and starry flounder (Phillips, 1987). These approaches are valuable and necessary; however, rapid, short-term assessments are also of value because they can be used to canvas a wide range of environments and to detect varied sources of toxicity.

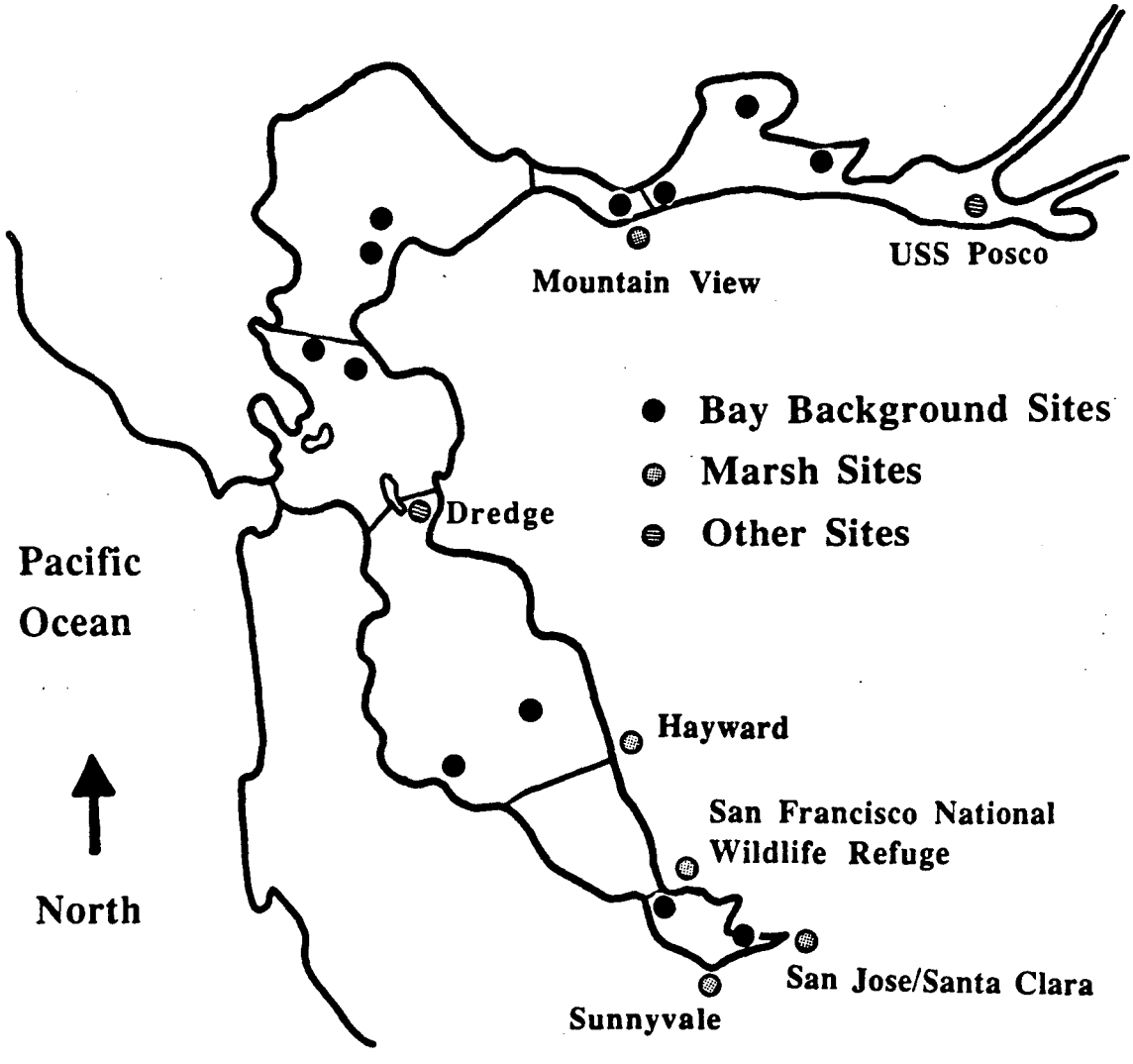
Use of short-term toxicity tests to assess ambient toxicity can be a powerful approach in directing toxicity control programs. This is because the methods can be used to rapidly evaluate: 1) spatial distribution of toxicity in varied environments 2) magnitude and temporal variation of varied sources of toxicity 3) distribution of toxic effects relative to specific discharges 4) toxicity reduction options and 5) toxicity associated with exceedances of criteria for specific chemicals. Consequently, direct assessment of the potential toxic effects may also provide more rapid development of effective control strategies than does extensive chemical-specific testing.

Evaluating the effects of contaminants in complex estuaries such as San Francisco Bay requires diverse efforts to assess concentrations of toxic substances as well as varying types of biological effects. Use of short-term toxicity tests is among the most effective tools available for rapid assessment, and this study provides insights into the potential for toxicity in San Francisco Bay waters and marsh environments.

1.2 PURPOSE AND APPROACH

The goal of this project was to evaluate the spatial and temporal distribution of toxicity in the waters of San Francisco Bay, with special emphasis on nearshore marsh habitats. There were three major program components. In the first component, toxicity at sites selected to be representative of Bay background conditions was evaluated (Fig.1). Our goal was to determine whether toxicity was observable in the open waters of the Bay, relatively remote from specific discharge sites. The survey was conducted at 12 stations distributed throughout Carquinez Straits, San Pablo Bay, Central Bay and South San Francisco Bay on a quarterly basis for one year. An ancillary goal of this component of the project was to determine whether any of the toxicity that might be observed was correlated with exceedances of water quality criteria for trace metals. Consequently, sampling was coordinated with another group that performed trace metals analyses.

Figure 1. Ambient Toxicity Survey Sites



The significance of the Bay Background component of the program is threefold. First, despite extensive speculation, there are few data available to determine whether there is widespread toxicity in waters of the Bay. Second, if toxicity is observed adjacent to specific discharges, there are few data available to aid in determining whether the observed toxicity contributes to a greater regional problem or whether it should be considered a strictly local concern. Third, no data exist correlating ambient toxicity in estuarine ecosystems with exceedances of specific-chemical criteria.

In the second program component, toxicity was evaluated in five Bay marshes (Fig. 1). This program component was significant because 1) no surveys have been performed to evaluate toxic effects in local marshes; although the remaining marshes are considered to have extraordinary biological significance and 2) because wastewater is discharged into the majority of these marshes with little knowledge of its potential for producing detrimental biological effects.

In the second program component, sites were selected to determine whether point-source discharges cause toxicity in local marshes. One site, the San Francisco Bay National Wildlife Refuge, was selected as a reference marsh. Two sites (Hayward Marsh and Mountain View Marsh) are marsh reclamation projects receiving wastewater input, and two sites are marshes sited adjacent to point source discharges. Attribution of the cause of toxicity was based on comparisons to ambient reference stations, to toxicity in laboratory control waters and, in some cases, to discharge dilution studies coupled with effluent and ambient toxicity testing. Toxicity test species were selected based on either their known sensitivity to the effluent, or based on salinity restrictions at the site. In addition, an evaluation of the cause of toxicity at the Hayward Marsh was performed.

The issue of toxicity in Bay marshes has broad significance. In general, national criteria for wetland management have not been developed, and few studies of water column toxicity in wetland ecosystems have been performed anywhere in the nation (Hammer, 1990; Godfrey et al., 1985; USEPA and USFWS, 1984). Rather, studies have focused on the fate and flux of individual contaminants or on the biomagnification of individual contaminants (Hammer, 1990). The options for managing mixed waste inputs have not been fully

recognized, except for the use of conventional parameters such as BOD and COD.

Short-term tests can be used as tools to evaluate the quality of wastewater discharged into marshes from wastewater treatment plants, landfills, and urban runoff. The tests can be used, with other tools, to guide engineering design and remediation in marsh environments. One example of this potential is provided in the toxicity reduction evaluations conducted during this study. In the San Francisco Bay area, where considerable experimentation with marsh design has taken place, as well as nationwide, important questions remain unanswered. Varied approaches are needed to answer the question "What is the capacity of wetland ecosystems to treat and to degrade toxicants?"

The third program component was comprised of two additional surveys that were conducted to evaluate specific toxicity issues. In the first survey, toxicity associated with dredging in the Oakland Harbor was studied, because local concern exists regarding the potential ecological hazards associated with this activity. Second, toxicity in the New York Slough area was investigated because reports of ambient toxicity had been obtained for this area.

1.3 SCOPE AND LIMITATIONS

Because this study focuses on water column toxicity and toxicity attributable to complex mixtures, these data do not provide insights into the potential for sediment toxicity, or the concentrations of contaminants in the Bay ecosystem. The toxicity tests may only predict the potential range of short-term chronic effects. The principle limitations of the survey design are that sampling was restricted in time, that most sites were sampled only once and that all potential sources of toxicity were not directly accounted for. Toxicity attributable to urban runoff and agricultural discharges was not specifically evaluated.

This report provides, first, those methods that are applicable to the entire project. Second are presented the specific design and results for each survey. These are followed by findings related to the toxicity test methodologies.

Chapter 2

METHODS

2.1 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.

2.1.1 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, 1988a; USEPA, 1989a). Fathead minnow, less than 24 hours old, were supplied by either Aquatic Resources (Sebastopol, CA) or Aquatox Inc. (Hot Springs, AK). Silverside minnow larvae, 5-8 days old upon arrival, were supplied by either Aquatic Indicators (St. Augustine, FL) or Cultured Aquatics (Northport, NY). Tests were initiated with 8-9 day old fish that were salinity acclimated at a rate of 5 ppt/day. For the fathead minnow test, we used Arrowhead brand mineral water as a dilution and control water and potassium dichromate at five concentrations (24, 12, 6, 3 and 1.5 mg/l as total chromium) as a reference toxicant. In the silverside minnow test, we used seawater collected at Bodega Bay Marine Laboratory as a natural seawater control and Arrowhead Brand mineral water with 40 Fathoms artificial sea salts added as a salinity-adjustment control. Copper sulfate was used as a reference toxicant in the following concentration range: 320, 160, 80, 40 and 20 ug/l as total copper.

2.1.2 *Ceriodaphnia* Survival and Reproduction Test

The water flea (*Ceriodaphnia dubia*) survival and reproduction test was performed using inhouse cultures and according to the EPA protocol (EPA,1989), with minor deviations

in the preparation of culturing, dilution and control waters and in the feeding of the test organisms. Culturing waters for both cladocerans and the algal food cultures differed from those specified in the protocol. We maintained stock cultures of cladocerans in one of four culture media: City of Davis, CA well water; a 10% Perrier water and 90% Arrowhead brand mineral water mixture bubbled overnight; a standard EPA moderately hard water mixture (USEPA, 1989a); or a mixture consisting of 20% Bold's Basel algal culture medium (Bischoff and Bold, 1963), 20% EPA moderately hard and 60% quartz distilled water. We maintained algal cultures in Bold's Basel medium (Stein, 1973). Vitamins (see below) were added to the algal culturing media used in all of the surveys and to the *Ceriodaphnia* culture water for the Mountain View and second Hayward marsh surveys.

With one exception, control and dilution waters used during testing were a mixture of 10% Perrier water and 90% Arrowhead brand mineral water bubbled overnight. In the USS Posco survey, however, we used City of Davis, CA well water for both the dilution and control water. For the Mountain View and second Hayward surveys, vitamins were added to both the control and dilution waters (see below). In all other surveys, vitamins were not used in the control or dilution waters. Sodium Chloride at 1, 2 and 3 g/l was used as a reference toxicant.

Stock solutions of thiamine, biotin and B_{12} in concentrations of 0.75 g/l, 0.0075 g/l and 0.01 g/l, respectively, were added to cladoceran and algal culture media in concentrations of 1 ml of each stock solution per liter medium. During testing, we also used these same stocks and concentrations in those surveys in which vitamins were added to dilution and control waters.

Stock cultures and test animals were fed one of two mixtures. In the Sunnyvale and first Hayward Marsh surveys, culture organisms were fed at a rate of 1ml/l each day, and test organisms were fed at a rate of 0.1 ml/15 ml per day of a tri-algal mixture comprised of *Ankistrodesmus palcatus*, *Chlamydomonas reinhardtii* and *Chlorella minutissima*. The combined cultures had an average density of 3×10^8 cells/ml, which resulted in concentrations of 2×10^6 cells/ml in the test cups. All other tests used the YCT mixture specified in the EPA cladoceran protocol (USEPA, 1989a) and *Selenastrum* with an average culture density of 1×10^7 cells/ml. The amount of YCT mixture and *Selenastrum* used

during testing varied between 0.1-0.3 ml/15 ml cup, resulting in final densities of 7×10^4 - 2×10^5 cells/ml. However, for the purposes of an interlaboratory comparison performed during the USS Posco survey, 1.5 ul of YCT was added to each cup, and the final concentration of *Selenastrum* in each cup was 3×10^5 cells/ml.

2.1.3 Mollusc Embryo Development Test

Mollusc embryo development tests, using either the bay mussel (*Mytilus edulis*) or the Pacific oyster (*Crassostrea gigas*), were conducted according to ASTM protocol (ASTM, 1987). Adult bay mussels were obtained from either Cove Mussel Co. (Marshall, CA) or Sea Farms West (Carlsbad, CA). Adult Pacific oysters were obtained from Intertidal Aquafarms (Marshall, CA).

Below are described deviations in spawning procedure and test containers used. Instead of holding the adults for two weeks prior to testing as prescribed in the protocol, we received them either the day before or the day of testing and held them dry in a cooler with ice at 12°C. We have found that mussels spawn more readily in water heated to 25°C instead of the ASTM maximum of 20°C. Once an individual commences spawning, it is then removed from the heated water and placed in an individual beaker containing water at the test temperature 16°C. This procedure ensures minimal exposure of gametes to water over 20°C. Tests were run in 20 ml glass scintillation vials instead of flasks. Tests were terminated by adding 1 ml of a 1% glutaraldehyde solution to each 10 ml test volume. Embryo suspensions were then concentrated twofold by removing 5.5 ml of supernatant from each vial. Counts were made after inverting the capped vials to resuspend the embryos.

Bodega Bay seawater was used as the natural seawater control, and either Arrowhead brand mineral or Sierra brand distilled water that was salinity adjusted with natural brine, was used for the brine control. Initially, we used sodium azide as a reference toxicant, with test concentrations ranging from 1-200 mg/l. However, after repeated tests, we utilized a narrower range (30, 20, 15, 10, and 5 mg/l sodium azide).

2.1.4 Echinoderm Fertilization Tests

The echinoderm sperm cell bioassay was conducted using either the purple sea urchin (*Stongylocentrotus purpuratus*) or the sand dollar (*Dendraster excentricus*), following the general approach of Dinnel (1987), while incorporating specific modifications either suggested in Cherr et al. (1987) or developed in our laboratory. Similar to Cherr et al. (1987), we collect sperm by dry spawning. However, rather than inverting males over a water-filled beaker, we collect sperm from the aboral surface using a syringe. Subsequently, sperm were stored in a container on ice. Our work has shown the sperm:egg ratio to be an important factor in the sensitivity of this bioassay. Therefore, like Cherr et al. (1987), we routinely conducted a sperm:egg ratio pre-test using a range of ratios to determine the lowest ratio that results in 80-90% fertilization in both the seawater and brine controls. Unlike Cherr et al. (1987) and Dinnel (1987) who suggest 20 minute (10 minute sperm exposure) and 80 minute (60 minute sperm exposure) test times, respectively; we used a 40 minute (20 minute sperm exposure) test. Based on a one-time comparison of 10, 20, and 60 minute sperm exposure times, we found a substantial decrease in fertilization using 60 minute exposure time. There was no difference in percent fertilization between the 10 and 20 minute exposure times. One exception is the sea urchin test conducted during our first survey at the San Francisco Bay National Wildlife Refuge. This test was conducted before the time comparison test and used a 30 minute sperm exposure time.

Procedures for the sperm cell bioassay using the sand dollar were identical to those using the sea urchin, except for the technique used in gamete collection. For the sand dollar, both males and females were rinsed with seawater after injection to remove traces of a toxic pigment, which is released by the adults in response to the KCl injection. Sperm is collected in the same fashion as with the sea urchin. However, instead of spawning females upside down into beakers, they were placed in petri dishes and covered with seawater. We then collected eggs, as they were released, using a pasteur pipette. The sand dollar eggs are more fragile than those of the sea urchin; consequently, it is not advisable to rinse them before counting.

For both the sea urchin and the sand dollar, Bodega Bay seawater was used as the natural seawater control and either Arrowhead brand mineral water or Sierra brand distilled water,

salinity adjusted with natural seawater brine, was used as the brine control. The pH of all test waters was 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. An additional concentration of 500 mg/l sodium azide was used when sand dollar assays were conducted, because they are generally less sensitive to this reference toxicant than are the sea urchins. Adult sea urchins were obtained from either Bodega Marine Laboratory (Bodega Bay, CA) or from Pacific Biomarine (Venice, CA). Adult sand dollars were obtained from either Pacific Biomarine (Venice, CA) or EA Engineering (Concord, CA). Animals were held for up to 2 weeks in 20-gallon aquaria with undergravel and recirculation filtration at 13°C.

2.1.5 Algal Growth Tests

Algal growth tests were performed on seawater samples using the *Skeletonema* bioassay. These tests were conducted by an independent laboratory following the guidelines in EPA Bioassay Procedures for the Ocean Disposal Permit Program (USEPA, 1978). All ambient and control waters used in the *Skeletonema* bioassays were filtered to 0.45 um prior to testing.

Algal growth tests were performed on freshwater samples using the *Selenastrum* bioassay. Three such tests were performed, two by independent laboratories and one inhouse. All tests were conducted according to EPA specifications (USEPA, 1989a). Ambient and control waters used in the Sunnyvale and Mountain View Marsh surveys were filtered to 0.45 um. Samples used in the USS Posco survey were filtered to 5 um. Ambient and control waters used in the Mountain View Marsh and USS Posco surveys were pH adjusted to 7.5 prior to test initiation. No pH adjustment was performed on samples used in the Sunnyvale Marsh survey.

2.1.6 Mysid Test

The Mysid (*Mysidopsis bahia*) short-term chronic test was performed only once in our study, using animals supplied by Aquatic Research Organisms (Hampton, NH). Tests were conducted according to the EPA protocol (USEPA, 1988a), with several deviations in test design. Due to supplier error, we used 8 to 11-day old mysids rather than the 7-day olds

specified in the protocol. While the protocol calls for 5 mysids per replicate chamber with 8 replicate chambers per treatment, we used 10 mysids per replicate chamber and 4 replicate chambers per treatment. We used a test volume of 200 ml instead of 150 ml to compensate for the increased mysid density in each replicate. Survivorship was the only endpoint measured.

We used Bodega Bay seawater diluted with Arrowhead brand mineral water as a natural control and Arrowhead brand mineral water salinity adjusted with natural brine as a brine control. Potassium chromate at three concentrations (0.43, 1.7 and 6.7 ug/l as total chromium) was used as a reference toxicant.

2.2 SAMPLE COLLECTION

Sample collection methods differed between the Bay Background surveys and the Marsh Toxicity surveys. For the Bay background surveys, all samples were collected from a boat using one of three types of apparatus. These were: 1) an "ultra clean" peristaltic pump system 2) an inert plastic bilge pump or 3) rinsed plastic cubitainers. In all cases, samples were taken from the side of the boat facing into the current to avoid contamination from the engine. Samples were stored in plastic cubitainers.

For the marsh surveys, all samples were collected from either a boat or on foot using one of two sampling procedures. In two of the surveys (San Francisco Bay National Wildlife Refuge and San Jose Wastewater Treatment Plant), water samples were pumped directly into a plastic cubitainer using a diaphragm pump fitted with Bev-A-Line brand tubing. In the remaining four surveys (Sunnyvale Wastewater Treatment Plant, two surveys at Hayward Marsh and Mountain View Sanitary District), we collected grab samples using plastic cubitainers.

