Watershed-scale Evaluation of Agricultural BMP Effectiveness in Protecting Critical Coastal Habitats:

Final Report on the Status of Three Central California Estuaries

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Executive Summary

Along California's Central Coast, the Pajaro, Salinas, and Santa Maria Rivers drain to coastal estuaries that provide essential habitat for early life stages of commercially and recreationally important marine fish species, threatened anadromous fish species, migratory birds, and other wildlife. These are the largest watersheds on the central coast and each contains year-round, intensively cultivated agricultural land that supports a $3.5 billion/year industry producing most of the nation's lettuce, artichokes, and crucifer crops. Runoff from irrigated agriculture constitutes a significant portion of river flow during most of the year, and a number of studies have documented pesticide occurrence and biological impacts in these watersheds.

Evidence of pesticide impacts has encouraged diverse stakeholders to begin implementing farm management practices (MPs) to reduce pesticide concentrations and toxicity in agricultural runoff. This project is designed to complement ongoing evaluations of individual MPs with measurements of pesticide concentrations throughout the estuarine environments, including the water column, sediments, and in resident biota. Biological effects were measured at the organism and community levels. Chemical analyses emphasized pesticide impacts because previous research in these watersheds has indicated these are the primary chemicals of concern impacting beneficial uses. A broad suite of pesticides were measured, including legacy organochlorines, widely-used organophosphates, increasingly-used pyrethroids, herbicides and fungicides.

Sampling in the three estuaries was conducted from January 2008 until October 2009. A total of fifteen sampling events were conducted in each estuary, and these were divided between eleven irrigation season events and four storm events. Storm and irrigation monitoring included water toxicity and chemistry analyses for pesticides. Samples were collected at multiple stations in the estuaries and in key tributaries flowing to the estuaries. In addition, sediment toxicity was assessed at eight estuary stations and the tributary stations during three irrigation season sampling events. Benthic community characterizations were also conducted in May and October 2008. All sediment samples were analyzed for pesticides, as well as grain size and total organic carbon. Pesticide analyses in resident fish and sand crab tissues were conducted once in each

estuary. The goal of this project was to establish a baseline of estuary conditions with respect to pesticide impacts as MPs are beginning to be implemented.

Water samples from the lower Pajaro River estuary were often toxic to the amphipod *H. azteca*. Fifty-five percent of irrigation season samples collected in the estuary were toxic to amphipods, while 25% of the storm water samples were toxic. The majority of toxicity was observed in water from the lower estuary. Twenty-seven samples were tested for toxicity to *C. dubia* in the Pajaro River watershed, and of these, four samples were significantly toxic (15%). The toxicity of these samples was sometimes accounted for by sum toxic units (TUs) of pesticides, which were calculated by comparing chemical concentrations to know toxicity thresholds. Many of the Pajaro River samples had detected concentrations of diazinon, chlorpyrifos and malathion. In February 2009 there were sufficient concentrations of diazinon and or chlorpyrifos to account for *C. dubia* mortality in samples from two Pajaro River tributaries, the Monterey Drainage Ditch and Watsonville Slough. Concentrations of malathion in October 2009 were sufficient to account for *C. dubia* mortality at the same stations.

Ten of 24 sediment samples collected from eight stations over 16 months in the Pajaro River estuary were toxic to amphipods *H. azteca* (42%). While a number of organophosphate, organochlorine, and pyrethroid pesticides were detected in sediments from the Pajaro River estuary and its tributaries, only the pyrethroid pesticide bifenthrin was detected at concentrations that could partly account for the observed toxicity. The magnitude of sediment toxicity was low in most of the samples demonstrating statistically significant amphipod mortality. Toxic concentrations of bifenthrin were detected in a subset of the toxic sediment samples. Toxicity was observed in five of the nine sediments collected from the Pajaro River tributaries (56%). As with the estuary stations, the magnitude of toxicity was relatively low in the tributary samples, with the exception of the October 2009 Monterey Drainage Ditch sample (0% survival).

Benthic community structure was characterized using the Relative Benthic Index (RBI). The benthic community structure was considered highly impacted at 4 of the 5 stations in May 2008, and was moderately impacted at the remaining station. Benthic conditions were considered highly impacted at all stations during November 2009 sampling. Stations with degraded benthos

had lower RBI scores due to the presence pollution tolerant polychaete species, the absence of positive indicator species, low overall abundances of invertebrates, and low taxa numbers.

Fish in the Pajaro estuary and sand crabs in the adjacent surf zone continue to be contaminated with DDE, the primary metabolite of the organochlorine pesticide DDT. In addition, fish from this estuary were contaminated with current use fungicides and herbicides. These include azoxystrobin and pyraclostrobin, and the herbicide boscalid. Current use organophosphate and pyrethroid pesticides were not detected in sand crab or fish from the Pajaro estuary.

Of the three estuaries characterized in this study, the Salinas River estuary was the least impacted by pesticides. Few of the water and sediment samples from this estuary were toxic. Despite this, benthic communities were characterized as being highly impacted. This was due to relatively low abundances of invertebrates, low taxa numbers, the presence of negative indicator species, and the absence of positive indicator species. When water toxicity was observed in this estuary, this was usually caused by chlorpyrifos. A greater percentage of water and sediment toxicity was observed in the two Salinas River estuary tributaries, Blanco Drain and the Salinas River at Davis Road. Water and sediment samples from the Blanco Drain were sometimes toxic, and water toxicity at this station was associated with elevated concentrations of chlorpyrifos. Toxic sediments from Blanco Drain contained mixtures of the pyrethroid pesticides bifenthrin, cypermethrin and cyhalothrin. Toxic sediments from the Davis Road station contained mixtures of chlorpyrifos and pyrethroid pesticides.

As with the Pajaro estuary, sand crabs from the surf zone adjacent to the Salinas River estuary were contaminated with p'p DDE, but no current use pesticides, herbicides, or fungicides. Total DDT concentrations in the Salinas estuary sand crabs were comparable to concentrations measured in sand crabs from the Santa Maria estuary mouth, and higher than those from the Pajaro estuary. Fish from this estuary were contaminated with DDE, and two fungicides, azoxystrobin and pyraclostrobin. No current use pesticides were detected in fish from the Salinas River estuary.

The Santa Maria River estuary was the most impacted water body in this study. The majority of water samples were highly toxic to invertebrates, and chemistry and TIE evidence suggests toxicity was caused by chlorpyrifos, pyrethroid pesticides, or mixtures containing both classes of pesticides. A high percentage of sediment samples were also toxic in this estuary, and sediment toxicity was associated with mixtures of chlorpyrifos and pyrethroid pesticides. Based on the Relative Benthic Index, all of the Santa Maria estuary stations where benthic communities were classified had a high degree of impact. Impacts in the Santa Maria River estuary were likely due to the proximity of this system to Orcutt Creek, the tributary which accounts for most of the flow to the lower Santa Maria River. Water and sediment samples from Orcutt Creek were highly toxic to invertebrates and toxicity was due to mixtures of the same pesticides measured in the estuary.

Sand crabs and fish collected in and adjacent to the Santa Maria estuary were contaminated with numerous fungicides, herbicides, and pesticides. The level of tissue contamination in biota from this estuary was much higher than those from the Pajaro and Salinas River Estuaries, and reflected the greater contamination of water and sediment in this system. Sand crabs from the surf zone adjacent to the Santa Maria estuary mouth continue to be contaminated with high concentrations of DDT. In addition, sand crab tissues contained seven current-use biocides, including the pyrethroid pesticides bifenthrin and cyfluthrin, the organophosphates diazinon and chlorpyrifos, and the fungicides azoxystrobin, pyraclostrobin, and boscalid. Thirteen current-use pesticides as well as DDT and its two primary degradation products were detected in fish collected from the Santa Maria River estuary. The organophosphate pesticides chlorpyrifos and diazinon were detected in all fish from this estuary, as was the pyrethroid pesticide, bifenthrin. As was observed in sand crabs, several fungicides were also detected in fish tissue, including azoxystrobin and pyraclostrobin.

This study provides data on the status of three ecologically important estuaries on California's Central Coast, and includes comprehensive characterizations of pesticide contamination in water, sediment and biota. The results indicate that toxicity and impacts on biological communities are highly variable among these three estuaries, and that these impacts are associated with varying levels of pesticide loading. As management practices are implemented on a comprehensive scale

in the watersheds influencing these water bodies, it is expected that pesticides loading will decrease, and the percentage of toxic water and sediment samples should also decrease. Data from the current project provide baseline information that will allow resource managers to track changes in toxicity and associated ecosystem structure with changes in pesticide contamination in the estuaries and their key tributaries.

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Introduction

Coastal estuaries are among the most ecologically important and critically threatened habitats in California. Less than 20% of the State's coastal wetlands remain from the time of European settlement, and many of these wetlands face threats from water quality degradation. Along California's Central Coast, rivers in the three largest watersheds, the Pajaro, Salinas, and Santa Maria, drain to coastal estuaries that provide essential habitat for early life stages of commercially and recreationally important marine fish species, threatened anadromous fish species, migratory birds, and other wildlife. Designated beneficial uses for these estuaries include recreation, salmonid migration corridors, cold and warm water fisheries, shellfisheries, recreational and commercial fishing, biological habitats of special significance, and estuarine habitat for wildlife, fish spawning, and rare, threatened, and endangered species.

Each of these watersheds contains year-round, intensively cultivated agricultural land that supports a $3.5 billion/year industry producing most of the nation's lettuce, artichokes, and crucifer crops. Runoff from irrigated agriculture constitutes a significant portion of river flow during most of the year, and a number of studies have documented pesticide occurrence and biological impacts in the rivers and estuaries of the Pajaro (e.g., (Hunt et al., 1999), Salinas (e.g., (Anderson et al., 2003b) and Santa Maria (e.g., (Anderson et al., 2006b).

Evidence of pesticide impacts has encouraged a number of diverse stakeholders to begin implementing farm management practices (MPs) to reduce pesticide concentrations and toxicity in agricultural runoff. Over the past decade, researchers at UC Davis have been coordinating with farm watershed groups, the Central Coast Regional Water Quality Control Board's (CCRWQCB) Agricultural Waiver program, the Monterey Bay National Marine Sanctuary Water Quality Protection Program, and the Resource Conservation Districts (RCDs) to identify water quality problems, and to evaluate the effectiveness of individual farm scale MPs to mitigate pesticide runoff. Practice effectiveness evaluations are continuing as part of MP implementation throughout these watersheds. A key component of these evaluations includes a watershed-wide assessment to determine the degree to which the cumulative implementation of MPs is improving water quality. The current project is designed to provide a comprehensive

baseline assessment to allow future evaluations of the watershed-wide effectiveness of agricultural MP implementation.

The goal of this project was to establish a baseline of estuary conditions with respect to pesticide impacts as MPs are beginning to be implemented. The project was designed to incorporate sufficient spatial and temporal replication to allow detection of changes in conditions over the next decade as farm management practices change. This project is designed to complement ongoing evaluations of individual MPs (funded separately) with measurements of pesticide concentrations throughout the estuarine environments, including the water column, sediments, fish and sand crab tissues. Biological effects were measured at the organism and community levels. Chemical analyses emphasized pesticide impacts because previous research in these watersheds has indicated these are the primary chemicals of concern impacting beneficial uses. A broad suite of pesticides were measured, including legacy organochlorines, widely-used organophosphates, increasingly-used pyrethroids, herbicides and fungicides.

There were four primary objectives:
1. Characterize the occurrence of pesticides in estuary water, sediment, fish and sand crab tissue.
2. Qualitatively compare the suites of pesticides detected in the estuaries with those found in key adjacent tributaries that convey agricultural runoff into the estuaries.
3. Determine the magnitude of biological effects in the estuaries, and use toxicity identification evaluations (TIEs), co-occurrence gradients, and benthic community responses to investigate relationships between pesticide occurrence and biological effects.
4. Link estuary condition with agricultural pesticide usage, number and coverage of MPs implemented in the watersheds, and effectiveness of individual MPs; and share information with growers, RCDs, and others who can use it to encourage, adapt, and improve MP implementation.

Estuary Descriptions

Pajaro River estuary

The Pajaro River watershed drains approximately 1,300 square miles and includes 60,815 acres in agriculture. The river forms the dividing line between Santa Cruz and Monterey counties.

Agriculture activities in the lower Pajaro River watershed are concentrated in fields near the city of Watsonville, and in the towns of Aromas and Pajaro. The main tributaries to the lower river and estuary include Corralitos and Salsipuedes Creeks, Watsonville Slough, and agriculture discharges from the Beach Street Ditch and the Monterey Drainage Ditch on the south side of the river.

Figure 1. Map of the Pajaro River estuary showing the 8 sediment sampling stations (diamonds 1-8), and the 5 benthic community sampling stations (diamonds 1-5). The upper and lower estuary water toxicity and chemistry stations are depicted with triangles. The three tributary stations, Watsonville Slough/Beach Street Ditch (WAT), the Monterey Drainage Ditch (MDD), and the Pajaro River at Thurwachter Bridge (THU), are depicted with circles.

