RMP EPISODIC AMBIENT WATER TOXICITY STUDY

2000-2001 Annual Report

"AMBIENT WATER TOXICITY IN THE SAN FRANCISCO ESTUARY"

R. Scott Ogle, Pacific EcoRisk, 835 Arnold Dr., Suite 104, Martinez, CA 94553
Phone: (925)313-8080 Fax: (925)313-8089 e-mail: scottogle@pacificecorisk.com

Andrew Gunther, Applied Marine Sciences, 4749 Bennett Dr., Suite L, Livermore, CA 94550
Phone: (925)373-7142 Fax: (925)373-7834 e-mail: gunther@amarine.com

Paul Salop, Applied Marine Sciences, 4749 Bennett Dr., Suite L, Livermore, CA 94550
Phone: (925)373-7142 Fax: (925)373-7834 e-mail: salop@amarine.com

David Bell, Applied Marine Sciences, 4749 Bennett Dr., Suite L, Livermore, CA 94550
Phone: (925)373-7142 Fax: (925)373-7834 e-mail: bell@amarine.com

Jeffrey Cotsifas, Pacific EcoRisk, 835 Arnold Dr., Suite 104, Martinez, CA 94553
Phone: (925)313-8080 Fax: (925)313-8089 e-mail: cotsifas@pacificecorisk.com

Jordan Gold, Applied Marine Sciences, 4749 Bennett Dr., Suite L, Livermore, CA 94550
Phone: (925)373-7142 Fax: (925)373-7834 e-mail: gold@amarine.com

AMBIENT WATER TOXICITY IN THE SAN FRANCISCO ESTUARY

ABSTRACT

Toxicity testing of ambient waters conducted by the San Francisco Bay Regional Monitoring Program for Trace Substances (RMP) has indicated that, in general, there does not appear to be any consistent, wide spread toxicity problems within the Bay's waters. A major exception to this have been the occurrences of significant toxicity in ambient water samples collected from just upstream of the confluence of the Sacramento and San Joaquin Rivers down to the mouth of the Napa River following major storm events in Jan-Feb of 1996 and 1997. It is believed that this toxicity was the result of pesticides in stormwater runoff from within the Sacramento and San Joaquin River watersheds. This suggested that significant ambient water toxicity is present in San Francisco Bay, occurring as "events" of short duration and/or of localized nature, probably the result of stormwater runoff and/or other surface water runoff events. To investigate the frequency and magnitude of these episodic events, we have been collecting and performing toxicity tests on ambient water samples at selected sites in San Francisco Bay following significant rainstorm events for the past four winter seasons. The results of these tests indicate that ambient water toxicity is present in parts of the Bay primarily following runoff events. Results of ELISA analyses suggest that some of this toxicity may be due to organophosphate (OP) pesticides, while the possible causes of other toxic water samples still remains unknown. Apparent reductions in the magnitude and frequency of ambient water toxicity to the test organism *Americamysis bahia* (formerly *Mysidopsis bahia*) over the past several years mirror reductions in the application of OP pesticides in the Estuary's watersheds. However, the application of other pesticides, such as pyrethroids, has increased over this same period, suggesting that changes in the types of ambient toxicity in the Estuary (e.g., increased sediment toxicity; potential fish toxicity) may be occurring, and that corresponding changes in the monitoring approach, e.g., the use of tests and test species that will be more sensitive to changing use patterns of pesticides and their fate and transport characteristics may be called for. It is critical that monitoring programs such as this maintain a vigilant awareness of changes in activities within the monitoring area watersheds and adapt the monitoring approach to reflect those changes.

Table of Contents

AMBIENT WATER TOXICITY IN THE SAN FRANCISCO ESTUARY

INTRODUCTION

The San Francisco Estuary (Figure 1), consisting primarily of the Sacramento and San Joaquin Rivers, the "Delta", and San Francisco Bay, is one of the most economically and ecologically important watershed drainage systems in the United States. Encompassing an area of roughly 1600 square miles, the Estuary receives approximately 47% of the surface water runoff in California from over 60,000 square miles of drainage, provides drinking water to over 22 million people, and supports one of the most productive agricultural regions in the world. Over 70% of the freshwater input into the Estuary is from the Sacramento River watershed, with a little less than 20% coming from the San Joaquin River watershed, and the remaining 10% from local watersheds.

As with most major estuaries, there have been, and continue to be, a wide variety of land uses along the Estuary's shorelines and in it's upstream watersheds. Historical mining activities, urbanization, industrial development, and agriculture have all had major impacts on the Estuary, including chemical contamination: metals from historical mining activities, pesticides from agricultural activities, and a variety of contaminants from industrial and urban discharges. This long-term and continuing contamination of the Estuary has been reflected in the population declines of numerous aquatic organisms over the past 150 years (Herbold et al. 1992).

The San Francisco Regional Monitoring Program for Trace Substances

Current information regarding the degree and nature of contamination in the Estuary is an essential first step in the management and eventual restoration of the Estuary by local, state, and federal regulatory agencies. To meet this need, the State's San Francisco Bay Regional Water Quality Control Board (SF Bay Regional Board) created the San Francisco Estuary Regional Monitoring Program for Trace Substances (the RMP) in 1983 (Thompson et al. 2000). The RMP is a collaborative multi-component monitoring and research program, managed by the SF Regional Board, regulated wastewater dischargers (who also fund the program), and the San

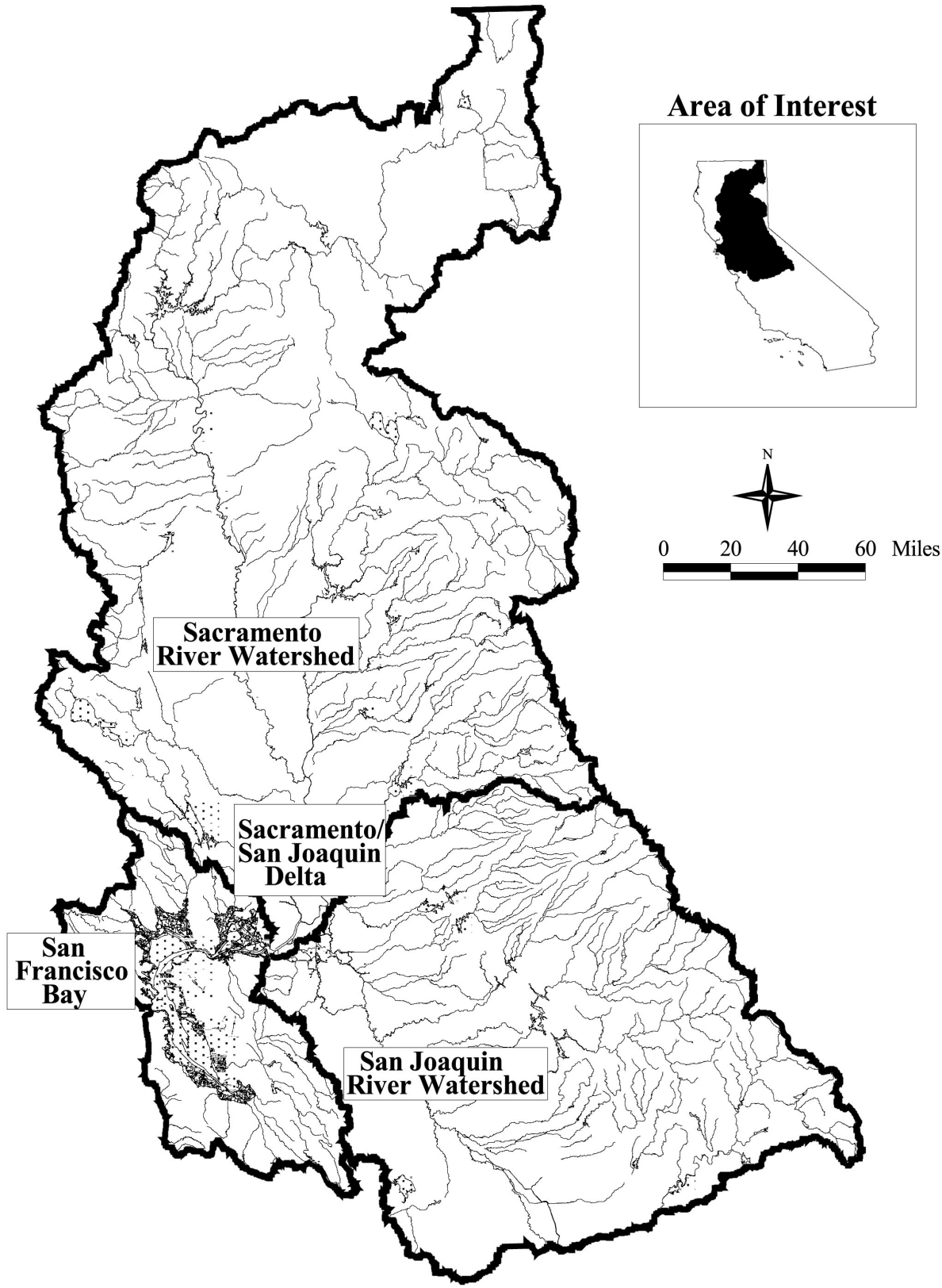


Figure 1. The San Francisco Estuary, including the Sacramento River, the San Joaquin River, the Delta and San Francisco Bay.

Francisco Estuary Institute, an independent, non-profit research organization. The RMP, and its team of academic, agency, and private sector scientists, have as their objectives:

- To describe the patterns and trends in contaminant concentrations and distributions;
- To describe contaminant loading into the Estuary;
- To measure the effect(s) of contaminants on selected parts of the Estuary;
- To compare the resulting monitoring information to relevant water quality objectives and other guidelines;
- To synthesize, integrate, and distribute information from a range of sources to more completely characterize the sources, distribution, fates, and effects of contaminants in the Estuary ecosystem.

RMP Program Elements

The main study elements, or baseline studies, of the RMP include:

- Chemical analyses of surface waters for selected metal and organic contaminants;
- Characterization of ambient surface water toxicity;
- Characterization of ambient sediment toxicity;
- Evaluation of contaminant bioaccumulation using *in situ* “bagged” bivalves.

As part of the Adaptive Management Strategy of the RMP, observations of significant trends and/or anomalies in the baseline studies, as well as suggestions for new monitoring tools and approaches, are used as the basis for follow-up or preliminary Pilot Studies to investigate selected aspects of contamination of the Estuary in greater detail.

RMP BASELINE AMBIENT WATER TOXICITY TESTING

Toxicity testing of ambient surface waters has been an integral baseline component of the RMP since its conception. The fundamental approach of toxicity testing is to expose selected aquatic organisms to samples of the Estuary's ambient waters under laboratory conditions, and to assess potential adverse effects, such as impaired reproduction or development, reduced growth, and/or mortality, that may result. These toxicity tests are performed with test organisms selected on the basis of: well-established US EPA testing guidelines; availability of organisms for testing; availability of published information regarding the toxicity of contaminants; and cost.

At selected stations located throughout San Francisco Bay (Figure 2), ambient water samples for baseline toxicity testing were collected via peristaltic pumping, using pre-cleaned silicon tubing, to deliver ambient water from approximately 1 m depth to pre-cleaned sample containers aboard the sampling vessel. During the first four years of monitoring (1993-1997), samples were collected into 20 L high-density polyethylene (HDPE) cubitainers; subsequently, samples have been collected into 20 L fluorocarbon-lined HDPE jerricans. For each sample collection, approximately 1-2 L of ambient water were initially pumped into each container, which were then vigorously shaken to provide a preliminary rinse with site water, after which the containers were emptied and then completely re-filled. A total of 40 L (two sample containers) of ambient water was collected at each site. The samples were then placed into ice chests and covered with ice for storage onboard the sampling vessel. Upon return to port, the samples were immediately transported to the testing laboratories in Concord and Martinez, CA.

Year One RMP Toxicity Testing

The first year of RMP toxicity testing included collection of ambient water samples from eight stations (BA20, BA40, BC10, BD20, BD50, BF20, BG20, and BG30; see Figure 2) in March, May, and September 1993. Each of these water samples was tested using two different toxicity tests: the bivalve embryo development test with *Mytilus sp.* and *Crassostrea gigas* (ASTM 1991), and the algal growth test using the diatom *Thalassiosira pseudonana* (ASTM 1990). Relative to the Laboratory Control treatments, no toxicity was observed in any of the water samples for either of the tests (SFEI 1994). The *Thalassiosira* actually exhibited biostimulation

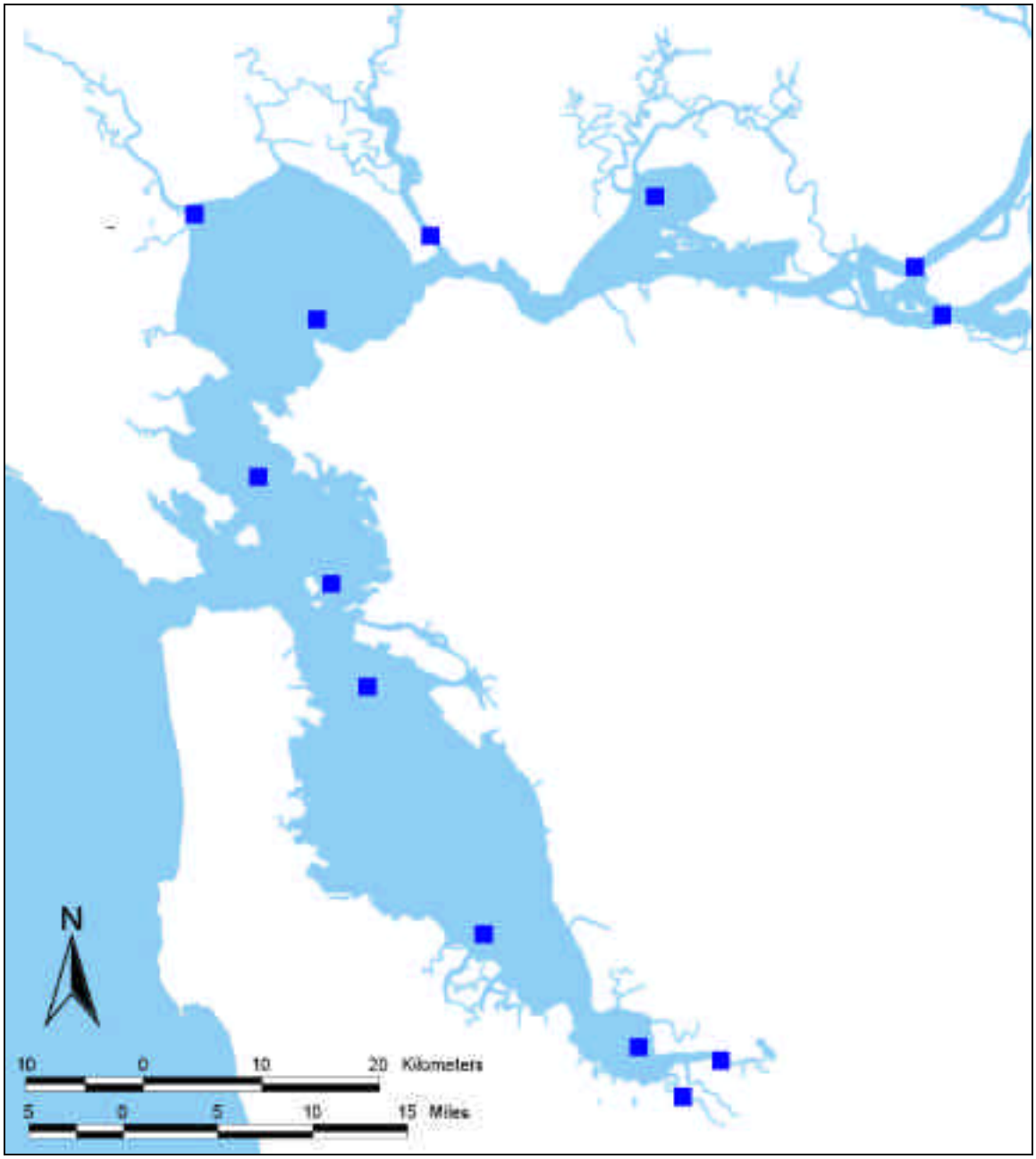


Figure 2. RMP “Baseline” ambient water sampling stations in San Francisco Bay.