Regardless of the sampling procedure used, all equipment was rinsed with sample water prior to sample collection. All samples were stored in coolers and chilled during transport.

2.3 SAMPLE PREPARATION AND WATER CHEMISTRY

When samples were not used immediately in testing, they were stored in refrigerators at 4-7°C. Generally, samples were held 12-24 hours prior to testing; however, there were two types of situations in which samples were held for greater than 24 hours. In the first case, because of fluctuating salinities in the ambient waters over the seven day collection period, sample salinity sometimes exceeded the salinity criteria of a particular bioassay. This necessitated the use of an older sample. This occurred at Sunnyvale Marsh where four of the samples used in the fathead minnow bioassay were held for greater than 24 hours (Guadaloupe 24-96 hours; Calabasas, Moffett Channel and Junction 24-48 hours) and in the *Ceriodaphnia* test in which samples were held either from 24-48 hours (Discharge T1, Discharge T2, Discharge Upstream, Baylands Forebay and Bioxidation Pond) or from 24-96 hours (Calabasas, Moffett Channel, Junction, and Guadaloupe).

The second situation in which samples were held for more than 24 hours before testing occurred when sampling was not conducted on a daily basis. In these cases, daily renewals were always performed, but they were performed using samples that were held for varying periods of time. During the four Bay background surveys, sampling at each site was conducted only once. Ambient waters used for the silverside minnow test in the first three surveys were held from 24-168 hours. In the first and third Bay Background surveys, waters for the sea urchin tests were held for 24-72 hours. For the second and fourth surveys, samples were held 24 hours or less. Ambient samples used in the mollusc test were held either less than 48 hours (first survey) or for 24-72 hours (second and third surveys). At the San Jose Wastewater Treatment Plant, we collected samples on three occasions, and waters used in the fathead minnow and *Ceriodaphnia* tests were held from 24-72 hours before use. For the dredge survey, samples used in the silverside minnow bioassay from the first collection date were held for 24-48 hours. Samples from three of the stations (Schnitzer Steel, Pacific Dry Dock and Treasure Island) collected on the second and third sampling dates were held for 24-48 and 24-72 hours, respectively. Samples collected on the second date during dredging operations were used for the remainder of the testing because the dredge was moved out of the area.

Ambient samples underwent two manipulations to prepare them for use in testing. First, they were shaken to resuspend particulates and filtered through a 37-um Nitex mesh to remove large particulates and predatory organisms. Secondly, samples frequently were salinity adjusted prior to testing to conform with salinity ranges specified in the various protocols. Salinities were reduced for fathead minnow and silverside minnow bioassays using Arrowhead Brand mineral water. For *Ceriodaphnia* bioassays, either a 10% Perrier/90% Arrowhead mineral water mixture or well water from Davis, CA was used. Salinities were increased for the sea urchin, mollusc, and mysid shrimp bioassays using brine made by concentrating Bodega Bay seawater by slow heating to approximately 80 ppt and for silverside minnow bioassays using Forty Fathoms or Marine Mix brands of artificial sea salts added to the ambient sample. Salinity adjustment in the *Skeletonema* assay was performed by the addition of a hypersaline solution, prepared with "Marine Environment" sea salts. When salinity alteration resulted in a dilution of the ambient sample, the final ambient concentrations are recorded as "Ambient %" in the results.

2.4 WATER QUALITY MEASUREMENTS

Water Quality measurements were made for each toxicity test according to specifications in EPA and ASTM protocols. Any deviations from the specified ranges are noted in the results.

2.5 REFERENCE TOXICANT TESTS

Reference toxicant tests were conducted for each echinoderm and mollusc test that was conducted. For all other species, reference toxicant tests were only conducted periodically as a quality assurance measure. In a few cases, a restricted number of doses were used. The reference toxicants used for each species are described in the preceeding subsections.

2.6 STATISTICS

All statistical analyses were performed using the TOXSTAT package produced by University of Wyoming, according to the specifications presented in the EPA and ASTM protocols for each species.

2.6.1 Proportional Data

Statistical analysis of survivorship and abnormality in the mollusc bioassay, reduced fertilization in the echinoderm bioassay, and survivorship in the *Mysidopsis*, silverside minnow and fathead minnow bioassays was performed in several steps. First, data were tested for normality using either a Chi-squared or Shapiro-Wilks test, followed by either a Bartletts or Hartley test for homogeneity. If data did not pass either test, then they were arcsine transformed and rerun. Arcsine transformed data are noted in the text. When data passed these two tests, they were analyzed for significance using a Dunnetts test. If transformed data did not pass the tests for homogeneity or normality, then a nonparametric Kruskal-Wallis test was used and is noted in the results section. Unless otherwise noted, all significance is determined by comparison to both brine and seawater controls and p-values less than 0.05. Two-tailed tests were used for all of the ambient test data.

All survival data for the *Ceriodaphnia* bioassay were analyzed using a Fisher's Exact test.

2.6.2 Nonproportional Data

Statistical analysis of growth in both *Skeletonema* and *Selenastrum*, reproduction in *Ceriodaphnia*, and larval weights in both the silverside and fathead minnow bioassays were performed in the same manner as described above for the proportional data, except that arcsine transformations were not made. In addition, statistical analysis of *Ceriodaphnia* reproduction data often required a Bonferroni T-test instead of a Dunnetts Test. This occurred when replicate sizes were unequal due to the presence of males, which are not included in the calculation of average of young per female.

Chapter 3

RESULTS OF BAY BACKGROUND SURVEYS

3.1 INTRODUCTION

The Bay Background component of the program consisted of four surveys that were designed to study seasonal and spatial differences in toxicity among sites located in Carquinez Straits, San Pablo Bay, Central Bay, and South San Francisco Bay. Each survey involved sampling at 12 fixed stations representing both nearshore and channel locations (Table 1 and Fig. 1). Moving northeast to southwest, the stations were: Port Chicago, Grizzly Bay, Pacheco Creek, Benicia/Martinez Bridge, Pinole Shoal Channel, Pinole Shoal Nearshore, Richmond Bridge Channel, Richmond Bridge Nearshore, San Bruno Shoal, Coyote Point, Dumbarton Bridge and Extreme South Bay (Fig.1 and Table 1). With the exception of the fourth survey, sampling was conducted in three groups over three successive days; consequently, individual samples were used for the duration of the test period.

The first three surveys were coordinated with University of California at Santa Cruz researchers who were analyzing the concentrations of trace metals at the same stations we used. Supplementary to their efforts, ancillary water quality measurements such as the determination of total suspended solids, dissolved organic carbon and total organic carbon were also made. This coordinated effort enabled us to consider the results of our bioassays in relation to the results of the metals analyses and the ancillary water quality measurements. The survey design, field conditions, and results for the individual surveys are described below chronologically.

Distribution and severity of toxicity in these four surveys varied both temporally and geographically. Toxic effects were observed at more stations using the sea urchin bioassay than with any other species tested.

TABLE 1. LOCATION OF SAMPLING STATIONS FOR BAY BACKGROUND SURVEYS

Station Name	Latitude	Longitude
NORTH BAY		
Port Chicago	38.03	122.01
Grizzly Bay	38.06	122.02
Pacheco	38.02	122.05
Benicia/Martinez Bridge	38.02	122.08
CENTRAL BAY		
Pinole Shoal Channel	38.03	122.19
Pinole Shoal Nearshore	38.01	122.19
Richmond Bridge Channel	37.55	122.26
Richmond Bridge Nearshore	37.55	122.24
SOUTH BAY		
San Bruno Shoal	37.37	122.17
Coyote Point	37.36	122.20
Dumbarton Bridge	37.30	122.07
Extreme South Bay	37.29	122.05

3.2 FIRST SURVEY -- SURVEY DESIGN AND FIELD CONDITIONS

Sampling for this survey was conducted on April 18, 19, and 20, 1989, during a period of wet weather and over a range of tidal conditions. We performed bioassays using three species: silverside minnow (*Menidia beryllina*), purple sea urchin (*Strongylocentrotus purpuratus*), and the bay mussel (*Mytilus edulis*). A reference toxicant series was run for both the sea urchin and mussel bioassays but not for the silverside minnow. However, since the average salinities of the north, central, and southern portions of the Bay were different, we ran three natural seawater salinity controls for the silverside minnow test.

3.3 FIRST SURVEY -- RESULTS

In the first survey, toxicity was observed using two of the four species tested (Table 2). All samples tested using the sea urchin bioassay were toxic. Samples from 10 of the 12 stations elicited significantly reduced fertilization ranging from 0-14% (arcsine transformed, $p < 0.05$). For the two remaining stations, both located in the North Bay region, fertilization was also significantly reduced and ranged from 21-30%. The "percent ambient sample", a measure of the dilution that occurred due to salinity adjustment of the sample, ranged from 52-92%. This test was conducted using a sperm:egg ratio of 1000:1, and due to an oversight, we used two rather than three replicates for the controls and reference toxicants.

A toxic response was also observed using the *Skeletonema* assay. Growth in all South Bay samples and one Central Bay sample (Pinole Shoal Channel) was significantly inhibited, ranging from 39-63%. No toxic effect was observed using either the silverside minnow or mollusc bioassays.

3.4 SECOND SURVEY -- SURVEY DESIGN AND FIELD CONDITIONS

Sampling was conducted on August 8, 9, and 10, 1989, over a range of tidal conditions and during a period representing dry, summer conditions. Bioassays were performed using three species: silverside minnow, sand dollar (*Dendraster excentricus*) and Pacific oyster (*Crassostrea gigas*). Reference toxicant tests and controls were similar to those used in the first survey.

TABLE 2. TOXICITY OBSERVED IN SAMPLES COLLECTED DURING THE FIRST BAY BACKGROUND SURVEY (APRIL 18-28, 1989)

STATION LOCATION	SILVERSIDE MINNOW		BAY MUSSEL			SEA URCHIN		SKELETONEMA[1]	
	Mean Surv.[2] (%)	Mean Wt. (mg)	Mean Surv. (%)	Mean Abnorm.[3] (%)	Amb.[4] (%)	Fert.[5] (%)	Amb. (%)	Mean Cells/ml	Inhib.[6] (%)
NORTH BAY									
Port Chicago	97	0.72	91	12	52	1*[7]	52	30,000	5
Grizzly Bay	100	0.76	91	11	52	21*	52	28,833	9
Pacheco	100	0.81	99	12	61	30*	61	22,333	29
Benicia/Martinez Bridge	100	0.79	95	15	62	3*	62	25,000	21
Seawater Control	100	0.83	100	9	NA	100	NA	31,500	NA
Salinity Adj. Control	-	-	97	12	NA	98	NA	26,000	18
CENTRAL BAY									
Pinole Shoal Channel	97	1.01	81	7	74	0*	75	23,667	63*
Pinole Shoal Nearshore	97	0.96	100	10	80	7*	81	32,667	48
Richmond Bridge Channel	100	0.96	90	10	80	1*	81	36,167	43
Richmond Bridge Nearshore	97	0.97	100	9	84	1*	85	52,833	17
Seawater Control	100	1.03	100	9	NA	100	NA	63,250	NA
Salintiy Adj. Control	-	-	97	12	NA	98	NA	-	-
SOUTH BAY									
San Bruno Shoal	97	0.82	88	11	90	3*	92	28,500	41*
Coyote Point	100	0.85	74	9	90	0*	92	29,500	39*
Dumbarton Bridge	93	0.84	83	8	87	14*	87	25,667	47*
Extreme South Bay	100	0.90	92	11	87	3*	87	21,333	56*
Seawater Control	93	0.93	100	9	NA	100	NA	48,000	NA
Salinity Adj. Control	-	-	97	12	NA	98	NA	-	-

[1]For Skeletonema all North Bay samples diluted to between 96-97% ambient.

[2]Survival endpoint.

[3]Abnormality endpoint.

[4]Ambient concentration tested after necessary salinity adjustment.

[5]Fertilization endpoint.

[6]Inhibition endpoint.

[7]* Indicates value is significantly different from all controls, $p < 0.05$.

3.5 SECOND SURVEY -- RESULTS

A toxic response was observed using two of the three species tested during the second survey (Table 3). Using the sand dollar bioassay, significantly reduced fertilization was not as widespread or as severe as in the first survey. Significantly reduced fertilization, ranging from 45-66%, was observed in samples from all of the South Bay stations; for this test a sperm:egg ratio of 500:1 was used. In addition, samples from Richmond Bridge Channel, a Central Bay station, elicited significantly reduced fertilization of 77% when compared to the seawater control only; for this test a sperm:egg ratio of 300:1 was used. The sample from this station was not significantly toxic when compared to the brine control. However, since this sample was subjected to minimal salinity adjustment (95% ambient), the seawater control is the more appropriate of the two controls.

Using the oyster bioassay, survivorship was slightly, but significantly, reduced to 77% in the sample from the Extreme South Bay. There was no significant toxicity at any of the stations using the abnormality endpoint. No toxicity was observed using the silverside minnow bioassay.

3.6 THIRD SURVEY -- SURVEY DESIGN AND FIELD CONDITIONS

Samples were collected on December 12, 13, and 14, 1989, during a period that was characterized by unusually dry winter conditions. Sampling was conducted over a range of tidal conditions. Bioassays were performed using three species: silverside minnow, sea urchin, and bay mussel. Reference toxicant tests and controls were similar to those in the first and second surveys.

3.7 THIRD SURVEY -- RESULTS

In the third survey, the sea urchin was the only species of the three tested that exhibited a toxic response to the sample waters (Table 4). Toxicity was widespread, occurring in waters collected from eight out of the 12 stations located throughout the Bay. When arcsine transformed data from the North Bay stations were compared to the brine control, an additional station (Grizzly Bay) was significant. However, fertilization in this sample was

TABLE 3. TOXICITY OBSERVED IN SAMPLES COLLECTED DURING THE SECOND BAY BACKGROUND SURVEY (AUGUST 9-16, 1989)

STATION LOCATION	SILVERSIDE MINNOW		PACIFIC OYSTER			DENDRASTER	
	Mean Surv.[1] (%)	Mean Wt. (mg)	Mean Surv. (%)	Mean Abnorm.[2] (%)	Amb.[3] (%)	Mean Fert.[4] (%)	Amb. (%)
NORTH BAY							
Port Chicago	97	0.69	91	10	77	95	77
Grizzly Bay	100	0.67	95	10	77	95	77
Pacheco	97	0.62	94	9	85	93	85
Benicia/Martinez Bridge	100	0.63	93	6	85	87	85
Seawater Control	100	0.62	96	13	NA	97	NA
Salinity Adj. Control	-	-	94	14	NA	96	NA
CENTRAL BAY							
Pinole Shoal Channel	100	0.68	79	5	88	92[5]	88
Pinole Shoal Nearshore	100	0.65	89	5	89	93	89
Richmond Bridge Channel	100	0.51	95	2	93	77[6]	95
Richmond Bridge Nearshore	100	0.53	90	5	96	84	99
Seawater Control	90	0.53	96	13	NA	98	NA
Salinity Adj. Control	-	-	94	14	NA	92	NA
SOUTH BAY							
San Bruno Shoal	100	0.79	79	6	100	56*[7]	100
Coyote Point	100	0.78	86	5	100	45*	100
Dumbarton Bridge	87	0.81	94	6	94	59*	98
Extreme South Bay	100	0.80	77*	6	96	66*	96
Seawater Control	97	0.80	96	13	NA	96	NA
Salinity Adj. Control	-	-	94	14	NA	99	NA

[1] Survival endpoint.

[2] Abnormality endpoint.

[3] Ambient concentration tested after necessary salinity adjustment

[4] Fertilization endpoint.

[5] Pinole Shoal Channel and Pinole Nearshore samples tested and statistically analyzed with the North Bay samples and control.

[6] Significantly different from seawater control only, $p < 0.05$

[7] * Indicates value is significantly different from all controls, $p < 0.05$

TABLE 4. TOXICITY OBSERVED IN SAMPLES COLLECTED DURING THE THIRD BAY BACKGROUND SURVEY (DECEMBER 12-20, 1989)

STATION LOCATION	SILVERSIDE MINNOW		BAY MUSSEL			SEA URCHIN	
	Mean Surv.[1] (%)	Mean Wt. (mg)	Mean Surv. (%)	Mean Abnorm.[2] (%)	Amb. (%)[3]	Mean Fert.[4] (%)	Amb. (%)
NORTH BAY							
Port Chicago	100	0.72	90	3	72	79*[5]	72
Grizzly Bay	100	0.64	98	3	73	90[6]	73
Pacheco	100	0.72	89	1	76	64*	76
Benicia/Martinez Bridge	100	0.72	81	5	74	62	74
Seawater Control	100	0.51	93	4	NA	96	NA
Salinity Adj. Control	-	-	89	3	NA	97	NA
CENTRAL BAY							
Pinole Shoal Channel	100	0.89	73	3	91	57*	91
Pinole Shoal Nearshore	100	0.82	73	4	91	61*	91
Richmond Bridge Channel	93	0.65	96	1	100	87	100
Richmond Bridge Nearshore	97	0.79	75	1	100	71*	100
Seawater Control	97	0.79	93	4	NA	96	NA
Salinity Adj. Control	_	-	89	3	NA	98	NA
SOUTH BAY							
San Bruno Shoal	100	1.06	82	5	100	78*	100
Coyote Point	97	0.98	72	3	100	95	100
Dumbarton Bridge	97	1.05	94	3	100	79*	100
Extreme South Bay	100	1.10	81	3	100	36*	100
Seawater Control	100	1.03	93	4	NA	96	NA
Salinity Adj. Control	-	-	89	3	NA	98	NA

[1]Survival endpoint.

[2]Abnormality endpoint.

[3]Ambient concentration tested after necessary salinity adjustment.

[4]Fertilization endpoint.

[5]* Indicates value is significantly different from all controls, $p<0.05$

[6]Value is significantly differently different from brine control only, $p<0.05$.

90%, a comparatively high value. At all stations, the severity of toxicity was less than that seen in the first survey, with fertilization ranging from 36-79%. We used a 700:1 sperm:egg ratio for this test. No toxicity was observed in either the silverside minnow or mollusc bioassays.

3.8 FOURTH SURVEY -- SURVEY DESIGN AND FIELD CONDITIONS

Samples were collected from the South Bay on April 17 and from the North and Central Bay on April 18, 1990. There were scattered, light showers before and during sample collection. The sea urchin was the only species tested. The South Bay samples were tested on the same day that samples were collected. Based on the results of this first bioassay, one sample was chosen for a Toxicity Identification Evaluation (TIE), based on EPA protocols USEPA, 1988b; USEPA, 1988c). This TIE was performed the day following sample collection (April 18). Samples collected from the Central and North Bay on April 18 were held 24 hours before testing.

3.9 FOURTH SURVEY -- RESULTS

Toxicity was observed in samples from nine out of 12 stations using the sea urchin bioassay (Table 5). No other species were tested. Samples from all four stations in the North Bay elicited significantly reduced fertilization ranging from 29-65%, as compared to both the seawater and brine controls. Samples from Pinole Shoal Channel and Pinole Shoal Nearshore in the Central Bay also elicited significantly reduced fertilizations of 68% and 54%, respectively, when compared to both the seawater and brine controls. When compared to the seawater control, fertilization in the sample from Richmond Bridge Channel (82%) was also significant. Samples from three stations in the South Bay (San Bruno Shoal, Extreme South Bay and Coyote Point) elicited significant toxicity compared to both the seawater and brine controls, with fertilization in the ambient samples ranging from 49-52%. A sperm:egg ratio of 200:1 was used for testing.