The Pajaro River estuary provides critical nesting and foraging habitat to resident and migratory shorebirds, including western snowy plovers, a federally-listed endangered species. The estuary and lagoon also provide critical nursery and foraging habitat for numerous marine and estuarine fish and invertebrate species, including tidewaters gobies, an endangered species. The Pajaro River estuary and some of its tributaries are also used by migrating adult steelhead trout, and the estuary provides foraging habitat for out-migrating steelhead smolts (ESA, 2001). Other fish

species commonly found in the estuary include staghorn sculpin, starry flounder, striped bass, topsmelt, arrow gobies, and surf perch (J. Smith, San Jose State University, personal communication).

In the lower Pajaro River and adjacent Watsonville Slough watersheds, several water bodies are currently listed as impaired by pesticides, fecal coliforms and/or nutrients under Clean Water Act §303[d], including Watsonville Slough, and the lower Pajaro River. Monitoring for pesticides and associated biological effects in the Pajaro River estuary has been limited. The State Water Resources Control Board's Toxic Substances Monitoring Program (TSMP) analyzed chemical contaminants in fish tissues from the estuary in 1992. In addition, The Bay Protection and Toxic Cleanup Program (BPTCP) assessed sediment toxicity and chemical contamination at one station in the estuary in the 1992 (Downing et al., 1998). Dugan et al. (Dugan et al., 2005) analyzed chemical contamination in sand crab tissues collected adjacent to the estuary mouth as part of CCRWQCB monitoring. In addition to this work, additional Regional Board studies have included pesticide and toxicity monitoring in the lower Pajaro River watershed (Hunt et al., 1999), and monitoring by the Central Coast Conditional Waiver Cooperative Monitoring Program (CMP).

For this study, the Pajaro River estuary was divided into eight sections and one station was sampled within each of the eight sections for sediment toxicity and chemistry. The sections were numbered from west to east, with station 1 being closest to the mouth of the estuary (see diamond symbols in Figure 1). Stations 3 and 4 were located in the channel that leads from the Watsonville Slough/Beach Street ditch inputs. Samples for benthic community characterizations were collected at Stations 1 – 5. In addition to these 8 sediment stations, two additional stations were selected for water toxicity testing and chemical analyses. One station was located in the lower estuary (Lower) and one was located in the upper estuary (Upper; see triangle symbols in Figure 1). Three additional tributary stations were located at key inputs into the estuary. The tributaries are indicated with circles in Figure 1, and these were in the channel at the confluence of the Watsonville Slough and Beach Street Ditches (WAT), in the Monterey Drainage Ditch (MDD), and in the Pajaro River at the Thurwachter Bridge crossing (THU).

Salinas River estuary

The Salinas River flows 155 miles from its headwaters in San Luis Obispo County, through the Salinas Valley and enters the Monterey Bay National Marine Sanctuary at the Salinas River National Wildlife Refuge. At 4,600 square miles, the Salinas River watershed is the largest on the central California coast. The Salinas River Valley is the heart of the most productive vegetable producing region in the country (California Farm Bureau; http://www.cfbf.com, 2005). This region contains year-round, intensively cultivated agricultural land supporting a nearly $3.5 billion/year industry producing most of the nation's salad greens, artichokes, and crucifer crops.

The Salinas River estuary provides critical nesting and foraging habitat to resident and migratory shorebirds, including brown pelican and western snowy plovers, a federally-listed endangered species. The estuary provides critical nursery and foraging habitat for numerous marine and estuarine fish and invertebrate species. The estuary and some of its tributaries are also used by migrating adult steelhead trout, and at certain times of the year, the estuary may provide foraging habitat for out-migrating steelhead smolts (USFWS, 2002). Other fish species commonly found in the estuary include staghorn sculpin, starry flounder, striped bass, topsmelt, croaker, and surf perch.

Runoff from irrigated agriculture constitutes a significant portion of stream flow in the northern Salinas Valley watershed during much of the year, and a number of studies have documented pesticide occurrence and biological impacts in watershed tributaries (Hunt et al., 2003) and in the Salinas River (Anderson et al., 2003a; Anderson et al., 2003b) . In the lower Salinas and adjacent Gabilan watersheds, thirteen water bodies are currently listed as impaired by pesticides and/or nutrients under Clean Water Act §303[d], including Gabilan, Natividad and Alisal Creeks, the lower Salinas River, and Tembladero Slough.

Monitoring for pesticides and associated biological effects in the Salinas River estuary have been limited. The TSMP analyzed chemical contaminants in fish tissues from the estuary in 1983 and 1999. In addition, The BPTCP assessed sediment toxicity and chemical contamination at one station in the estuary in the 1992 (Downing et al., 1998). Dugan et al. (Dugan et al., 2005)

analyzed chemical contamination in sand crab tissues collected adjacent to the Salinas River estuary mouth.

Figure 2. Map of the Salinas River estuary showing the 8 sediment sampling stations (diamonds 1-8), and the 5 benthic community sampling stations (diamonds 1-5). The upper and lower estuary water toxicity and chemistry stations are depicted with triangles.

As with the Pajaro estuary, the Salinas River estuary was divided into eight sections and one station was sampled within each of the eight sections for sediment toxicity and chemistry. The sections were numbered from west to east, with station 1 being closest to the mouth of the estuary (see diamond symbols in Figure 2). Samples for benthic community characterizations were collected at Stations 1 – 5. In addition to these 8 sediment stations, two additional stations were selected for water toxicity testing and chemical analyses. One station was located in the lower estuary (Lower) and one was located in the upper estuary (Upper; triangle symbols in Figure 2). Two additional tributary stations were located at key inputs into the estuary. The tributaries are indicated with circles in Figure 3 and these were located in the Blanco Drain at Cooper Road (BLA), and in the Salinas River channel at the Davis Road crossing (DAV).

Figure 3. Map of the two Salinas River tributary stations (circles), Blanco Drain at Cooper Road (BLA), and the Salinas River at Davis Road (DAV).

Santa Maria River estuary

The Santa Maria River watershed drains approximately 1,880 square miles comprising 1,203,000 acres on California's central coast. The river forms the dividing line between Santa Barbara and San Luis Obispo counties. The watershed includes the Cuyama and Sisquoc Rivers which join to form the Santa Maria River. Orcutt Creek drains approximately 50,000 acres of land southeast of the Santa Maria River estuary. This creek drains much of the agricultural land in the lower river basin and it enters the river just upstream of the estuary (SAIC, 2004). Upstream of the Highway 1 Bridge, the river flows underground for most of the year, and the river bed is often dry from here to the Sisquoc River input. Flows from the Cuyama River are controlled by the Twitchell dam. Downstream of Highway 1, the river flows freely and is comprised of river flow and agriculture tailwater discharges. Inputs to the Santa Maria River estuary are dominated by Orcutt Creek and a drainage ditch that enters the river near the entrance to the Rancho Guadalupe

Dunes Preserve. Together flows from these two sources comprise 92% of the total input into the estuary (SAIC, 2004).

The Santa Maria River estuary provides critical nesting and foraging habitat to resident and migratory shorebirds, including western snowy plovers. The estuary is recognized as a globally important wetland along the Pacific Flyway in the western hemisphere (SAIC, 2004). The estuary and lagoon also provide critical nursery and foraging habitat for numerous marine and estuarine fish and invertebrate species, including tidewater gobies. Other fish species found in the estuary include staghorn sculpin, starry flounder, three spine stickleback, fathead minnows, arroyo chub, and mosquitofish. Although there have been no recent surveys of steelhead in the lower river or estuary, there is potential for adult and juvenile steelhead to use the estuary, particularly during wet years (SAIC, 2004). The Santa Maria River estuary and its tributaries have been designated as critical habitat for steelhead trout by NOAA Fisheries (SAIC, 2004).

In the lower Santa Maria and adjacent Oso Flaco Creek watersheds, several water bodies are currently listed as impaired by pesticides and/or nutrients under Clean Water Act §303[d], including Orcutt-Solomon Creek, the lower Santa Maria River, and Oso Flaco Creek. Monitoring for pesticides and associated biological effects in the Santa Maria River estuary have been limited. The TSMP analyzed chemical contaminants in fish tissues from the estuary in 1992 and 1999. In addition, the BPTCP assessed sediment toxicity and chemical contamination at one station in the estuary in the 1993 (Downing et al., 1998). Duggan et al. (Dugan et al., 2005) analyzed chemical contamination in sand crab tissues collected adjacent to the Santa Maria estuary mouth. Additional Regional Board studies have included pesticide and toxicity monitoring in the lower Santa Maria River watershed (Anderson et al., 2006b; Phillips et al., 2006; Phillips et al., 2010), and monitoring by the Central Coast Conditional Waiver Cooperative Monitoring Program (CMP).

Figure 4. Map of the Santa Maria River estuary showing the 8 sediment sampling stations (diamonds 1-8), and the 5 benthic community sampling stations (diamonds 1-5). The upper and lower estuary water toxicity and chemistry stations are depicted with triangles. The tributary station at Orcutt Creek (ORC) is depicted with a circle.

As with the other two estuaries, the Santa Maria River estuary was divided into eight sections and one station was sampled within each of the eight for sediment toxicity and chemistry. The sections were numbered from west to east, with station 1 being closest to the mouth of the estuary (see diamond symbols in Figure 4). Samples for benthic community characterizations were collected at Stations 1 – 5. In addition to these eight sediment stations, two additional stations were selected for water toxicity testing and chemical analyses. One station was located in the lower estuary (Lower) and one was located in the upper estuary (Upper; triangle symbols in Figure 4). An additional tributary station was located in Orcutt Creek, a key input into the estuary. This station, ORC, is indicated with a circle in Figure 4 and was located at the sand plant, where the creek crosses under the road to the Guadalupe Dunes Reserve.

Methods

Evaluation of the three estuaries was conducted from January 2008 until October 2009 (Table 1). A total of fifteen sampling events were conducted in each estuary, and these were divided between eleven irrigation season events and four storm events. Storm events were defined as rainfall greater than or equal to 0.5 inches within 24h preceding sampling. For all sampling events, the following parameters were monitored at the upper and lower estuary stations: water toxicity using either *Hyalella azteca* (96h)*, Ceriodaphnia dubia* (96h), or both species (depending on conductivity, see below), water chemistry analyses for pesticides (GC/MS, described below), and conventional water chemistry (nitrates, phosphates, dissolved oxygen, pH, turbidity, conductivity). All of these parameters were also analyzed during nine sampling events conducted concurrently at the tributary stations (Table 1). The tributary monitoring also included flow measures and analyses of pesticides associated with suspended sediments collected during the storm events (described below). These latter measures were intended to characterize instantaneous loading of pesticides associated with water versus those associated with suspended sediments.

In addition, sediment toxicity (*H. azteca* 10d) was assessed at the eight estuary stations and the tributary stations on three irrigation season samples which were collected in May and October 2008, and again in October 2009. Benthic community characterizations were also conducted during the May and October 2008 sediment sampling events. All sediment samples were analyzed for pesticides using GC/MS, as well as grain size and total organic carbon.

Pesticide analyses in resident fish and sand crab tissues were conducted once in each estuary. Fish for tissue analyses were collected in October, 2008, and sand crabs were collected in August, 2008. In addition to these analyses, plasma vitellogenin was measured in male and female fathead minnows (*Pimephales promelas*) in laboratory experiments. These experiments assessed the potential endocrine disruption in fish using samples from Orcutt Creek, the Santa Maria River, and the Santa Maria estuary.

Table 1. Sampling months and parameters measured in three central coast estuaries in 2008 – 2009 during 15 separate sampling runs (R). W = water column sampling for toxicity and chemistry; S = sediment sampling for toxicity and chemistry, B = benthic community sampling, C = sand crab sampling, F = fish sampling, X = suspended sediment sampling (see text for details).