(growth in the site waters was greater than the Laboratory Control treatment) at almost every station, a phenomenon that is common in non-toxic ambient waters, typically due to higher nutrient concentrations in the ambient water samples and/or nutrient limitation in the Control treatment media.

Year Two RMP Toxicity Testing

Year Two (1994) of the Program saw several adaptive changes to the RMP baseline toxicity testing. Sampling of ambient waters for toxicity testing was reduced to two events for the year (February and September) with the number of sampling sites being increased to 13 Stations: C-1-3, C-3-0, BA10, BA40, BB70, BC10, BC60, BD30, BD15, BD50, GF20, BG20, and BG30 (Figure 2). The toxicity testing approach itself also saw adaptive changes, with the *Thalassiosira* test being discontinued due to the potential confounding interference of biostimulation, and the addition of the U.S. EPA 7-day mysid shrimp *Americamysis bahia* survival and growth test. *Americamysis bahia* (formerly *Mysidopsis bahia*) is a small mysid shrimp native to the Gulf of Mexico (Price 1982); it is not resident to San Francisco Bay, but is a readily obtained EPA standard test species that is employed throughout the United States, which is known to be very sensitive to a wide variety of contaminants.

As in 1993, there was no toxicity to bivalve embryo development in the ambient water samples collected from the 13 stations during the two sampling periods (SFEI 1995). In February, *A. bahia* exhibited slight, but statistically significant reductions in survival at the Napa River (BD50) and Red Rock (BC60) stations (less than 40% reduction in survival relative to the Control treatments at both stations); no mysid toxicity was observed for any of the other February water samples nor in any water samples collected in September (SFEI 1995)

Year Three RMP Toxicity Testing

The toxicity testing approach for Year 3 (1995) was similar to the previous year: water samples were collected from the same 13 stations in February and August, and were assessed for toxicity using the mussel (*Mytilus sp*.) embryo development test and the mysid (*A. bahia)* survival and growth test. As in the previous years, there was no toxicity to bivalve embryo development in the

ambient water samples (SFEI 1996). In February, *A. bahia* exhibited a slight, but statistically significant reduction in survival at the San Joaquin River (BG30) station (less than 20% reduction in survival relative to the Control treatment); no mysid toxicity was observed for any of the other February water samples, nor in any water samples collected in August (SFEI 1996).

Year Four RMP Toxicity Testing: Significant Toxicity in Northern Bay System

Year 4 (1996) of the RMP saw dramatic and significant developments in the baseline ambient water toxicity testing. Samples were collected at the same 13 stations in February and July, and were assessed for toxicity using the mussel (*Mytilus sp*.) embryo development test and the mysid (*A. bahia)* survival and growth test. As in the previous years, there was no significant toxicity to bivalve embryo development present in any of the ambient water samples (SFEI 1997). However, in February, there was region-wide toxicity to the mysid with dramatic and significant reductions in survival at the four northern-most stations: Napa River, Grizzly Bay, the Sacramento River, and the San Joaquin River (Figure 3). No mysid toxicity was observed for any of the other February water samples. Again in July, there was region-wide toxicity to the mysid with slight, but statistically significant reductions in survival at the Grizzly Bay, the Sacramento River, and the San Joaquin River stations (Figure 3).

Year Five RMP Toxicity Testing: A Repeat of Episodic Toxicity

Year 5 (1997) of the RMP also saw dramatic toxicity in the baseline ambient water toxicity testing. Samples were collected at 10 stations (Stations BB70, BC10, BC60 were dropped from the 13 stations sampled in 1995 and 1996) in January and August, and were assessed for toxicity using the bivalve (*Mytilus sp*.) embryo development test and the mysid (*A. bahia)* survival and growth test.

Unlike previous years, statistically significant toxicity to bivalve embryo development was evident in the Grizzly Bay, Pinole Point, Redwood Creek, and Sunnyvale ambient water samples, although there was less than a 5% reduction in normal development relative to the

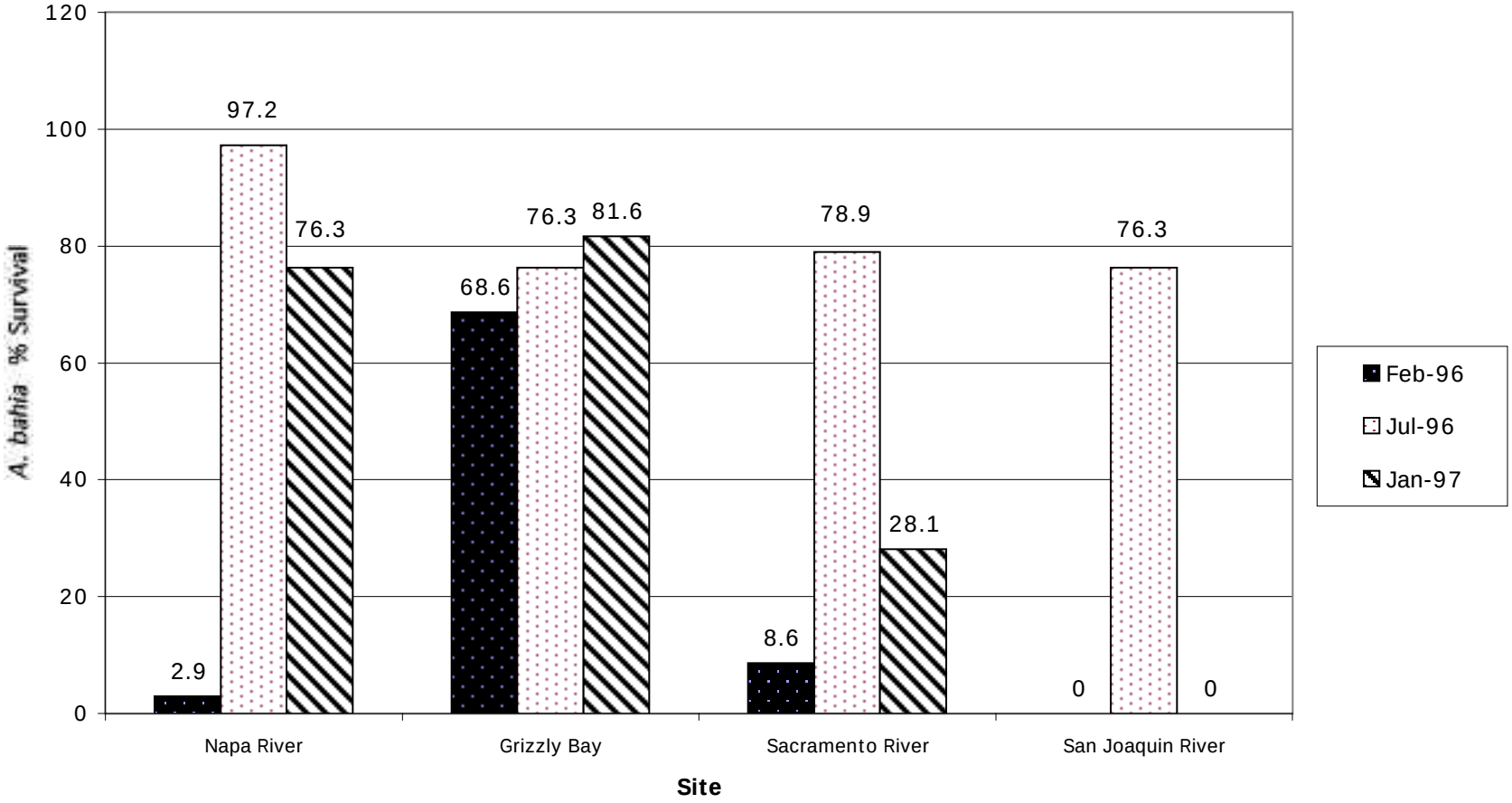


Figure 3. Toxicity of RMP Baseline ambient water samples to *Americamysis bahia:* (a) February 1996; (b) July 1996; (c) January 1997. Percent survival data have been normalized to the corresponding Control treatment survival response. With the exception of the July 1996 Napa River sample, each sample resulted in statistically significant reductions in mysid survival relative to the Control.

Control for each of the samples, suggesting that these differences are not toxicologically significant. There was no toxicity to the bivalves in the July/August samples.

In February, there was once again region-wide toxicity to the mysid with significant reductions in survival at the four northern-most stations: Napa River, Grizzly Bay, the Sacramento River, and the San Joaquin River (Figure 3). While the North Bay station water samples collected in July/August were not toxic to mysids, there was significant toxicity in the waters collected from the four southernmost stations, with complete mortality observed in the samples from Coyote Creek, San Jose, and Sunnyvale.

HYPOTHESIS: AMBIENT WATER TOXICITY IN SAN FRANCISCO ESTUARY IS EVENT-BASED AND EPISODIC IN NATURE

Interestingly, the February 1996 sampling was the first time that the RMP baseline sampling had occurred following significant rainfall in the Estuary's watersheds. Earlier studies had already reported that agricultural runoff in the San Joaquin and Sacramento River watersheds was frequently toxic to aquatic organisms, particularly following significant rainfall events (Foe and Connor 1991; Foe 1995; Werner et al. 2000). In 1988, the Central Valley Regional Water Quality Control Board began conducting monitoring studies of ambient water toxicity in the San Joaquin River basin. They found that much of the San Joaquin River was toxic "about half the time" to *Ceriodaphnia dubia*, a freshwater planktonic invertebrate (Foe and Connor 1991). It was hypothesized that pesticides in agricultural runoff were causing the observed toxicity; concurrent monitoring of agriculturally-dominated tributaries of the river revealed similar toxicity problems (Foe and Connor 1991). Follow-up monitoring in 1991-92 observed that 22% of the water samples collected from the San Joaquin Basin were toxic to *Ceriodaphnia* (Foe 1995). Most of the observed toxicity could be attributed to the concentrations of four pesticides: diazinon, chlorpyrifos, fonofos, and carbaryl, although other pesticides were also detected in the water samples. When the pesticide concentrations were normalized to their toxicity to *Ceriodaphnia* (in a Toxic Units approach), it was found that diazinon, chlorpyrifos, and parathion accounted for over 90% of all toxicity. More recent ambient water toxicity monitoring the Sacramento River watershed and in the Delta revealed significant toxicity to *Ceriodaphnia*,

and concurrent Toxicity Identification Evaluations (TIEs) demonstrated that diazinon, chlorpyrifos, and carbofuran were the main causes of this toxicity (Werner et al. 2000).

Chemical monitoring of the off-site transport of dormant spray pesticides (from orchards) in the Estuary's ambient waters immediately following rainstorm events (Kuivila and Foe 1995) clearly demonstrated that pulses of pesticides (e.g., diazinon) are flowing down through the watersheds and into and through the northern San Francisco Bay system (Figure 4).

Toxic ambient waters are not limited to stormwater runoff from agricultural land. Pesticides are also sold 'over the counter' and are commonly applied as part of routine home maintenance and gardening practices. Numerous stormwater runoff studies in urban areas have also observed surface water toxicity to *Ceriodaphnia* which chemical analyses and TIEs have attributed to diazinon and/or chlorpyrifos (BAASMA 1996; Werner et al. 2000; Bailey et al. 2000).

All of these studies combined indicate that there are event-based, pulsed introductions of contaminants into the surface water that flows through San Francisco Bay (e.g., stormwater runoff), and that such episodic pulses are a likely cause of the ambient water toxicity observed for February 1996 (and January 1997) RMP baseline toxicity testing. Based upon the information from these various studies, the SF Bay Regional Board placed most of San Francisco Bay on the impaired bodies of water (303d) list due to OP pesticides.