3.10 PRELIMINARY TIE STUDY -- RESULTS

Preliminary attempts to identify the potential causes of toxicity in water collected from San

TABLE 5. TOXICITY OBSERVED USING THE SEA URCHIN BIOASSAY IN SAMPLES COLLECTED DURING THE FOURTH BAY BACKGROUND SURVEY (APRIL 17-18, 1990)

STATION LOCATION	SEA URCHIN	
	Mean Fert.[1] (%)	Amb. (%)[2]
NORTH BAY		
Port Chicago	65*[3]	71
Grizzly Bay	29*	71
Pacheco	42*	71
Benicia/Martinez Bridge	53*	73
Seawater Control	99	NA
Salinity Adj. Control	85	NA
CENTRAL BAY		
Pinole Shoal Channel	68*	85
Pinole Shoal Nearshore	54*	85
Richmond Bridge Channel	82[4]	92
Richmond Bridge Nearshore	93	92
Seawater Control	99	NA
Salinity Adj. Control	85	NA
SOUTH BAY		
San Bruno Shoal	52*	95
Coyote Point	49*	97
Dumbarton Bridge	83	90
Extreme South Bay	50*	87
Seawater Control	93	NA
Salinity Adj. Control	86	NA

[1] Fertilization endpoint.

[2] Ambient concentration tested after necessary salinity adjustment.

[3] * Indicates value is significantly different from all controls, $p<0.05$.

[4] Significantly different from seawater control only, $p<0.05$.

Bruno Shoal in the South Bay, indicated only that toxicity was transitory. Using a sea urchin bioassay, this sample was identified as toxic within 12 hours of collection. A TIE was performed the following day on this sample, and a second sea urchin test was run on both the fractionated and unfractionated portions within 30 hours of initial sample collection. Unfortunately, in the second sea urchin test, no toxicity was observed in the unfractionated samples, making it impossible to assess the effects of fractionation. A similar decrease in toxicity was observed for the sample collected at Port Chicago. At 24 hours, 65% fertilization was observed; whereas at 48 hours, mean percent fertilization was 95%.

3.11 METALS SURVEY -- RESULTS

The Bay Background survey was designed to determine whether ambient toxicity was correlated with trace metals concentrations that exceeded EPA Criteria for the protection of aquatic life. The actual trace metal data for nickel, cadmium, silver, lead, zinc, and copper will be reported elsewhere by UC Santa Cruz researchers. However, we have determined, from preliminary data, that there is no observable relationship between the observed sea urchin toxicity and exceedances of EPA metals criteria. This is primarily due to the fact that there were far fewer exceedances of metals criteria than observations of toxicity using the urchin test. In addition, there was no widespread increase in metals concentrations during the first survey when the greatest toxicity was observed.

3.12 SUMMARY OF BAY BACKGROUND SURVEY FINDINGS

Echinoderm fertilization data for the four Bay Background surveys are summarized in Figure 2. The data indicate that when comparisons among surveys are made, spatial patterns are not clearly discernable. However, data from the first survey demonstrate much more severe toxicity than do data from the other three surveys, indicating that toxicity may vary widely over time. The potential implications of these findings are discussed in Chapter 7.

Figure 2. Echinoderm Fertilization Success in Four Bay Background Surveys

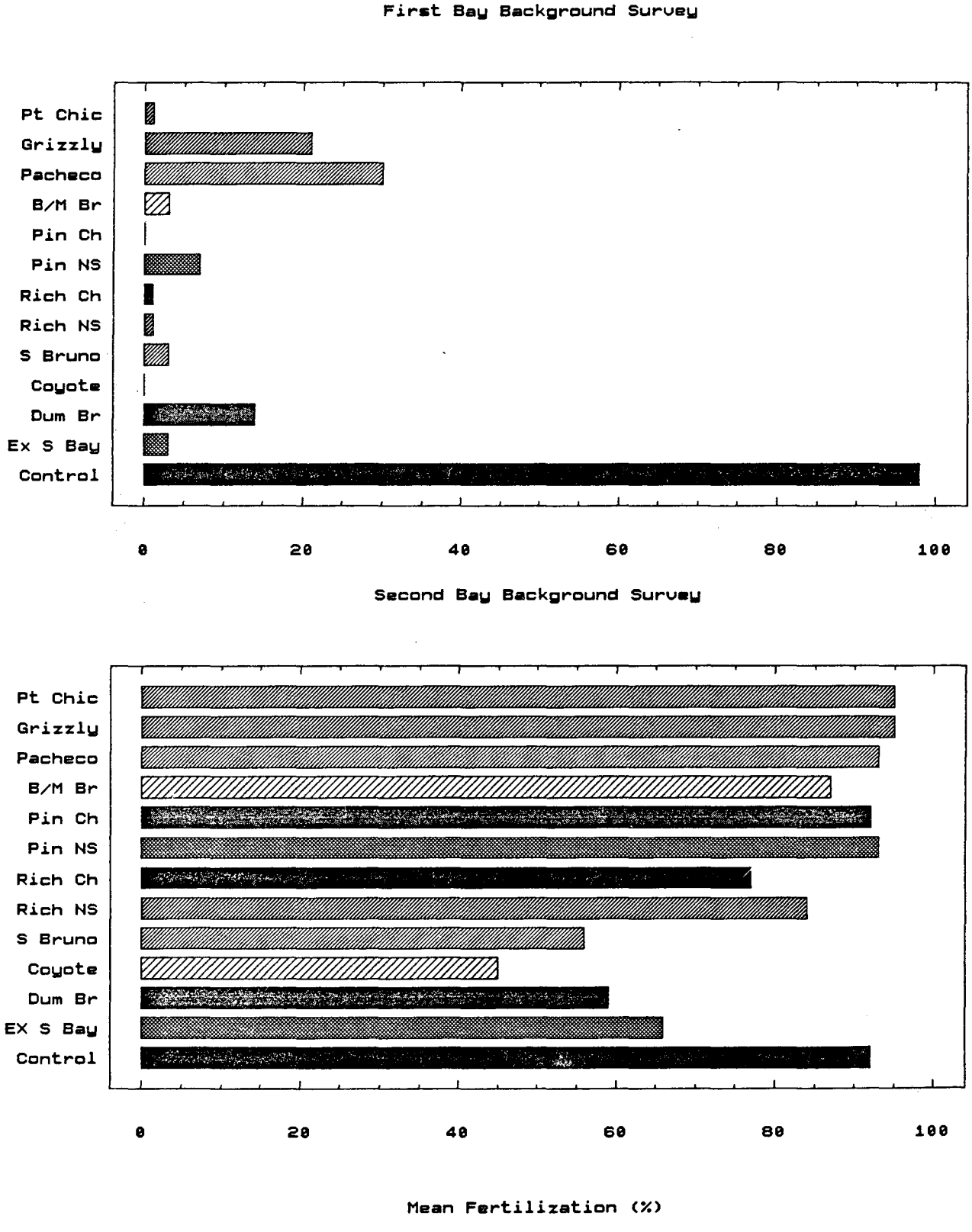
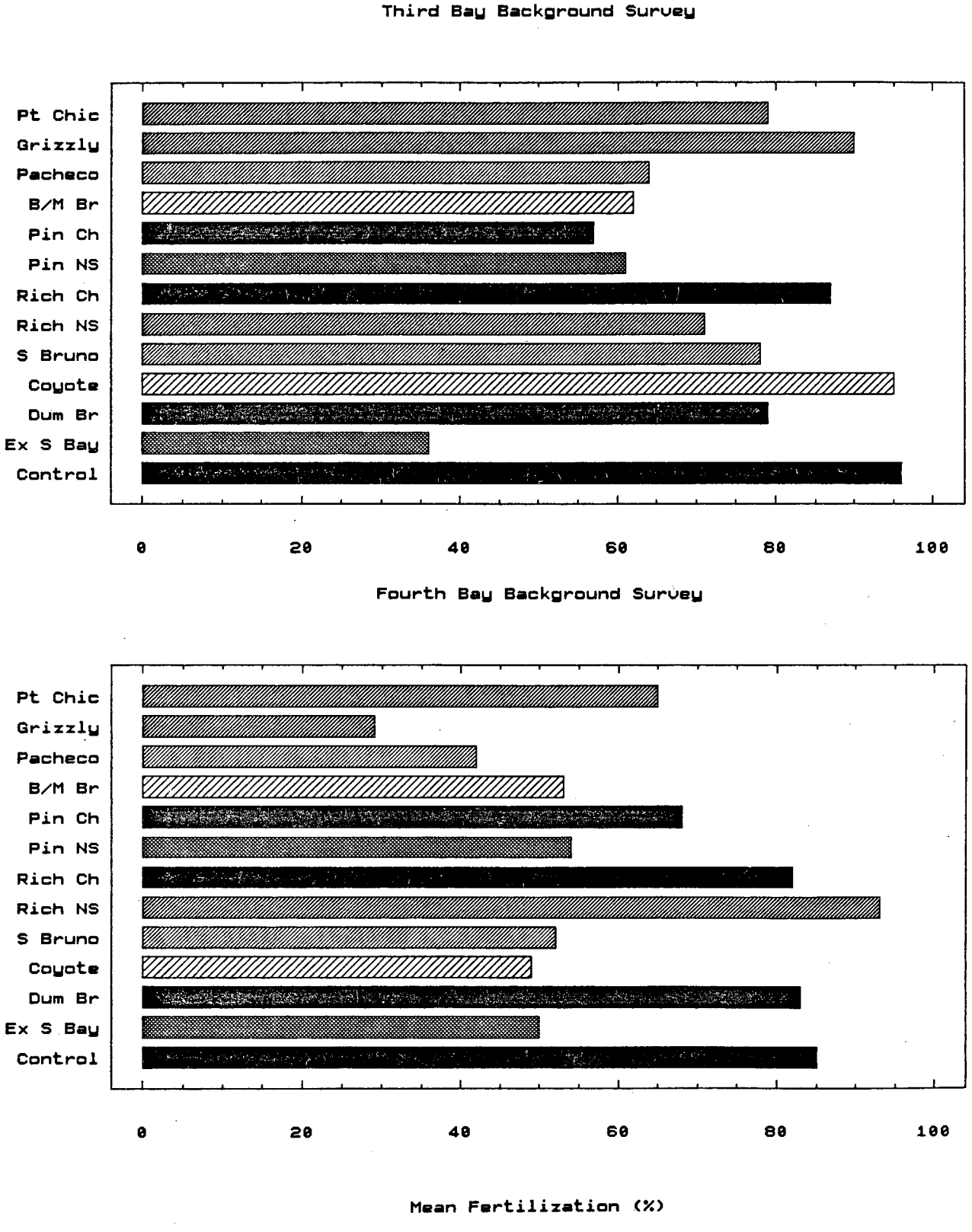


Figure 2. Continued



Chapter 4

RESULTS OF BAY MARSH SURVEYS

4.1 INTRODUCTION

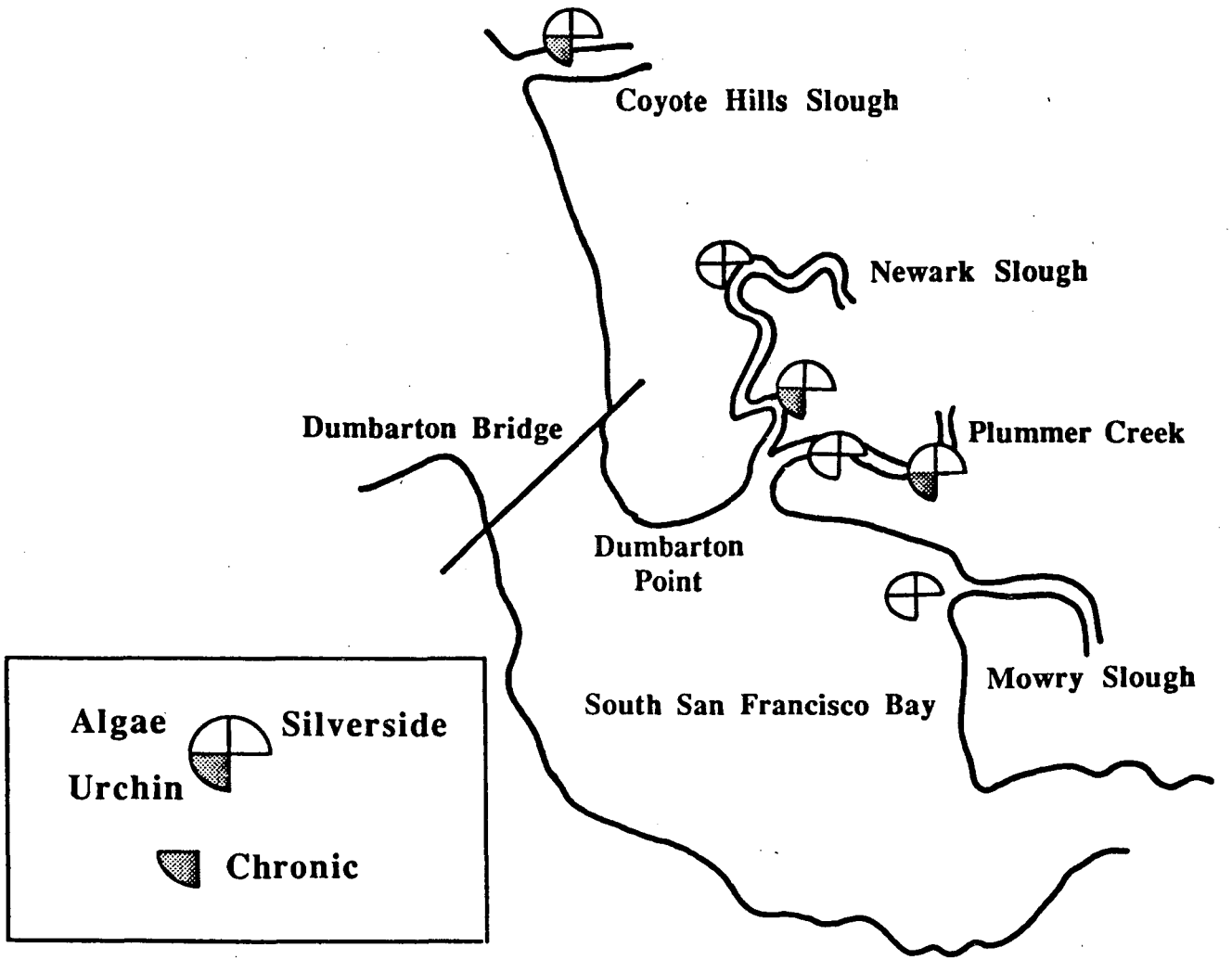
The surveys of toxicity in Bay marshes were conducted at one reference site, two marsh reclamation projects and at two marshes adjacent to discharge sites. In addition, studies were conducted to identify the cause of toxicity observed at the Hayward Marsh. The purpose of these surveys was to determine the spatial distribution of toxicity at varied marsh sites, involving a broad range of management practices. A second goal was to determine whether levels of toxicity observed in ambient waters were predictable from effluent toxicity data. The survey design and field conditions varied among sites, and these are described below.

Extensive toxicity was observed in one of the reclamation marshes (Hayward Marsh) as well as in one marsh adjacent to a discharge (Sunnyvale Wastewater Treatment Plant); however, some toxicity was observed in four of the five marshes tested. First, the results of testing in the reference marsh located at the San Francisco Bay National Wildlife Refuge are described. Second, results of studies at the two reclamation marshes are given; these include extensive data on investigations of the cause of toxicity at the Hayward Marsh. Third, data on toxicity at the two sites adjacent to municipal discharges are presented in relation to observed effluent toxicity.

4.2 SAN FRANCISCO BAY NATIONAL WILDLIFE REFUGE -- SURVEY DESIGN AND FIELD CONDITIONS

San Francisco Bay National Wildlife Refuge, located in the South Bay (Fig.3), was chosen as our reference marsh, because it is among the marshes least influenced by point-source discharges. A single industrial discharge (FMC Corporation with a discharge rate of approximately 0.1 mgd) enters Plummer Creek. Tidal flushing comprises a major source of water exchange to all marshes and sloughs; although minor freshwater input occurs at

Figure 3. San Francisco Bay National Wildlife Refuge Ambient Toxicity Survey



Plummer Creek, Coyote Hills Slough, and Newark Slough. We sampled at six sites that represent upstream and downstream reaches of the major marsh waterways. Over a 7-day period, March 15-21, 1989, sampling was conducted daily during the morning high tide to facilitate boat access into the channels and sloughs. There was sporadic rain before and during the sampling period, with heavy showers on day four. We conducted bioassays using three species. These were the silverside minnow, the purple sea urchin and the alga, *Skeletonema*. In addition to performing tests on the ambient samples, reference toxicant tests were conducted for all species tested. Natural seawater control and reference toxicant waters for the silverside minnow test were adjusted to a salinity that was the average of the ambient salinities on day one.

4.3 SAN FRANCISCO BAY NATIONAL WILDLIFE REFUGE -- RESULTS

A toxic response was elicited by only one of the three species tested (Fig. 3 and Table 6). Using the sea urchin bioassay with a 1000:1 sperm:egg ratio, samples from three stations (Coyote Hills Slough, Lower Newark, and Upper Plummer) elicited significantly reduced fertilization of 45-75% as compared to the seawater control. When compared to both the brine and seawater controls, only the Coyote Hills Slough sample showed significant toxicity (45% fertilization). However, given that the ambient samples were diluted by no more than 10% with brine, the seawater control is potentially an appropriate control for comparison. The three stations at which significant toxicity was observed are the three stations most plausibly influenced by urban runoff during a storm event. Coyote Hills Slough receives urban runoff, the Lower Newark station is adjacent to a highway, and Upper Plummer Creek also receives some urban drainage.

One minor deviation from the sea urchin protocol, which may have had an effect on fertilization in the ambient samples, was that samples were not pH adjusted to 8.0 prior to testing. We did not observe toxicity in the samples from any stations using either the silverside minnow or *Skeletonema* bioassays.

TABLE 6. TOXICITY OBSERVED IN SAMPLES COLLECTED FROM THE SAN FRANCISCO BAY NATIONAL WILDLIFE REFUGE (MARCH 15-21, 1989)

STATION LOCATION	SILVERSIDE MINNOW		SEA URCHIN		SKELETONEMA	
	Mean Surv.[1] (%)	Mean Wt. (mg)	Mean Fert.[2] (%)	Amb.[3] (%)	Mean Cells/ml	Mean inhib.[4] (%)
Coyote Hills Slough	87	0.79	45*[5]	95	51,200	NA
Upper Newark Slough	93	0.69	83	90	48,133[6]	NA
Lower Newark Slough	97	0.64	69+[7]	95	32,533	20
Upper Plummer Creek	93	0.71	75+	93	26,667	34
Lower Plummer Creek	83	0.77	86	95	35,067	14
Mowry Slough	90	0.78	88	95	46,933	NA
Seawater Control	93	0.74	100	NA	40,533	NA
Salinity Adj. Control	-	-	78	NA	-	-

[1]Survival endpoint.

[2]Fertilization endpoint.

[3]Ambient concentration tested after necessary salinity adjustment.

[4]Inhibition endpoint.

[5]* Indicates value is significantly different from all controls, $p < 0.05$.

[6]This sample was diluted to 96% ambient for the Skeletonema test.

[7]+ Indicates value is significantly different from the seawater control, $p < 0.05$.

4.4 HAYWARD MARSH RECLAMATION PROJECT -- SURVEY DESIGN AND FIELD CONDITIONS

Hayward Marsh, formerly the site of salt evaporation ponds, is one of two wastewater marsh reclamation projects constructed in the San Francisco Bay region. This survey was designed to evaluate the potential toxicological effects associated with a reclamation project that relies upon secondarily treated wastewater from the Union Sanitary District as its sole source of freshwater. The approximate current discharge rate into the marsh is 10 mgd. The marsh is divided into a series of 5 interconnected ponds, the last two of which are tidally influenced (Fig. 4). We sampled along a gradient moving away from the effluent discharge and at a site chosen to represent the background conditions of the Bay.