	2008								2009						
	R1 Jan	R2 Feb	R3 Apr	R4 Apr	R5 May	R6 Jul	R7 Aug	R8 Sep	R9 Oct	R10 Feb	R11 Aug	R12 Aug	R13 Sept	R14 Sept	R15 Oct
Station	Storm 1	Storm 2	Irrigation 1	Irrigation 2	Irrigation 3	Irrigation 4	Irrigation 5	Irrigation 6	Irrigation 7	Storm 3	Irrigation 8	Irrigation 9	Irrigation 10	Irrigation 11	Storm 4
Santa Maria															
estuary 1	W	W	W	W	WSB	W	W C	W	WSBF	W	WS	W	W	W	W
estuary 2	W	W	W	W	WSB	W	W	W	WSB	W	WS	W	W	W	W
estuary 3					SB				SB		S				
estuary 4					SB				SB		S				
estuary 5					SB				SB		S				
estuary 6					S				S		S				
estuary 7					S				S		S				
estuary 8					S				S		S				
Orcutt Creek	WX	WX		W		WS			S	WX	WS		W	W	WX
Salinas															
estuary 1	W	W	W	W	WSB	W	W C	W	WSBF	W	WS	W	W	W	W
estuary 2	W	W	W	W	WSB	W	W	W	WSB	W	WS	W	W	W	W
estuary 3					SB				SB		S				
estuary 4					SB				SB		S				
estuary 5					SB				SB		S				
estuary 6					S				S		S				
estuary 7					S				S		S				
estuary 8					S				S		S				
Blanco Drain	WX	WX		W		WS			S	WX	WS		W	W	WX
Davis Road	WX	WX		W		WS			S	WX	WS		W	W	WX
Pajaro															
estuary 1	W	W	W	W	WSB	W	W C	W	WSBF	W	WS	W	W	W	W
estuary 2	W	W	W	W	WSB	W	W	W	WSB	W	WS	W	W	W	W
estuary 3					SB				SB		S				
estuary 4					SB				SB		S				
estuary 5					SB				SB		S				
estuary 6					S				S		S				
estuary 7					S				S		S				
estuary 8					S				S		S				
MDD	WX	WX		W		WS			S	WX	WS		W	W	WX
Thurwachter	WX	WX		W		WS			S	WX	WS		W	W	WX
Watsonville	WX	WX		W		WS			S	WX	WS		W	W	WX

Water and Sediment Collection

Water samples were collected in 2.5-liter amber glass bottles. Bottles were rinsed three times with site water before filling, and were filled at least one cm below the surface to avoid the surface microlayer. Bottles were immediately placed in coolers with sufficient wet ice to adjust and maintain the temperature at 4 ± 3°C during transport to Marine Pollution Studies Laboratory (MPSL). Water samples were stored at 4 ± 3°C for no longer than 48 hours prior to toxicity test initiation. Water samples for toxicity testing were homogenized and poured through a 25-µm pre-cleaned Nitex® screen to remove fauna and larger buoyant particulates. A separate screen was used for each sample. Samples were placed in a constant temperature room at test temperature to acclimate for 24 hours prior to the initiation of the toxicity test.

Bed sediment was collected to a maximum depth of 5 cm using either a polycarbonate core tube or a Petit Ponar grab sampler, depending on water depth. Samples were homogenized in a stainless steel container and placed in 2L glass jars and stored in iced coolers for transport. Testing was initiated within two weeks of sample collection. Additional containers of water and sediment were collected for chemical analysis and shipped or delivered to the U.S. Geological Survey's (USGS) analytical laboratory in Sacramento, CA.

Suspended Sediment Collection and Instantaneous Loading

At each river/tributary site during storm events, large-volume water samples were collected for isolation of sufficient quantities of suspended material for sediment-associated pesticide analyses. Samples were collected from multiple points and depths along a stream transect or from one point using a high-volume peristaltic pump fitted with Teflon tubing. The collected water was pumped into pre-cleaned 20-L stainless steel soda kegs. The volume collected from each site ranged from about 400 to 1000 L, depending on suspended-sediment concentrations. The objective was to process a sufficient volume of water to obtain at least 20 grams of suspended sediment per site. The large volume water sample was pumped through a Westphalia continuous-flow centrifuge operating at 9,500g at the rate of 2 L/min to segregate the liquid and solid phases and concentrate the suspended sediments (> 0.3 µm) into slurry. The centrifuge flow rate is based on a study of particle trapping efficiency (Horowitz et al., 1989) who found that 2 L/min using the Westphalia centrifuge yields in the optimum particle trapping efficiency.

The water exiting the centrifuge represents the liquid phase and was analyzed for dissolved pesticides. The sediment slurry remaining in the centrifuge was further dewatered in the laboratory using a high-speed refrigerated centrifuge operating at 10,000 revolutions per minute (RPM). The segregated water and sediment samples were stored at 4ºC and -20ºC, respectively prior to analysis. All water samples were extracted within 48 hours of collection and all sediment samples remained frozen for no longer than one year prior to extraction and analysis.

Instantaneous pesticide loads were calculated for all tributaries in each watershed except Watsonville Slough to determine when the influx of dissolved and sediment bound pesticides to the estuaries was the highest. Stream discharge was measured at each un-gauged tributary site following methods established by USGS protocols (Buchanan and Somers, 1969), and instantaneous pesticide loads were calculated for all compounds detected. Loading of dissolved pesticides was calculated during the four storm events and four dry season/irrigation sampling events. However, loading of pesticides associated with suspended sediments was only calculated during the four storm events (3 during winter and 1 during application).

Very little flow was measured in the smaller tributaries during most of the dry season and flow tended to decrease as the irrigation season progressed with the exception of Orcutt Creek whose flows tended to vary during. Conversely there was little to no flow in the larger tributaries during most of the irrigation season and both the Salinas and Pajaro Rivers were ponded throughout the summer. Although the pesticide loads were low compared to storm events there still is a consistent flux of pesticides into the estuaries during the dry season.

Benthic Community Assessment

Benthic invertebrates were sampled in May and September/October of 2008. The lower five sediment sampling sites of each estuary were considered spatially representative and were targeted for sampling. These sites corresponded to the most downstream sediment sampling sites. For each site, a 0.1 m^2 area was sampled, using either a Petit Ponar grab sampler or polycarbonate cores. Sample depth was 5 cm. Samples were deposited into a 1 mm sieve and swirled gently in a few inches of water to screen out sediment (Figure 5). Samples were stored in plastic jars and fixed in the field with borate-buffered 10% formalin. After a period of 3 days

to 2 weeks, samples were rinsed with water and stored in 70% ethanol. Samples were shipped to Weston Solutions in Carlsbad, CA for analysis.

Figure 5. Benthic community sampling in the Santa Maria estuary.

Sand Crab Collection

Sand crabs (*Emerita analoga*) were collected in August 2008. Ovigerous crabs with visible eggs were collected in the surf zone at three stations per estuary (Dugan et al., 2005). Sampling stations were chosen based on observed outflow from the estuaries into the ocean, and 50 m north and 50 m south of the outflow site. Fifty crabs were collected at each station by scooping sand into dip nets with a stainless steel shovel (Figure 6). The shovel was cleaned with methanol between each site. On average, crabs were present at a depth of 10-20 cm and were collected in the lower intertidal zone in active surf. Representative lengths (cm) were recorded (5 were measured from each site), and crabs were stored in methanol-rinsed aluminum foil, in plastic bags, on dry ice. Samples were cryofrozen and then shipped on ice to the USGS, analytical laboratory in Sacramento CA. Samples were homogenized in a stainless steel blender and sub-

sampled in the laboratory for extraction and analysis. Samples were analyzed for 89 current-use and 3 legacy organochlorine (DDE, DDD and DDT) pesticides.

Figure 6. Sampling sand crabs on beach adjacent to the Santa Maria estuary mouth.

Fish Collection

Fish sampling was conducted in October 2008 using a 33 m beach seine deployed from a small boat (Figure 7). Five to ten fish were collected in each estuary depending on their size. An effort was made to collect the same species in each estuary for comparative purposes. Targeted species were starry flounder (*Platichthys stellatus*), staghorn sculpin (*Leptocottus armatus*), and topsmelt (*Atherinops affinis*). In addition to these species, white croaker (*Genyonemus lineatus*) was collected in the Salinas River estuary. Once collected, whole fish were wrapped in methanol-rinsed aluminum foil, placed in plastic bags, then transported on ice to USGS analytical laboratory.

Figure 7. Sampling fish in the Salinas River estuary.

Toxicity Testing

Water Toxicity Testing

Water toxicity in the estuary samples was assessed using 96h exposures with the amphipod *H. azteca* (USEPA, 2002). Water toxicity in the tributary samples was assessed using 96h exposures with the water flea *C. dubia* using similar procedures. Tributary samples with conductivities exceeding 3,000 µS/cm were tested with *H. azteca*, and estuary samples with conductivities less than 3,000 µS/cm were also tested with *C. dubia*. Amphipod exposures were conducted in 300 mL beakers containing 100 mL of test solution and ten organisms. Daphnid tests were conducted in 50 mL glass beakers, each containing 30 mL of test solution and five organisms. Both exposures consisted of five replicate beakers. Test solutions were renewed at 48h, at which time both organisms were fed YCT (yeast, cerophyll and trout chow mixture).

Dissolved oxygen, pH, and conductivity were measured with an Accumet meter and appropriate electrodes (Fisher Scientific, Pittsburgh, PA). Un-ionized ammonia was measured using a Hach 2010 spectrophotometer (Hach, Loveland, CO). Water temperature was recorded with a continuous recording thermometer (Onset Computer Corporation, Pocasset, MA). Additional daily temperatures were measured using a glass spirit thermometer.

Water Toxicity Identification Evaluations

Water column TIEs with *H. azteca* were conducted three toxic samples: one from Pajaro River estuary and two from the Santa Maria River estuary. TIE exposures were conducted in 20 mL glass scintillation vials (3 replicates) containing 15 mL treated sample and five amphipods. Amphipods were exposed for 96h. Several characterization and identification treatments designed to identify organic and pesticide toxicity were performed on a dilution series of the water samples (USEPA, 1991). Sample concentrations were 0 (treatment blank), 25, 50, and 100%. Treatment blanks consisted of control water that underwent the same treatment as the sample. The Baseline was untreated sample that was tested to determine the magnitude of toxicity. The Amberlite treatment consisted of Amberlite XAD-4® (Rohm and Haas, Spring House, PA, USA) carbonaceous resin added to the sample to reduce bioavailability of non-polar organic chemicals (Kosian et al., 1999). Approximately 4g of resin was activated by rinsing with 4 mL of methanol and then thoroughly rinsed with Nanopure® water. The resin was then added to 120 mL of sample, stirred, and allowed to equilibrate for 24 hours. The resin was then separated from the sample and the sample was diluted for testing. The resin was eluted by placing it in a syringe and passing 10 mL of acetone through the resin at a rate of 1 mL/min. The acetone was evaporated to 1.2 mL and added to 120 mL of clean water for dilution and testing.

Samples were centrifuged (30 min at 2500G and 4ºC) to remove toxicity that might be caused by particulates. Samples were also passed through an organic solid-phase extraction (SPE) column to remove potentially toxic non-polar organic compounds. Oasis® HLB columns were used for all treatments (Hydrophilic-Lipophilic Balance®, 6 mL, 500 mg, Waters Corporation, Milford, MA, USA). All column treatments followed the manufacturer's suggested generic method for conditioning and loading. The column and pump apparatus was constructed by placing a column in a ring stand clamp, attaching tubing to the outlet of the column, and then passing the tubing

through a peristaltic pump. Prior to attachment to the column, the tubing was cleaned by passing 10 mL 1N hydrochloric acid, 25 mL Nanopure, 25 mL methanol, and 25 mL Nanopure. After attaching tubing to the columns, they were conditioned by passing two column volumes of acetone, two column volumes of methanol, and one column volume of Nanopure. After conditioning, columns were immediately loaded. A separatory funnel was clamped above the column and filled with 120 mL control water. The control water was dripped into the column and pumped through at a rate of one mL per minute. After control water had passed through the column, 120 mL of sample was pumped through. Test concentrations were prepared by combining the post-column samples. After extracting the sample, the columns were eluted by first washing with 4 mL Nanopure, followed by 12 mL of acetone. Solvent fractions were evaporated to 1.2 mL and reconstituted in 120 mL clean water. Toxicity of the eluates was tested to assess whether toxic concentrations of organic chemicals were recovered from the columns. Test concentrations were prepared by combining reconstituted fractions with control water containing similar concentrations of solvent.

Samples also underwent recently developed treatments for the characterization and identification of pyrethroid pesticide toxicity. The addition of carboxylesterase enzyme (Sigma-Aldrich, St. Louis, MO, USA) to the sample hydrolyzes ester-containing compounds, such as pyrethroid pesticides to their corresponding acid and alcohol, which are generally not toxic (Wheelock et al., 2004). A bovine serum albumin (BSA) protein-addition control was conducted with this treatment to account for reduction of contaminant bioavailability due to complexation by the enzyme addition. Piperonyl butoxide (250 µg/L PBO, Sigma-Aldrich, St. Louis, MO, USA) is a metabolic inhibitor and was added to block the metabolic activation of acetylcholinesterase-inhibiting organophosphate pesticides (Ankley et al., 1991b). It is also a potent synergist of pyrethroid toxicity, because it inhibits their metabolism (Kakko *et al*., 2000; Ware and Whitacre, 2004). PBO was added to water samples to reduce toxicity caused by organophosphate pesticides and increase toxicity caused by pyrethroids.

Sediment Toxicity Testing

Sediment toxicity was assessed three times at eight stations in each estuary and at the tributary stations. Sediment toxicity was assessed using the 10d growth and survival toxicity test with *H.*

azteca, a resident epibenthic amphipod (USEPA, 2000). Each sample was thoroughly homogenized on a sediment roller and divided among eight laboratory replicates, each with ten 7- to 14-day-old amphipods. The amphipods were exposed to 100 mL of sediment in 300 mL beakers, each containing 175 mL of overlying water. The test temperature was 23 ± 1°C. Water quality parameters, including dissolved oxygen, pH, conductivity, and ammonia, were measured at the beginning of each test. Hardness and alkalinity were measured at the beginning of each test. Overlying water was renewed twice daily, and 1.5 mL YCT was added daily to each test container. The containers were not aerated unless dissolved oxygen decreased below 2.5 mg/L. Negative control reference sediment was created using equal parts sediment from a reference site in the Salinas River (Monterey County, California, USA), and clean, kiln-dried sand (#60, RMC Pacific Materials, Monterey, CA, USA). The sediment was amended with organic peat moss (Uni-Gro, Chino, CA, USA). One kilogram (dry weight) of formulated sediment was prepared by combining 496.25g reference sediment, 496.25g sand, and 7.50g peat with 350 mL laboratory well water.