THE ECOLOGICAL IMPLICATIONS OF EVENT-BASED EPISODIC AMBIENT WATER TOXICITY

While the studies reporting event-based episodic toxicity suggest that pesticides in surface water runoff may cause toxicity to invertebrates in waters within the Sacramento-San Joaquin River basins and the San Francisco Estuary, no link has yet been conclusively established. Long-term studies of zooplankton distribution and abundance in the Sacramento-San Joaquin Delta have reported a significant decline in the number of zooplankton species in the freshwater parts of the estuary (Obrebski et al. 1992), with recent zooplankton density being 1-2 orders of magnitude lower than in the early 1970s. Use of organophosphate (OP) pesticides like diazinon and chlorpyrifos has increased substantially since their introduction in the 1950s and 1960s, suggesting a possible link between pesticide toxicity and zooplankton declines. Similar adverse

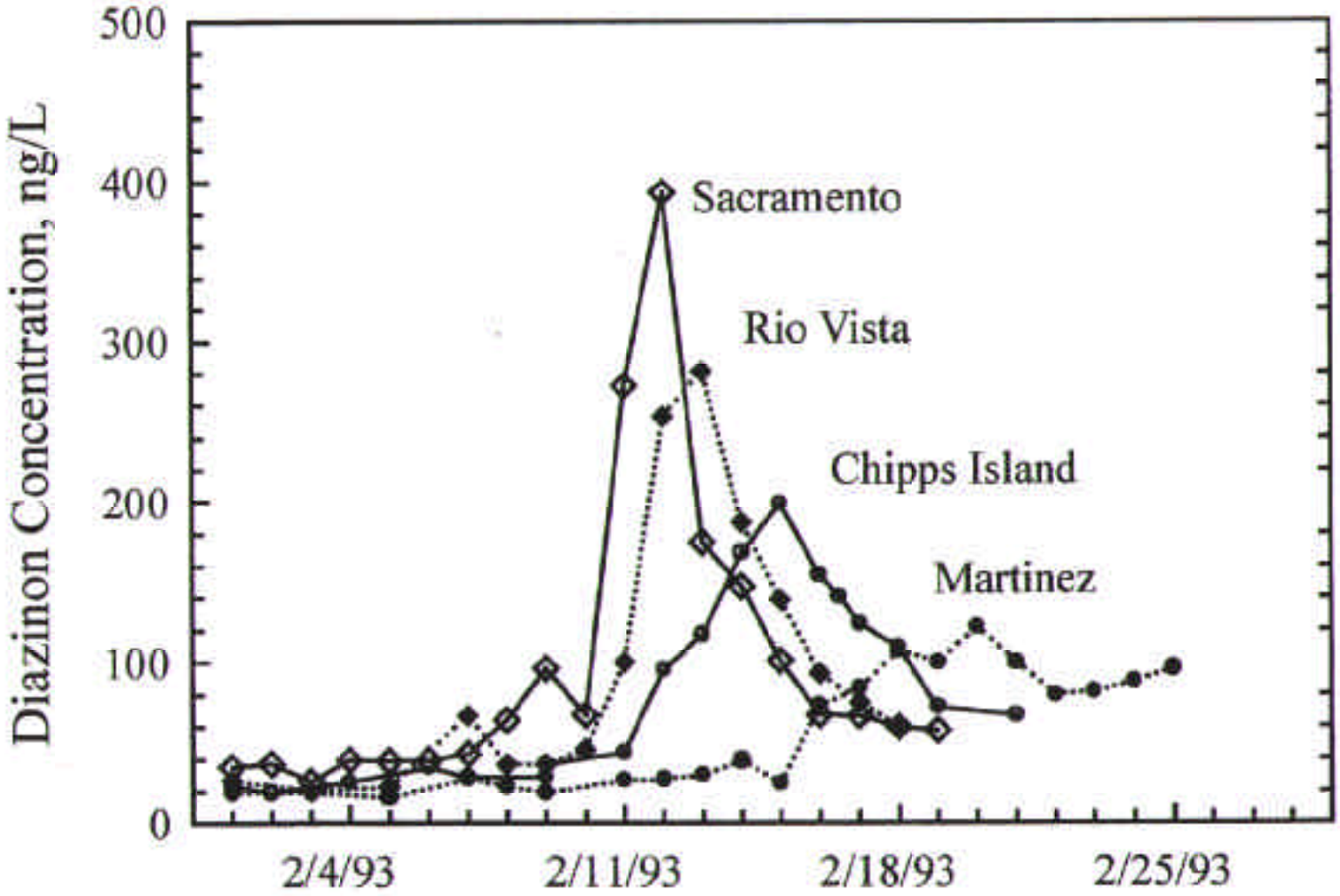


Figure 4. Concentrations of diazinon measured in ambient waters from Sacramento to San Francisco Bay, February 1993 (printed with permission, from Kuivila and Foe (1995)).

declines in benthic invertebrates may also have taken place over the past several decades; recent monitoring of benthic invertebrate resources in the Sacramento-San Joaquin basins by the US EPA Environmental Monitoring & Assessment Program (EMAP) have observed lower invertebrate abundance and diversity than expected from similar studies conducted elsewhere in the United States (Peter Husby, US EPA Region 9, personal communication). However, it should be noted that these population-level indicators of ecosystem condition could also be driven by other natural and human influences, such as the introduction of invasive species, which make it difficult, if not impossible to establish single cause-and-effect relationships.

While maintaining healthy, viable invertebrate communities in the Estuary is and should be an objective in and of itself, it can be argued that an even more important role for these invertebrate resources is as food for resident fish populations. Numerous studies have documented that virtually all of the priority fishery populations in the San Francisco Estuary rely upon these invertebrates, particularly during their vulnerable early life stages (Heubach et al. 1963; Eldridge et al. 1982; Schaffter et al. 1982; Brown 1992; Moyle et al. 1992; Meng and Moyle 1996). If pulses of pesticides through these aquatic ecosystems diminish the available invertebrate resources at critical periods, such as when fish fry are obligately using the invertebrates for food, then adverse effects on the fish populations may occur. This potential problem is of paramount importance as the period of high pesticide concentrations in these waters (January-June) coincides with the presence of early life stages of most of the fishery populations currently in decline. This includes delta smelt (*Hypomesus transpacificus*), chinook salmon (*Oncorhynchus tshawytscha*), longfin smelt (*Spirinchus thaleichthys*), splittail (*Pogonichthys macrolepidotus*), steelhead trout (*Oncorhynchus mykiss*), and green sturgeon (*Acipenser medirostris*), all of which have been identified as "Priority Species" by the CALFED Bay-Delta program.

Finally, it should be noted that the San Francisco Estuary serves as habitat for several State and Federal Threatened and Endangered species. Delta smelt, splittail, and the California freshwater shrimp are examples of threatened and/or endangered species that reside in these waters. The potential for ambient water toxicity to adversely affect the recovery of these species will be critically important information for the environmental scientists and managers responsible for the husbandry of these resources.

THE RMP EPISODIC TOXICITY PILOT STUDY: METHODS

Based upon the hypothesis that stormwater runoff and other surface water runoff events were the primary sources of toxicity in the Estuary's ambient waters, the RMP initiated a Pilot Study to investigate event-based episodic ambient water toxicity in greater detail. Beginning in the winter of 1996-1997, samples were collected at selected sites (Figure 5) to assess the potential for toxicity to enter the Estuary following stormwater runoff events.

Event Selection Rationale - In order to cover the temporal extent of potential sources of contaminant input (e.g., first flush [Oct-Dec], dormant spray runoff [Dec-Feb], row crop runoff [Mar-June], urban gardening [Apr-June]), stormwater runoff events were sampled during winter-spring season. The following factors were considered in deciding when to sample:

a. The magnitude of the storm event should produce significant runoff (i.e., >0.5 inches in a 24-hr period, using "real –time" data posted on the Internet;
b. Sampled storm events shall be separated temporally so as to allow for significant interim anthropogenic activities to take place in the watershed;
c. All stations need not necessarily be sampled during the same precipitation event;
d. Some sampling events shall follow upstream activities (e.g. dormant spraying) known to result in pesticide contamination of runoff.

Collection of Ambient Water Samples

Event-based Sampling at Guadalupe Slough, Pacheco Slough, and Napa River

Event-based sampling was conducted at Guadalupe Slough and Alviso Slough in South San Francisco Bay (1996-98), Pacheco Slough (1997-2000), and the Napa River (at JFK Landing, 1996-1997 and1998-2000) (Figure 5). The first two locations drain predominantly urbanized watersheds, while the Napa River drains urban regions and a large predominantly agricultural watershed. These sites were purposely located below head-of-tide to document toxicity in estuarine rather than fresh waters. Long-term sampling was conducted at Mallard Island, located

Figure 5. RMP Episodic Toxicity Study ambient water sampling stations.

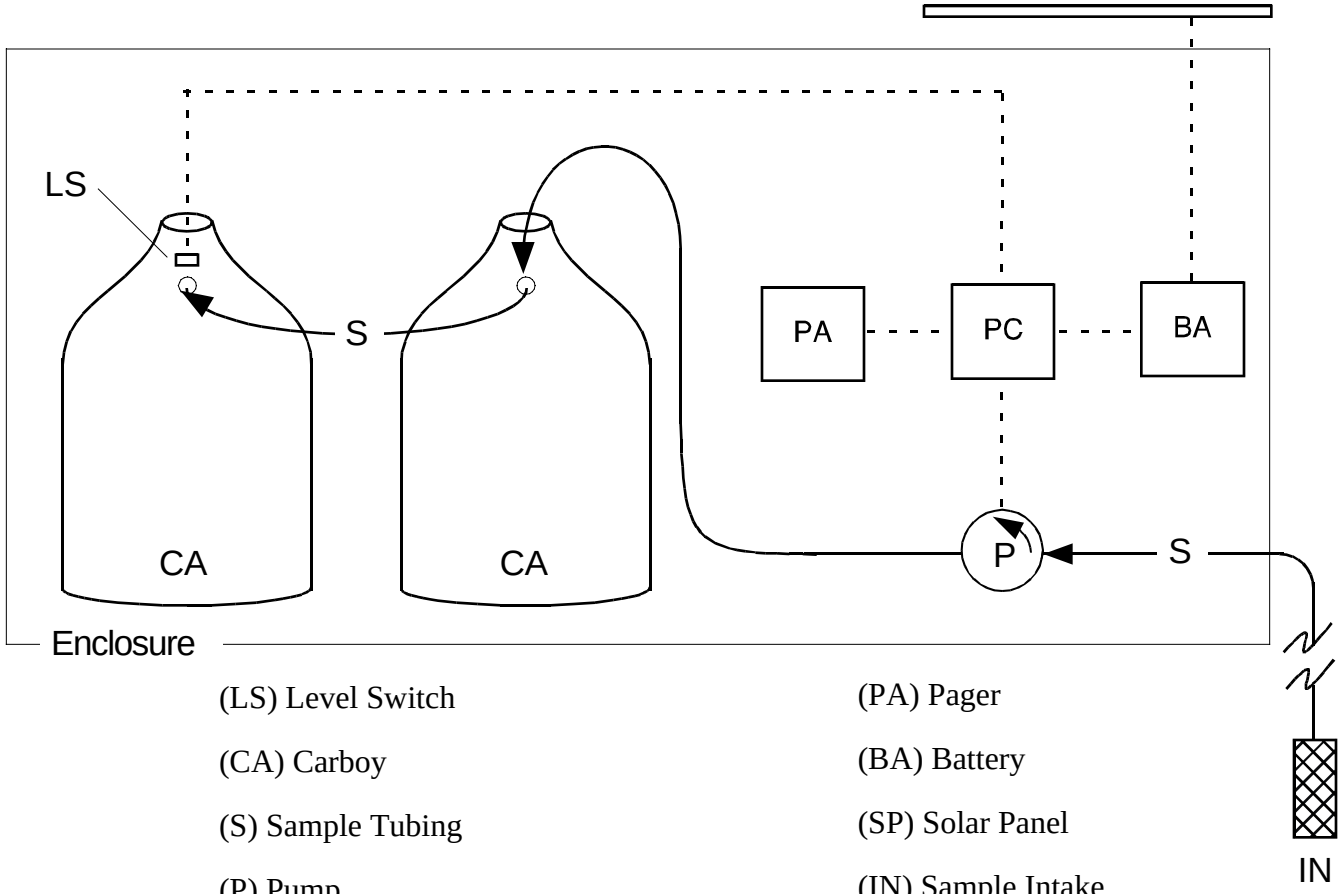
just downstream of the confluence of the Sacramento and San Joaquin Rivers (Figure 5) in order to characterize episodic toxicity resulting primarily from upstream activities.

During the first two years of the episodic toxicity study, ambient water samples were collected by manually placing pre-cleaned 4 L amber glass bottles approximately 10-15 cm below the water surface with the mouth of the bottle oriented upstream; the bottle cap was quickly removed and the bottle allowed to fill, after which it was retrieved and sealed. Approximately 24 L of ambient water (six 4-L bottles) was sampled for each event, with the samples being immediately transported, on ice, to the toxicity-testing laboratory.

As measurements of pesticide concentrations in stormwater runoff during these initial two years indicated variable concentrations over relatively short time scales, a time-composited sample was preferred. Since it was not practical to have trained staff standing-by in anticipation of "predicted" storm events, a collection system was required that could be activated remotely and left unattended for long periods of time. No automated sampling system was commercially available that would collect the large samples (40 L) required for both toxicity tests and follow-up Toxicity Identification/Evaluations (TIEs), and so an automatic sampler was custom-designed (Fig 6).

The sampler consists of a power supply, computer processor, pager, peristaltic pumping system, sample containers, and a weatherproof protective enclosure (Figure 6). The water samplers are powered either by AC line voltage or photovoltaic collectors, depending on power accessibility at the site. A phone call to the pager activated the computer processor that controls a pre-determined sample collection program (e.g., 500 mL collected every 15 minutes), including reverse-flow purging of unwanted water from the peristaltic pumping system prior to collection of any water. Sample tubing (pre-cleaned silicon) was placed below the mean lower low water line and the intake was screened to prevent fouling. Samples were collected into two interconnected 20 L HDPE carboys, which were retrieved and transferred to the laboratory within 24 hours of activating the sampler.

The automatic water sampler allowed remote initiation of sample collection (with controlled compositing over a period of several hours) at any time of day or night. Performance of the



(LS) Level Switch

(CA) Carboy

(S) Sample Tubing

(P) Pump

(PC) Programmable Computer

(PA) Pager

(BA) Battery

(SP) Solar Panel

(IN) Sample Intake

--- Electrical Wiring

Figure 6. Schematic diagram of remote-operated ambient water autosampler

samplers has been mixed. The sampler powered by AC line voltage has been moderately reliable. The sampler powered by photovoltaic has been less reliable, with problems associated with inadequate power supplied by the solar collectors. This problem has been corrected by increasing the size of the solar panels for 2000-2001 sampling year. When, during the sample collection immediately following a storm event, it was observed that the autosamplers failed to collect an acceptable sample, a grab sample was collected by manually-placing pre-cleaned 20 L HDPE containers approximately 10-15 cm below the water surface with the mouth of the container oriented upstream; the container cap was quickly removed and the carboy allowed to fill, after which it was retrieved and sealed. Two such containers (~40 L) of ambient water were sampled for each event, with the samples being immediately transported, on ice, to the testing laboratory.