In the first survey, samples were collected daily over a 7-day period (July 11-17, 1989), at six stations -- the outflows of four of the ponds, a mixing channel and the bay background water (Fig. 4). There were no precipitation events during the sampling period. Testing was conducted on all samples using the silverside minnow and the sea urchin. Reference toxicant tests were conducted for all species tested. For the silverside minnow test, all ambient samples (except the Bay background), reference toxicant final solutions, and the salinity adjustment control were salinity adjusted to 22 ppt. The natural seawater control was run at 32 ppt.

A second survey was conducted to characterize the persistence and possible causes of toxicity observed during the first survey. Nine stations (Fig. 5), including three not sampled in the first survey, were sampled daily over a 7-day period (November 15-21, 1989). Similar to the first survey, there was no precipitation during the sampling period. Silverside minnow and sea urchin bioassays were conducted on all samples. Fathead minnow and *Ceriodaphnia* bioassays were conducted using samples from the first three ponds. Reference toxicant tests were run for all species except the fathead minnow. For the silverside minnow test, all ambient samples (except Bay background), the salinity adjustment control, and a seawater control were adjusted to 10 ppt. The reference toxicants and an additional seawater control were run at 21 ppt. For the *Ceriodaphnia* test, the sample from Basin 2B Outfall was diluted to a seven-day average of 89% ambient water to reduce the conductivity

Figure 4. Hayward Marsh Ambient Toxicity Survey I

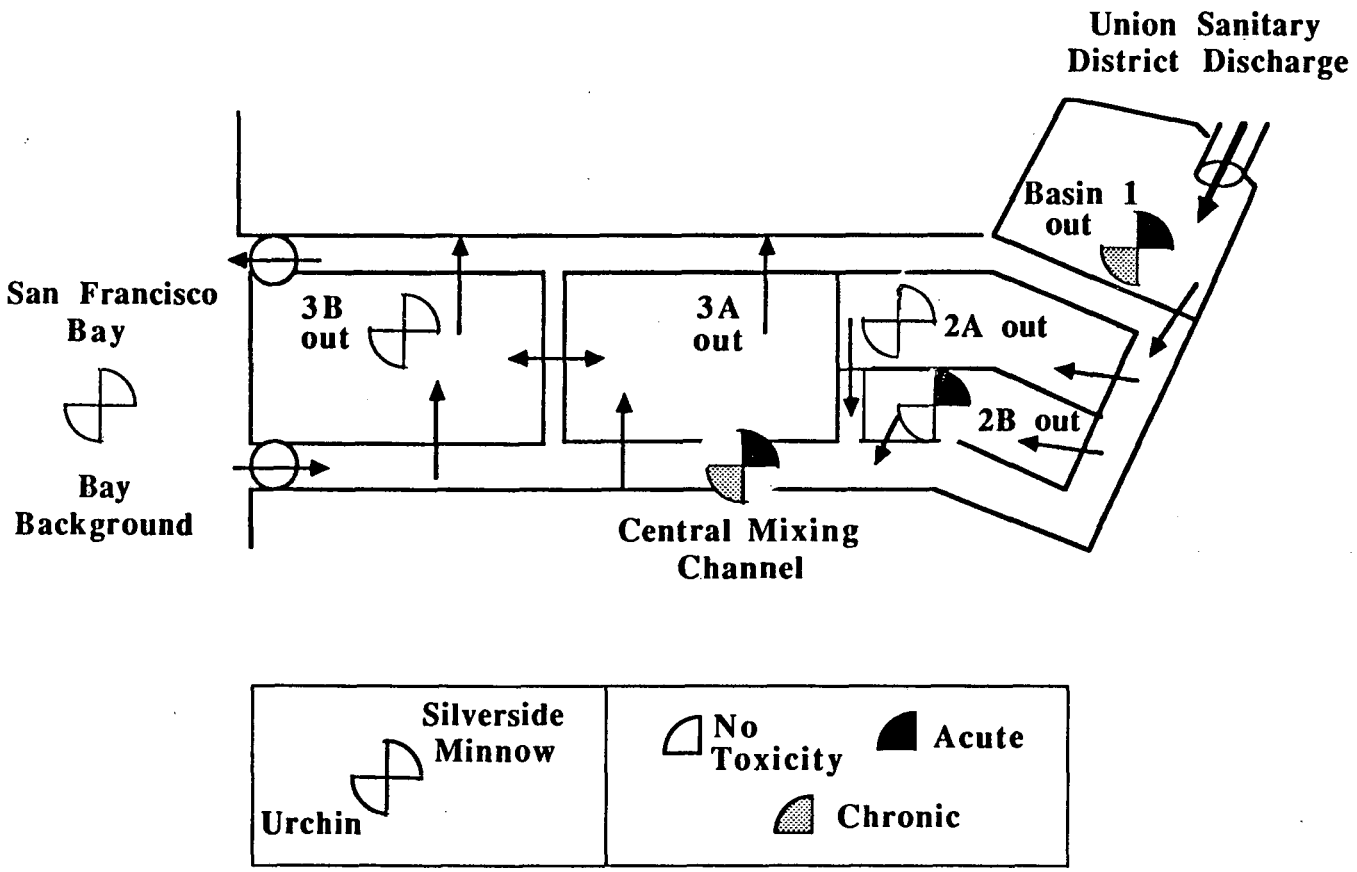
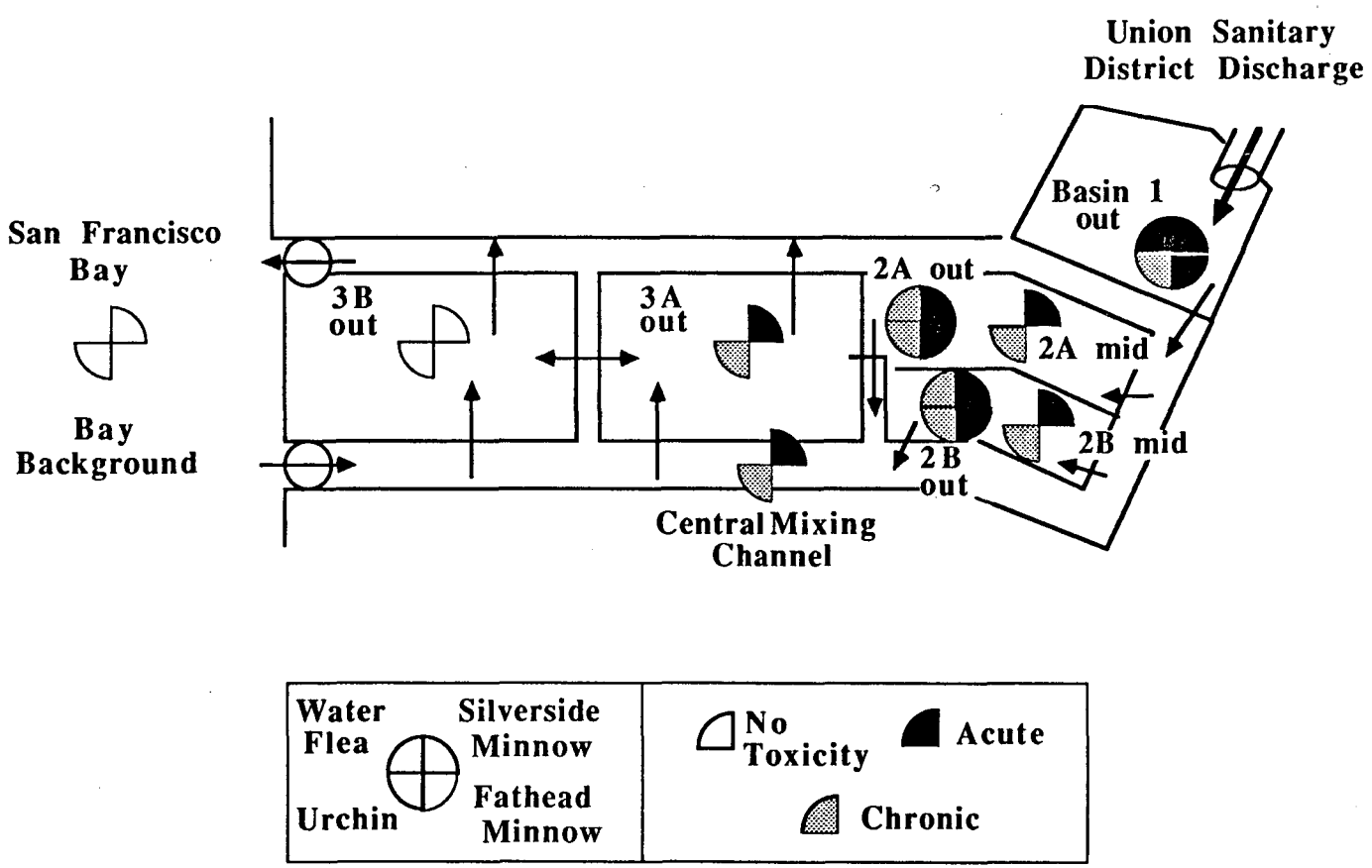


Figure 5. Hayward Marsh Ambient Toxicity Survey II



below 2000 umhos/cm^2.

Due to the fact that extensive toxicity was observed in both surveys, toxicity correlation and toxicity identification studies are also described below.

4.5 HAYWARD MARSH RECLAMATION PROJECT - TOXICITY CHARACTERIZATION RESULTS

In the first Hayward Survey conducted July 11-17, 1989, toxic responses were observed using both species tested (Fig. 4, Table 7), and toxicity generally occurred in basins closest to the outfall. Using the silverside minnow test, samples from three stations (Basin 1 Outfall, Basin 2B Outfall, Central Mixing Channel) nearest to the outfall resulted in a toxic response ranging from 0-43% survival. Times to mortality in the three basins were 100% mortality in 24h, 60% mortality in 24h and 70% mortality in 48h, respectively. Using the sea urchin test, samples from two stations elicited signifcant toxicity using a sperm:egg ratio of 500:1; fertilization in Basin 1 Outfall and the Central Mixing Channel treatments were 74% and 83%, respectively (arcsine transformed, $p < 0.05$).

In the second Hayward Marsh survey (November 15-21, 1989), in which three additional stations were sampled and additional species were studied, we observed toxic effects in ambient waters with all of the four species tested (Fig. 5, Table 8). Acute lethal effects were observed with three species (silverside minnow, water flea and fathead minnow). For the silverside minnow, water from all of the sites significantly reduced survivorship, ranging from 0-67%, with the exception of the two stations furthest from the outfall (Basin 3B Outfall and Bay Background).

Seven of the sites identified as toxic using the silverside minnow assay were also toxic in comparison to both the brine and seawater controls using the sea urchin test (a sperm:egg ratio of 700:1). Levels of toxicity did not exhibit a clearcut decrease with increasing distance from the outfall. Fertilization ranged from 2-61% at these sites. The sample from one additional station (Basin 3B Outfall) was significant when compared to the seawater control only. However, since samples were diluted to between 62-68% for salinity adjustment, the brine control is the more appropriate control.

TABLE 7. TOXICITY OBSERVED IN THE FIRST SURVEY CONDUCTED AT THE HAYWARD MARSH (JULY 11-17, 1989)

STATION LOCATION	SILVERSIDE MINNOW		SEA URCHIN	
	Mean Surv.[1] (%)	Mean Wt. (mg)	Mean Fert.[2] (%)	Amb. (%)
Basin 1 Outfall	0*[3]	-	74*	69
Basin 2A Outfall	70	0.49	98	69
Basin 2B Outfall	43*	-	93	69
Central Mixing Channel	43*	-	83*	73
Basin 3B Outfall	80	0.51	92	74
Bay Background	93	0.49	99	84
Fresh/Seawater Control	90	0.45	100	NA
Salinity Adj. Control	90	0.42	99	NA

[1]Survival endpoint.

[2]Fertilization endpoint.

[3]* Indicates value is significantly different from all controls, $p < 0.05$.

TABLE 8. TOXICITY OBSERVED IN THE SECOND SURVEY CONDUCTED AT THE HAYWARD MARSH (NOVEMBER 15-21, 1989)

STATION LOCATION	SILVERSIDE MINNOW		FATHEAD MINNOW		SEA URCHIN		CERIODAPHNIA		
	Mean Surv.[1] (%)	Mean Wt. (mg)	Mean Surv. (%)	Mean Wt. (mg)	Mean Fert.[2] (%)	Amb. (%)[3]	Surv. (%)	Avg. Repro.[4]	Amb. (%)
Basin 1 Outfall	3*[5]	-	0*	-	16*	62	0*	-	100
Basin 2A Midway	0*	-	-	-	35*	62	-	-	-
Basin 2B Midway	53*	-	-	-	7*	62	-	-	-
Basin 2A Outfall	0*	-	0*	-	43*	62	70	3*	100
Basin 2B Outfall	17*	-	0*	-	2*	62	80	6*	89
Central Mixing Channel	27*	-	-	-	45*	62	-	-	-
Basin 3A Outfall	67*	-	-	-	61*	68	-	-	-
Basin 3B Outfall	90	0.47	-	-	82	68	-	-	-
Bay Background	93	0.59	-	-	90	95	-	-	-
Seawater Control	97/97[6]	0.66/0.69	-	-	94	NA	-	-	-
Freshwater Control	-	-	100	0.53	-	-	100	17	NA
Salinity Adj. Control	100	0.74	-	-	88	NA	-	-	-

[1]Survival endpoint.

[2]Fertilization endpoint.

[3]Ambient concentration tested after necessary salinity adjustment.

[4]Average number of young per female.

[5]* Indicates value is significantly different from all controls, $p < 0.05$.

[6]Controls run at 10 ppt and 21 ppt, respectively

Toxicity tests using *Ceriodaphnia* and fathead minnow could only be conducted in basins closest to the outfall because of increasing salinities in the outer basins. Of the three sites tested using the *Ceriodaphnia* bioassay, only samples from Basin 1 elicited significantly reduced survivorship. The Basin 2A and 2B Outfall stations exhibited significantly reduced reproduction. At these same three stations, 100% mortality occurred within 72 hours in the fathead minnow test. The toxicity characterization data for the second Hayward Marsh survey clearly indicate a decrease in toxicity with distance from the outfall for the majority of species tested.

There were some minor deviations from protocol standards and sample adjustments in both Hayward surveys which may have influenced the outcome of the testing. In the first survey, both the seawater and the salinity adjustment controls for the silverside minnow test had slightly lower than acceptable larval weights (0.45 mg and 0.42 mg, respectively). In the second Hayward survey, a 10 ppt seawater control was started one day late using nine-day instead of eight-day old larvae. On the second day of testing, the sample from Basin 1 required aeration prior to being used in both the silverside minnow and *Ceriodaphnia* bioassays; although this could have reduced the toxicity of volatile compounds present in the sample, significant acute mortality observed in both species at other stations indicates that aeration did not markedly affect the toxicity observed.

4.6 HAYWARD MARSH RECLAMATION PROJECT- RESULTS OF TOXICITY CORRELATIONS

To determine the causes of toxicity observed in the Hayward Marsh, we first evaluated the potential contributions of unionized ammonia, chlorine and heavy metals. This evaluation consisted of comparing observed concentrations of ammonia, chlorine and heavy metals to levels of these substances known to cause toxicity. The majority of the data address the contribution of unionized ammonia.

Preliminary data obtained in the first Hayward Marsh Survey indicated that unionized ammonia occurred at levels exceeding 1mg/l, which is sufficient to cause toxicity in many species including fathead minnow, silverside minnow, and potentially, *Ceriodaphnia*

(USEPA, 1985; USEPA, 1989b). Consequently, for the second Hayward survey, measurements of free ammonia in ambient waters used in the silverside minnow, fathead minnow, *Ceriodaphnia* and sea urchin tests were made during the testing period.

The most dramatic patterns of mortality that might be correlated with ammonia toxicity were documented using the fathead and silverside minnow tests. We observed complete mortality of fathead minnow after two days of exposure to unionized ammonia concentrations that, with the exception of basin 1 on the first day (0.55 mg/l NH_3), ranged from 1.44-2.63 mg/l NH_3 (Table 9). The LC50 for larval fathead minnow, at pH 7.64-8.1, is 0.75-1.59 mg/l NH3 (USEPA, 1985). Consequently, in the laboratory-adjusted waters, all samples except one contained sufficiently high ammonia concentrations to explain the toxicity observed. Under field conditions of temperature and pH (Table 9), lethal values of NH_3 were observable on the second day but not on the first day. These data indicate that toxicity could occur under both laboratory and field conditions.

Patterns of survivorship in the silverside minnow test also indicate that ammonia is the principle cause of toxicity. Seven-day average calculations of free ammonia in the laboratory waters that were temperature and salinity adjusted (Table 10, laboratory adjusted) indicated that lethal concentrations of unionized ammonia were observed in adjusted samples from all stations, except the Bay Background station. The lethal value for silverside minnow larvae is approximately 0.9 mg/l at pH 7 to 8 (USEPA, 1989b). Calculated 7-day average values for the ambient, unadjusted waters (Table 10, ambient) ranged from 1.02-0.61 mg/l, indicating that conditions were less toxic in the marsh itself than were the samples tested under laboratory conditions. Nevertheless, ammonia levels sufficient to cause ambient toxicity were observed in one 7-day average value.

A more detailed analysis of daily cumulative survivorship in relation to free ammonia concentrations in each basin (Table 11) further revealed the presence of lethal levels in adjusted samples from several basins. This was paticularly noticeable on the second day of the survey, in which the most rapid mortality occurred. On day two, free ammonia concentrations in adjusted samples from all basins of the marsh ranged from 2.58 to 4.19 mg/l. Mortality was observed in samples from all basins that contained free ammonia concentrations exceeding 3 mg/l, with the exception of Basin 2B midway. These levels of

TABLE 9. DAILY CUMULATIVE SURVIVORSHIP AND FREE AMMONIA CONCENTRATIONS FOR THE FATHEAD MINNOW BIOASSAY IN THE SECOND HAYWARD MARSH SURVEY

STATION LOCATION	DAY 1			DAY 2		
	Mean Surv. (%)[1]	Laboratory NH_3 (mg/l)	Ambient[2] NH_3 (mg/l)	Mean Surv. (%)	Laboratory NH_3 (mg/l)	Ambient NH_3 (mg/l)
Basin 1 Outfall	10	0.55	0.36	1	1.49	0.98
Basin 2A Outfall	10	1.44	0.69	0	2.53	1.28
Basin 2B Outfall	10	1.44	0.64	0	2.63	1.34

[1]Survival endpoint.

[2]Free ammonia calculated for pH and temperatures measured in the field.

TABLE 10. SEVEN-DAY AVERAGE CALULATIONS OF FREE AMMONIA FROM THE SECOND HAYWARD MARSH SURVEY

STATION LOCATION	AMBIENT[1,2]				LABORATORY UNADJUSTED[3]				LABORATORY ADJUSTED[4]			
	Temp (C)	pH	Total N (mg/l)	NH_3 (mg/l)	Temp (C)	pH	Total N (mg/l)	NH_3 (mg/l)	Temp (C)	pH	Total N (mg/l)	NH_3 (mg/l)
Basin 1 Outfall	19	7.64	31	0.61	25	7.64	31	0.89	25	7.77	31	1.40
Basin 2A Midway	16	7.80	29	0.64	25	7.80	29	1.20	25	8.08[5]	29[5]	2.53[5]
Basin 2B Midway	15	7.95	27	0.78	25	7.95	27	1.50	25	8.04	27	1.83
Basin 2A Outfall	15	7.97	29	0.83	25	7.97	29	1.62	25	8.16[5]	29[5]	2.84[5]
Basin 2B Outfall	15	7.93	28	0.74	25	7.93	28	1.46	25	8.03	28	1.77
Central Mixing Channel	15	7.96	27	0.77	25	7.96	27	1.52	25	8.08	27	1.89
Basin 3A Outfall	15	8.23	21	1.02	25	8.23	21	2.08	25	8.33	21	2.25
Basin 3B Outfall	15	8.12	22	0.83	25	8.11	22	1.72	25	8.13	22	1.77
Bay Background	13	7.83	0.63	0.01	25	7.83	0.63	0.03	25	7.82[6]	0.63[6]	0.03[6]

[1] Free ammonia calculated for temperatures measured in the field.