Sediment Toxicity Identification Evaluation

A whole-sediment TIE was conducted on composite samples from the upper Santa Maria estuary in December 2008, using samples collected in October 2008. Samples from Stations 6, 7, and 8 were combined to provide sufficient sediment for the TIE. The whole sediment TIE consisted of five replicate 250 mL beakers containing 50 mL sediment and approximately 175 mL overlying water and ten amphipods. Sediment TIE treatments included 10% Amberlite addition to reduce the bioavailability of organic contaminants (USEPA, 2007). The addition of carboxylesterase enzyme to hydrolyze pyrethroid pesticides (Wheelock et al., 2004). A bovine serum albumin (BSA) protein-addition control was conducted with this treatment to account for reduction of contaminant bioavailability due to complexation by the enzyme addition. Piperonyl butoxide was used to block the metabolic activation of acetylcholinesterase-inhibiting organophosphate pesticides (Ankley et al., 1991b), and synergize toxicity caused by pyrethroid pesticides.(Kakko et al., 2000; Ware and Whitacre, 2004).

Assessment of Endocrine Disruption

The potential for exposure endocrine disrupting chemicals in the Santa Maria estuary was assessed using measurements of plasma vitellogenin in fathead minnows exposed to water and sediment samples. Detection of vitellogenin, an egg yolk precursor, in male fish is an indication of exposure to these chemicals. Samples were collected from the Orcutt Creek and Lower Santa Maria River sites on April 13, 2010 using methods described above. Discharge and suspended sediment loads were elevated at both stations because sampling occurred soon after a significant rain event.

Adult fathead minnows were obtained from the commercial supplier Aquatic Research Organisms (Hampton, NH) and held at MPSL before initiation of the exposure test. Tanks were maintained on flow-through MPSL well water at ambient temperature (~17 °C) under fluorescent lighting (10-20 μE/M^2/s).

Exposure System

A 6-day static-renewal test was conducted with four replicate aquaria containing 2 cm of site sediment and 8L of site water. Control replicates consisted of reference sediment and well water. Aquaria were renewed daily by pumping approximately 2.5L of fresh site water into each replicate at a rate of 25 mL/min. Excess water was allowed to flow through a screened port on the aquarium. Water temperature was maintained at 25C ±1, and lighting was 16L:8D. All aquaria were aerated to maintain sufficient concentrations of dissolved oxygen. Each aquarium contained two male and two female fish. All fish were sexually mature adults, approximately one year old. Fish were fed daily with wet flake food slurry (Zeigler Bros., Inc., Gardiners, PA). Dissolved oxygen was measured daily, and pH, conductivity and ammonia were measured at test initiation and termination. Alkalinity and hardness measurements were taken at test initiation.

Plasma Vitellogenin

At test termination fish were anaesthetized with MS-222, and the caudal peduncle was partially severed with micro-dissection scissors. Blood was collected from the caudal vein with a heparinized microhematocrit capillary tube. Plasma was immediately isolated by centrifugation

for 3 min at 14,000 rpm and stored at -80ºC until analysis. Vitellogenin was measured by enzyme-linked immunosorbent assay (ELISA) using the commercially available quantitative fathead minnow vitellogenin assay kit per manufacturer's instructions (Biosense Laboratories, Inc., Bergen, Norway). Samples from female fish were analyzed at two dilutions (1:5000 and 1:500,000) in duplicate. Samples from male fish were analyzed at three dilutions (1:50, 1:5000, 1:500,000) in duplicate. Normal levels of vitellogenin in female fish are orders of magnitude higher than those of males, so the lowest dilution is not necessary to capture changes in female vitellogenin levels. Raw absorbance values were converted to concentrations (ng/ml) according to data analysis guidance provided by the kit manufacturer. Mean absorbance values were averaged and multiplied by the dilution factor for final concentrations. Concentrations were compared to a log-log standard curve, formed with a power fit. Samples that did not fall within the standard curve were considered out of range. Mean concentrations were calculated for the males of each treatment by calculating a grand mean (mean of the replicate means). When values were out of range of the standard curve, either the lowest or highest detection level was substituted in the analysis in order to represent an order of magnitude value. A separate-variance t-test was conducted between the grand means to determine significant difference.

Benthic Community Characterization

Samples with very high organism abundances were split into one half or one quarter volumes before sorting (after Watt, Weston Solution unpublished report). The samples were then sorted into major taxonomic groups and other minor phyla and identified to the species level, where possible. Secondary taxonomic identification was provided on 10% of the samples for QA purposes. Species lists were then tabulated and community indices were calculated. This included calculation of the Relative Benthic Index (RBI) for each sample. RBIs were calculated by Ananda Ranasinghe (Southern California Coastal Water Research Project).

Methods for applying the RBI to central coast lagoon habitats are discussed in Barnett et al. (Barnett et al., 2008). These are based on methods developed as part of the Bay Protection and Toxic Cleanup Program (BPTCP), and were originally developed by Jim Oakden, John Oliver, and Peter Slattery (Moss Landing Marine Laboratories). The RBI was developed for application to California bay and estuarine habitats. A detailed description of the methods used to calculate

the RBI for central coast estuaries is provided in Hunt et al. (Hunt et al., 2001). The community pattern metrics used in the RBI include number of species and individuals (total number of all taxa, total number of mollusc species and individuals, and total number of crustacean species and individuals), the abundance of species indicative of relatively disturbed benthic habitats, and the abundance of species indicative of relatively undisturbed benthic habitats. Negative indicators included *Capitella* sp. complex and oligochaetes. Positive indicators included the amphipods *Grandifoxus grandis* and *Eohaustorius estuarius* and the bivalve *Tellina modesta* (as suggested to A. Ranasinghe by Jim Oakden, Moss Landing Marine Laboratories). The overall RBI was calculated by summing the values of the total fauna, total molluscs, crustacean species, and indicator species and standardizing it to the total range. This resulted in a range of values from 0.00 (most impacted) to 1.00 (least impacted). The RBI is scaled from 0 to 1 based on the range of values in the development dataset (Ranasinghe et al., in press). The scaling is based on the Habitat E dataset (coastal wetlands and estuaries; personal communication, A. Ranasinghe, Southern California Coastal Water Research Project). During application to the present data, if the raw RBI value is less than the minimum in the development dataset, the result is a negative scaled value. This occurred in several of the estuary samples presented below.

RBI developer thresholds were based on the distribution of index values, following Hunt et al. (2001). The RBI values were subdivided into four categories: (1) Unaffected - a community that would occur at a reference site for that habitat; (2) Marginal deviation from reference - a community that exhibits some indication of stress, but might be within measurement variability of reference condition; (3) Affected - a community that exhibits clear evidence of physical, chemical, natural, or anthropogenic stress; (4) Severely Affected - a community exhibiting a high magnitude of stress. Affected and severely affected communities are those believed to be showing clear evidence of disturbance, while unaffected and marginal communities do not. Disturbed communities could be due to the effects of one or more types of anthropogenic or natural stress while undisturbed communities likely indicate minimal stress of all types (Ranasinghe et al., In Press).

The RBI is the only index available for use in west coast wetlands and estuaries, designated Habitat E in Ranasinghe et al. (in press). These are low salinity coastal wetlands and estuaries

ranging from southern California to Puget Sound, Washington. We note that the application of the RBI in Habitat E has not been validated or evaluated relative to other indexes (e.g., the Benthic Response Index). The RBI has been validated for use in Habitats C (southern California marine bays) and D (polyhaline central San Francisco Bay) and was used to evaluate central California sites as part of a California statewide analysis using various benthic community index methods (Ranasinghe et al., in press).

Chemistry Methods

Water

Filtered water samples were analyzed for a suite of 59 pesticides by extracting one liter of sample water onto Oasis HLB SPE cartridges. Prior to extraction, all water samples were filtered using either the continuous-flow centrifuge (described above) or a 0.7 µm glass fiber filter (GF/F) to remove/separate suspended material. All samples were spiked with ^{13}C-atrazine, and ^{13}C-diazinon as recovery surrogates. Following extraction, the SPE cartridges were dried with carbon dioxide, eluted with 12 mL of ethyl acetate, reduced to 200 µL and deuterated internal standards were added. All sample extracts were analyzed on a Varian Saturn 2000 (Walnut Creek, CA, USA) gas chromatograph/ion trap mass spectrometer (GC/ITMS). Additional details are given in Hladik et al. (Hladik et al., 2008).

Bedded and Suspended Sediment

Sediment samples were extracted based on methods described in Smalling and Kuivila (Smalling and Kuivila, 2008). Briefly, sediment samples were extracted by pressurize liquid extraction (PLE) using a Dionex 200 Accelerated Solvent Extractor (ASE) with dichloromethane. Sample matrix was removed using stacked pre-packed Carbon/Alumina SPE cartridges. Finally, sulfur was removed using a gel-permeation/high-pressure liquid chromatography system (GPC/HPLC). Sample extracts were analyzed for current-use pesticides by GC/ITMS. In addition, moisture content, percent organic carbon, and percent nitrogen were measured for each sediment sample (Smalling et al., 2005).

Tissue

Fish and sand crab samples were extracted using procedures described in Riedel et al. (Riedel et al., 2002) with minor modifications. Briefly, approximately 5 grams weight tissue was extracted with dichloromethane using a Dionex Model 200 accelerated solvent extractor (ASE) at 100ºC and 1500 psi. Extractable lipid on a wet-tissue basis was determined gravimetrically on each sample to the nearest 0.001g using a microbalance. Lipids and other interferences were removed using GPC/HPLC followed by florisil packed column chromatography. All data was normalized to total extractable lipids and was reported on a µg/kg lipid weight basis in order to compare data between species.

Sample extracts (1 µL injection volume) were analyzed by GC/ITMS. Analyte separation on the GC/MS was achieved using a 30 m x 0.25 mm i.d., 0.25 µm DB-5ms fused silica column (Agilent Technologies, Folsom, CA, USA) with helium as the carrier gas. The temperature of the splitless injector was held constant at 275ºC. Because of the number of compounds and instrument limitations, samples were injected twice using two different temperature programs: a short (30 min) and a long (61 min) GC temperature gradient. The fungicides and organochlorine pesticides were separated from the other compounds because of greater ease in setting selected ion storage (SIS) windows. The short temperature program was 80ºC (hold 1 min) with and increase to 300ºC at 10ºC/min (hold 10 min). The long temperature program was 80ºC (hold 0.5 min), increase to 120ºC at 10ºC/min, increase to 200ºC at 3ºC/min (hold 5 min), followed by a third increase to 219ºC at 3ºC/min, and a final increase to 300ºC at 10ºC/min (hold 10 min). The transfer line and ion trap temperatures were 280ºC and 220ºC, respectively. The MS was operated in electron ionization (EI) mode with an emission current of 15 µA and no offset when run in full scan mode, and an emission current of 45 µA with a multiplier offset of 300 volts when using SIS windows. Data was collected in full scan and SIS modes. Complete details of the analytical method are described elsewhere (Crepeau et al., 2000; LeBlanc et al., 2004; Smalling and Kuivila, 2008).

Calibration of the GC/ITMS was achieved using calibration standards that spanned the linear range of instrument response (0.025 to 5.0 ng/µL). The response of the instrument was monitored every 6-8 samples with mid-level check standards.

The limit of detection (LOD) was calculated as the amount of analyte in the spiked sample that produced a signal greater than three times the background signal and was calculated. Method detection limits (MDLs) were determined by spiking seven replicates of representative sediment with a mixture of pesticides at a concentration of approximately 10 µg/kg (dry weight) (USEPA, 1992). Analytes identified at concentrations less than the MDL or LOD were reported as estimated values.

The MDL was calculated for each pesticide using the following equation:

MDL = S x *t* (n-1, 1-α = 0.99), where:

MDL = method detection limit (µg/kg)

S = standard deviation of replicate samples

n = number of replicates

t = value of Student's t statistic at 6 degrees of freedom and 99 % confidence level

Quality Assurance

To ensure the integrity of the data collected, quality assurance/quality control (QA/QC) procedures were conducted. Pesticide concentrations in all matrices (water, sediment and biota) were validated against a comprehensive set of quality control parameters including laboratory and field blanks, matrix spikes, replicate samples, certified reference material and surrogate recovery. Environmental and QA/QC data met or exceeded applicable Surface Water Ambient Monitoring Program (SWAMP) guidelines and was reviewed by project staff and the USGS CAWSC Water Quality Specialist. All quality assurance guidelines including are outlined in a SWAMP compatible Quality Assurance Program Plan (SWAMP, 2008).