Long-Term Sampling at Mallard Island

Event-based samples were also collected from the State of California's Department of Water Resources Monitoring Station at Mallard Island. In addition, regular sampling 2-3 times weekly from January-February through June was implemented annually in 1998. Water samples were collected through pre-cleaned silicon tubing via peristaltic pumping. When the peristaltic pumping system was unavailable or when the hydraulic head was too great for the peristaltic pump to overcome, samples were collected from the plumbed pipeline system that delivers ambient water directly into the sampling station lab. During the first two years of the Episodic Toxicity Study, the water samples were collected into pre-cleaned 4 L amber glass bottles, which were immediately transported, back to the testing lab; approximately 24 L of ambient water was collected at each sampling event. Subsequent year's samples were collected into pre-cleaned 12 or 23 L fluorocarbon-lined HDPE jerricans, which were similarly immediately transported to the testing lab.

Receipt and Handling of the Ambient Water Samples at the Testing Lab

Upon receipt of each water sample at the testing lab, the samples were logged in, and a small aliquot of each sample was collected and analyzed for determination of routine water quality characteristics (temperature, pH, D.O., salinity, and total ammonia). An additional 250 mL aliquot was transferred into a pre-cleaned Teflon bottle, which was stored at 4˚C for later

analyses of diazinon and chlorpyrifos via ELISA. The samples were then stored at 4˚C within a sample refrigerator until being used for preparation of toxicity testing test solutions.

Ambient Water Toxicity Testing

Toxicity Testing with Americamysis bahia **-** Each of the water samples was evaluated using the short-term chronic *A. bahia* testing approach, as per the US EPA testing guidelines (US EPA 1994), with survival as the test endpoint.

The Control treatment water for these tests consisted of reverse-osmosis de-ionized water to which a commercial artificial sea salt (Forty Fathoms®, bioassay grade) was added to bring the salinity up to 20 ppt. Similarly, each day of each test, an aliquot of the ambient water sample had the salinity adjusted up of 20 ppt using the same artificial sea salt. The toxicity of each salinity-adjusted ambient water was tested at the 100% concentration only. Routine "new" water quality characteristics (pH, D.O., and salinity) were measured for each freshly prepared test solution prior to use in this test.

There were eight replicates for each test treatment, each replicate consisting of 200 mL of water in a 400 mL glass beaker. The tests were initiated by the random allocation of five 7-day old mysids into each replicate. The test replicates were then placed within a temperature-controlled water bath at 26°C.

The mysids were fed brine shrimp nauplii, *ad libitum*, twice each day (once in the a.m. and once in the p.m.). Each day, fresh test solutions were prepared and water quality characteristics determined as before. The test replicates were then removed from the water bath and each was examined to determine the number of surviving organisms. Then, any dead animals, uneaten food, and waste material were removed while replacing approximately 80-90% of the test media within each replicate with fresh test solution, after which the test replicates were replaced into the water bath.

After seven days exposure, each replicate was examined and the final number of surviving organisms determined. The resulting percentage survival data for each ambient water sample was

compared to the corresponding Control treatment to determine whether any differences were statistically significant at the $p<0.05$ level; all statistical analyses were performed following the US EPA (1994) statistical flowchart, using ToxCalc® software (Version 5.0, TidePool Scientific, McKinleyville, CA).

Toxicity Testing with Menidia beryllina – During the 2000-2001 rainfall season, water samples from the Napa River and Pacheco Slough were also evaluated using the short-term chronic *Menidia* testing approach, also following the US EPA testing guidelines (US EPA 1994), with survival as the test endpoint.

The Control treatment water for these bioassays was prepared by salting up reverse-osmosis, de-ionized (RO-DI) water to a salinity of 20 ppt using a commercial artificial sea salt (Forty Fathoms®, bioassay grade). Similarly, each day of each test, an aliquot of the ambient water sample had the salinity adjusted up to 20 ppt using the same artificial sea salt. The toxicity of each salinity-adjusted ambient water was tested at the 100% concentration only. Routine "new" water quality characteristics (pH, D.O., and salinity) were measured for each freshly prepared test solution prior to use in this test.

There were four replicates at each treatment level, each replicate consisting of 400 mL of test media in a 600 mL glass beaker. Each test was initiated by randomly allocating ten larval (7-11 days old) *Menidia beryllina*, obtained from a commercial supplier, into each of the replicate beakers. The beakers were placed in a temperature-controlled water bath at 25°C under a 16L:8D photoperiod. The fish were fed freshly-hatched brine shrimp nauplii twice each day. Each day, each replicate container was examined, and the number of live fish was recorded. Then, approximately 80% of the old media in each replicate container was carefully drained and replaced with fresh media. Routine "old" water quality characteristics (pH, D.O., and salinity) were measured and recorded for the old test media from one randomly selected replicate per treatment each day.

After seven days exposure, the number of live fish in each replicate beaker was recorded. The resulting percentage survival data for each ambient water sample was compared to the corresponding Control treatment to determine whether any differences were statistically

significant at the $p<0.05$ level; all statistical analyses were performed following the US EPA (1994) statistical flowchart, using ToxCalc® software.

ELISA Analyses of Ambient Water Samples for Diazinon and Chlorpyrifos

Because earlier studies in upstream waters had indicated the OP pesticides diazinon and chlorpyrifos as suspected causes for much of the observed toxicity to *Ceriodaphnia*, ambient water samples collected following stormwater runoff events were analyzed for these two OP pesticides using Enzyme-linked immunosorbent assays (ELISA). The ELISA analyses were performed as per the instructions provided by the ELISA kit manufacturer (Strategic Diagnostics, Newark, DE).

The ELISA method is the same for diazinon and chlorpyrifos, although due to the specificity of the antibodies for individual compounds, separate analyses are performed for each compound. For example, one antibody will be specific for chlorpyrifos and will not 'react' with diazinon, while a different antibody (from a different kit) specific for only diazinon is used in the diazinon analysis. The method described below is for diazinon, and is the same as is performed for chlorpyrifos.

ELISA is based upon the use of polyclonal antibodies, which bind to specific substrates [(*e.g.*, either diazinon or chlorpyrifos (or a diazinon- or chlorpyrifos-enzyme conjugate)]. Antibodies specific for diazinon are immobilized onto the walls of the plate's test wells. An aliquot of the sample and a small amount of the diazinon-enzyme conjugate are added to each test well. Any diazinon in the water sample competes with the diazinon-enzyme conjugate for the antibody binding sites on the wall. The addition of an indicator-dye that reacts only with the enzyme conjugate causes the sample to change color. The resulting color formation is therefore inversely proportional to the diazinon concentration.

For each set of sample analyses, a set of QA/QC samples, including laboratory blanks, sample duplicates, and laboratory and sample matrix spikes were also analyzed. For this study, the reporting limits (RLs) were arbitrarily established as equal to the lowest standard concentration used in establishing the calibration curve. Prior to June of 2000, this corresponded to 30 ng/L for

diazinon and 60 ng/L for chlorpyrifos. Subsequent to June 2000, this corresponded to 62.5 ng/L for both compounds.

For the duplicate analyses, QA/QC performance is based on the relative percent difference (RPD), which must be $\leq$ 20%. Matrix values below the detection limits are considered to be unknown, since the actual concentration can fall somewhere between zero and the detection limit; therefore, for sample concentrations that are measured as 'below detection limits; QA/QC duplicate analysis cannot be accurately determined.

EPISODIC TOXICITY STUDY RESULTS

Year One (1996-97) Results

The unusual rainfall pattern during the winter of 1996-97 impacted the progress of this initial year of the study (see below). The results of the Year One study are summarized in Table 2 below.

Table 2a. Summary of RMP Episodic Toxicity Pilot Study, 1996-1997

Year One (1996-1997)	Guadalupe Slough Area	Napa River	Mallard Island
# Tests	16	2	4*
# Tests w/ Significant Toxicity	3 (19%)	0 (0%)	0* (0%)

* - Tests for sampling conducted in response to rainstorm events; other non-storm related sampling is not reported in this table.

Table 2b. RMP Episodic Toxicity Pilot Project, 1996-1997: Toxic samples.

Sample	% Mysid Survival		ELISA Analyses (ng/L)	
	Control	Site Water	Diazinon	Chlorpyrifos
Guadalupe Slough (10/29/96)	97.5	0	392	145
Guadalupe Slough (4/19/97)	95	0	<R.L.	78
Guadalupe Slough (5/23/97)	97.5	47.5	<R.L.	70

R.L. = reporting limit (30 ng/L for diazinon and 60 ng/L for chlorpyrifos).

Guadalupe Slough/Alviso Slough - Toxicity was observed in three out of the 16 Guadalupe Slough area samples. ELISA analyses of the OP pesticides diazinon and chlorpyrifos revealed that the concentrations of chlorpyrifos exceeded the reported acute LC50 of 35 ng/L for *A. bahia* for all three of the toxic samples (Table 2b); however, three other samples which also had measured chlorpyrifos levels exceeding the reported LC50 did not exhibit significant mysid mortalities, confounding the interpretation of the measured concentrations of chlorpyrifos as indications of chlorpyrifos as the probable cause of observed toxicity.

North Bay (Napa River & Mallard Island) - The heavy rains and major flooding in the Sacramento and San Joaquin River watersheds during the winter of 1996-97 disrupted the planned sampling at the Mallard Island station. This precluded the collection of samples that might have been impacted by upstream agricultural and urban activities (e.g., pesticide spraying) that might have otherwise taken place in a normal rainfall year. None of the Napa River or Mallard Island samples that were collected in Year One were toxic. All ELISA measurements of diazinon and chlorpyrifos were below the reporting limits.

However, ambient water collected from the northern San Francisco Bay sites during the January 1997 RMP cruise all exhibited significant toxicity (Figure 3), indicating that surface water runoff from the Sacramento and San Joaquin River watersheds was contributing significant toxicity to the ambient waters in the Bay on an episodic basis. Unfortunately, due to the unusual rainfall, no Mallard Island samples were collected at the same time as the cruise samples for comparative purposes.

Year Two (1997-98) Results

The mysid toxicity test results of the Year Two study, performed during the winter of 1997-98, are summarized in Tables 3a and 3b, below.

Guadalupe Slough - Two out of 14 samples collected at Guadalupe Slough resulted in significant toxicity (both with $\geq$50% mortality relative to the Control treatment). Of the 14 water samples collected, eight had elevated concentrations of diazinon and/or chlorpyrifos. However, only one of the toxic Guadalupe Slough water samples had measured diazinon or chlorpyrifos

concentrations exceeding the reported acute LC50 for *A. bahia*, suggesting that other contaminants were responsible for the observed toxicity.

Pacheco Slough - Five out of 13 samples collected at Pacheco Slough resulted in statistically significant mortality, although only one toxic sample exhibited greater than 50% mortality relative to the Control. Of the 13 water samples collected, 10 had measurable concentrations of diazinon and/or chlorpyrifos. However, only one of the water samples had measured diazinon or chlorpyrifos concentrations exceeding the reported acute LC50 for *A. bahia*, again suggesting that other contaminants were responsible for some of the observed toxicity.

Table 3a. Summary of RMP Episodic Toxicity Pilot Study, 1997-1998

Year Two (1997-1998)	Guadalupe Slough	Pacheco Slough	Mallard Island
# of Tests	14	13	70
# Tests w/ Significant Toxicity	2 (14%)	5 (38%)	10 (14%)

Table 3b. RMP Episodic Toxicity Pilot Project,1997-1998: Toxic samples.				
Sample	% Mysid Survival		ELISA Analyses (ng/L)	
	Control	Site Water	Diazinon	Chlorpyrifos
Guadalupe Slough (4/3/98)	100	30	345	50
Guadalupe Slough (5/12/98)	97.5	50	342	<R.L.
Pacheco Slough (12/7/97)	95	77.5	257	<R.L.
Pacheco Slough (1/4/98)	100	70	278	<R.L.
Pacheco Slough (3/24/98)	100	85	258	<R.L.
Pacheco Slough (5/4/98)	95	5	54	73
Pacheco Slough (5/13/98)	97.5	60	<R.L.	<R.L.
Mallard Island (11/20/97)	100	30	<R.L.	<R.L.
Mallard Island (1/5/98)	100	82.5	<R.L.	<R.L.
Mallard Island (1/30/98)	90	22.5	<R.L.	<R.L.
Mallard Island (2/12/98	97.5	50	114	<R.L.
Mallard Island (2/14/98)	100	40	<R.L.	<R.L.
Mallard Island (2/17/98)	95	80	<R.L.	<R.L.
Mallard Island (5/5/98)	95	17.5	<R.L.	69
Mallard Island (5/7/98)	97.5	22.5	<R.L.	57
Mallard Island (5/9/98)	95	35	<R.L.	<R.L.
Mallard Island (5/14/98)	97.5	50	<R.L.	<R.L.

R.L. = reporting limit (30 ng/L for diazinon and 60 ng/L for chlorpyrifos).

Mallard Island - As described above, ambient water samples were collected at Mallard Island:

(1) on an episodic basis, following significant storm events; and

(2) on a continuous basis, biweekly from January through May.