[2] Seven-day average NH_3 concentrations were calculated from daily NH_3 values. Consequently, individual values cannot be directly recalculated using the average values reported; daily values are required.

[3] Free ammonia calculated at the laboratory test temperature for samples that were not salinity adjusted because they were used for freshwater tests.

[4] Free ammonia calculated at the laboratory test temperature for samples that were salinity adjusted to 10 ppt for the silverside minnow test. The Bay Background sample was unadjusted.

[5] Three-day average.

[6] Five-day average

TABLE 11. DAILY CUMULATIVE SURVIVORSHIP AND ADJUSTED FREE AMMONIA CONCENTRATIONS FOR THE SILVERSIDE MINNOW IN THE SECOND HAYWARD MARSH SURVEY

STATION LOCATION	DAY 1		DAY 2		DAY 3		DAY 4		DAY 5		DAY 6		DAY 7	
	Mean Surv. (%)[1]	NH_3 (mg/l)	Mean Surv. (%)	NH_3 (mg/l)	Mean Surv. (%)	NH_3 (mg/l)	Mean Surv. (%)	NH_3 (mg/l)	Mean Surv. (%)	NH_3 (mg/l)	Mean Surv. (%)	NH_3 (mg/l)	Mean Surv. (%)	NH_3 (mg/l)
Basin 1 Outfall	56	1.00	10	4.07	10	1.30	3	1.30	3	0.68	3	1.02	3	0.42
Basin 2A Midway	90	1.46	6	4.07	0	2.08	0	-	0	-	0	-	0	-
Basin 2B Midway	93	1.36	73	3.48	60	2.06	53	1.96	53	1.10	53	1.67	53	1.21
Basin 2A Outfall	73	2.04	0	4.19	0	2.30	0	-	0	-	0	-	0	-
Basin 2B Outfall	93	1.74	20	3.29	16	1.78	16	1.49	16	1.30	16	1.17	16	1.08
Central Mixing Channel	80	1.70	37	3.23	37	1.59	37	1.82	33	1.75	27	1.93	27	1.22
Basin 3A Outfall	100	2.04	90	2.58	90	2.34	87	2.19	83	2.00	67	2.19	67	2.44
Basin 3B Outfall	100	1.24	93	2.95	93	1.24	93	1.59	93	1.40	93	1.93	90	2.05
Bay Background	97	0.02	93	0.04	93	0.03	93	0.02	93	-	93	0.03	93	-

[1]Survival endpoint.

NH_3 would also have resulted in lethal levels under field conditions of temperature and pH.

Nevertheless, ammonia toxicity does not explain all of the toxicity observed in all of the basins, nor is there toxicity in all of the basins containing potentially lethal concentrations of free ammonia. For example, on the first test day, silverside minnow mortality was not significant in any of the basins except Basin 1. Significantly, free ammonia levels in this basin, at 1 mg/l, were lower than in any of the other basins of the marsh which ranged from 1.24-2.04 mg/l (Table 11), leading us to conclude that free ammonia was not the principle cause of toxicity in this basin on day one. Alternatively, concentrations in excess of 2 mg/l persisted in Basin 3A outfall for seven days, with relatively low mortality rates. These data indicate that relatively high free ammonia concentrations alone may not explain all of the toxicity observed or that other factors mitigating ammonia toxicity in the bioassays were not fully explained.

Toxicity observed using the sea urchin bioassay was comparatively moderate, and unionized ammonia was also documented to be at comparatively moderate levels (Table 12). Due to the low temperature used in the sea urchin bioassay and the low total ammonia levels on day 5 of the survey (the date the sea urchin test was conducted), unionized ammonia did not reach high levels. To our knowledge, the concentrations of free ammonia that cause toxicity in this test have not been documented; as such, the potential interrelationships between toxicity and ammonia levels cannot be thoroughly evaluated.

During the second Hayward Marsh survey, measurements of residual chlorine were also made on both salinity-adjusted and unadjusted samples. All unadjusted samples from the first three collection dates were analyzed, and concentrations did not exceed 0.05 mg/l as Cl_2. Subsequent measurements, limited to Basin 1, had similarly low chlorine levels. Based on low chlorine concentrations in the unadjusted samples, we only analyzed the salinity-adjusted samples from Basin 1 on second and third test dates. Neither of these measurements exceeded 0.01 mg/l Cl_2.

Samples collected on the first sample day from Basins 1A, 2A Outfall and 2B Outfall were analysed by staff of the East Bay Dischargers Authority (EBDA) for concentrations of total

TABLE 12. FREE AMMONIA IN THE SEA URCHIN BIOASSAY FOR THE SECOND HAYWARD MARSH SURVEY

STATION LOCATION[1]	pH	TOTAL N (MG/L)	NH_3 (MG/L)
Basin 1 Outfall	7.99	30	0.79
Basin 2A Midway	7.97	26	0.67
Basin 2B Midway	7.98	24	0.62
Basin 2A Outfall	7.97	24	0.62
Basin 2B Outfall	7.98	23	0.60
Central Mixing Channel	7.98	23	0.60
Basin 3A Outfall	7.98	19	0.49
Basin 3B Outfall	7.98	20	0.52
Bay Background	7.99	0.65	0.002

[1]Water used in testing collected November 19, 1989.

copper, nickel, chromium, lead, arsenic, mercury, cadmium, silver, zinc, cyanide and selenium. Concentrations of these metals in the three basins were compared to EPA freshwater criterion values (4-day or 24-h average) for these substances. Exceedances were noted for copper and zinc. The mean concentration of copper in the three basins was 49 ppb, as compared to the freshwater criterion value of 6.5 ppb. The mean concentration of zinc was 63 ppb, as compared to a freshwater criterion of 58 ppb. Nickel concentrations exceeded the marine criterion (7.1 ppb) but not the freshwater criterion values. The mean of the nickel concentrations in the three basins was 9.3 ppb. Values for lead, mercury, cadmium and silver were reported using detection limits that exceeded the freshwater criterion values. The copper data, in particular, indicate that high ambient metals concentrations may be one of the causes of unexplained toxicity in the basins.

4.7 HAYWARD MARSH RECLAMATION PROJECT -- RESULTS OF TOXICITY IDENTIFICATION EVALUATIONS

Data obtained in the toxicity correlation phase, described above, indicated that unionized ammonia was one of the principle causes of toxicity in the marsh. Consequently, two additional studies were conducted to further evaluate this possibility. First, pH-adjustment tests were conducted; and secondly, ammonia removal experiments were performed to evaluate the potential role of unionized ammonia in contributing to the toxicity observed.

In the pH-adjustment tests, seven-day old fathead minnow larvae were exposed to water collected on the last sampling date (November 21) from Basin 2A Outfall. The following four treatments were prepared: treatment 1 was pH adjusted to one unit above the ambient level, treatment 2 was pH adjusted and maintained one unit below the ambient level, and treatments 3 and 4 were two unadjusted treatments in which the pH was allowed to drift.

The pH of a treatment appeared to have a significant influence on larval survival due to its effect on concentration of free ammonia (Table 13). The most dramatic response was seen in treatment #1, in which pH was adjusted from the ambient pH of 7.94 to 8.94. In this treatment, complete mortality occurred in less than two hours. Complete mortality also occurred after approximately 17 hours in the two unadjusted samples (#3 and #4) because pH was allowed to drift from 7.94 to 8.17 and 8.37, respectively. In contrast, there

TABLE 13. HAYWARD MARSH TIE--RESULTS OF PH ADJUSTMENT AND AMMONIA REMOVAL TESTS USING FATHEAD MINNOW

TREATMENT[1]	INITIAL PH	TOTAL N	NH_3 AT INITIAL PH (mg/l)	FINAL PH	NH_3 AT FINAL PH (mg/l)	TIME TO MORT. (hrs)
1. +1 pH	8.94	28	9.46	8.81	7.93	2
2. -1 pH	6.93	28	0.14	7.03	0.52	-
3. No pH adj.	7.93	28	1.36	8.37	3.56	19
4. No pH adj.	7.94	28	1.39	8.17	2.45	17
5. No pH adj. no NH_3 removal	7.67	43	1.29	8.20	4.02	24
6. No pH adj. NH_3 removal	7.69	10	0.31	8.17	0.87	-
7. +1 pH no NH_3 removal	8.70	43	10.19	8.68	9.83	16
8. +1 pH NH_3 removal	8.68	10	2.29	8.52	1.61	-

[1] No mortality was exhibited in the zeolite blank or the mineral water control.

was no mortality in the treatment #2 for which pH was reduced from 7.94 to 6.93 and maintained over a 24-hour period. Initial and final concentrations of free ammonia in the different treatments are presented in Table 13. Results of these sample manipulations further indicated that free ammonia was at least one explanation for the toxicity observed.

The ammonia removal experiments were conducted using Clinoptilolite, an ammonia-stripping natural zeolite resin. This procedure for ammonia removal generally followed that outlined in the EPA Methods for Aquatic Toxicity Identification Evaluations: Phase II Toxicity Identification (USEPA, 1988b), although there were some exceptions. Rather than filtering an ambient sample through a zeolite-packed column, the zeolite was added directly to the sample and stirred for 3-5 minutes. Alternately, portions of zeolite (10 gms in total) were added, and ammonia measurements were taken using a specific ion probe until ammonia concentrations reached 10 ppt as total N. The zeolite was then removed by filtering the sample to 37 um.

Six treatments of Basin 2a Outfall water (collected November 29, 1989) were prepared and tested in 48-hour bioassays, using *Ceriodaphnia* and fathead minnow larvae (< 48h-old). The ammonia-stripped sample was divided into two portions, one pH-adjusted back to the initial ambient level and the other pH-adjusted one unit above the ambient level. Two additional treatments from which no ammonia had been stripped were also tested; one was raised one pH unit, the other was left at ambient pH. Two controls, Arrowhead brand mineral water and a zeolite blank, were also tested. Each treatment contained only one replicate with five fish per replicate. We made no attempt to maintain constant pH values during the test period in any of the treatments.

The removal of ammonia had a significant effect on fathead minnow larval survival (Table 13). In the fathead minnow test, we observed complete mortality within 16 hours in treatment #7 in which no ammonia was removed and the pH was adjusted to from 7.67 to 8.70. Complete mortality was also observed within 24 hours in treatment #5 in which no ammonia was removed and the pH rose from 7.67 to 8.20. There was no mortality after 48 hours in either treatments #6 or #8, from which ammonia had been removed, or in the controls. These data further indicate that unionized ammonia is a major cause of toxicity in the marsh.

4.8 MOUNTAIN VIEW SANITARY DISTRICT MARSH RECLAMATION PROJECT -- SURVEY DESIGN AND FIELD CONDITIONS

Similar in design to the Hayward Marsh system, the Mountain View marsh relies upon treated wastewater from the adjacent Mountain View Sanitary District as its primary source of freshwater input. Unlike the Union Sanitary District, a component of the secondary treatment facilities at Mountain View includes advanced biological oxidation of ammonia prior to the discharge of effluent into the marsh.

Samples were collected from the five major ponds (Fig. 6), the effluent discharge point, and immediately downstream of the point where the marsh discharges into Peyton Slough. Two additional samples were also collected; one from a point upstream in Peyton Slough and the other from an adjacent downstream marsh (Shell Marsh). The latter marsh was the site of a major oil spill in 1988. All samples were collected daily, over a 7 day period, January 30 - February 5, 1990, with rain events occurring prior to the third and sixth samplings. We conducted ambient toxicity tests and reference toxicant tests using fathead minnow, *Ceriodaphnia, Selenastrum* and sea urchins.

4.9 MOUNTAIN VIEW SANITARY DISTRICT MARSH RECLAMATION PROJECT -- RESULTS

A toxic response was observed using three out of the four species tested in samples from the reclamation marsh at Mountain View (Fig. 6 and Table 14); although the magnitude of toxicity observed was much less than that observed at Hayward Marsh. Using the fathead minnow bioassay, only exposure to the sample from the Effluent Box elicited minor but significantly reduced survivorship (80%) and larval weight (0.45 mg). Using a sea urchin bioassay, exposure to samples from three stations (Effluent Box, Basin B Outfall and Basin D) resulted in significant toxicity as compared to the brine control, with fertilizations ranging from 4-40% (arcsine, $p=0.05$). In comparison to the seawater control, one additional station (Lower Peyton Slough) was significant. However, because all of the samples were diluted with brine to 64% prior to testing, the brine control is more representative of the ambient concentration. In this test, we used a 400:1 sperm:egg ratio.

Figure 6. Ambient Toxicity Survey at Mountain View Sanitary District Marsh

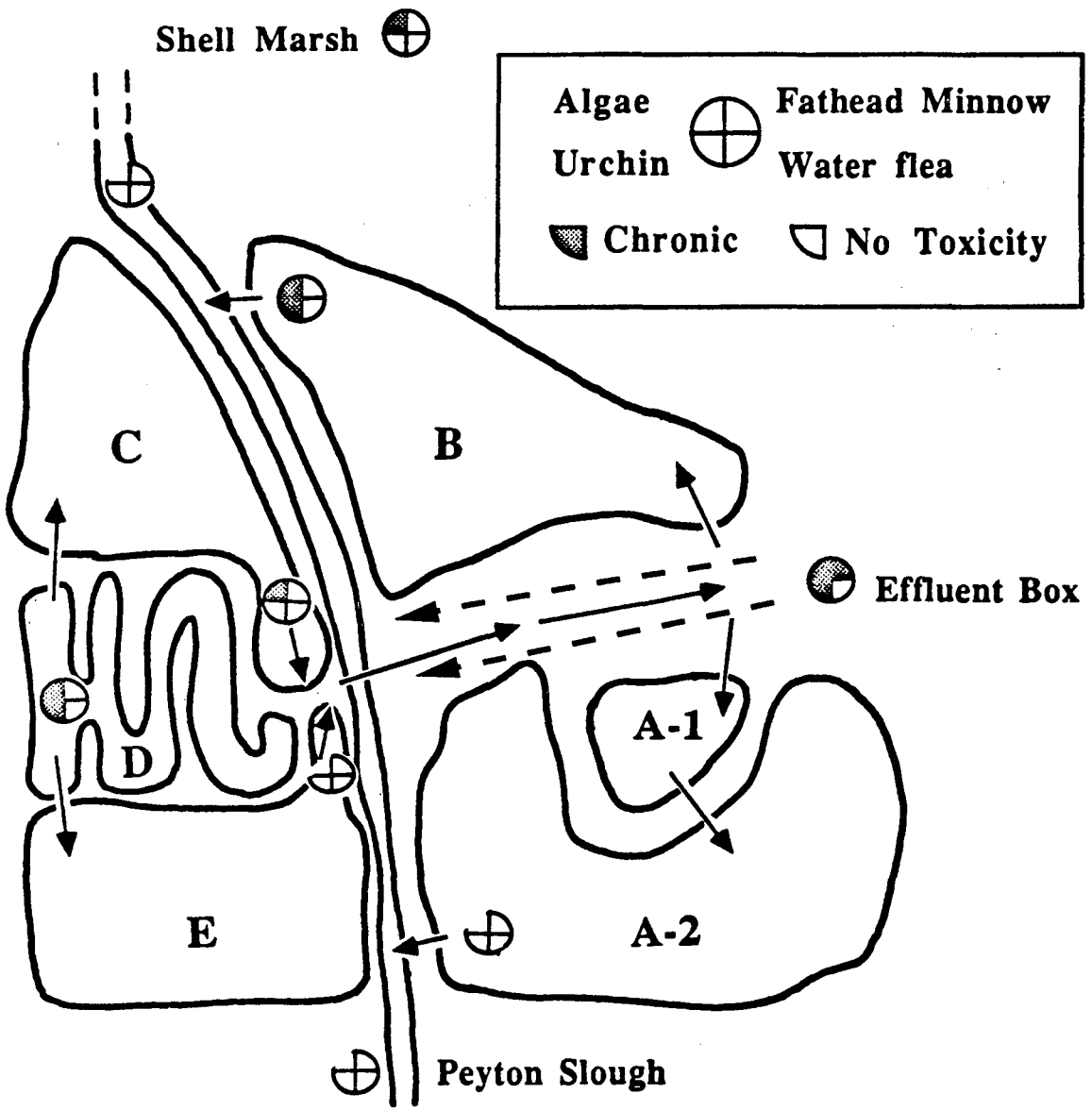


TABLE 14. TOXICITY OBSERVED IN SAMPLES COLLECTED FROM MOUNTAIN VIEW SANITARY DISTRICT MARSH (JANUARY 30- FEBRUARY 5, 1990)

STATION LOCATION	FATHEAD MINNOW		SEA URCHIN		CERIODAPHNIA		SELENASTRUM	
	Mean Surv.[1] (%)	Mean Wt. (mg)	Mean Fert.[2] (%)	Amb. (%)	Mean Surv. (%)	Mean Repro[3]	Mean Cells/ml	Mean Inhib.[4] (%)
Effluent Box	80*[5]	0.45*	4*	64	100	21	1,151,142	49*
Basin D Midway	100	0.56	11*	64	100	33	1,261,666	44*
Basin C Outfall	100	0.63	98	64	100	35	790,500	65*
Basin E Outfall	100	0.63	99	64	90	34	-	-
Basin A2 Outfall	100	0.73	93	64	70	23	-	-
Basin B Outfall	97	0.60	40*	64	80	32	676,136	70*
Lower Peyton Slough	100	0.61	77	64	100	32	-	-
Shell Marsh	90	0.62	99	64	100	31	673,111	70*
Upper Peyton Slough	93	0.71	97	64	100	29	-	-
Fresh/Seawater Control	97	0.73	93	NA	60	17	2,390,000	NA
Salinity Adj. Control	-	-	63	NA	-	-	-	-

[1] Survival endpoint.

[2] Fertilization endpoint.

[3] Number of young per female.

[4] Growth inhibition endpoint.

[5] * Indicates value is significantly different from all controls, $p < 0.05$.

Using the *Selenastrum* assay, samples from all of the stations tested (Shell Marsh, Basin D Midway, Basin C Outfall, Basin B Outfall and Effluent Box) elicited a toxic response. We saw no toxic response using the *Ceriodaphnia* bioassay.

Several characteristics of the fathead minnow test should be noted. On day 2 of testing, the sample from Basin D was aerated slowly for 40 minutes to raise the dissolved oxygen to 6.4 mg/l. This aeration may have decreased the concentrations of toxic volatile compounds; however, since no toxicity was observed in the other basins which were not aerated, this is unlikely. Final dissolved oxygen levels in the Effluent Box treatment which were at or below 40% saturation on days three, four and five may account for all of the mortality in this treatment.

There were two minor deviations in protocol for the *Ceriodaphnia* bioassay, neither of which influenced the interpretation of the results. As was necessary for the fathead minnow test, the sample from Basin D required 40 minutes of aeration on day 2. In addition, control survival (60%) was lower than the protocol specification of 80%. However, this is not considered problematic since survival in all treatments exceeded 70%.