Data Interpretation

Samples were defined as toxic if the following two criteria were met: 1) there was a significant difference ($p<0.05$) in mean organism response (e.g., percent survival) between a sample and the

negative laboratory control, as determined using a separate-variance *t*-test, and 2) the difference in organism response between the sample and control was greater than 20% (Phillips et al., 2001). The acceptability of each TIE treatment was evaluated by checking for adequate survival in each treatment blank. Water TIE treatments were evaluated using toxic units based on the results of the dilution series. These units were calculated by dividing 100 by the treatment-specific median lethal concentration (LC50, as percent sample) calculated from the sample dilution series. Comparing toxic units among the treatments provided better resolution than simply comparing single concentrations from the various dilution series.

Chemistry data in water and sediment were compared to known toxicity thresholds, where available, and to other water quality criteria. The thresholds used for assessing the potential for pesticide toxicity to *C. dubia* and *H. azteca* in water are provided in Table 2. Pesticide toxic units for *C. dubia* and *H. azteca* were calculated by dividing the measured chemical concentration by the organism-specific LC50s. Total organic carbon concentrations in the sediment were used to normalize total sediment chemical concentrations to organic carbon-corrected concentrations using the following equation:

Chemical Concentration (µg/g dry wt.) ÷ TOC (%) = OC-Corrected Concentration (µg/g OC)

Corrected concentrations are considered to be more representative of the bioavailable fraction of contaminants in sediment. Higher concentrations of TOC can reduce the bioavailability of sediment contaminants.

Previous research has shown that chlorpyrifos and diazinon are additive when they occur in mixtures (Bailey et al., 1997), as are some mixtures of pyrethroids (Weston and Jackson, 2009). Based on this, and the fact that all of the pesticides that were measured at toxic concentrations are neurotoxins, the toxic units for these pesticides were added to calculate a total TU value for each sample. TUs for *H. azteca* were calculated by adding individual TUs from chlorpyrifos, diazinon, bifenthrin, permethrin, and the total sum of DDT metabolites. Sum TUs for *C. dubia* were calculated by adding individual TUs from chlorpyrifos, diazinon, malathion, bifenthrin, and permethrin.

In addition, concentrations of unionized ammonia were also measured in water and these were compared to LC50s for toxicity to *C. dubia* and *H. azteca*. No unionized ammonia concentrations exceeded these LC50s in any of the samples during this study.

Sediment toxicity thresholds and sediment quality guideline values used to assess the sediment chemistry data are provided in Table 3. In addition, concentrations of unionized ammonia were also measured in sediment overlying water and these were compared to the LC50 for toxicity to *H. azteca*. No unionized ammonia concentrations exceeded this LC50s in any of the samples during this study.

Table 2. Water chemistry evaluation thresholds. LC50 indicates median lethal concentration. CMC indicates criterion maximum concentration (1- hour average not to be exceeded more than once in a three year period). CCC indicates criterion continuous concentration (96-hour average not to be exceeded more than once in a three year period).

Chemical	Endpoint/Criterion	Concentration (ng/L)	Reference
Organophosphates			
Chlorpyrifos	*C. dubia* LC50	53	(Bailey et al., 1997)
	H. azteca LC50	86	(Phipps et al., 1995)
	CMC	83	Squirt Water Quality Criteria
	CCC	41	Squirt Water Quality Criteria
	CMC	25	Santa Maria Numeric Target
	CCC	15	Santa Maria Numeric Target
Diazinon	*C. dubia* LC50	320	(Bailey et al., 1997)
	H. azteca LC50	6,510	(Ankley et al., 1995)
	CMC	100	Squirt Water Quality Criteria
	CCC	100	Squirt Water Quality Criteria
	CMC	160	Santa Maria Numeric Target
	CCC	100	Santa Maria Numeric Target
Dichlorvos	*C. dubia* LC50	130	(Ankley et al., 1991a)
Malathion	*C. dubia* LC50	2120	(Ankley et al., 1991a)
	CCC	100	Squirt Water Quality Criteria
Pyrethroids			
Bifenthrin	*C. dubia* LC50	142	(Wheelock et al., 2004)
	H. azteca EC50	3.3	(Weston and Jackson, 2009)
	H. azteca LC50	9.3	(Anderson et al., 2006a)
Cyfluthrin	*C. dubia* LC50	344	(Wheelock et al., 2004)
	H. azteca EC50	1.9	(Weston and Jackson, 2009)
Cypermethrin	*C. dubia* LC50	683	(Wheelock et al., 2004)
	H. azteca EC50	1.7	(Weston and Jackson, 2009)
Lambda Cyhalothrin	*C. dubia* LC50	200	(Wheelock et al., 2004)
	H. azteca EC50	2.3	(Weston and Jackson, 2009)
Permethrin	*C. dubia* LC50	250	(Wheelock et al., 2004)
	H. azteca LC50	21.1	(Anderson et al., 2006a)
Organochlorines			
pp DDT	*H. azteca* LC50	70	(Phipps et al., 1995)
pp DDD	*H. azteca* LC50	170	(Phipps et al., 1995)
pp DDE	*H. azteca* LC50	1390	(Phipps et al., 1995)
Dieldrin	*H. azteca* LC50	7600	(Phipps et al., 1995)

Table 3. Sediment chemistry evaluation thresholds. LC50 indicates median lethal concentration. TEL indicates threshold effects level. PEL indicates probable effects level. PEC indicates probable effects concentration.

Chemical	ng/g	µg/g oc	Endpoint	Reference
Organophosphates				
Chlorpyrifos	399	1.77	LC50	(Brown et al., 1997; Amweg and Weston, 2007)
Pyrethroids				
Bifenthrin	12.9	0.52	LC50	(Amweg et al., 2005)
Cyfluthrin	13.7	1.08	LC50	(Amweg et al., 2005)
Cypermethrin	14.87	0.38	LC50	(Maund et al., 2002) mean value
Esfenvalerate	41.8	1.54	LC50	(Amweg et al., 2005)
Lambda-Cyhalothrin	5.6	0.45	LC50	(Amweg et al., 2005)
Permethrin	200.7	10.83	LC50	(Amweg et al., 2005)
Organochlorines				
Dieldrin		2000	Mean LC50	(USEPA, 2003)
	2.85		TEL	Squirt Water Quality Criteria
	6.67		PEL	Squirt Water Quality Criteria
Total Chlordane	17.6		PEC	(Macdonald, 2000)
	4.5		TEL	Squirt Water Quality Criteria
	8.9		PEL	Squirt Water Quality Criteria
Total DDT	572		PEC	(Macdonald, 2000)
	11000	367	LC50	(Nebeker et al., 1989) 3% TOC
	49700	473	LC50	(Nebeker et al., 1989) 10.5% TOC
		2580	LC50	(Swartz et al., 1994)
	6.98		TEL	Squirt Water Quality Criteria
	4450		PEL	Squirt Water Quality Criteria
DDD		1300	LC50	Predicted in Weston et al. 1994 (Amweg et al., 2005)
4,4'-DDD	3.54		TEL	Squirt Water Quality Criteria
	8.51		PEL	Squirt Water Quality Criteria
DDE		8300	LC50	Predicted in Weston et al. 1994
4,4'-DDE	1.42		TEL	Squirt Water Quality Criteria
	6.75		PEL	Squirt Water Quality Criteria

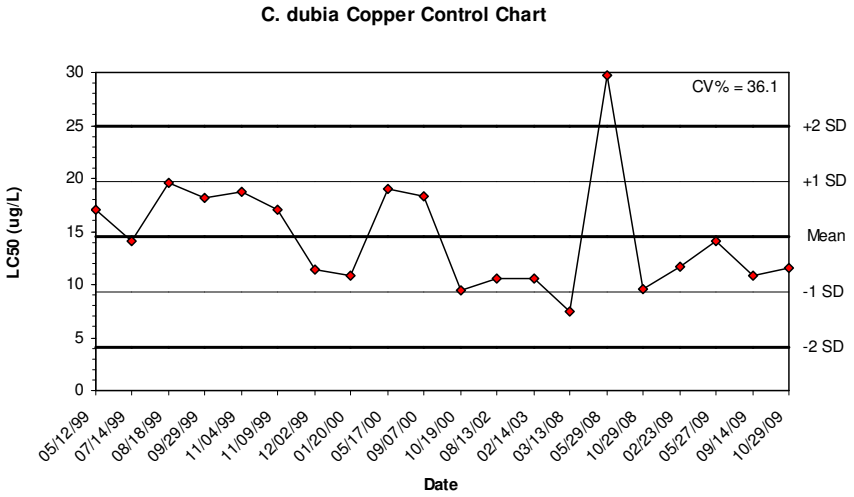
Results and Discussion

Quality Assurance

Toxicity

All toxicity test controls had acceptable survival (>90%) based on the criteria set forth in the U.S. EPA protocols. Toxicity testing precision was evaluated with reference toxicant tests and with field duplicates. Concurrent reference toxicant tests were evaluated in relation to past test performance. Reference toxicant tests were conducted using the standard protocol on a dilution series of copper for *C. dubia* and cadmium for *H. azteca*. Both the *C. dubia* and *H. azteca* responses, measured as LC50s, were within the control chart confidence limits (Figure 8), indicating that test organisms responded to the toxicant in a manner consistent with previous tests.

Eighteen field duplicates were tested for water toxicity (nine with *H. azteca* and nine with *C. dubia*), and six field duplicate was tested for sediment toxicity. Three samples had relative percent differences (RPDs) greater than 30%. Two of these samples and their duplicates exhibited a high magnitude of toxicity. The third sample was moderately toxic, but its duplicate was not. All other water sample RPDs were less than 30%. All sediment RPDs were less than 10% with the exception of one sample from the Santa Maria River estuary. The sample was not significantly different from the control but the duplicate was. This difference was likely do to sample heterogeneity in the field.



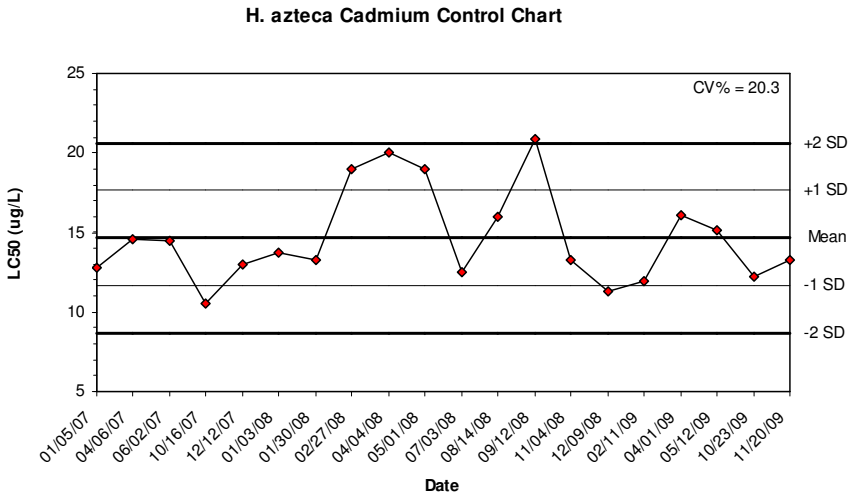


Figure 8. *C. dubia* and *H. azteca* reference toxicant control charts.

Water Chemistry

Field blanks analyzed with all water samples did not contain any detectable levels of pesticides. Mean recoveries of surrogates in surface water samples were 89 $\pm$ 12% and 95 $\pm$ 10% for ^{13}C-atrazine and ^{13}C-diazinon, respectively. Twelve matrix spiked samples were analyzed and recoveries for all compounds ranged from 75 to 120%. Of the compounds detected in the eight replicate samples collected, relative standard deviations ranged from 0.4 to 23.5%. The relative standard deviation for four matrix spiked replicate samples were less than 25% and ranged from 0.3 to 22%. Mean recoveries of the 35 compounds in NWQL 2033 (National Water Quality Laboratory, Denver, CO) added to sample water as a certified reference material, were 94 $\pm$ 13.8%.

Sediment and Tissue Chemistry

Laboratory blanks consisting of approximately 5g baked sodium sulfate (Na_2SO_4) carried through the extraction and cleanup steps did not contain any detectable levels of pesticides. Final method recoveries in spiked sediment ranged from 72 to 124%, whereas final method recoveries in spiked fish and sand crab tissues ranged from 78 to 95%. Mean recoveries of surrogates in the environmental samples were 91 $\pm$ 13%, 96 $\pm$12% and 91 $\pm$ 11% for ^{13}C-trifluralin, *p,p*'- DDE and cis-permethrin, respectively. Of the compounds detected in the 8 laboratory replicate samples, relative standard deviations ranged from 0.1 to 25%. The relative

standard deviations for four matrix spiked replicate samples ranged from 0 to 25%. Mean recoveries (± standard deviation) of *p,p-* DDD and *p,p-* DDE in SRM 1941b (Organics in marine sediment, NIST, Gaithersburg, MD) way were 93 ± 5.6 and 103 ± 7.6%, respectively.