Of the 70 water samples collected, 10 resulted in significant mysid mortality (eight of which exhibited >50% mortality relative to the Control). More importantly, there were two periods of time, February 12-17 and May 5-9, during which three consecutive water samples were toxic (Figure 7a), suggesting that the ambient waters in North Bay were similarly toxic for at least two extended periods of time during this monitoring. In order to save costs, ELISA analysis were not performed on the 'routine' biweekly water samples collected from Mallard Island (it is expected that the greatest likelihood of elevated pesticide concentrations in these ambient waters will be during stormwater runoff events, and therefore, diazinon and chlorpyrifos were measured in the Mallard Island water samples only following significant rainstorms and at the same time that Guadalupe Slough and Pacheco Slough water samples were being analyzed). Surprisingly, only

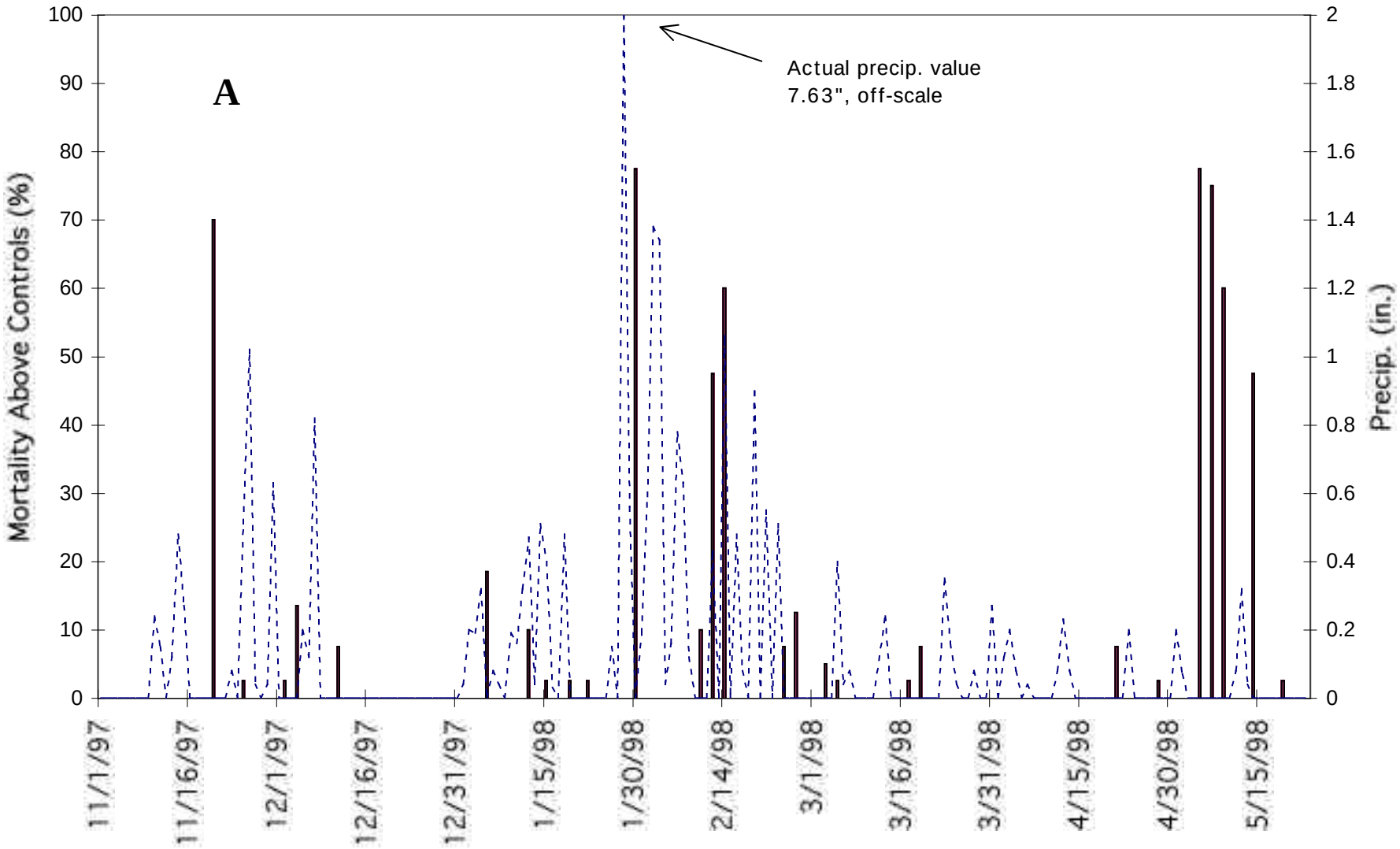
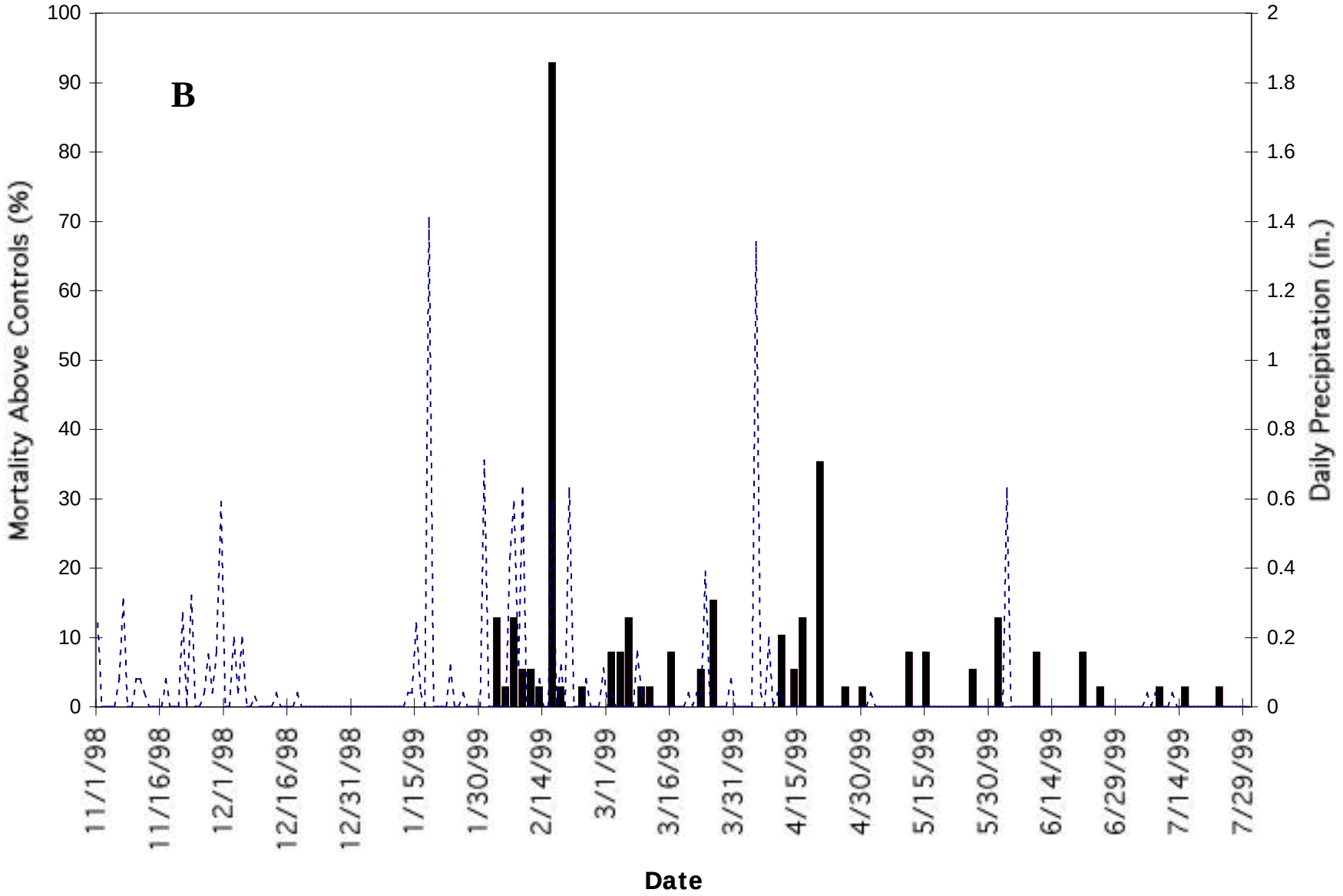
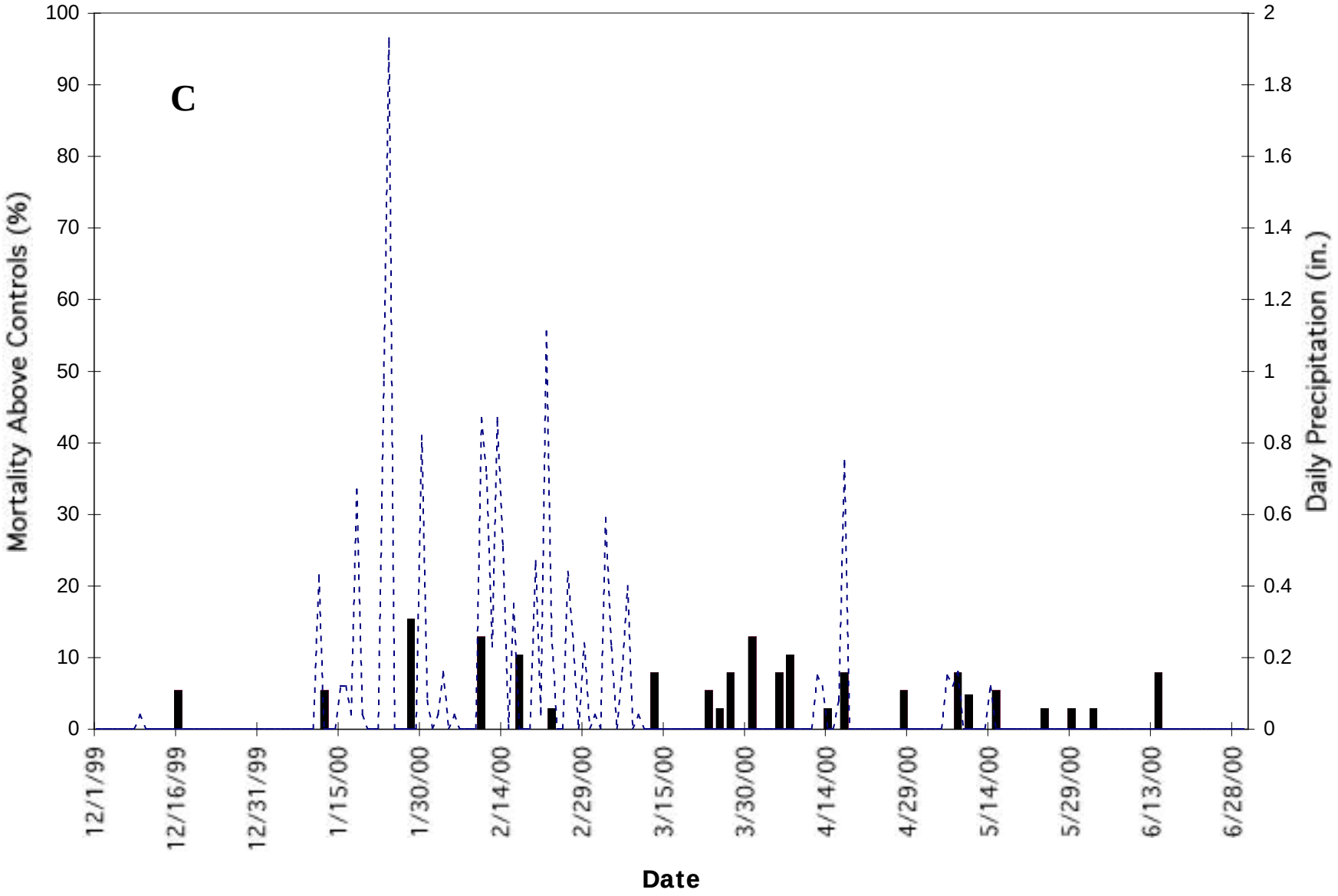
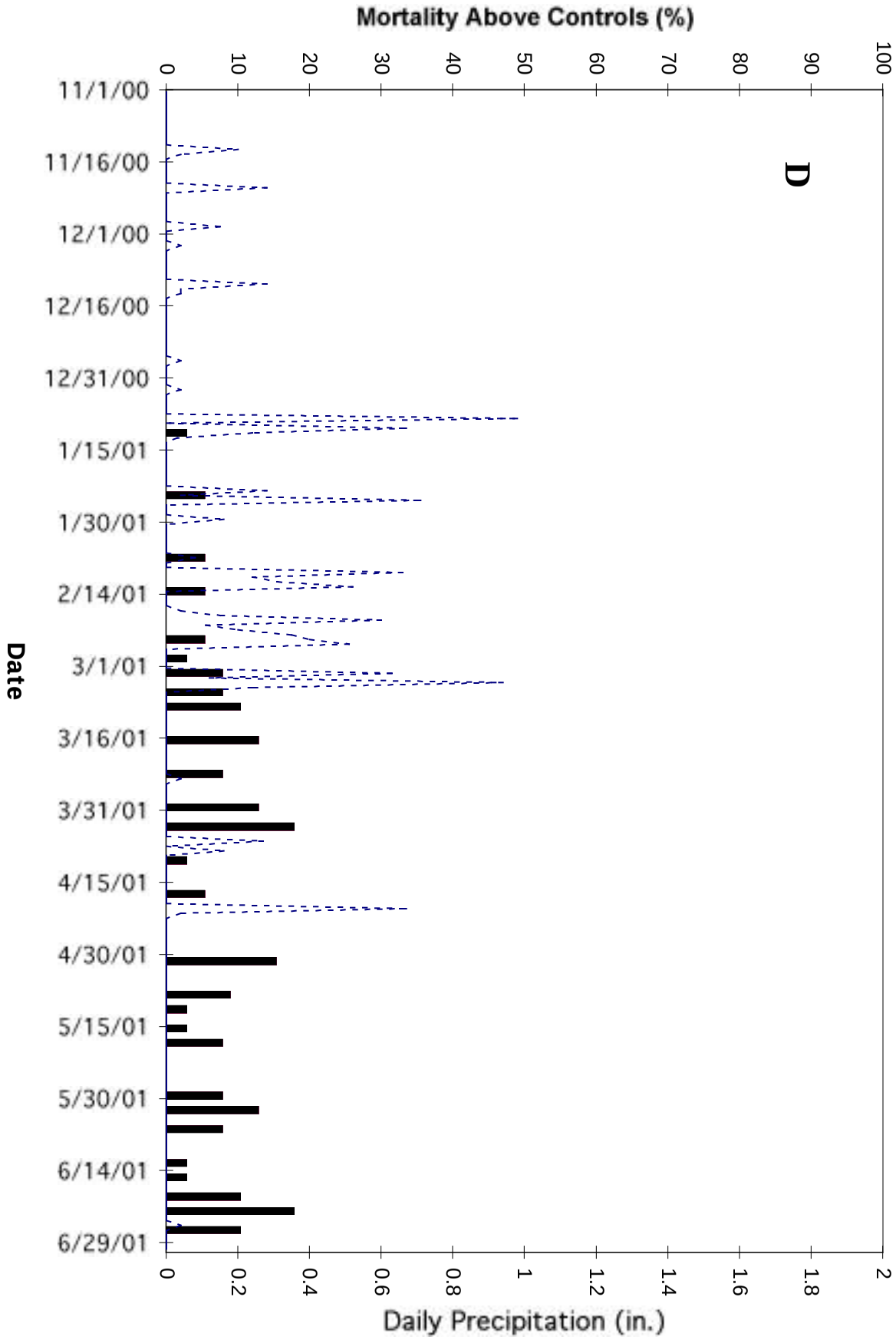


Figure 7. Toxicity of ambient Mallard Island water samples: (a) 1997-1998; (b) 1998-1999; (c) 1999-2000; (d) 2000-2001.