There was one deviation in the sea urchin bioassay which had no significant effect on the conclusions of the survey. Brine control fertilization was low (63%) due to abnormalities in fertilization. In all of the treatments, we observed some embryos with a fertilization envelope which had not "lifted off". Consequently, it was unclear whether or not they should be scored as fertilized or unfertilized. Our decision to score these embryos as unfertilized resulted in a brine control fertilization of 63%, which was below the level of 70% specified in the protocols.

4.10 SUNNYVALE MARSH AND WASTEWATER TREATMENT PLANT -- SURVEY DESIGN AND FIELD CONDITIONS

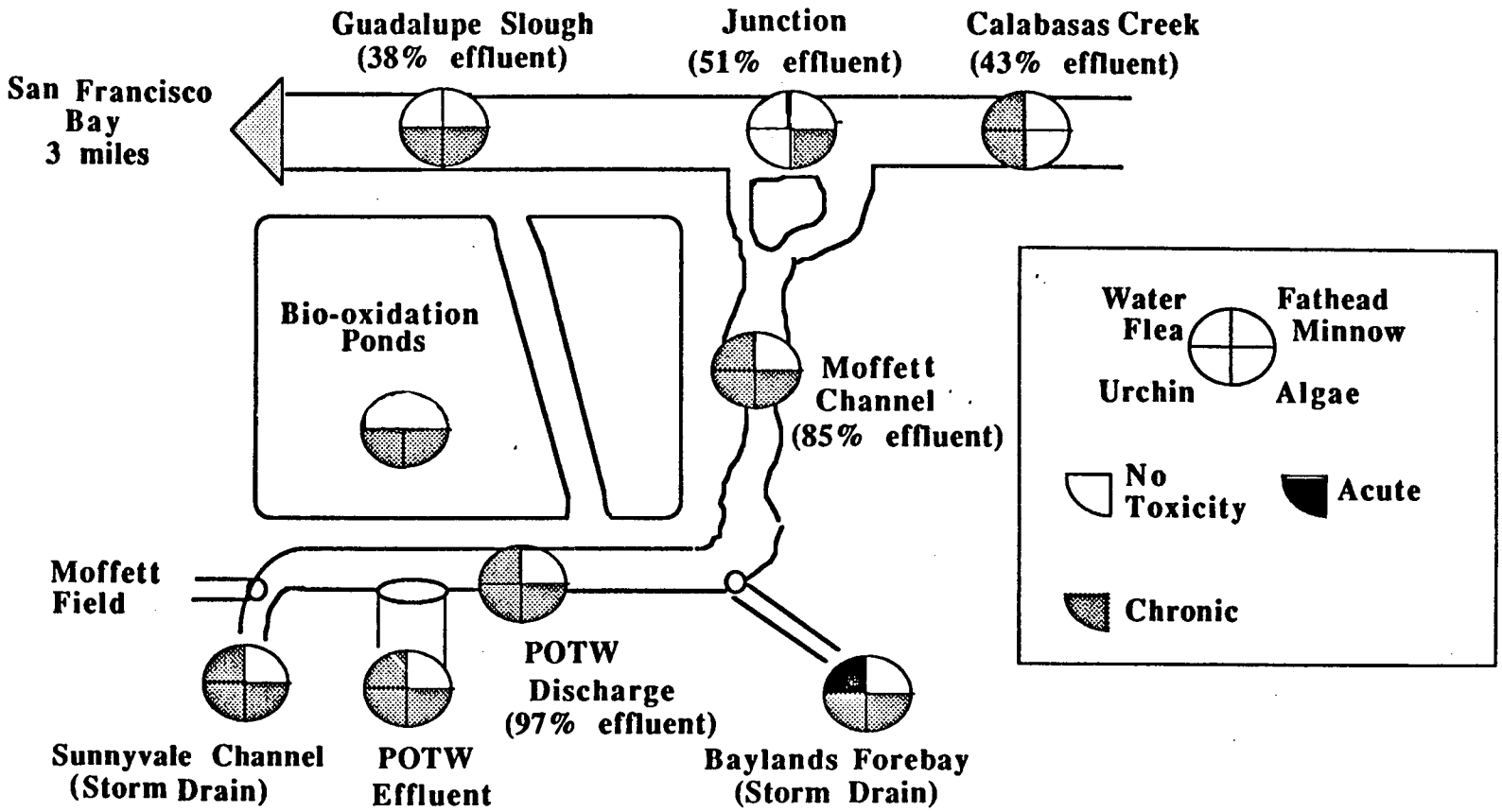
The marshes adjacent to the Sunnyvale Wastewater Treatment Plant (Sunnyvale Marsh) exemplify a system that receives much of its freshwater input from a wastewater treatment plant, although it is not specifically managed as a marsh reclamation project. This site was selected because of its marsh system but also because effluent toxicity had been

documented to be comparatively high. *Ceriodaphnia* was known to be a sensitive test species at this site. We studied toxicity associated with the effluent discharge, a storm drain discharge and the flow of an urban creek. The latter two represent additional inputs into the marsh. To determine the spatial and temporal distribution of the effluent, within the marsh system, this survey was conducted in conjunction with modelling and dye-dilution studies, performed by contractors to the Sunnyvale Wastewater Treatment Plant. Effluent toxicity was also evaluated by private contractors to the plant.

Evaluations of ambient toxicity were performed using fathead minnow, *Ceriodaphnia, Selenastrum* and purple sea urchin. Samples were collected at the following 8 stations (Fig. 7): one station adjacent to the discharge point sampled at the beginning and end of each sampling date (Discharge T1 and T2), four stations located on adjacent waterways subject to the direct or tidal influence of effluent discharged from the Sunnyvale Wastewater Treatment Plant (Moffett Channel, Calabasas Creek, Junction of Calabasas Creek and Moffett Channel, Guadaloupe Slough and a site upstream, but within the tidal influence of, the discharge), one station receiving water from an urban storm drain (Baylands Forebay), and one station in the biological oxidation pond of the Sunnyvale Wastewater Treatment Plant and representing a secondary stage in the treatment process. An additional *Ceriodaphnia* bioassay was performed using samples from all of the stations by an independant laboratory (contracted by the San Francisco Bay Regional Water Quality Control Board). The purpose of conducting this assay in two different laboratories was to provide a quality assurance check on our data for this critical test species.

Generally, samples were collected daily over a 7-day period, June 12-18, 1989, at high slack tide; with the exception of four stations that were only accessible on four of the sampling days. There was no precipitation immediately before or during the sampling period. Reference toxicant tests and rhodamine controls were conducted for each of the species tested. Ambient samples from the Calabasas, Junction, Guadaloupe and Baylands Forebay stations were tested at a 50% dilution in either one or both of the *Ceriodaphnia* bioassays; because initial conductivities exceeded 2000 umhos/cm2, which in our experience, is the upper range of salt tolerance for this species.

Figure 7. Sunnyvale Ambient Toxicity Survey



4.11 SUNNYVALE MARSH AND WASTEWATER TREATMENT PLANT -- RESULTS

A toxic response was observed using three of the four species tested in water from Sunnyvale Marsh (Fig. 7 and Table 15). However, the tests using *Ceriodaphnia* are the only tests that demonstrate a relationship between effluent toxicity and ambient toxicity. In both the testing conducted inhouse and by an independent laboratory, reproduction was almost completely inhibited at the sites closest to the discharge (97% effluent). The inhouse testing also demonstrated a significant decrease in reproduction in Moffett Channel, the next site downstream of the plant (83%effluent). However, this observation was not confirmed by the independent laboratory.

Findings at two sites downstream (Calabasas and Guadaloupe) indicated that toxicity attributable to high conductivities in some samples was likely. For example, toxicity observed in the 100% ambient sample collected at the Calabasas site may be attributed to high conductivities (3250 umhos/cm^2). Acute toxic effects were observed in the Baylands forebay urban stormdrain that were not confirmed by the independent laboratory.

Synoptic measurements of effluent toxicity using *Ceriodaphnia* corroborate the findings reported in our ambient study. Reproduction in 100% effluent was almost totally inhibited (1.5 young per female as opposed to 19.2 young per female in the controls). At 75%, 50% and 25% effluent respectively, reproductive output was 5.5, 10.9 and 16.2 young per female (NOEC=50%; Kruskal Wallis, $p=0.05$). Significantly, no toxicity was observed in the biological oxidation pond in our testing; and consequently, wastewater in the final effluent was significantly more toxic than was the wastewater in this secondary stage of treatment.

In the *Selenastrum* bioassay, significant inhibition of growth ranging from 79-100% occurred in all samples, with the exception of Calabasas Creek (Fig. 7, Table 15). Because such extreme inhibition was observed in the majority of samples, conclusions cannot be drawn regarding the relationship of the toxicity observed to effluent concentration. If ambient samples had been tested at varying dilutions, relative toxicity might have been discernable. No toxicity was observed using the fathead minnow bioassay at any of the sites.

TABLE 15. TOXICITY OBSERVED IN A SURVEY CONDUCTED IN MARSHES ADJACENT TO THE SUNNYVALE WASTEWATER TREATMENT PLANT (JUNE 12-18, 1989)

STATION LOCATION	FATHEAD MINNOW		SEA URCHIN		CERIODAPHNIA 1[1]			CERIODAPHNIA 2[2]			SELENASTRUM	
	Mean Surv.[3] (%)	Mean Wt. (mg)	Mean Fert.[4] (%)	Amb (%)[5]	Mean Surv. (%)	Mean Repro.[6]	Amb. (%)	Mean Surv. (%)	Mean Repro.	Amb. (%)	Mean Cells/ml	Mean Inhib (%)[7]
Discharge T1	97	0.66	49*[8]	72	90	1*	100	90	0*	100	-	-
Discharge T2	100	0.65	-	-	100	0*	100	80	2*	100	20,000	98*
Sunnyvale Channel	93	0.65	48*	77	100	4*	100	90	2*	100	41,300	96*
Moffett Channel	97	0.69	48*	72	100	5*	100	90	14	100	38,700	96*
Junction	97	0.69	85	72	100	24	50	60	11	100	180,000	82*
Calabasas Creek	100	0.69	33*	72	90/100	8*/17[9]	100/50	100	18	50	488,000	50
Guadaloupe Slough	97	0.67	81*	72	90	10	50	90	15	50	201,000	79*
Baylands Forebay	93	0.71	63*	72	50*/ 40*	-/-	100/50	80	13	50	1,300	100*
Bioxidation Pond	100	0.87	50*	72	100	24	100	100	20	100	9,300	100*
Sea/Freshwater Control	97	0.55	100	NA	100	14	NA	90	16	NA	971,000	NA
Salinity Adj. Control	-	-	98	NA	-	-	-	-	-	-	-	-
Rhodamine Control	97	0.56	92	NA	100	10	NA	-	-	-	-	-

[1] Test performed in-house.
[2] Test performed by an independent laboratory.
[3] Survival endpoint.
[4] Fertilization endpoint.
[5] Ambient concentrations tested after necessary salinity adjustment.
[6] Number of young per female.
[7] Growth inhibition endpoint.
[8] * Indicates value is significantly different from all controls, $p<0.05$.
[9] Represents the two dilutions tested: 100% / 50%

Toxicity was exhibited in the sea urchin bioassay at seven of the eight sites tested (using a sperm:egg ratio of 500:1). Significantly reduced fertilization ranging from 33-81% was seen in all samples except the Junction station (Table 15). Three effluent concentrations (10, 25 and 50%) were tested resulting in a fertilization NOEC of 10% effluent and percent fertilizations of 99, 92 and 73% respectively. These urchin data generally indicate more toxic conditions adjacent to the discharge and in Calabasas Creek, but the toxicity in the effluent was relatively low and would not be expected to produce discernable differences among sites in the receiving water.

4.12 SAN JOSE/SANTA CLARA MARSH AND WASTEWATER TREATMENT PLANT -- SURVEY DESIGN AND FIELD CONDITIONS

San Jose Wastewater Treatment Plant is similar to the Sunnyvale treatment plant, as it is the primary source of freshwater to an adjacent marsh system. Artesian Slough, a tidally-influenced channel comprised mainly of effluent from the San Jose plant, connects with Coyote Creek and Mud Slough, all flowing into South San Francisco Bay.

We sampled at five stations along Artesian Slough, moving away from the discharge point (Table 16, Artesian 1 station is closest to outfall) and at two stations downstream from the junction of Artesian Slough and Coyote Creek (Fig. 8). Three additional upstream stations, located on Coyote Creek, Upper Coyote Creek and Coyote Slough, were also sampled.

Sampling was conducted at all sites on three separate occasions, August 25, 27, and 29, 1989, approximately 2-3 hours after the morning (maximum) low tide. The first few days of sampling were scheduled to coincide with a dye-dilution study to determine the distribution of effluent within the marshes and sloughs. The latter study was conducted by a contractor to the San Jose Wastewater Treatment Plant. In addition, effluent toxicity was also assessed by a private laboratory. Weather conditions during the sampling period were mostly sunny, although one rain event occurred on the night before the last sampling date.

Bioassays conducted on the ambient samples were the silverside minnow test, the sand dollar fertilization test, and a *Mysidopsis bahia* 7-day survivorship test. Rhodamine controls

Figure 8. Ambient Toxicity Survey at San Jose Marsh

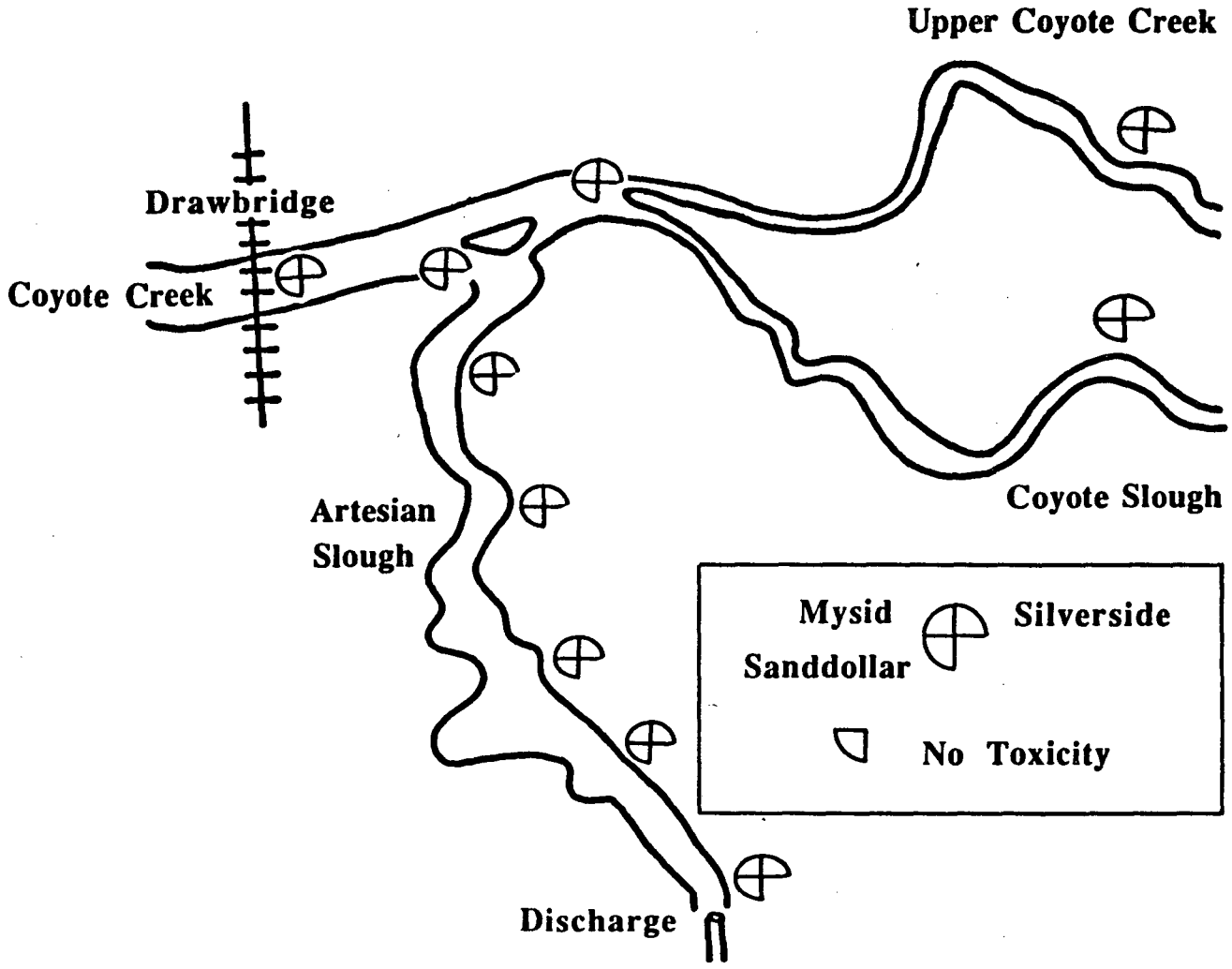


TABLE 16. TOXICITY OBSERVED IN A SURVEY CONDUCTED IN MARSHES ADJACENT TO THE SAN JOSE/SANTA CLARA WASTEWATER TREATMENT PLANT (JUNE 13-19, 1989)

STATION LOCATION	SILVERSIDE MINNOW		MYSIDOPSIS		DENDRASTER	
	Mean Surv.[1] (%)	Mean Wt. (mg)	Mean Surv. (%)	Amb. (%)[2]	Mean Fert.[3] (%)	Amb. (%)
Artesian 1	93	0.92	90	68	64	58
Artesian 2	100	0.90	94	68	71	60
Artesian 3	97	0.92	98	69	72	60
Artesian 4	90	0.85	98	80	69	62
Artesian 5	97	0.88	92	85	83	66
Artesian Junction	100	0.93	94	85	85	68
Railroad Bridge	100	0.87	94	86	76	68
Coyote Junction	93	0.90	96	87	80	70
Lower Coyote Creek	100	0.96	88	88	65	74
Upper Coyote Creek	97	0.99	90	88	69	74
Seawater Control	87	0.87	92	NA	94	NA
Salinity Adj. Control	97	0.92	92	NA	75	NA
Rhodamine Control	97	0.86	98	NA	-	-

[1]Survival endpoint.

[2]Ambient concentrations tested after necessary salinity adjustment.

[3]Fertilization endpoint. No significant toxicity when compared to the brine control.

were run for all the species except the sand dollar. Reference toxicant tests were conducted for all the species except the silverside minnow. Ambient water from three stations, with initial salinities less than 5 ppt, was adjusted to 10 ppt for the silverside minnow test. Controls for the silverside minnow test were as follows: the salinity adjustment and rhodamine controls were run at the average ambient salinity; the seawater control was run at a higher salinity than the average of the ambient samples due to an initial oversight.

4.13 SAN JOSE/SANTA CLARA MARSH AND WASTEWATER TREATMENT PLANT -- RESULTS

No toxicity was observed in the samples from San Jose/Santa Clara Marsh using silverside minnow, *Ceriodaphnia, Mysidopsis* and sand dollar bioassays (Fig. 8 and Table 16). For the sand dollar bioassay (using a sperm:egg ratio of 1000:1), if treatments are compared to the brine controls, no significant differences were detected. However, seven stations exhibited significantly reduced fertilization as compared to the seawater control. Since ambient samples were diluted to between 58-74% with brine, statistics based on comparisons to the brine control are more appropriate. No significant toxicity was observed in effluent samples during synoptic testing using silverside minnow and *Ceriodaphnia.*

4.14 SUMMARY OF MARSH SURVEY RESULTS

Toxic effects were observable in samples from four of the five marshes surveyed (Fig.9). Only at San Jose Marsh was no toxicity observed. Hayward Marsh was the most toxic of the two reclamation marshes, with acute and chronic effects at several stations and with effects observable using a variety of species. Toxicity at this site is apparently largely attributable to unionized ammonia; although unionized ammonia does not explain all of the toxicity observed. Mountain View Marsh, with improved ammonia removal, exhibited only comparatively minor toxicity at a restricted number of stations. Samples from Sunnyvale Marsh elicited chronic effects adjacent to the wastewater treatment plant and in nearby waters. Toxic responses were elicited by three of the four species tested, but only the *Ceriodaphnia* test data indicated a clear relationship between toxicity and the concentration of effluent in the receiving water. Samples from the reference site, San Francisco Bay National Wildlife Refuge, elicited toxicity in the sea urchin bioassay at three stations. The

cause of the toxicity is unknown, but toxicity attributable to urban runoff is a possibility at this site.