Pajaro River estuary and Tributaries

Water Toxicity Testing

Water samples collected in the lower Pajaro River estuary were often toxic to the amphipod *H. azteca* (Table 4). Fifty-five percent of irrigation season samples collected in the estuary were toxic to amphipods, while 25% of the storm water samples were toxic. The majority of toxicity was observed in water from the lower estuary. Of the three tributaries monitored, only water from the Pajaro River at Thurwachter Bridge was toxic to *H. azteca*, and toxicity was observed on only one occasion. Two of the estuary samples were toxic to *C. dubia* (Table 4). Toxicity to *C. dubia* was observed on two occasions in samples from the Monterey Drainage Ditch and from Watsonville Slough. No clear spatial or temporal relationships between tributary and estuary toxicity in these results. This study lacked synoptic toxicity testing with both species at all of the tributary stations. For example, because a greater proportion of the water samples were toxic to *H. azteca*, testing of all the tributaries with this species might have helped track toxicity from these sources. *H. azteca* is considerably more sensitive than *C. dubia* to pyrethroid pesticides and because of the increased use of this class of pesticide in agriculture and urban watersheds, this species is being recommended for increased use as a water toxicity monitoring species.

The relationships between pesticides measured in water and water toxicity to *H. azteca* and *C. dubia* was less clear in the Pajaro estuary samples than those from the Salinas and Santa Maria estuaries. As described above, sum toxic units (TUs) were calculated for chemicals based on their respective LC50s for toxicity to the two test species. The TUs were then used to help account for toxicity in the samples from the three estuaries and their tributaries. In the Pajaro River watershed, the toxicity of 41 samples was assessed using *H. azteca*. Of these, ten samples were toxic (24%), but toxicity of only three of these samples could be explained by sum TUs (Table 5). In this analysis, a threshold of 0.5 sum TUs was used as a cutoff to assess the potential for toxicity since this is calculated to be equal to the LC25 for each chemical (1 TU = chemical concentration at the LC50, 0.5 TU = chemical concentration at the LC25). Seven of

the samples from the Pajaro River estuary were toxic to *H. azteca* but had sum TUs less than 0.5. Toxicity of these samples remains unexplained based on analyses of water chemistry. In May 2008, there were toxic concentrations of bifenthrin in water from the upper and lower Pajaro River estuary, but neither sample was toxic to *H. azteca*. In February 2009 there were sufficient TUs of bifenthrin in water samples from the upper estuary, the Monterey Drainage Ditch, and Watsonville Slough to account for the observed *H. azteca* mortality. The measured pesticide concentrations reflect both dissolved and particle bound phases in these water samples. Therefore, observances of low mortality in samples having pesticide concentrations that exceeded known toxicity thresholds are likely due to factors influencing bioavailability, such as dissolved organic carbon. Dissolved organic carbon was not measured in these samples, so it is not possible to determine the degree to which this constituent may have influenced bifenthrin bioavailability.

Twenty-seven samples were tested for toxicity to *C. dubia* in the Pajaro River watershed, and of these, four samples were significantly toxic (15%). The toxicity of these samples could be explained by sum TUs (Table 5). Many of the samples had detected concentrations of diazinon, chlorpyrifos and malathion. In February 2009 there were sufficient concentrations of diazinon and or chlorpyrifos to account for *C. dubia* mortality in samples from the Monterey Drainage Ditch and Watsonville Slough. Concentrations of malathion in October 2009 were sufficient to account for *C. dubia* mortality at the same stations.

Table 4. Mean percent survival (standard deviation) of amphipods *H. azteca* and cladocerans *C. dubia* in Pajaro River upper and lower estuary and tributary samples. Grey shading indicates survival significantly lower than the controls.

	Run 1 Storm 1 1/7/08	Run 2 Storm 2 2/26/08	Run 3 Irrigation 4/4/08	Run 4 Irrigation 4/30/08	Run 5 Irrigation 5/30/08	Run 6 Irrigation 7/3/08	Run 7 Irrigation 8/14/08	Run 8 Irrigation 9/12/08	Run 9 Irrigation 10/10/08	Run 10 Storm 3 2/9/09	Run 11 Irrigation 9/10/09	Run 12 Irrigation 9/24/09	Run 13 Irrigation 10/16/09	Run 14 Irrigation 10/23/09	Run 15 Storm 4 10/30/09
H. azteca	Percent Survival (standard deviation)														
Pajaro Upper	66 (18)	92 (8)	94 (9)	90 (7)	92 (5)	84 (15)	94 (9)	82 (13)	94 (5)	16 (15)	50 (38) [1]	70 (37) [1]	46 (11)	100 (0)	96 (6)
Pajaro Lower	86 (9)	88 (8)	94 (6)	90 (10)	75 (13) [1]	36 (23) [1]	0 (0)	34 (27) [1]	25 (29)	82 (15)	34 (38) [2]	70 (34[2]	82 (8)	82 (8) [1]	80 (24) [1]
Monterey Ditch													0 (0)		
Thurwachter						92 (8)						8 (8) [3]		73 (15) [2]	82 (24) [2]
Watsonville	84 (6)					78 (23)						90 (0) [4]	0 (0)	98 (5)	82 (11)
C. dubia															
Pajaro Upper	100 (0)	80 (25)		92 (11)						84 (17)			88 (11)		
Pajaro Lower	96 (9)	88 (11)								88 (27)			92 (11)		
Monterey Ditch	100 (0)	92 (11)		100 (0)		100 (0)				44 (33)		100 (0)	0 (0)	92 (11)	100 (0)
Thurwachter	96 (9)	96 (9)		100 (0)						100 (0)			96 (9)		
Watsonville		100 (0)		96 (8)						20 (45)			0 (0)		
Dilutions					[1]90%	[1] Bad Cond. Cont.		[1]60%			[1]69% [2]50%	[1]85% [2]54% [3]54% [4]66%		[1]40% [2]75%	[1]75% [2]75%
Notes															

Table 5. Relationships between water toxicity to *H. azteca* and *C. dubia* and major detected chemicals in samples from the Pajaro River estuary and its tributaries. Toxicity is mean percent survival (standard deviation). Shading indicates toxic sample, sum TUs > 0.5, and chemicals that exceed either the *H. azteca* or *C. dubia* LC50. Sum TUs for *H. azteca* were calculated by adding individual TUs from chlorpyrifos, diazinon, bifenthrin, permethrin, and the DDT metabolites. Sum TUs for *C. dubia* were calculated by adding individual TUs from chlorpyrifos, diazinon, malathion, bifenthrin, and permethrin.

Station	Run	*H. azteca* % Surv.	Toxic Units	*C. dubia* % Surv.	Toxic Units	Chlorpyrifos	Diazinon	Malathion	Bifenthrin	Fenpropathrin	Permethrin	Fipronil	p p' DDE	p p' DDD	p p' DDT
Pajaro Upper	1	66 (33)	0.02	100 (0)	0.46		149						2.50		
Pajaro Lower	1	86 (9)	0.02	96 (9)	0.42		133						2.15		
Monterey Ditch	1			100 (0)	0.05		16.6						9.8		
Thurwachter	1			96 (9)	0.49		156					4.8			
Watsonville	1	84 (6)	0.01				25.4						13.2		
Pajaro Upper	2	92 (8)	0.02	80 (25)	0.41		131					1.8	2.8		
Pajaro Lower	2	88 (8)	0.02	88 (11)	0.28		90.2					1.4	2.6		
Monterey Ditch	2			92 (11)	0.30	7.0	54.0						12.4	4.4	9.2
Thurwachter	2			96 (9)	0.68		219					1.2	2.2		
Watsonville	2			100 (0)	0.20		64.0						8.8		
Pajaro Upper	3	94 (9)	0.24			18.6	9.6						30.4		
Pajaro Lower	3	94 (6)	0.43			33.8	46.4	12.4					40.0		
Pajaro Upper	4	90 (7)	0.01	92 (11)	0.09		30.2						1.0		
Pajaro Lower	4	88 (8)	0										0.6		
Monterey Ditch	4			100 (0)	0.05		13.4				1.0		4.6	2.6	
Thurwachter	4			100 (0)	0.10		27				4.4		0.4		
Watsonville	4			97 (8)	0.08		25.1						1.8		
Pajaro Upper	5	92 (5)	2.01				35.8		12.0		15.0		3.4		
Pajaro Lower	5	75 (13)	1.21			7.4	28.2	16.8	8.8		3.4		18.4		
Pajaro Upper	6	84 (15)	0				15.8								
Pajaro Lower	6	36 (23)	0												
Monterey Ditch	6			100 (0)	0.03		10.6						3.1	2.0	
Thurwachter	6	92 (8)	0				16.2								
Watsonville	6	78 (23)	0										1.0		
Pajaro Upper	7	94 (9)	0				30.2								
Pajaro Lower	7	0 (0)	0												

Station	Run	*H. azteca* % Surv.	Toxic Units	*C. dubia* % Surv.	Toxic Units	Chlorpyrifos	Diazinon	Malathion	Bifenthrin	Fenpropathrin	Permethrin	Fipronil	p p' DDE	p p' DDD	p p' DDT
Pajaro Upper	8	82 (13)	0.01				9.2						7.0		
Pajaro Lower	8	34 (27)	0												
Pajaro Upper	9	94 (5)	0												
Pajaro Lower	9	25 (29)	0												
Pajaro Upper	10	16 (15)	0.03	84 (17)	0.18	2.0	44.6						3.6		
Pajaro Lower	10	82 (15)	0.06	88 (27)	0.29	4.0	61.4	43.2					3.6		
Monterey Ditch	10			44 (33)	0.60	3.2	160	77.0					29.2		
Thurwachter	10			100 (0)	0.14	1.8	32.6						4.2		
Watsonville	10			20 (45)	1.45	31.0	270	36.0					8.6		
Pajaro Upper	11	50 (38)	0												
Pajaro Lower	11	34 (38)	0												
Pajaro Upper	12	70 (37)	0												
Pajaro Lower	12	70 (34)	0												
Monterey Ditch	12			100 (0)	0								3.0		
Thurwachter	12	8 (8)	0												
Watsonville	12	90 (0)	0												
Pajaro Upper	13	46 (11)	1.90	88 (11)	0.24			357	9.6				58.8		57.5
Pajaro Lower	13	82 (8)	0.48	92 (11)	0.10			213					36.3		31.6
Monterey Ditch	13	0 (0)	3.98	0 (0)	1.35	11.2		2019	25.4	49.8	3.0	4	75.2	11.8	59.8
Thurwachter	13			96 (9)	0.32			510	11.0				92.8	18.5	97.6
Watsonville	13	0 (0)	1.44	0 (0)	2.66			5462	12.3				23.3		7.1
Pajaro Upper	14	100 (0)	0				15.5								
Pajaro Lower	14	82 (8)	0												
Monterey Ditch	14			92 (11)	0								7.1		
Thurwachter	14	73 (15)	0												
Watsonville	14	98 (5)	0										6.0		
Pajaro Upper	15	96 (6)	0												
Pajaro Lower	15	80 (24)	0												
Monterey Ditch	15			100 (0)	0								4.2		
Thurwachter	15	82 (24)	0												
Watsonville	15	82 (11)	0										1.6		

Upper Pajaro estuary Water TIE

A TIE was conducted on water collected from the upper Pajaro River estuary in February 2009. The magnitude of toxicity was assessed with *H. azteca* using a dilution series of the sample, and a percent sample LC50 was calculated. The dilutions were 0 (control water), 10%, 25%, 50%, and 100% upper estuary water. Based on the LC50, toxic units in the baseline (untreated) sample were calculated. Note that the sample toxicity LC50 and corresponding TU calculations differ from the chemical toxic units discussed above. The TIE treatments may either lower or increase sample TUs relative to the untreated sample and these changes in toxicity provide evidence of the cause of toxicity.

The untreated upper estuary sample contained 1.7 TUs, and amphipod survival in 100% (undiluted) sample was 13% (Table 6). The sample was subjected to a number of TIE treatments using methods described above, and several treatments reduced toxicity. Toxicity was reduced by treating the sample with Amberlite, a carbonaceous resin that reduces bioavailability of organic chemicals (<1TU). In addition, toxicity was reduced by centrifuging the sample to remove particles and particle-bound chemicals (<1 TU). Toxicity was also reduced by passing the sample through an HLB SPE column (<1 TU). Like Amberlite, the HLB column removes organic chemicals. The column was eluted with acetone and the acetone was then added to clean water to create an eluate treatment. This treatment determines whether chemicals bound to the column are recovered in toxic amounts. The HLB eluate was toxic (1.5 TUs), indicating that organic chemicals were responsible for toxicity of this sample. In addition to these treatments, the sample was subjected to additional steps that are specific for identifying toxicity due to pyrethroid pesticides. Addition of a carboxylesterase enzyme reduced toxicity (<1 TU), but addition of BSA did not. The enzyme hydrolyzes the ester bond in pyrethroid pesticides and toxicity reduction with enzyme addition provides evidence of pyrethroid toxicity. These results support the conclusion that toxicity was caused by a pyrethroid by allowing differentiation between reductions of toxicity due to ester hydrolysis, versus toxicity reduction due to sorption of pesticides to the protein base of the enzyme. There was also a large increase in amphipod mortality with the addition of the metabolic inhibitor PBO (14.3 TUs). Increased toxicity with PBO addition provides additional evidence of pyrethroid toxicity. To provide an additional line of evidence, the Amberlite resin was eluted with acetone, and the solvent was added to clean

water to make an eluate treatment. The resin eluate was toxic and amphipod survival was 27% (data not shown in Table 6). A separate sample of the eluate was analyzed for the presence of organophosphate and pyrethroid pesticides. No organophosphate pesticides were detected in the eluate using ELISA. No pesticides were detected in the eluate using GC/MS. This sample had been subjected to a liquid-liquid extraction prior to GC analysis as a dehydration step, and it is possible chemicals were lost during this process. Previous experience with acetone eluates containing pyrethroids have shown that direct injection of the eluate into the GC yields better recovery (Anderson et al., 2010). This is particularly important when they are at concentrations on the low end of the toxic range.