B
Mortality Above Controls (%)
Daily Precipitation (in.)
Date
100
90
80
70
60
50
40
30
20
10
0
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
11/1/98
11/16/98
12/1/98
12/16/98
12/31/98
1/15/99
1/30/99
2/14/99
3/1/99
3/16/99
3/31/99
4/15/99
4/30/99
5/15/99
5/30/99
6/14/99
6/29/99
7/14/99
7/29/99


C
Mortality Above Controls (%)
Daily Precipitation (in.)
Date
100
90
80
70
60
50
40
30
20
10
0
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
12/1/99
12/16/99
12/31/99
1/15/00
1/30/00
2/14/00
2/29/00
3/15/00
3/30/00
4/14/00
4/29/00
5/14/00
5/29/00
6/13/00
6/28/00


Mortality Above Controls (%)
0
10
20
30
40
50
60
70
80
90
100
D
Date
11/1/00
11/16/00
12/1/00
12/16/00
12/31/00
1/15/01
1/30/01
2/14/01
3/1/01
3/16/01
3/31/01
4/15/01
4/30/01
5/15/01
5/30/01
6/14/01
6/29/01
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
Daily Precipitation (in.)

two of the toxic water samples from Mallard Island had diazinon or chlorpyrifos concentrations that exceeded the reported LC50; in six of the toxic water samples, including two of the three consecutively toxic samples in February, both diazinon and chlorpyrifos were below the ELISA reporting limits, suggesting that other contaminants were responsible for the observed toxicity.

Year Three (1998-99) Results

The results of the Year Three study, performed during the winter of 1998-99, are summarized in Tables 4a and 4b, below.

Table 4a. Summary of RMP Episodic Toxicity Pilot Study, 1998-1999

Year Three (1998-1999)	Napa River	Pacheco Slough	Mallard Island
# of Tests	10	11	61
# Tests w/ Significant Toxicity	2 (20%)	3 (27%)	3 (5%)

Table 4b. RMP Episodic Toxicity Pilot Project, 1998-1999: Toxic samples.

Sample	% Mysid Survival		ELISA Analyses (ng/L)	
	Control	Site Water	Diazinon	Chlorpyrifos
Napa River (3/9/99)	92.5	62.5*	52	<R.L.
Napa River (5/3/99)	95	0*	<R.L.	<R.L.
Pacheco Slough (2/7/99)	90	0*	295	<R.L.
Pacheco Slough (4/5/99)	97.5	85*	<R.L.	<R.L.
Pacheco Slough (5/3/99)	95	67.5*	<R.L.	<R.L.
Mallard Island (2/16/99)	95	2.5*	<R.L.	<R.L.
Mallard Island (3/26/99)	100	85*	<R.L.	<R.L.
Mallard Island (6/1/99)	97.5	85*	<R.L.	<R.L.

R.L. = reporting limit (30 ng/L for diazinon and 60 ng/L for chlorpyrifos).

Napa River - Two of the 10 Napa River samples were toxic to *A. bahia*. The toxic sample collected on 5/7/99 resulted in 100% mysid mortality within 48 hrs; a re-test of that same sample with new test organisms resulted in complete mortality within 24 hrs. Both of these samples had measured concentrations of diazinon and chlorpyrifos that were either below the reported LC50 value or which were below the reporting limits, suggesting that other contaminants were responsible for the observed toxicity.

Pacheco Slough - Three of the 11 samples collected from Pacheco Slough resulted in significant mysid mortalities. All three of these samples had measured concentrations of diazinon and chlorpyrifos which were either below the reported LC50 value or which were below the reporting limits.

Mallard Island - Only three of the 61 water samples collected through the end of June at Mallard Island resulted in significant reductions in *A. bahia* survival. For all three samples, the measured concentrations of diazinon and chlorpyrifos were either below the reporting limits. Unlike the previous year, there were no sets of consecutively toxic samples indicative of an extended duration of ambient water toxicity (Figure 7b).

Year Four (1999-2000) Results

The results of the Year Three study, performed during the winter of 1999-2000, are summarized in Table 5a below.

Table 5a. Summary of RMP Episodic Toxicity Pilot Study, 1999-2000

Year Four (1999-2000)	Napa River	Pacheco Slough	Mallard Island
# Tests	11	12	56
# Tests w/ Significant Toxicity	2 (18%)	3 (25%)	2 (4%)

Table 5b. RMP Episodic Toxicity Testing Pilot Project,1999-2000: Toxic samples.

Sample	% Mysid Survival		ELISA Analyses (ng/L)	
	Control	Site Water	Diazinon	Chlorpyrifos
Napa River (4/17/00)	97.5	87.5	<R.L.	<R.L.
Napa River (5/7/00)	97.5	17.5	46	63
Pacheco Slough (11/8/99)	92.5	67.5	540	<R.L.
Pacheco Slough (1/18/00)	97.5	80	135	85
Pacheco Slough (5/7/00)	97.5	87.5	<R.L.	<R.L.
Mallard Island (1/28/00)	92.5	77.5	73	63
Mallard Island (2/10/00)	100	87.5	<R.L.	<R.L.

R.L. = reporting limit (30 ng/L for diazinon and 60 ng/L for chlorpyrifos).

Napa River - Two of the 11 Napa River samples were toxic. However, only one of these samples exhibited measured diazinon and/or chlorpyrifos concentrations exceeding the reported LC50; the concentrations of these OPs were below the reporting limits in the remaining toxic sample.

Pacheco Slough - Three out of the 12 Pacheco Slough samples were toxic, with one of these samples having measured chlorpyrifos concentrations that exceeded the reported LC50. The remaining samples had diazinon and chlorpyrifos concentrations which were either below the reported LC50 values and/or which were below the reporting limits.

Mallard Island - Only two of the 56 Mallard Island samples were toxic; one of these samples exhibited measured chlorpyrifos concentrations exceeding the reported LC50. The remaining sample had diazinon and chlorpyrifos concentrations which were either below the reported LC50 values and/or which were below the reporting limits. There were no sets of consecutively toxic samples indicative of an extended duration of ambient water toxicity (Figure 7c).

Year Five (2000-2001) Results

The results of the Year Five study, performed during the winter of 2000-2001, are summarized in Tables 6a & 6b below.

Table 6a. Summary of RMP Episodic Toxicity Pilot Study, 2000-2001

Year Five (2000-2001)	Napa River	Pacheco Slough	Mallard Island
Americamysis bahia tests			
# of Tests	14	12	53
# Tests w/ Significant Toxicity	0	1	3
Menidia beryllina tests			
# of Tests	14	9	-
# Tests w/ Significant Toxicity	0	0	-

Table 6b. RMP Episodic Toxicity Testing Pilot Project, 2000-2001: Toxic samples.						
Sample ID	% Mysid Survival		% *Menidia* Survival		ELISA Analyses (ng/L)	
	Control	Site Water	Control	Site Water	Diazinon	Chlorpyrifos
Pacheco Slough (4/6/01)	90	75 *	100	100	<R.L.	<R.L.
Mallard Island (3/16/01)	97.5	85 *	-	-	-	-
Mallard Island (4/3/01)	97.5	80 *	-	-	<R.L.	<R.L.
Mallard Island (6/1/01)	97.5	85 *	-	-	-	<R.L.

* - Significantly less than the Control treatment response at $p < 0.05$.
R.L. = reporting limit (62.5 ng/L for both diazinon and chlorpyrifos).

Napa River - None of the 14 Napa River samples collected between October 2000 through June 2001 were toxic to *A. bahia*.

In a separate study, "non-stormwater" ambient water samples were collected weekly from the Napa River and were tested for toxicity to *A. bahia* and *Menidia*. A number of these samples were highly toxic to *Menidia*, causing complete mortality in as short as 24 hrs (Table 7). In other samples from this study, sub-lethal toxicity to both fish and mysids was observed.
A very limited preliminary Toxicity Identification Evaluation (TIE) performed on one of these toxic Napa River water samples suggested that toxicity was associated with the suspended particulates (Table 8), presumably the result of contaminants associated with the particulates. However, the actual cause(s) of this extreme toxicity is currently unknown.

In an independent study, testing of Napa River water samples collected following a rain event in February 2001 similarly indicated that suspended particulates collected from Napa River water were toxic to a larval aquatic insect (*Chironomus tentans*), and that apparent desorption of the toxicant from the suspended particulates in 48-hr elutriates resulted in chronic toxicity to *Ceriodaphnia dubia* (Gunnarson et al. 2001).

Table 7. Ambient water toxicity testing: Napa River				
Sample Date	*Mysidopsis bahia* % Survival		*Menidia beryllina* % Survival	
	Control Water	Napa River	Control	Napa River
9-2-99	95	92.5	100	12.5*
re-test			100	0*[a]
9-17-99	100	100	100	0*[a]
Re-test #1			97.5	42.4*[b]
Re-test #2			100	0*[a]
Re-test #3			100	0*[a]
8-23-00	75	82.5	100	0[b]
re-test			100	0[b]

* - Significantly less than the Control at $p < 0.05$.
a – complete mortality within 48 hrs.
b – Test terminated after 5 days to conserve water sample for TIE.

Table 8. TIE evaluation of Napa River Water toxicity to *Menidia beryllina*.		
TIE Treatment	% Survival	Toxicity Removal?
Control	100	
Baseline (Untreated Sample)	0	
Aeration	0	No
EDTA	0	No
STS	0	No
Filtration	90	Yes
Centrifugation	100	Yes
Centrifugation + C_{18}SPE	100	Yes
Conclusion: Toxicity associated with suspended particulates.		

Pacheco Slough - Three out of the 12 Pacheco Slough samples were toxic, with one of these samples having chlorpyrifos concentrations that exceeded the reported LC50 (the cause of toxicity in the remaining two samples is unknown).

Mallard Island - Only two of the 56 Mallard Island samples were toxic; as with the other stations, only one of these samples exhibited diazinon and/or chlorpyrifos concentrations exceeding the LC50 (the cause of toxicity in the remaining sample is unknown). There were no sets of consecutively toxic samples indicative of an extended duration of ambient water toxicity (Figure 7d).

DISCUSSION

Episodic monitoring for aquatic toxicity has demonstrated that ambient water toxicity occurs in the San Francisco Estuary on the timescale of days. These toxic events, which appear to be caused by agricultural and urban runoff following rainstorms, or from other surface water releases following application of pesticides in agricultural areas, could easily go undetected in monitoring programs that sample ambient waters according to a fixed schedule.

Potential Causes of Observed Ambient Water Toxicity

During the first several years of episodic monitoring (1996-98), much of the toxicity that was observed could be attributed to the measured concentrations of the OP pesticides diazinon and chlorpyrifos, which is consistent with similar monitoring upstream in the watershed: ambient water toxicity in the Sacramento and San Joaquin River watersheds has been most commonly linked to diazinon and chlorpyrifos (Foe and Connor 1991; Foe 1995; Werner et al. 2000; Bailey et al. 2000; deVlaming et al. 2000). It has been demonstrated that 'pulses' of these pesticides from the Sacramento and San Joaquin River watersheds enter northern San Francisco Bay (Kuivila and Foe 1995) (Figure 4). In the current study, these pesticides were measured at concentrations that exceed the reported acute LC50's in some toxic ambient water samples, suggesting that OP pesticides may have caused some of the ambient water toxicity that we observed. Similar episodic toxicity with links to OP pesticides has also recently been reported for the Pajaro River Estuary in Central California (Hunt et al. 1999).

However, the results of this study also suggest that the cause(s) of the observed toxicity is more complex. Some ambient water samples with measured chlorpyrifos concentrations at, or exceeding, the reported acute LC50 did not result in significant *Americamysis bahia* mortalities. This may be a function of bioavailability, such that under certain conditions, the ELISA method measures some chlorpyrifos that otherwise would not be bioavailable (e.g., the ELISA antibodies may 'scavenge' chlorpyrifos molecules which are sorbed to particulates or dissolved ligands). More importantly, there have been several toxic ambient water samples, including all of the toxic samples collected in the 1998-1999 monitoring and most of the toxic 1999-2000 samples, in which the measured diazinon and chlorpyrifos concentrations were either well below toxic

thresholds or were below the reporting limits of the ELISA method. While chlorpyrifos could potentially cause toxicity at measured concentrations <RL (i.e., >LC50 of 35 ng/L, but still below 60-62.5 ng/L), this suggests that the OP pesticides may be causing toxicity due to an additive effect (as shown in laboratory testing by Bailey et al. [1997]), or that other contaminants are responsible or may be playing a contributory role.

For instance, recent studies have reported that low levels (i.e., several orders of magnitude below their acute toxicity concentrations) of triazine herbicides (e.g. atrazine) can synergistically potentiate the toxicity of diazinon and chlorpyrifos (Beldon and Lydy 2000; Jin-Clark et al. 2002). With the application of between 38,000 to 60,000 lbs, annually, of atrazine in California during 1991-1998 (Kegley et al. 2001), such interactions and potentiation of diazinon and/or chlorpyrifos toxicity may be occurring. Furthermore, a recent study of several urban streams across the United States reported that atrazine, diazinon, and chlorpyrifos were among the 11 most commonly occurring pesticides in urban runoff (Hoffman et al. 2000), suggesting that such toxic interactions may be a widespread occurrence.