Figure 9. Summary of Findings at Five Marsh Sites

SAN FRANCISCO BAY NATIONAL WILDLIFE REFUGE

Silverside Minnow: No Toxicity Observed
Echinoderm: Toxic Effects at 50% of Stations/ 45-75% Fertilization
Algae: No Toxicity Observed

HAYWARD MARSH SURVEY I

Silverside Minnow: Toxic Effects at 50% of Stations/ 57-100% Mortality
Echinoderm: Toxic Effects at 33% of Stations/ 74-83% Fertilization

HAYWARD MARSH SURVEY II

Fathead Minnow: Toxic Effects at 100% of Stations/ 100% Mortality
Silverside Minnow: Toxic Effects at 78% of Stations/ 33-100% Mortality
Echinoderm: Toxic Effects at 78% of Stations/ 2-61% Fertiliztion
Water flea: Toxic Effects at 100% of Stations/ 100% Mortality at 1 station and Decreased Reproduction at 2 stations

MOUNTAIN VIEW MARSH

Fathead Minnow: Toxic Effects Observed at 11% of Stations/ 20% Mortality and Decreased Growth
Echinoderm: Toxic Effects at 33% of Stations/ 4-40% Fertilization
Water Flea: No Toxicity Observed
Algae: Toxic Effects Observed at 100% of Stations/ 44-70% Inhibition of Growth

SUNNYVALE MARSH

Fathead Minnow: No Toxicity Observed
Echinoderm: Toxic Effects at 78% of Stations/ 33-63% Fertilization
Water Flea: Toxic Effects at 67% of Stations/ 50% Mortality at 1 Station and Decreased Reproduction at 5 Stations
Algae: Toxic Effects Observed at 78% of Stations/ 82-100% Inhibition of Growth

SAN JOSE/SANTA CLARA MARSH

Silverside Minnow: No Toxicity Observed
Echinoderm: No Toxicity Observed
Mysid Shrimp: No Toxicity Observed

Chapter 5

RESULTS OF ANCILLARY SURVEYS

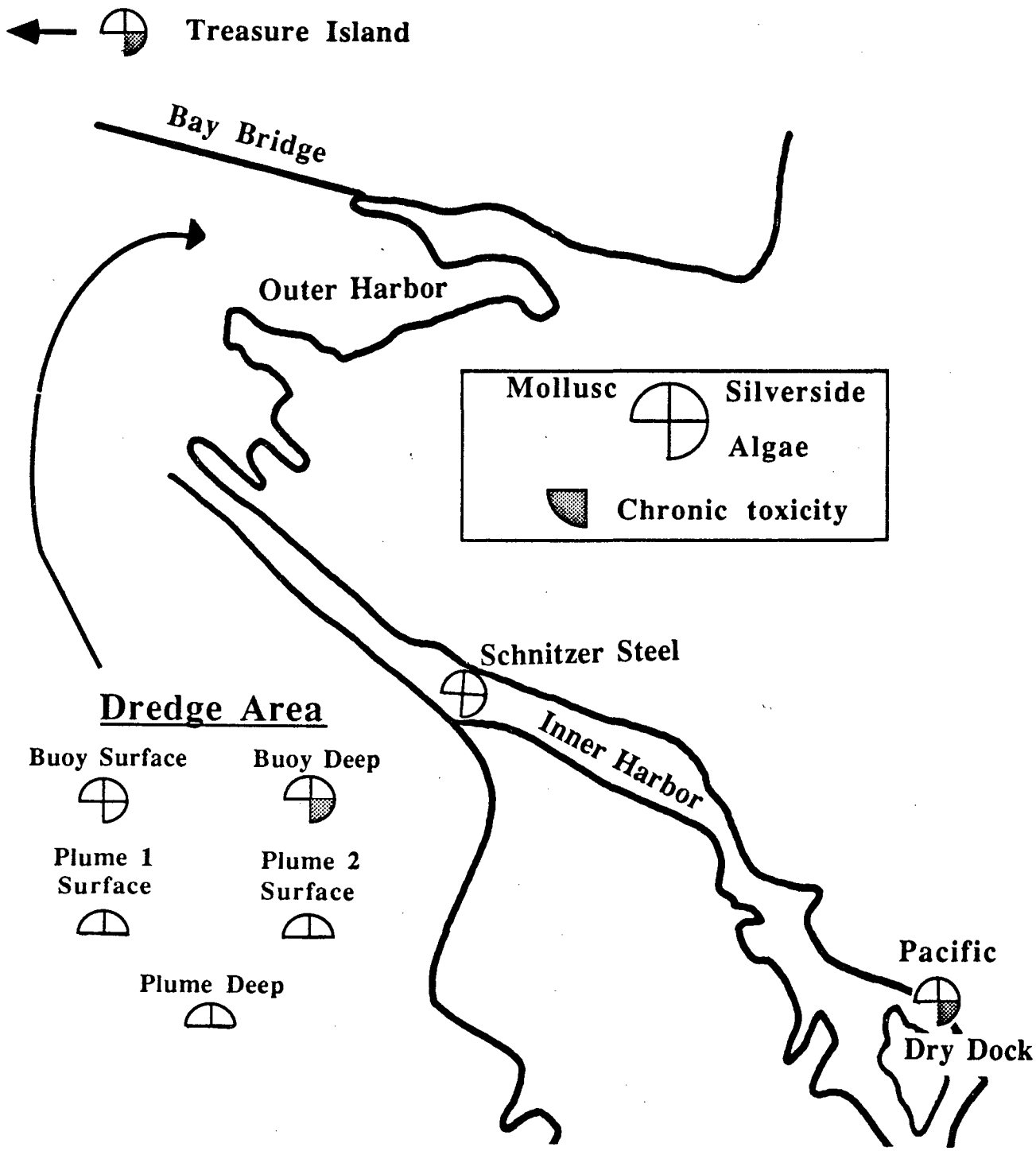
5.1 INTRODUCTION

Two additional surveys were conducted to investigate specific toxicity issues of concern in the Bay Area. Neither of these surveys were directly related to the Bay Background component of the program or to the Marsh Survey component of the program; consequently, they are presented separately in this report. The Oakland Inner Harbor and Dredge survey was a unique opportunity to evaluate both possible toxicity associated with waterfront industries and local dredging activities. The USS Posco Refinery survey was designed to specifically determine the degree to which USS Posco and adjacent industry contribute to documented toxicity in the New York Slough area. Results, survey design, and field conditions are presented below.

5.2 OAKLAND HARBOR AND DREDGE SURVEY -- SURVEY DESIGN AND FIELD CONDITIONS

Although sediment toxicity has been observed in Oakland Inner Harbor, no studies have been conducted to determine the potential for water column toxicity associated with dredging activities in this area. The purpose of this survey was to quantify toxicity associated with both dredging activitity and the practices of two industries located in the Oakland Inner Harbor. Unfortunately, changes in the Army Corps of Engineers dredge schedule necessitated changing our survey design. The dredge was moved to the Outer Harbor on the first day of our sampling; consequently, we assessed toxicity associated with dredging activities in the Outer Harbor rather than the Inner Harbor. The evaluation of toxicity at the two industrial sites progressed as planned. Water samples in the Inner Harbor were collected from Schnitzer Steel, a metals handling and recycling facility in operation for over 10 years, and Pacific Dry Dock, a ship repair facility in operation for over 20 years (Fig. 10).

Figure 10. Dredge and Oakland Harbor Ambient Toxicity Survey



Five samples falling into two categories were collected near an Army Corps of Engineers hopper dredge operation in the Oakland Outer harbor. First, there were two samples taken near a fixed buoy after the dredge had passed. One was taken from the surface and the other, within a meter of the bottom (Outer Buoy 3 surface and Outer Buoy 3 depth). The second set of dredge samples was taken from the center of the dredge plume (Mid-plume surface, Mid-plume 2 surface and Mid-plume depth). A sample was taken from the Treasure Island Mussel Watch station, representing Bay background conditions.

Sampling at all sites was conducted on April 5 and 7, 1989. As of April 8, 1989, dredge operations had been moved out of Oakland Outer Harbor, and sampling was conducted only at sites that were not related to the dredging activity (Schnitzer Steel, Pacific Dry Dock, Treasure Island). There was no precipitation before or during the sampling period.

Ambient toxicity and reference toxicant tests were conducted using the silverside minnow, *Skeletonema*, and bay mussel. The seawater control for the silverside minnow test was adjusted to the mean ambient salinity.

5.3 OAKLAND HARBOR AND DREDGE SURVEY -- RESULTS

Only one of three species tested exhibited a toxic response to samples from the Oakland Inner Harbor and Dredge Survey (Fig. 10, Table 17). In the *Skeletonema* bioassay, significant inhibition of growth ranging from 28-47% occurred in samples from three of the five stations tested. These stations included one related to the dredging (Outer Buoy 3 Depth), one related to industry (Pacific Dry Dock) and the reference station at Treasure Island. No toxic effects occurred in any of the samples using the silverside minnow and mollusc bioassays. However, low larval control weights averaging 0.35 mg in the silverside minnow test may have obscured a toxic response in the treatments.

5.4 USS POSCO STEEL REFINERY -- SURVEY DESIGN AND FIELD CONDITIONS

The purpose of this survey was to evaluate the toxicity of the USS Posco effluent relative to waters upstream and downstream from the point of intake (Fig.11). Other unpublished

TABLE 17. TOXICITY OBSERVED IN SAMPLES COLLECTED IN CONJUNCTION WITH DREDGING ACTIVITES CONDUCTED IN THE OAKLAND OUTER HARBOR (APRIL 6-13, 1989)

STATION LOCATION	SILVERSIDE MINNOW		BAY MUSSEL			SKELETONEMA	
	Mean Surv.[1] (%)	Mean Wt. (mg)	Mean Surv. (%)	Mean Abnorm.[2] (%)	Amb. (%)	Mean Cells/ml	Mean Inhib.[3] (%)
Schnitzer Steel	100	0.52	94	10	91	27,378	18
Pacific Dry Dock	97	0.45	94	11	93	24,178	28*[4]
Outer Buoy 3 Surface	90	0.49	99	13	91	25,200	25
Outer Buoy 3 Depth	93	0.64	95	12	91	18,641	44*
Mid-plume Surface 1	90	0.47	75	12	89	-	-
Mid-plume Surface 2	100	0.48	99	10	91	-	-
Mid-plume Depth	97	0.44	87	8	91	-	-
Treasure Island	93	0.47	91	14	89	17,600	47*
Seawater Control	93	0.35	100	14	NA	33,422	NA
Salinity Adj. Control	-	-	91	10	NA	-	-

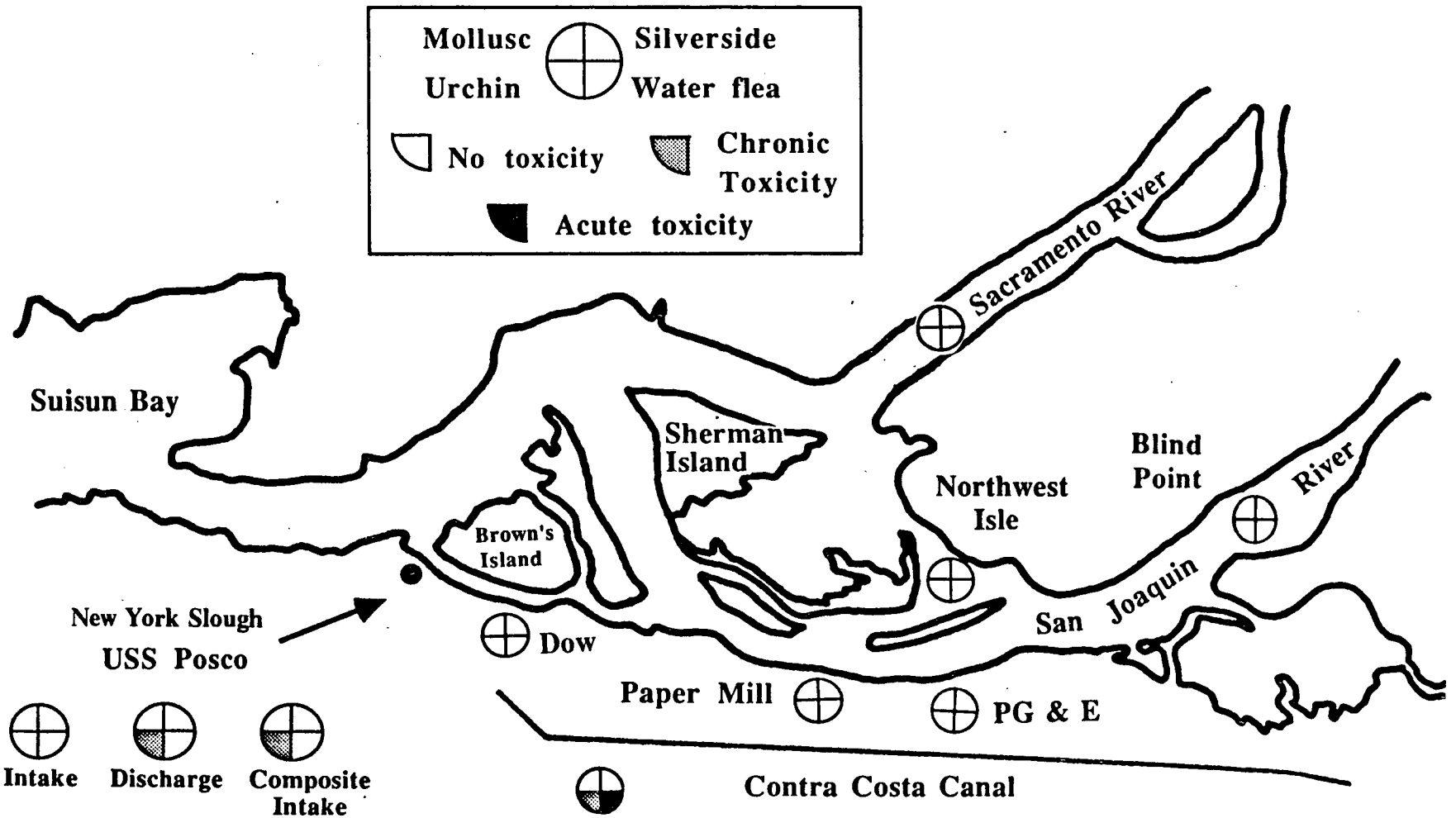
[1] Survival endpoint.

[2] Abnormality endpoint.

[3] Growth inhibition endpoint.

[4] * Indicates value is significantly different from all controls, $p < 0.05$.

Figure 11. USS Posco Ambient Toxicity Survey



reports indicated that toxicity in the effluent of this steel refinery may be attributable to ambient toxicity at the intakes within New York Slough or the Contra Costa Canal. The latter is a water conveyance system with an intake at Rock Slough, several miles east in the Delta region. Approximately 75% of the effluent is comprised of once-through cooling water; two thirds of this is from an intake in New York Slough and one third is from an intake in Contra Costa Canal. Samples were collected at seven stations located upstream of the USS Posco intake (PG&E/Antioch, Blind Point/San Joaquin River, North West Island, Sacramento River, Dow Chemical, and Simpson Paper Mill). Two samples were collected adjacent to the Refinery intake and discharge points in New York Slough (Posco intake and Posco discharge). Two additional 24-hour composite samples were provided by Posco Refinery staff (Contra Costa Canal, and a 24-hour composite at the intake). Samples were collected daily over a seven-day period, February 19-25, 1990, during which time there was no precipitation.

Bioassays were conducted on all ambient samples using the following species: silverside minnow, water flea, sea urchin and bay mussel and *Selenastrum*. For the silverside minnow test, all ambient samples, reference toxicant tests, and controls were conducted at a salinity of 8 ppt. In the *Ceriodaphnia* test, samples often required dilution to bring the conductivity to the acceptable limit of 2000 umhos/cm^2. Reference toxicant tests were conducted for all species tested except the algae.

Synoptic testing was also conducted by an independant laboratory contracted to USS Posco. Tests were conducted on 24-hour composite samples of New York Slough intake water, effluent, and Contra Costa Canal intake water using *Ceriodaphnia*, fathead minnow, silverside minnow, sea urchin and algae.

5.5 USS POSCO STEEL REFINERY -- RESULTS

We observed significant toxicity in samples from the USS Posco Refinery using two of the five species tested (Fig. 11 and Table 18); although general patterns of toxicity were not discernable. The most remarkable finding was the presence of acute toxic effects in the Contra Costa Canal. This finding supported previous reports that sporadic toxic events are

TABLE 18. TOXICITY OBSERVED IN WATERWAYS ADJACENT TO THE USS POSCO SITE (FEBRUARY 19-25, 1990)

STATION LOCATION	SILVERSIDE MINNOW		BAY MUSSEL			SEA URCHIN		CERIODAPHNIA			SELENASTRUM	
	Mean Surv. (%)[1]	Mean Wt. (mg)	Mean Surv. (%)	Mean Abnorm.[2] (%)	Amb.[3] (%)	Mean Fert.[4] (%)	Amb. (%)	Mean Surv. (%)	Mean Repro.[5]	Mean Amb.[6] (%)	48hr/96hr (Mean Cells/ml) ($x10^3/x10^6$)	48hr/96hr Inhib.[7] (%)
POSCO Discharge	100	0.65	98	15	66	46*[8]	63	90	24	32	277/5.28	1/NA
POSCO Intake	97	0.77	100	19	67	66	66	90	24	29	475/5.82	NA
24-hr Comp. Intake	100	0.75	100	22	66	30*	63	100	25	27	351/5.29	NA
24-hr Comp. Effl.	-	-	-	-	-	-	-	-	-	-	295/4.34	NA
Contra Costa Canal	100	0.66	100	22	61	24*	61	0*	-	100	310/4.58	NA
Dow Chemical	100	0.72	92	19	64	79	63	100	26	35	409/5.27	NA
North West Isle.	97	0.77	98	29	61	79	61	90	30	87	479/4.95	NA
Paper Mill	100	0.68	96	17	61	66	63	70	24	75	461/5.12	NA
PG&E/Antioch	100	0.74	96	24	61	86	62	70	23	80	307/4.44	NA
Sacto. River	100	0.71	99	21	61	70	63	70	24	60	498/5.21	NA
San Joaquin River	100	0.68	98	24	61	83	61	90	27	95	432/5.62	NA
Fresh/Seawater cont.	100	0.66	98	18	NA	90	NA	90	21	NA	281/1.30	NA
Salinity Adj. cont.	100	0.60	99	24	NA	66	NA	-	-	-	-	-

[1] Survival endpoint.

[2] Abnormality endpoint.

[3] Ambient concentrations tested after necessary salinity adjustment.

[4] Fertilization endpoint.

[5] Average number of young per female.

[6] The average dilution over a seven-day period after salinity adjustment.

[7] Inhibition endpoint.

[8] * Indicates value is significantly different from all controls, $p<0.05$.

observable in this area but that they may be difficult to characterize without frequent monitoring.

Using the sea urchin bioassay, samples from the three stations associated with the effluent and cooling water intakes (Posco discharge, Posco 24-hour composite intake and Contra Costa Canal) exhibited significantly reduced fertilization as compared to the brine control using a sperm:egg ratio of 400:1. Levels of fertilization ranged from 24-46%, with the discharge sample being the least toxic of the three samples. When comparisons were made to the seawater control, four additional samples were identified as toxic. However, since all samples were diluted during salinity adjustment to between 61-66%, the brine is the more suitable of the controls. It should be noted that the brine control fertilization (66%) was slightly low relative to protocol specifications.