The evidence from this TIE suggests that amphipod mortality in the upper Pajaro estuary was caused by a pyrethroid pesticide. As discussed above, no pyrethroids were detected in this water sample, but this may have been due to filtering the analytical sample with a glass fiber filter prior to GC/MS analysis. As discussed below, bifenthrin was detected at a toxic concentration in sediment from the Monterey Drainage Ditch, and at lower concentrations in sediments from three estuary stations (Table 8). This confirms the presence of this pyrethroid in the lower estuary and one of its key tributaries.

Table 6. Mean percent survival (standard deviation) of *H. azteca* from a TIE using water collected from the Upper Pajaro estuary. Toxic units are based on the LC50 of the treatment dilution series. Detected chemicals were measured by GC/MS.

	Mean Percent (SD) Survival					Toxic	Detected	Concentration	LC50
Treatment	Control	10%	25%	50%	100%	Units	Chemicals	ng/L	ng/L
Baseline	93 (12)	70 (17)	67 (23)	63 (15)	13 (23)	1.7	Chlorpyrifos	2	86
Amberlite	100 (0)	100 (0)	93 (12)	87 (12)	60 (53)	<1	Diazinon	44.6	6510
Centrifuge	87 (12)	80 (20)	87 (23)	93 (12)	93 (12)	<1			
HLB	100 (0)	87 (23)	73 (12)	100 (0)	80 (20)	<1			
HLB Eluate	100 (0)	93 (12)	80 (0)	67 (12)	27 (12)	1.5			
Enzyme	93 (12)	87 (12)	67 (31)	93 (12)	60 (53)	<1			
BSA	100 (0)	100 (0)	100 (0)	87 (12)	0 (0)	1.6			
PBO (D4)	93 (12)	27 (31)	0 (0)	0 (0)	0 (0)	14.3			

Sediment Toxicity

Ten of 24 sediment samples collected from 8 stations over 16 months in the Pajaro River estuary were toxic to amphipods *H. azteca* (42%). The majority of sediment toxicity was observed in the

October 2008 and October 2009 sampling events; only one station was toxic in June 2008 (Table 7). The magnitude of sediment toxicity was generally low, and survival ranged from 44% to 75% in samples that were significantly toxic. Amphipod growth results are listed in Table A1. While a number of organophosphate, organochlorine, and pyrethroid pesticides were detected in sediments from the Pajaro River estuary and its tributaries, only the pyrethroid pesticide bifenthrin was detected at concentrations that could partly account for the observed toxicity (Table 8). No metals were detected at concentrations exceeding established toxicity thresholds (Table A2). Organic carbon-corrected toxic units of bifenthrin were calculated. Sediment collected at Pajaro River estuary Station 7 contained 0.144 TUs bifenthrin in May 2008, but this sample was not toxic. Sediment from Stations 5 and 6 contained approximately 0.25 TU of bifenthrin in Oct 2009. Both of these samples were toxic. Sediment from Station 3 contained 0.07 ng/g bifenthrin in October 2008 (0.07 TUs). While this sample was significantly toxic, the concentration of bifenthrin measured in this sample did not appear to be sufficient to account for the observed mortality. No toxicity was observed in the two lowest estuary stations (stations 1 and 2) or at the highest estuary station (station 8).

Toxicity was observed in five of the nine sediments collected from the Pajaro River tributaries (56%). As with the estuary stations, the magnitude of toxicity was relatively low in these samples, with the exception of the October 2009 Monterey Drainage Ditch sample (0% survival). This sample contained 1.049 TUs of bifenthrin and fenpropathrin, which was sufficient to account for the observed amphipod mortality (Table 7).

Table 7. Mean percent survival (standard deviation) and organic carbon-corrected toxic unit (TU) sums for Pajaro River sediment tests. The chemicals driving the sum TU are listed for sum TU > 0.1. Bif = bifenthrin and Fen = fenpropathrin. Shading indicates significant toxicity or sum TU values > 0.5.

	Jun-08			Oct-08			Oct-09		
Station	Survival Mean	OC-Corrected Sum TU	Sum TU Chemicals	Survival Mean	OC-Corrected Sum TU	Sum TU Chemicals	Survival Mean	OC-Corrected Sum TU	Sum TU Chemicals
Pajaro 1	86 (20)			98 (5)			94 (9)		
Pajaro 2	91 (11)	0.001		95 (8)	0.003		93 (5)	0.001	
Pajaro 3	44 (29)			48 (30)	0.065		71 (15)		
Pajaro 4	80 (17)			73 (15)	0.009		81 (11)	0.001	
Pajaro 5	81 (14)	0.001		75 (15)			56 (14)	0.209	Bif
Pajaro 6	94 (7)	0.001		60 (20)	0.004		66 (14)	0.320	Bif
Pajaro 7	85 (25)	0.056		53 (21)	0.005		60 (21)	0.003	
Pajaro 8	99 (4)	0.003		100 (0)	0.001		89 (10)		
MDD	75 (32)	0.028		84 (7)	0.001		0 (0)	1.049	Bif, Fen
Thurwachter	73 (28)	0.003		70 (35)	0.001		85 (13)	0.028	
Watsonville	41 (17)			91 (6)	0.006		89 (14)	0.002	

Table 8. Concentrations of total organic carbon (%) and detected organic chemicals (ng/g) in Pajaro River estuary and tributary sediments.

June 2008	**TOC (%)**	Chlorpyrifos	Bifenthrin	Fenpropathrin	Permethrin	p p' DDD	p p' DDE	p p' DDT	Oxyfluorfen	Napropamide	3,5 DCA	Carbaryl	Prometryn	Trifluralin
Pajaro 1	0.2													
Pajaro 2	1.01					1.7		0.7						
Pajaro 3	3.71					2.3		0.0						
Pajaro 4	3.77					4.3		1.1						
Pajaro 5	6.54					9.3		4.1						
Pajaro 6	0.85					2.9		1.5						
Pajaro 7	6.55		1.9			23.3		12.2						
Pajaro 8	0.56					3.7		2.8						
MDD	2.97					46.2	234	30.0						
Thurwachter	1.45					2.8	11.8							
Watsonville	6.75					2.9			13.5	99.8				
October 2008														
Pajaro 1	0.52						0.4						2.8	
Pajaro 2	2.07					4.9	15.5							
Pajaro 3	2.07		0.7						1.9	17.7		11.7		
Pajaro 4	2.72					8.2	82.2							
Pajaro 5	1.2									7.6				
Pajaro 6	1.75					3.7	19.2							
Pajaro 7	2.57					8.9	30.5	4.7			22.7			0.2
Pajaro 8	0.54					1.1			2.7	7.8	2.6			
MDD	1.66					7.8			22.6	83.5		5.8		
Thurwachter	2.91					2.1	9.5				4.8			
Watsonville	2.68					4.3	56.6				19.4			
October 2009														
Pajaro 1	0.52						0.7							
Pajaro 2	1.17					1.0	3.4	1.4						
Pajaro 3	4.65						8.0							
Pajaro 4	5.24					2.8	23.8							
Pajaro 5	3.02		3.2			4.7	22.8	6.2		11.6				
Pajaro 6	2.22		3.6			4.9	19.1	7.6		0.9				
Pajaro 7	4.86					8.3	30.1	8.8						
Pajaro 8	3.91													
MDD	2.35	0.7	10.4	29.0	2.7	35.2	200	52.3	27.45					
Thurwachter	0.81					12.3	37.9	32.9						
Watsonville	4.74					2.6	24.9							

Pesticides in Sand Crabs

Ovigerous crabs (*Emerita analoga*) were collected in the surf zone at three stations per estuary during August 2008, similar to the collection period of a previous study (Dugan et al., 2005). The most widespread group of pesticides detected in sand crab tissue from the three estuaries was the DDTs. These chemicals have been banned in the US since 1970, but are highly persistent. DDE, the primary degradation product of DDT was detected in every sample with concentrations ranging from 121 to 1754 ng/g lipid weight. In sand crab samples from the Pajaro River estuary only DDE was detected with concentrations ranging from 235 to 348 ng/g lipid weight (Table 9). Note: Total DDT is comprised solely of pp DDE in these samples). These results suggest that comparable concentrations of DDTs persist and are biologically available decades after their use was banned.

Table 9. Concentrations (in ng/g lipid weight) of pesticides detected in sand crabs collected in August 2008 from the Pajaro River estuary mouth. All sand crab samples were homogenates of 50 gravid and non-gravid female sand crabs collected from 3 stations. The north and south stations were 50m in either direction from the mouth of the estuary. ND indicates non-detect.

Location	% Lipid	Azoxystrobin	Bifenthrin	Boscalid	Chlorpyrifos	Cyfluthrin	Diazinon	Pyraclostrobin	Σ DDTs
Mouth	3.45	ND	ND	ND	ND	ND	ND	ND	348
North	3.72	ND	ND	ND	ND	ND	ND	ND	256
South	3.51	ND	ND	ND	ND	ND	ND	ND	235

Pesticides in Fish Tissue

In addition to DDTs, two fungicides and an herbicide were detected in fish collected from the Pajaro estuary. These include azoxystrobin and pyraclostrobin, and the herbicide boscalid (Table 10). Concentrations of current-use fungicides and herbicides have not been measured or reported previously in central coast estuaries, but DDT and it degradation compounds have been detected in previous samples from this estuary, as part of the Toxic Substances Monitoring program. Current use organophosphate and pyrethroid pesticides were not detected in fish during the current project (Table 10).

Table 10. Concentrations (in ng/g lipid weight) of pesticides detected in fish collected in October 2008 from the Pajaro River estuary. ND indicates non-detect.

Species	ID #	% lipid	Azoxystrobin	Bifenthrin	Boscalid	Chlorpyrifos	Cyfluthrin	Diazinon	Pyraclostrobin	Σ DDTs
Smelt	1-5	4.61	19.0	ND	25.1	ND	ND	ND	70.6	1523
Smelt	6-10	5.7	ND	ND	ND	ND	ND	ND	ND	1347
Scuplin	11-15	7.69	13.3	ND	ND	ND	ND	ND	28.9	1025
Starry flounder	19	2.51	72.1	ND	ND	ND	ND	ND	33.4	6219

Benthic Community Characterization

The benthic community structure at four of the five stations sampled for benthos in the Pajaro River estuary exhibited a high magnitude of stress in May 2008 (Table 11). All but Station 2 had extremely low RBI scores. Station 2 samples had a slightly higher RBI score but were still considered moderately affected. Most of the stations were described as severely affected based on the presence of negative indicator species, and the complete absence of positive indicator species. In addition, these samples had few taxa, and relatively low abundances relative to the uncontaminated stations from Habitat E areas described in Ranasinghe et al. (in press).

The majority of samples had relatively large numbers of amphipods (*Americorophium sp.*), compared to all other taxa, and the number of species counted in these samples ranged from 8 to 17. Abundances ranged from 335 to 543 individuals. The amphipods *Americorophium stimpsoni, A. spinicorne* and *Eogammarus confervicolus* were all found in the Pajaro River. These species are common in the Habitat E assemblages described in Ranasinghe et al. (in press). None of the positive indicators used in the current study (*Tellina modesta, Grandifoxus grandis, Eohaustorius estuarius*) were found in the Pajaro estuary samples, and none of these were listed as abundant in Habitat E assemblages in Ranasinghe et al. (in press). The amphipod *E. estuarius* is considered rare in central coast estuaries (personal communication, Jim Oakden, Moss Landing Marine Laboratories), and its absence may not be indicative of pollution impacts. There is little pollution tolerance information for the three most common amphipod species found in these samples (*Americorophium stimpsoni, A. spinicorne* and *Eogammarus confervicolus).* Amphipod species from the genus *Americorophium* are found to occur at the least impacted stations in the San Francisco estuary, and were listed as sensitive taxa in tidal freshwater habitats in Thompson et al. (2010). It should be noted that there is much

disagreement among west coast benthic ecologists about indicator taxa in mesohaline and tidal freshwater habitats (Thompson et al., 2010).