Ambient Water Toxicity, as Measured by *A. bahia*, Appears to be Declining

Despite such potential toxic interactions, relative to the 1997-98 results, the 1998-2001 monitoring results suggest a reduction in the frequency and magnitude of toxicity. Only 27% of the Pacheco Slough samples were significantly toxic in 1998-99, relative to 38% toxicity frequency observed in 1997-98. This difference was even more pronounced for the ambient water samples collected at Mallard Island, with only 4-5% of the samples being toxic in 1998-99 and 1999-2000, relative to 14% toxicity frequency observed in 1997-98. In addition, the 1998-2001 monitoring at Mallard Island did not document any sets of consecutively toxic samples indicative of an extended period of ambient water toxicity, such as were observed in February and May of 1998 (Figure 7a). Moreover, the magnitude of toxicity (as reflected by the degree [or percentage] of test organism mortality) is also markedly reduced in the later years (Figure 7b, 7c, 7d), again suggesting a reduction in the degree of ambient water toxicity.

The California State Water Resources Control Board and the California Department of Pesticide Regulation (DPR) have developed a Water Quality Control Plan to control ambient water toxicity caused by OP pesticides. The first action taken pursuant to the plan was to ask pesticides

users (e.g., growers) to voluntarily take measures to reduce contamination of ambient water by OP pesticides (Bennett et al. 1998). Recent pesticide use data suggest that this action has successfully reduced the amount of OP pesticides applied in recent years (Epstein et al. 2000). More recently (June and December of 2000, respectively), the EPA announced a ban on the use of chlorpyrifos for most residential applications and some agricultural applications, and a phase-out of diazinon for residential applications and ban on application to several agricultural crops. Although there is limited recent ambient surface water pesticide chemistry data available, these suggest that the reduced use of OP pesticides is translating into reduced concentrations in the estuary. Analyses of ambient waters at Vernalis (where the San Joaquin River enters the Delta) following stormwater runoff events during the dormant spraying season detected OP pesticides at concentrations approximately an order of magnitude lower in 1998 than the concentrations that were measured in 1992-94 (Kathryn Kuivila, USGS, personal communication).

Pesticide runoff into ambient waters, particularly the dormant sprays applied from mid-December through mid-March (Epstein et al. 2000), will be, in part, a function of rainfall. In order for such runoff to occur, it must be dry enough during the dormant spray season for farmers to move their equipment through their fields, but with intermittent rainfall of enough magnitude to effectively wash off surficial pesticide residues into adjacent ambient waters. Comparison of rainfall vs. observed toxicity during the winter of 1997-98 (Figure 7a) indicates several intermittent storms of relatively significant magnitude coinciding with significant toxicity that followed within a few days to a week (the time it would typically take for the pesticide runoff to flow downstream through the watershed and reach the Mallard Island sampling station).

Other incidences of significant toxicity, particularly later in the spring, appear to be independent of rainfall. One notable example is the significant toxicity observed during the period of April 5-14 in 1998 (Figure 7a). The April 5th & 7th Mallard Island water samples both had measured concentrations of chlorpyrifos that exceeded the reported 96-hr LC50 for *A. bahia* (Table 10). This is typically a time of the year during which chlorpyrifos is being applied to alfalfa crops, particularly on acreage within the Delta, with periodic release of irrigation tailwater into adjacent ambient waters. Such non-stormwater events may also be contributing significant toxicity to the Estuary's waters, although as with dormant spraying, there may be a concomitant decrease in OP pesticide applications on these other crops as well.

The State's water quality control plan appears to be achieving some degree of success, with reduction in the application of the OP pesticides (Figure 8), and a concomitant reduction in the ambient water toxicity that we have observed. This is particularly evident in the 1999-2000 and 2000-2001 monitoring during which several stormwater events were not accompanied by significant ambient water toxicity.

However, this decrease in OP pesticide use has been mirrored by increases in the application of other pesticides, e.g., pyrethroid pesticides (Figure 8), in the San Francisco Estuary watershed, both in agricultural use (Epstein et al. 2000), and in urban use (Moran 2002). This change in pesticide use may have important implications for ambient water toxicity in the San Francisco Estuary.

Whereas the OP pesticides diazinon and chlorpyrifos are very toxic to arthropods, particularly the crustaceans, they are generally much less toxic to fish (Novartis 1997; Giesy et al. 1997). A comparison of LC/EC50 data for diazinon toxicity to aquatic organisms indicates a ratio value of 165 for the 10^{th} percentile LC/EC50 values for fish relative to arthropods (Table 9); a similar comparison for chlorpyrifos reveals a ratio value of 97.4 for freshwater vertebrates relative to freshwater arthropods and 55.5 for saltwater fish relative to saltwater vertebrates (Table 9).

The pyrethroid pesticides are also very toxic to arthropods (again, particularly the crustaceans), however, the difference in toxicity to fish vs. toxicity to arthropod invertebrates is considerably less (Table 9). For cypermethrin, comparison of LC/EC50 data for aquatic organisms indicates a ratio value of 59.4 for the 10^{th} percentile LC/EC50 values for fish relative to arthropods. Similar comparisons yield ratio values of 21 for permethrin and 18.8 for fenvalerate (Table 9).

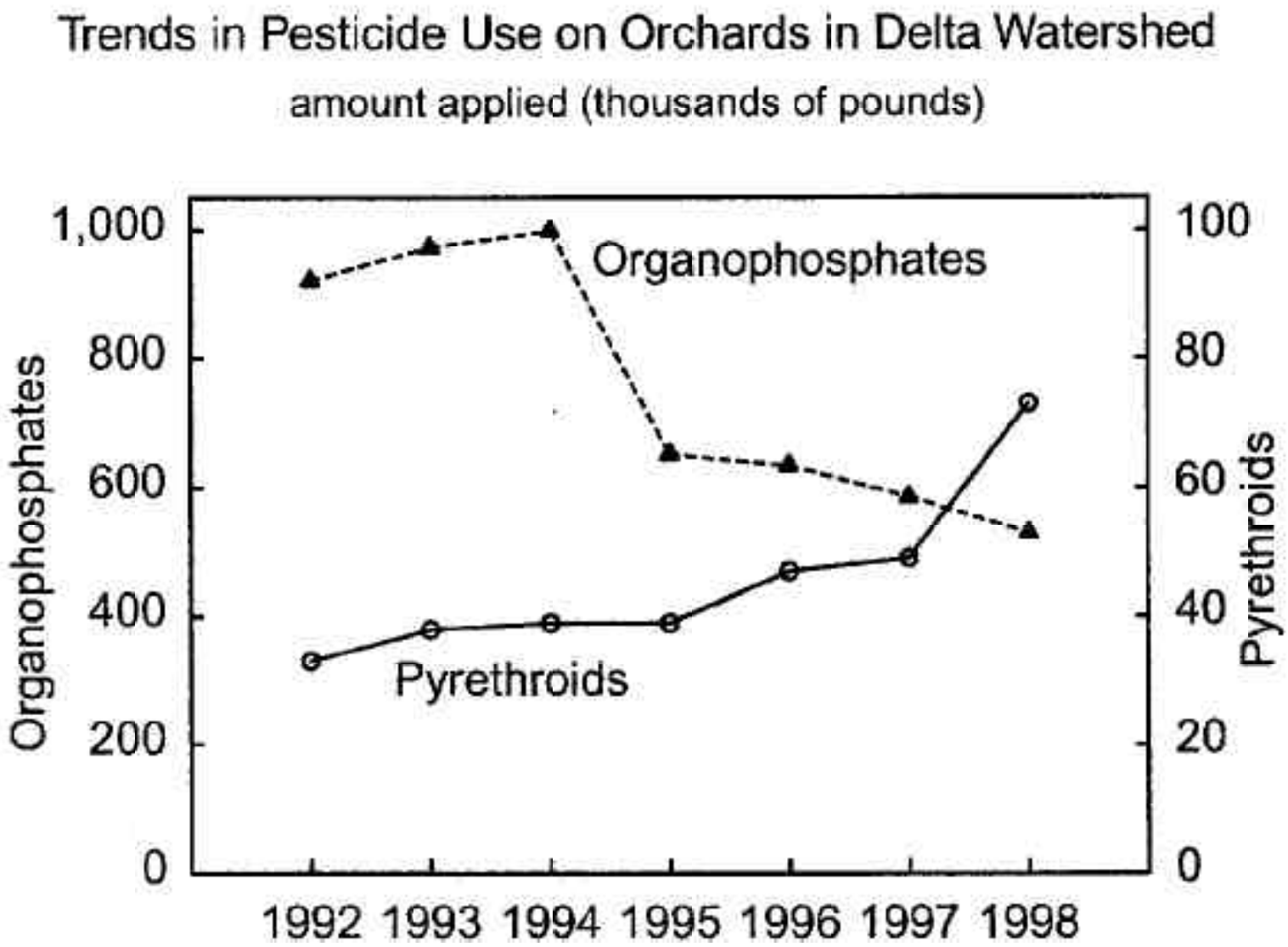


Figure 8. Recent trends in the usage of organophosphate (OP) pesticides and pyrethroid pesticide in the Delta (graph provided courtesy of K. Kuivila, U.S. Geological Survey, Sacramento, CA).

Table 9. Relative toxicity of the OP pesticides and pyrethroid pesticides to arthropods and fish.					
Pesticide	Taxa (aquatic organisms)	10^{th} percentile of LC/EC50 values (ng/L)	r^2	N	Ratio of 10^{th} percentile for fish vs. arthropods
Diazinon[a]	arthropods	483	0.96	23	165
	fish	79,900	0.98	29	
Chlorpyrifos[b]	FW arthropods	55	0.96	60	97.4
	FW vertebrates	5,358	0.94	19	
	SW arthropods	15	0.94	9	55.5
	SW vertebrates	832	0.92	11	
Cypermethrin[c]	Arthropods	6.4	0.98	42	59.4
	Vertebrates	380	0.83	17	
Permethrin[c]	Arthropods	76	0.96	36	21.0
	Vertebrates	1,600	0.88	24	
Fenvalerate[c]	Arthropods	8	0.90	16	18.8
	Fish	150	0.87	20	

FW – freshwater; SW – saltwater; a. Novartis (1997); b. Giesy et al. (1997); c. Solomon et al. (2001).

This difference was evident in a recent investigation performed in the Central Valley comparing the toxicity of the OP pesticide diazinon to the pyrethroid pesticide esfenvalerate in orchard runoff (Werner et al. 2002). In this study, the pesticides were applied in normal fashion to sections of an orchard, and samples of surface water runoff were collected from within the orchard following a rainstorm that occurred two days after application. The diazinon-contaminated samples were much more toxic to *Ceriodaphnia* than were the esfenvalerate-contaminated samples (400-800 Toxic Units [= 100/LOEC] for diazinon, relative to 10-20 Toxic Units for esfenvalerate). However, the reverse was true for toxicity to fathead minnows: for diazinon-contaminated water, there was <5-26% mortality within 96 hrs, whereas 96-hr mortality for fathead minnows ranged from 93-100% for the esfenvalerate-contaminated waters.

It should be noted that the Werner et al. study represents a "worst-case" scenario in which the water samples were collected directly from within the orchards. Before such water reaches the San Francisco Bay system, it will have come into contact with both surface soils and suspended particulates. While some sorption of OP pesticides to particulates and other materials can be

expected, significant concentrations can remain dissolved in the water, whereas pyrethroid pesticides tend to strongly sorb to sediment particulates with a relatively long half-life in sediments (Schimmel et al. 1983; Muir et al. 1985). It may well be that the type of "ambient" toxicity that might result from the pyrethroids differs from that due to the OPs. Increases in the frequency and magnitude of particulate-associated (e.g., sediments) toxicity might be expected consequences of increased use of pyrethroid pesticides. Given our reliance upon testing water as the primary exposure medium, we may not be effectively monitoring pyrethroid-related episodic toxicity. Assessment of toxicity associated with suspended particulates may help to better address any adverse ecological impacts associated with the increased use of pyrethroids (or other strongly-sorbing contaminants).

Potential impacts on Resident Aquatic Organisms

Long-term studies have reported significant declines in zooplankton abundance in the estuary, with recent zooplankton densities being 1-2 orders of magnitude lower than in the early 1970s (Obrebski et al. 1992). Use of pesticides has increased substantially over the last several decades, suggesting a possible link between pesticide toxicity and zooplankton declines. Numerous studies have indicated that laboratory toxicity tests such as the EPA 7-day *A. bahia* test that we employed are accurate predictors of adverse effects on the resident aquatic organisms community (reviewed in deVlaming and Norberg-King 1999).

The reported diazinon LC50 values for *A. bahia* and *Neomysis mercedis*, an important invertebrate resident to the Estuary, are remarkably similar (Table 10), which suggests that testing with *A. bahia* may be a satisfactory indicator of toxicity to resident invertebrate species. However, at least one important resident invertebrate, the crustacean *Palaemon macrodactylus*, is reported to be much more sensitive to OP pesticides than is *A. bahia* (Table 10). Such comparative toxicity information is lacking for most of the resident invertebrates, and it is quite possible that one or more of the resident invertebrate populations are experiencing severe toxic impacts as a result of episodic input of one or more contaminants in surface water runoff from urban and/or agricultural areas.

Table 10. Comparative toxicity of diazinon and chlorpyrifos to selected invertebrates.		
Species	Diazinon LC50 (ng/L)	Chlorpyrifos LC50 (ng/L)
Neomysis mercedis	4,100 [a,b]	140 [d]
Americamysis bahia	4,500 [c]	35 [e]
Palaemon macrodactylus	-	10 [f]

a. CDGF 1992a; b. CDFG 1992c; c. Nimmo et al. 1981; d. CDFG 1993; e. Schimmel et al. 1983; f. US EPA 1986.

This is particularly important as there is at least one State and Federal Endangered invertebrate, the California freshwater shrimp (*Syncaris pacifica*), that uses the surface waters in the Napa River watershed as habitat. Our observations of Napa River ambient water toxicity to *A. bahia* are an alarming "red flag" that such toxicity may well be adversely affecting the taxonomically-related California shrimp.

It can also be argued that if contaminant impacts on resident invertebrate populations are occurring, then impacts on fish may be taking place as a result of reduced food resources. Numerous studies have documented that virtually all of the priority fishery populations in the San Francisco Estuary rely upon these invertebrates, particularly during their vulnerable early life stages (Heubach et al. 1963; Eldridge et al. 1982; Schaffter et al. 1982; Brown 1992; Moyle et al. 1992; Meng and Moyle 1996). If pulses of pesticides through the Estuary's ecosystems diminish the available invertebrate resources at critical periods, such as when fish fry are obligately using the invertebrates for food, then adverse effects on the fish populations can be expected. This potential problem is of paramount importance as the period of high pesticide concentrations in these waters (January-June) coincides with the presence of early life stages of most of the fishery populations currently in decline (Moon et al., 2000). This includes delta smelt, chinook salmon, longfin smelt, splittail, steelhead trout, and green sturgeon, all of which have been identified as "Priority Species" of special concern within the San Francisco Estuary.