Using the mollusc bioassay, significant abnormality was only elicited in the sample from North West Isle, when compared to the seawater control. There was no significant toxicity in any of the samples when comparisons were made to the brine control which, for the same reasons as applied with the sea urchin test, was the more appropriate of the two controls.

In the *Ceriodaphnia* bioassay, nearly complete mortality occurred after 24 hours of exposure to a sample from the Contra Costa Canal collected on day 5. No significant decrease in survivorship or reproduction was observed at any other sites. No significant toxicity was seen using either the silverside minnow or *Selenastrum* assays.

Concurrant tests on samples from the Contra Costa Canal, the 24-hour intake composite, and the 24-hour effluent composite were performed by an independant laboratory using sea urchin, *Ceriodaphnia*, fathead minnow and silverside minnow assays. Using the sea urchin bioassay, samples from the Contra Costa Canal and a 24-hour composite intake sample diluted to 67% ambient had significantly reduced fertilizations of 45% and 80%, respectively. A 500:1 rather than a 400:1 sperm:egg ratio was used in the independant laboratory test and may account for fertilization higher than that observed for the same samples in house. In addition, no significant reduction in fertilization was reported for a 67% 24-hour composite effluent sample. In the *Ceriodaphnia* bioassay, catastrophic

mortality occured in the Contra Costa Canal sample on day 2 of testing. It is interesting to note that we observed a similar pattern of mortality at the same station on day 5 of the inhouse test; this discrepancy has not been resolved. No significant effects were observed in either the 24-hour composite intake or the 24-hour effluent composite. No significant effects were observed using either a silverside minnow bioassay or a fathead minnow test (run only on the 24-hour effluent composite sample).

In general, data from our laboratory and the independent laboratory do not corroborate previous findings of more extreme ambient toxicity in the vicinity of New York Slough. In addition, toxicity of the USS Posco effluent was comparatively low. The differences between our observations and those previously reported cannot be definitively explained; however, the differences are most likely attributable to temporal variation at this site. Temporal variation cannot be characterized in a single survey. The most significant finding is the observation of acute toxicity in the Contra Costa Canal; however, unexplained differences between the observations made in our laboratory and the independant contractors should be resolved by further monitoring at this site.

Chapter 6

METHODOLOGICAL FINDINGS

6.1 INTRODUCTION

There are selected technical issues relating to the bioassay protocols which could effect the characterization of the toxicity observed. These issues include intralaboratory precision of the toxicity tests, salinity adjustment of samples, and sperm:egg ratios used in the echinoderm assay.

6.2 INTRALABORATORY PRECISION

Intralaboratory variability for silverside, mollusc, and echinoderm tests was determined by evaluating the coefficient of variation (CV) of IC_{50} values for laboratory reference toxicant tests (Table 19). Intralaboratory variability for four silverside minnow tests was 34.4%, based on the survival endpoint. A CV could not be calculated for the growth endpoint because a 50% reduction in growth was only achieved in two tests. For the mollusc test, the CV of IC_{50} values was 38.2%. Laboratory variability for 14 echinoderm tests, using two echinoderm species, resulted in a CV of 39.7%. These estimates of test precision were similar to those reported by Schimmel et al. (1989), who reported CV values of 41% for the sea urchin (*Arbacia punctulata)* test and 20.6% for silverside minnow survival. Anderson and Norberg (1990) have reported on the interlaboratory precison of the toxicity tests, based on the results of a survey of 10 laboratories conducted in the San Francisco Bay area. For the mollusc test, a CV of 38% was reported; for the echinoderm test, in which four different species were used, a CV of 74% was reported. The silverside minnow test was not conducted in the latter survey. Both Schimmel et al. (1989) and Anderson and Norberg (1990) used reference toxicants that varied from those used in this studied, and this difference may account for some of the variations observed among studies.

TABLE 19. INTRALABORATORY PRECISION FOR THE ECHINODERM, SILVERSIDE MINNOW, AND MOLLUSC TESTS

SILVERSIDE MINNOW SURVIVAL	
Survey	IC_{50} (ug/l Cu)
S.F. Wildlife Refuge	80
Hayward 1	89
Hayward 2	35
Dredge	68
CV (%)	34

MOLLUSC PROPORTION NORMAL	
Survey	IC_{50} (mg/l Na azide)
Bay Background 1	41
Bay Background 2	17
Bay Background 3	20
Dredge	37
USS Posco	23
CV (%)	38

ECHINODERM FERTILIZATION	
Survey	IC_{50} (mg/l Na azide)
Bay Background 1	260
Bay Background 2--#1	313
Bay Background 2--#2	291
Bay Background 2--#3	307
Bay Background 3	88
Bay Background 4--#1	265
Bay Background 4--#2	146
S.F. Wildlife Refuge	196
Hayward 1	230
Hayward 2	142
Mountain View	113
Sunnyvale	182
San Jose	145
USS Posco	104
CV (%)	40

6.3 SALINITY ADJUSTMENT

The potential effects of salinity adjustment on performance of laboratory controls were evaluated for three species; silverside minnow, echinoderm, and mollusc. Samples used in the silverside minnow bioassay frequently required salinity adjustment using artificial seasalts. To evaluate the potential for toxicity attributable to the use of commercial sea salts, we compared natural seawater and salinity-adjustment controls for both the survivorship and growth endpoints (Table 20). We did not observe any significant difference in either growth or survivorship between the two controls. These data indicate that artificial seasalts are an effective means of salinity adjustment for this species.

In contrast to the silverside minnow bioassay, salinity adjustment in the sea urchin tests was often problematic. Use of artificial seasalts resulted in poor control fertilization, necessitating the use of natural brine for salinity adjustment. However, fertilization success in brine controls varied among surveys (Table 21). In six out of 11 tests, the brine controls were significantly different from the seawater controls. Through much trial and error, we have found that there are two factors that influence brine quality--the holding time of the seawater used to make the brine and the presence of precipitated salts. Brine should be made using recently collected seawater filtered to 0.45 um. In order reduce the possibility of salts falling out of solution, water was heated slowly at 60°C, stirred continuously, and not allowed to exceed approx 80 ppt. Since salt precipitation was more likely in brine held for longer than two weeks, new brine was made before each test.

In the mollusc bioassay, natural brine was used for all salinity adjustment. No systematic effort was made to determine whether artificial seasalts should be used in this test. Natural brine, however, routinely resulted in excellent control performance (Table 22). No significant differences were observed between the performance of natural seawater and brine controls.

6.4 SPERM:EGG RATIO IN THE ECHINODERM TEST

Early in the study, we observed that high sperm:egg ratios in the echinoderm test could obscure toxic effects and that the optimal ratio varied within the spawning season.

TABLE 20. COMPARISON BETWEEN SEAWATER AND ARTIFICIAL SEASALTS USING THE SILVERSIDE MINNOW GROWTH AND SURVIVAL TEST[1]

SURVEY	MEAN SURVIVAL (%)		MEAN GROWTH (mg)	
	Seawater	40 Fathoms	Seawater	40 Fathoms
Bay Background 1	90	100	0.53	0.62
Hayward Marsh 1	93	90	0.45	0.42
Hayward Marsh 2	97	100	0.66	0.74
San Jose	87	97	0.87	0.92

[1]No significant differences were observed between treatments

TABLE 21. COMPARISON BETWEEN SEAWATER AND NATURAL BRINE USING THE ECHINODERM TEST

SURVEY	MEAN FERTILIZATION(%)	
	Seawater	Brine
Bay Background 1	100	98
Bay Background 2	97	97
Bay Background 3	96	98
Bay Background 4	96	86*[1]
S.F. Wildlife Refuge	100	78**[2]
Hayward 1	100	99
Hayward 2	94	88*
Mountain View	93	63**
Sunnyvale	100	98
San Jose	94	75**
Posco	90	66**

[1] * Indicates seawater and brine controls were signifcantly different at the $p<0.05$ level.

[2] ** Indicates seawater and brine contols were significantly different at the $p<0.01$ level.

TABLE 22. COMPARISON BETWEEN SEAWATER AND NATURAL BRINE USING THE MOLLUSC EMBRYO DEVELOPMENT TEST[1]

SURVEY	ABNORMAL (%)	
	Seawater	Brine
Bay Background I	8.7	12.0
Bay Background II	13.1	14.4
Bay Background III	3.6	2.9
Dredge	14.6	10.4

[1]NSD between Bodega and brine-adjusted mineral water

Consequently, as the study progressed, we incorporated a pretest into our protocol to determine the lowest sperm:egg ratio which gave acceptable control fertilization. Both a seawater and brine control were tested with a minimum of three different ratios which, depending on the season, ranged from 100:1 to 1000:1. An example of the results of one such pretest are presented in Table 23. The addition of the pretest to the procedure can increase the sensitivity of the assay.

TABLE 23. EXAMPLE OF SEA URCHIN SPERM:EGG RATIO TEST

SODIUM AZIDE CONCENTRATION (mg/l)	MEAN FERTILIZATION (%)	
	100:1 Sperm:Egg	500:1 Sperm:Egg
Control	99	100
100	73	96
200	69	74
300	11	37
400	2	10

Chapter 7

DISCUSSION AND CONCLUSIONS

7.1 POTENTIAL SIGNIFICANCE OF TOXICITY OBSERVED IN BAY BACKGROUND SURVEYS

The key finding of the Bay Background surveys was that, with few exceptions, toxicity was only observable using the echinoderm fertilization assay. These data are summarized in section 3.12. The preliminary TIE, conducted on one date at two stations, indicated only that the toxicity observed on that particular day was not persistent. Significantly, holding times were not constant among stations for surveys 1 and 3, and this factor could have affected the levels of toxicity observed from station to station.

Nevertheless, almost complete inhibition of fertilization was observed in the first survey, and varying levels of inhibition were observed at all sites in subsequent surveys. This finding may indicate one of two things. The first possible interpretation of the observed toxicity using the echinoderm assay is that moderate toxicity exists throughout the Bay; and that toxic events, such as that observed during the First Bay Background survey can occur periodically. The widespread nature of the toxicity observed (using only one species) is perplexing but not unprecedented. In the Sacramento and San Joaquin Rivers, toxic effects have been documented along river stretches up to 75 miles in length (Foe and Connor, 1989) using ambient toxicity testing. Interestingly, the toxicity at those sites was only observed using one species, *Ceriodaphnia.*

The second possible interpretation of these data is that there may be positive interferences in the application of this test to Bay waters. This possibility is speculative but cannot be ruled out in such a complex system as the San Francisco Bay and Delta. The nature of potential positive interferences is unknown but could include any of a range of speculative possibilities such as the toxic effects of substances excreted by marine bacteria, to the physical effects of colloidal matter, to subtle changes in ionic content of sample waters.

Possibilities of this nature could be addressed experimentally.

The interpretation of negative findings using species other than echinoderms must include recognition of the numerous factors that are not explored in a general background survey. For example, the timing of sampling activities was limited in nature and was not scheduled to characterize specific events. In addition, the surveys were not conducted adjacent to specific sources of toxicity such as permitted discharge locations, sites of urban runoff, or agricultural discharge. Significant toxicity has been documented in all of these sources throughout the Bay area (Anderson et al., 1990; Woodward and Clyde/Kinnetics, Inc., 1989).

Sites at which low initial dilution of specific toxic inputs occurs will be the most vulnerable to toxic effects. The fact that more marked toxicity was observed during the marsh surveys than during the background surveys underscores this fact. We conclude that regional studies, such as the marsh surveys discussed below, are more likely to detect water quality impairment than the widespread Bay Background-type surveys. Nevertheless, the toxicity observed using the echinoderm assay must be viewed as significant and should be further explored. A repetition of the results obtained in the first survey would warrant the implementation of further experimental TIE efforts. To date, we know of no TIE efforts in the nation that have used the echinoderm assay.

7.2 POTENTIAL SIGNIFICANCE OF TOXICITY OBSERVED IN BAY MARSH SURVEYS

Comparison of the findings obtained during the Hayward Marsh surveys and the Sunnyvale Marsh survey elucidates some of the major advantages of the use of ambient toxicity testing. Results of these surveys clearly demonstrate that the tools can be applied to a variety of complex systems to elucidate potential instream effects. They also demonstrate that some marsh ecosystems could be better managed if the toxicity of effluent sources, and of the ambient waters, were better characterized. Clearly, these tools can be used to improve our knowledge of the potential effects of wastewater discharges into marsh ecosystems.

Short-term toxicity tests can also be used to identify the potential causes of toxicity in marsh

ecosystems. After sources have been characterized, experimentation can be conducted to identify the substances causing toxicity. At the Hayward site, the majority of toxicity is attributable to unionized ammonia, but not all toxicity could be explained. At the Sunnyvale site, TIE studies were not conducted but would have been more difficult to perform. The more subtle toxic effects would require much more detailed efforts than those invoked at Hayward. The fact that less toxicity was observed in the biological oxidation pond at Sunnyvale than was observed in the final effluent, also indicates that management options and source characterization must be considered as TIE efforts progress at more complex sites.

The question "What is the capacity of wetland ecosystems to treat and to degrade toxicants?" is not a new question. However, it has almost exclusively been answered from an engineering perspective. It is well known that carefully engineered marshes result in decreased BOD and COD in wastewater in the marsh (Hammer, 1990; WPCF,1990). In addition, sequestration of toxic substances has been evaluated (Hammer, 1990). It is widely believed that wetland ecosystems are sinks for many heavy metals. Recent research, however, has demonstrated that complex interrelationships between deposition in sediment and seasonal cycles of plant uptake are only remotely understood (Beeftink et al., 1982; Simpson et al., 1983; Dubinski et al., 1986). Consequently, it must be recognized that fluxes between these environmental compartments occur and that a sink may only serve a temporary storage function. In effect, it is as yet unresolved whether accumulation of toxic substances in these systems will eventually pose more harm than benefit.

Our findings reaffirm the conclusions of others--that engineering concerns must be coupled with ecotoxicogical investigation (Hicks and Stober, 1990; Brennan, 1985). Godfrey et al. (1985) have stated "While there is evidence that the negative impacts on wetland values from applications of wastewater are not severe, at least in the short term, breadth of knowledge about ecosystem effects lags behind sophistication of engineering design. This situation creates a motivational impetus that could lead to widespread use of the technique in advance of reasonably complete knowledge about environmental consequences". Thus, it seems reasonable to conclude that further implementation of marsh reclamation projects should, at a minimum, be coupled with well designed *post hoc* monitoring.

Short-term toxicity tests have been shown to be useful tools for ambient toxicity evaluations (e.g. Schimmel et al., 1989). Consequently, it is surprising that only a few studies have utilized laboratory or *in situ* toxicity testing. Woodward et al. (1988) have evaluated the toxicity of drilling fluids in arctic tundra wetland habitat. Johnson (1986) evaluated the potential impacts of selected agricultural chemical contaminants on a northern prairie wetland, and Lee et al. (1982) evaluated phytotoxicity of arsenic in flooded and upland disposal scenarios for contaminated dredge material.

Improved assessment of the ecological integrity of natural and humanmade wetland ecosystems may highlight many complex issues related to the competing uses of these habitats and the thorny policy questions that must be addressed to resolve these conflicts. Important questions include: 1) Should natural marshes be "traded" for humanmade marshes in marsh mitigation proceedings? 2) Should humanmade marshes be required to meet water quality criteria even if no marsh would exist in the absence of the wastewater input? 3) How can criteria for wetland functions be linked to toxicological criteria? The answers to these question will only come after concerted efforts between scientists and policymakers.

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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. and **T.J. Norberg.** 1990. Editorial: Precision of short-term toxicity tests in the real world. *Environ. Toxicol. Chem.* in press.

Anderson, S.L., M.P. Carlin, and **L. Suer.** 1990. Status of the effluent and ambient toxicity characterization program. Report of the San Francisco Bay Regional Water Quality Control Board (3/20/90). Oakland, CA.

Beeftink, W.G., J. Nieuwenhuize, M. Stoeppler and **C. Mohl.** 1982. Heavy-metal accumulation in salt marshes from the western and eastern Scheldt. *The Science of the Total Environment* **25**:199-223.

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.

Dubinski, B.J., R.L. Simpson and **R.E. Good.** 1986. The retention of heavy metals in sewage sludge applied to a freshwater tidal wetland. *Estuaries* **9**:102-111.

Foe, C. and **V. Connor.** 1989. Rice Season Toxicity Monitoring Results. Memorandum of the Central Valley Regional Water Quality Control Board (10/19/89). Sacramento, CA.

Glickman, A.H., K.A. Fletcher and **R.S. Caldwell.** 1988. Toxicological assessment of an oil refinery effluent using both resident and surrogate species tests. Abstract of the Society of Environmental Toxicology and Chemistry. Arlington, VA.

Godfrey, P.J., E.R. Kaynor, S. Pelczarski and **J. Benforado.** 1985. *Ecological Considerations in Wetlands Treatment of Municipal Wastewaters.* Van Nostrand Reinhold Co., New York, NY.

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

Hemond, H.F. and **J. Benoit.** 1988. Cumulative impacts on water quality functions of wetlands. *Environmental Management* **12**:639-653.

Hicks, D.B. and **Q.J. Stober.** 1990. Monitoring of constructed wetlands for wastewater. In D.A. Hammer, ed. *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.

McKim, J. 1977. Evaluation of tests with early life stages of fish for predicting long-term toxicity. *J. Fish. Res. Bd Can.* **34**: 1148.

Nelson, W. and **C. Weller.** 1984. Better rational for wetland management. *Environmental Management* 8:295-308.

Phillips, D.J.H. 1987. Toxic Contaminants in the San Francisco Bay-Delta and Their Possible Biological Effects. Report of the Aquatic Habitat Institute. Richmond, CA.

Race, M.S. and **D.R. Christie.** 1982. Coastal zone development: Mitigation, marsh creation, and decision-making. *Environmental Management* 6:317-328.

Schimmel, S.C., G.E. Morrison and M.A. Heber. 1989. Marine complex effluent toxicity program: test sensitivity, repeatability and relevance to receiving water toxicity. *Environ. Toxicol. Chem.* 8:739-746.

Simpson, R.L., R.E. Good, R. Walker and **B.R. Frasco.** 1983. The role of Delaware River freshwater tidal wetlands in the retention of nutrients and heavy metals. *J. Environ. Qual.* 12:41-48.

Stein, J.R. 1973. *The Handbook of Phycological Methods: Culture, Methods and Growth Measurement.* Cambridge University Press, Cambridge, England.

U.S. Environmental Protection Agency. 1978. Bioassay procedures for the Ocean Disposal Permit Program. Gulf Breeze, Fla.

U.S. Environmental Protection Agency. 1985. Ambient water quality criteria for ammonia-1984. EPA-440/5-85-001. Washington, DC.

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

U.S. Environmental Protection Agency. 1988c. Methods for aquatic toxicity identification evaluations: Phase II toxicity identification procedures. EPA-600/3-88-035. Duluth, MN.

U.S. Environmental Protection Agency. 1989a. 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.

U.S. Environmental Protection Agency. 1989b. Ambient water quality criteria for ammonia (saltwater). EPA-440/5-88-004. Washington, D.C.

U.S. Environmental Protection Agency and **U.S. Fish and Wildlife Service.** 1984. The ecological impacts of wastewater in wetlands: An annotated bibliography. EPA-905/3-84-002. Chicago, Il.

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 and Kinnetics, Inc. 1989. Santa Clara Valley Nonpoint Source Study, Volume 1: Loads Assessment Report. Report submitted to the San Francisco Bay Regional Water Quality Control Board. Oakland, CA.

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