Furthermore, episodic ambient water toxicity may directly impact fish. "Non-stormwater" ambient water samples collected from the Napa River at Cuttings Wharf (Figure 5) as part of the monitoring effort in 2001, as from another independent study, were shown to cause complete mortality to larval *Menidia beryllina* (Table 7), whereas these same water samples had no effect

on *A. bahia*. These test results illustrate the significant differences in toxicant sensitivity of different organisms, and again, indicates the possibility that samples that were not toxic to *A. bahia* in the current study may well have been toxic to other organisms, including fish. As with the California freshwater shrimp, the San Francisco Estuary serves as habitat for several State and Federal Threatened and Endangered fish species, such as Delta smelt and splittail. The potential for ambient water toxicity to adversely affect the recovery of these species will be critically important information for the environmental scientists and managers responsible for the husbandry of these resources.

The Need for Adaptive Strategies in Toxicity Monitoring Programs

Prior to this study, the RMP had monitored for toxicity in the San Francisco Estuary using periodic testing at set sampling sites and set sampling dates. This periodic monitoring did not document significant toxicity in the estuary. The results of this project document that adapting the conceptual model underlying the monitoring program, and the consequent modification of sampling protocols, can greatly improve the usefulness of the program to water quality managers. Similarly, adaptive changes in the type of toxicity tests utilized can also change the results of the program, as documented by the shift in the San Francisco Estuary from the use of the algal growth bioassay to the *A. bahia* test (Thompson *et al*., 2000).

Ambient surface water toxicity monitoring programs should be aware of changes in landscape activities (e.g., pesticide use) in the watersheds being studied, and must adapt the monitoring tools (e.g., sampling design, toxicity tests, and chemical analyses) to reflect those changes. For example, knowing that diazinon and chlorpyrifos had been linked to ambient water toxicity in upstream waters, and that OP pesticides can remain dissolved in the water, are very toxic to crustaceans, and are relatively non-toxic to fish, we believe that the currently used approach of ambient water sampling and toxicity testing with *A. bahia* is an appropriate monitoring approach. However, the fate and effects of the pyrethroid pesticides are different than the OP pesticides. This suggests that transitions in pesticide use (or use of other chemicals) in the Estuary watershed may need to be reflected in changes in the way we monitor for ambient toxicity. The current water sampling approaches, currently recommended suite of chemical analytes, and toxicity testing with *Ceriodaphnia* and mysids may not be the optimal approach for assessment of the effects of “new” contaminants, such as the pyrethroids, on the San Francisco Estuary

aquatic ecosystems. New approaches, such as assessment of the toxicity associated with suspended particulates and freshly-deposited surficial sediments may be more appropriate for the current- and emerging-use pesticides.

ACKNOWLEDGEMENTS

The authors are indebted to the staff of the San Francisco Estuary Institute for their valuable assistance with many aspects of this project: continual review and suggestions during the course of the overall study, and of this report, were provided by Drs. Rainer Hoenicke and Bruce Thompson, additional editorial review of this report was provided by Sarah Lowe, and administrative support was provided by Mr. Eric Wittner, Ms. Gabrielle Marek and Ms. Elizabeth Hartman. The project also benefited from the advice of Dr. Robert Spies and Mr. Dane Hardin of Applied Marine Sciences. Toxicity testing assistance was provided by Kathie Evans of Pacific EcoRisk. The administrative assistance of Ms. Susanna Chase, Ms. Deborah Florer, and Ms. Diane Stafford is also noted.

REFERENCES

Bailey HC, DiGiorgio C, Kroll K, Miller JL, Hinton DE, Starrett G (1996) Development of Procedures for Identifying Pesticide Toxicity in Ambient Waters: Carbofuran, Diazinon, Chlorpyrifos. Environmental Toxicology and Chemistry 15(6): 837-845.

Bailey H, Miller J, Miller M, Wiborg L, Deanovic L, Shed T (1997) Joint toxicity of diazinon and chlorpyrifos to *Ceriodaphnia dubia*. Environmental Toxicology and Chemistry 16:2304-2308.

Bailey HC, Deanovic L, Reyes E, Kimball T, Larson K, Cortright V, Connor V, Hinton DE (2000) Diazinon and chlorpyrifos in urban waterways in northern California, USA. Environmental Toxicology and Chemistry 19(1):82-87.

BASMAA (1996) San Francisco Bay Area Stormwater Runoff Monitoring Data Analysis 1988-1995. Prepared by Woodward Clyde Consultants for the Bay Area Stormwater Management Agencies Association, Regional Water Quality Control Board, Oakland, CA.

Belden JB, Lydy MJ (2000) Impact of atrazine on organophosphate pesticide toxicity. Environmental Toxicology Chemistry 19(9):2266–2274.

Bennett KP, Nordmark CE, Schuette J, 1998. Occurrence of aquatic toxicity and dormant-spray pesticide detections in the San Joaquin River watershed, Winter 1996-1997. Environmental Hazards Assessment Program EH-98-02. CA Dept. Pesticide Regulation, Sacramento, CA.

Brown RL (1992) Bay/Delta fish resources. California Dept. Water Resources Exhibit WRINT-DWR-30 to the State Water Resources Control Board. July 1992. 46 pp.

CDFG. 1992a. Test No. 162. California Department of Fish & Game, Aquatic Toxicology Laboratory, Environmental Services Division, Elk Grove, CA.

CDFG. 1992b. Toxicity Test No. 163. California Department of Fish & Game, Aquatic Toxicology Laboratory, Environmental Services Division, Elk Grove, CA.

CDFG. 1992c. Test No. 168. California Department of Fish & Game, Aquatic Toxicology Laboratory, Environmental Services Division, Elk Grove, CA.

CDFG. 1993. Hazard assessment of the insecticide chlorpyrifos to aquatic organisms in the Sacramento-San Joaquin River system. Admin Rept 93-1, California Department of Fish & Game, Environmental Services Division, Sacramento, CA.

Connor V (1995) Status of urban storm runoff projects. Central Valley Regional Water Quality Control Board, Sacramento, CA. Staff Memorandum.

DeVlaming V, Norberg-King TJ (1999) A review of single species toxicity tests:Are the tests reliable predictors of aquatic ecosystem community responses. EPA 600/R-97/11. Technical Report. U.S. Environmental Protection Agency, Duluth, MN.

DeVlaming, V, Connor V, diGiorgio C, Bailey HC, Deanovic LA, Hinton DE (2000) Application of whole effluent toxicity test procedures to ambient water quality assessment. Environmental Toxicology and Chemistry 19(1)42-62.

Eldrirge MB, Whipple JA, Bowers MJ (1982) Bioenergetics and growth of striped bass, *Morone saxatilis*, embryos and larvae. U.S. National Mar Fish Serv Fishery Bull 80:461-474.

Epstein L, Bassein S, Zalom FG (2000) Almond and stone fruit growers reduce OP increase pyrethroid use in dormant sprays. California Agriculture 54(6):14-19.

Foe C (1995) Insecticide concentrations and invertebrate bioassay mortality in agricultural return water from the San Joaquin basin. Central Valley Regional Water Quality Control Board, Sacramento, CA. Staff Report.

Foe C, Connor V (1991) San Joaquin watershed bioassay results, 1988-90. Central Valley Regional Water Quality Control Board, Sacramento, CA. Staff Memorandum.

Giesy JP, Solomon KR, Coats JR, Dixon K, Giddings J, Kenaga E, Kendall RJ (1997) Ecological risk assessment of chlorpyrifos in North American aquatic environments. Report of an expert panel. DowElanco, Indianapolis, IN.

Herbold B, Jassby AD, Moyle PB (1992) Status and Trends Report on Aquatic Resources in the San Francisco Estuary. San Francisco Estuary Project, Oakland, CA.

Hoffman RS, Capel PD, Larson SJ (2000) Comparison of pesticides in eight U.S. urban streams. Environmental Toxicology Chemistry 19(9):2249–2258.

Hunt JW, Anderson BS, Phillips BM, Tjeerdema RS, Puckett HM, deVlaming V (1999) Patterns of aquatic toxicity in an agriculturally dominated coastal watershed in California. Agriculture, Ecosystems, and Environment 75:75-91.

Jin-Clark Y, Zhu KY, Lydy MJ (2002) Effects of atrazine and cyanizine on chlorpyrifos toxicity in *Chironomus tentans* (Diptera: Chironomidae). Environmental Toxicology Chemistry 21(3):598–603.

Kay J (2000) Groups Challenge Waiver on Pesticides: Focus on Exemption for Irrigation Water. San Francisco Chronicle. San Francisco, CA: A21 & A24.

Kegley S, Orme S, Neumeister L (2000) Hooked on Poison: Pesticide Use in California, 1991-1998. Pesticide Action Network, San Francisco, CA.

Kuivila KM, Foe CG (1995) Concentrations, transport, and biological effects of dormant spray pesticides in the San Francisco Estuary, California. Environmental Toxicology and Chemistry 14(7):1141-1150.

Meng L, Moyle PB (1995) Status of the splittail in the Sacramento-San Joaquin estuary. Transactions American Fisheries Society 124:538-549.

Moon GE, Kuivila K, Rohl C, Schoellhamer D (2000) Exposure of Delta smelt to dissolved pesticides in 1998 and 1999. IEP Newsletter 13(4)27-33, Sacramento, CA.

Moran KD (2002) Pesticide market trends and potential water quality implications – Selection of pesticides for detailed review. Memorandum, April 29, 2002. *tdc* environmental, San Mateo, CA.

Moyle PB, Herbold B, Stevens DE, Miller LW (1992) Life history and status of Delta smelt in the Sacramento-San Joaquin estuary, California. Transactions American Fisheries Society 121:67-77.

Muir DCG, Rawn GP, Grift NP (1985) Fate of the synthetic pyrethroid insecticide deltamethrin in small ponds: A mass balance study. J Agric Food Chem 33:603-609.

Nimmo DR, Hamaker TL, Metthews E, Moore JC. 1981. An overview of the acute and chronic effects of first and second generation pesticides on an estuarine mysid. In Vernberg FJ, Calabrese A, Thurberg FP, Vernberg WB, eds, *Biological Monitoring of Marine Pollutants. Academic Press*, New York, NY, USA, pages 3-19.

Novartis (1997) An ecological risk assessment of diazinon in the Sacramento and San Joaquin River Basins. Technical Report. Novartis Crop Protection, Inc., Greensboro, NC.

Obrebski S, Orsi JJ, Kimmerer W (1992) Long-term trends in zooplankton distributions and abundance in the Sacramento-San Joaquin Estuary. Technical Report 32, Interagency Ecological Program (IEP), CA Department of Water Resources, Sacramento, CA.

Ogle S, Gunther A (1997) Episodic Toxicity in the San Francisco Bay System. San Francisco Estuary Institute, Richmond. 1996 Annual Report of the Regional Monitoring Program for Toxic Substances in the San Francisco Estuary.

Ogle S, Gunther, A (2000) Episodic Toxicity in the San Francisco Bay System. San Francisco Estuary Institute, Richmond. .

Ogle S, Gunther A, Hoenicke R (1998) Episodic Toxicity in the San Francisco Bay System. Interagency Ecological Program Newsletter 11(2): 14-17.

Price WW (1982) Key to the shallow water Mysidacea of the Texas coast with notes on their ecology. Hydrobiologia 93:9-21.

Schimmel SC, Garnas RL, Patrick Jr. JM, Moore JC (1983) Acute toxicity, bioconcentration, and persistence of AC 222,705, benthiocarb, chlorpyrifos, fenvalerate, methyl parathion, and permethrin in the estuarine environment. J Agric Food Chem 31:104-113.

SFEI (1994) 1993 Annual Report for the Regional Monitoring Program for Trace Substances in the San Francisco Estuary. San Francisco Estuary Institute, Richmond, CA. .

SFEI (1996) 1994 Annual Report for the Regional Monitoring Program for Trace Substances in the San Francisco Estuary. San Francisco Estuary Institute, Richmond, CA. .

SFEI (1997) 1995 Annual Report for the Regional Monitoring Program for Trace Substances in the San Francisco Estuary. San Francisco Estuary Institute, Richmond, CA. .

SFEI (1998) 1996 Annual Report for the Regional Monitoring Program for Trace Substances in the San Francisco Estuary. San Francisco Estuary Institute, Richmond, CA. .

SRH&A (1994) Identification and control of toxicity in stormwater discharges to urban creeks. Final Report prepared for the Alameda County Urban Runoff Clean Water Program.

Solomon KR, Giddings JM, Maund SJ (2001) Probabilistic risk assessment of cotton pyrethroids: 1. Distributional analysis of laboratory aquatic toxicity data. Env Toxicol Chem 20(3):652-659.

Thompson B., Hoenicke R., Davis JA, Gunther AJ (2000) An overview of contaminant-related issues identified by monitoring in San Francisco Bay. Environmental Monitoring and Assessment 64: 409-419.

US EPA. 1986. Ambient water quality criteria for chlorpyrifos – 1986. EPA 440/5-86-005. U.S. Environmental Protection Agency, Office of Water, Regulations and Standards, Washington, D.C., USA.

US EPA (1994) Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms. Second Edition. EPA-600-4-91-003. U.S. EPA, Environmental Monitoring Systems Laboratory, Cincinnati, OH.

Werner I, Deanovic LA, Connor V, deVlaming V, Bailey HC, Hinton DE (2000) Insecticide-caused toxicity to *Ceriodaphnia dubia* (Cladocera) in the Sacramento-San Joaquin River Delta, California, USA. Environmental Toxicology Chemistry 19(1):215-227.

Werner I, Deanovic LA, Hinton DE, Henderson JD, de Oliveira GH, Wilson BW, Krueger W, Wallender WW, Oliver MN, Zalom FG (2002) Toxicity of stormwater runoff after dormant spray application of diazinon and Esfenvalerate (Asana®) in a french prune orchard, Glenn County, California, USA. Bull. Environ. Contam. Toxicol. 68:29-36.