Benthic conditions were also highly degraded during November 2009 sampling at all Pajaro estuary stations. No macroinvertebrates were found at station 4. In addition to the absence of positive indicator species in these samples, some contained the negative indicator *Capitella*. These samples also had few taxa, low abundances, and few molluscan and crustacean taxa and individuals. Ranasinghe et al. (in press) found that samples from uncontaminated Habitat E stations had an average of 15.9 taxa per sample, higher than all but one of the Pajaro River estuary samples in the current study. Although all Pajaro River estuary stations were described as highly impacted based on the RBI, a relatively high magnitude of sediment toxicity was observed at only two of the stations with degraded benthos, stations 3 and 5.

Table 11. Benthic community indices for five Pajaro River estuary stations monitored in May and November 2008. Relative Benthic Index (RBI) is scaled from 0 (most degraded) to 1 (least degraded).

Station	Number Taxa	Abundance	Number Mollusc Taxa	Number Crustacea Taxa	Number Crustacea	Number Amphipod Taxa	Number *Capitella*	Number Oligochaeta	Number *Tellina*	Number *Grandifoxus*	Number *Eohaustorius*	RBI Score
May 2008												
Pajaro 1	8	339	1	4	300	2	0	35	0	0	0	0.08
Pajaro 2	17	379	2	4	274	3	0	0	0	0	0	0.18
Pajaro 3	10	531	1	4	80	3	0	23	0	0	0	0.09
Pajaro 4	11	543	1	4	139	4	0	31	0	0	0	0.10
Pajaro 5	13	335	1	5	117	3	1	89	0	0	0	0.10
November 2008												
Pajaro 1	8	68	1	3	5	1	0	1	0	0	0	0.07
Pajaro 2	5	274	1	0	0	0	27	2	0	0	0	-0.03
Pajaro 3	4	39	0	0	0	0	22	1	0	0	0	-0.06
Pajaro 4	0	0	0	0	0	0	0	0	0	0	0	NC
Pajaro 5	5	23	2	1	4	1	1	0	0	0	0	0.04

Instantaneous Pesticide Loading

Discharge measurements were collected for the Monterey Drainage Ditch tributary during four storm events and four times during the irrigation season, when sufficient flow allowed. Discharge was measured at the Thurwachter tributary during three winter storm events as flow allowed. Discharge measurements during the two 2008 storms were similar for both sites indicating that the magnitude of the storms were similar. As expected, the magnitude of the storm events influenced the instantaneous dissolved pesticide loads entering the Pajaro River estuary. Even though flows in the tributaries were similar between the two storms sampled in 2008, the pesticide loads were higher in the first storm (2491 g/d) compared to the second storm (1330 g/d). The storm sampled in January 2008 was thus considered the first flush and therefore the mass of pesticides entering the estuaries were higher compared to the February 2008 storm (Appendix, Table A3). Diazinon was one of the most frequently detected organophosphate pesticides in water, and diazinon loading at the Thurwachter site for the 2008 storm events was 289 and 385 g/d (Table A3). The river conveyed the majority of the dissolved pesticide load to the estuary during the storm events. Loading from the Monterey Drainage Ditch was an order of magnitude lower for most of the compounds detected (Table A3). This pattern was true for all storm events accept for the one sampled in October of 2009. This storm was sampled during the end of the growing season, when pesticides are being actively applied to the fields, and yielded a much higher pesticide load particularly for this smaller tributary. In this case, total pesticide loads from Monterey Drainage Ditch were three times higher than from Thurwachter (Table A3). Also loading of boscalid, a frequently detected fungicide, was about five times higher in Monterey Drainage Ditch (2292 g/d) compared to Thurwachter (467 g/d). Loads of other pesticides and fungicides (malathion and azoxystrobin) were similar at the two sites.

In 2008, inputs of the most frequently detected pesticides associated with suspended sediments was much lower compared to the 2009 winter and summer storm events (Table A3). Although the flows were higher in 2008, the concentration of suspended sediments was lower, particularly at the Thurwachter site (Table A3) compared to both 2009 storms. Total suspended sediment pesticide loads from the 2009 storms were approximately an order of magnitude higher

compared to 2008. The highest loads of total DDTs to the estuary were observed during the October 2009 storm (Table A3).

Salinas River estuary and Tributaries

Water Toxicity Testing

Intermittent toxicity to amphipods was observed in water samples from the Salinas River estuary and its tributaries (Table 12). Two of the four storm water samples from the upper estuary were toxic to *H. azteca* and one of four storm water samples were toxic in the lower estuary. Only one of the eleven irrigation season water samples was toxic to amphipods in the estuary. In addition, one tributary water sample from Davis Road and one from Blanco Drain was toxic to amphipods. The cause of toxicity to four of these samples remains unaccounted for based on analysis of the summed chemical toxic units. These include a sample from the Blanco Drain in April 2008, a sample from the upper Salinas estuary in February 2009, a sample from the lower Salinas estuary in September 2009, and one from the Salinas River at Davis Road in October 2009. Of the 35 water samples tested for toxicity with *H. azteca*, six were significantly toxic (17%). The toxicity of two of these samples could be explained by sum TUs (TU>0.5). When TUs accounted for water toxicity to *H. azteca*, this was usually due to chlorpyrifos, for example in the upper and lower Salinas River estuary in February 2008 (Table 13).

The single estuary water sample that was tested with *C. dubia* was toxic (February 2009). A greater proportion of samples from the Blanco Drain were toxic to this species. Three of the four storm water samples from the Blanco Drain were toxic to *C. dubia*, and five of nine irrigation and storm water samples from this site were toxic. Only one of the nine samples from the Davis Road station was toxic to *C. dubia*. When there were sufficient chemical TUs to account for water toxicity to *C. dubia,* this was usually due to mixtures of chlorpyrifos and diazinon (Blanco Drain in January and February 2008 and the upper Salinas estuary in February 2009). Toxicity of Blanco Drain water in October 2009 was accounted for by sum TUs of chlorpyrifos and malathion (Table 13).

Table 12. Mean percent survival (standard deviation) of amphipods *H. azteca* and cladocerans *C. dubia* in upper and lower Salinas River estuary and tributary samples. Grey shading indicates survival significantly lower than the controls.

	Run 1 Storm 1 1/7/08	Run 2 Storm 2 2/26/08	Run 3 Irrigation 4/4/08	Run 4 Irrigation 4/30/08	Run 5 Irrigation 5/30/08	Run 6 Irrigation 7/3/08	Run 7 Irrigation 8/14/08	Run 8 Irrigation 9/12/08	Run 9 Irrigation 10/10/08	Run 10 Storm 3 2/9/09	Run 11 Irrigation 9/10/09	Run 12 Irrigation 9/24/09	Run 13 Irrigation 10/16/09	Run 14 Irrigation 10/23/09	Run 15 Storm 4 10/30/09
H. azteca	Percent Survival (Standard Deviation)														
Salinas Upper	92 (8)	2 (5)	92 (8)	94 (9)	96 (9)	82 (13)	94 (6)	92 (13)	96 (9)	48 (15)	94 (6)	98 (5)	96 (6)	98 (5)	96 (6)
Salinas Lower	78 (5)[1]	18 (8)	74 (17)	90 (10)[1]	80 (16)[1]	92 (5)	92 (8)	82 (13)	94 (6)	92 (8)	10 (12)	92 (5)	96 (6)	96 (6)	96 (6)
Blanco	96 (6)	83 (21)		14 (11)						76 (6)					
Davis													0 (0)		
C. dubia															
Salinas Upper										24 (22)					
Salinas Lower															
Blanco	60 (25)	0 (0)		80 (14)		100 (0)				4 (9)		88 (18)	28 (27)	68 (23)	92 (18)
Davis	80 (14)	92 (11)		92 (11)		96 (9)				92 (11)		100 (0)	96 (9)	0 (0)	100 (0)
Dilutions	[1]50%			[1]45%	[1]60%										

Table 13. Relationships between water toxicity to *H. azteca* and *C. dubia* and major detected chemicals in samples from the Salinas River estuary and its tributaries. Toxicity is mean percent survival (standard deviation). Shading indicates toxic sample, TUs > 0.5, and chemicals that exceed either the *H. azteca* or *C. dubia* LC50. Sum TUs for *H. azteca* were calculated by adding individual TUs from chlorpyrifos, diazinon, bifenthrin, permethrin, and the DDT metabolites. Sum TUs for *C. d*ubia were calculated by adding individual TUs from chlorpyrifos, diazinon, malathion, bifenthrin, and permethrin.

Station	Run	*H. azteca* % Surv.	Toxic Units	*C. dubia* % Surv	Toxic Units	Chlorpyrifos	Diazinon	Malathion	Bifenthrin	Permethrin	Piperonyl Butoxide	Fipronil	p p' DDE	p p' DDD	p p' DDT
Salinas Upper	1	92 (8)	0												
Salinas Lower	1	78 (5)	0												
Blanco	1	96 (6)	0.16	60 (25)	0.55	11.6	105						7.40		
Davis	1			80 (14)	0.23	8.20	25.6						1.20		
Salinas Upper	2	2 (5)	0.86			70.6	216						3.6		
Salinas Lower	2	18 (18)	0.57			48.2	48.6						2.2		
Blanco	2	83 (21)	0.25	0 (0)	0.66	14.6	123						8.4	5.4	1.6
Davis	2			92 (11)	0.72	28.0	59.8						6.8		
Salinas Upper	3	92 (8)	0.25			21.2									
Salinas Lower	3	74 (17)	0.45			38.6	13.4								
Salinas Upper	4	94 (9)	0.77				72.8		3.6	7.8			1.8		
Salinas Lower	4	90 (10)	0												
Blanco	4	14 (11)	0.03	80 (14)	0.06		19.2						5.2	4.3	
Davis	4			92 (11)	0.13		36.4			4.4					
Salinas Upper	5	96 (9)	0				13.4								
Salinas Lower	5	80 (16)	0				16.6								
Salinas Upper	6	82 (13)	0				25.8								
Salinas Lower	6	92 (5)	0				11.8								
Blanco	6			100 (0)	0.27		84.9						3.7	1.6	
Davis	6			96 (9)	0.02		7.8								
Salinas Upper	7	94 (6)	0				16.8								
Salinas Lower	7	92 (8)	0												
Salinas Upper	8	92 (13)	0				18.4								
Salinas Lower	8	82 (13)	0												
Salinas Upper	9	96 (9)	0				18.4								
Salinas Lower	9	94 (6)	0				14.7								

Station	Run	*H. azteca* % Surv.	Toxic Units	*C. dubia* % Surv	Toxic Units	Chlorpyrifos	Diazinon	Malathion	Bifenthrin	Permethrin	Piperonyl Butoxide	Fipronil	p p' DDE	p p' DDD	p p' DDT
Salinas Upper	10	48 (15)	0.25	24 (22)	0.71	19.6	109					9.2	1.2		
Salinas Lower	10	92 (8)	0.03			1.8	59.6						0.6		
Blanco	10	76 (6)	0.10	4 (9)	0.19	8.0	13.8						12.6		
Davis	10			92 (11)	0.10	5.1							0.42		
Salinas Upper	11	94 (6)	0												
Salinas Lower	11	10 (12)	0												
Salinas Upper	12	98 (5)	0				10.9								
Salinas Lower	12	92 (5)	0												
Blanco	12			88 (18)	0.04	0.8	8.0						3.4		
Davis	12			100 (0)	0										
Salinas Upper	13	96 (6)	0					21.2					6.6		
Salinas Lower	13	96 (6)	0												
Blanco	13			28 (27)	0.77	5.2		1064	22.4	3.2	60.8		25.0		10.6
Davis	13	0 (0)	0.33	96 (9)	0.03					6.9			1.8		
Salinas Upper	14	98 (5)	0												
Salinas Lower	14	96 (5)	0												
Blanco	14			68 (23)	0.07	3.7							4.7		
Davis	14			0 (0)	0							0.96			
Salinas Upper	15	96 (6)	0												
Salinas Lower	15	96 (6)	0												
Blanco	15			92 (18)	0								2.4		
Davis	15			100 (0)	0										

Sediment Toxicity

As was observed with the water toxicity testing, little sediment toxicity was observed in the Salinas River estuary during this study. Only three of the 24 samples collected from the eight estuary stations were toxic to *H. azteca*, and the magnitude of toxicity was low in the samples demonstrating statistically significant amphipod mortality (Table 14). Amphipod growth data is presented in Table A1. Fifty percent of the tributary sediment samples were toxic to amphipods. Higher magnitude sediment toxicity was observed in samples from the Salinas River at Davis Road in October 2008 and October 2009 and in one sediment sample from the Blanco Road station in October, 2009. These were also the stations where intermittent water toxicity was observed.

While a number of legacy and current-use pesticides, herbicides, and fungicides were detected in sediments from the Salinas River estuary and its tributaries, none of these chemicals were present at sufficient concentrations to account for the observed toxicity (Table 15). A number of pyrethroids were detected in Blanco Drain sediment in October 2008. The combined toxic units of bifenthrin, cypermethrin and cyhalothrin in this sample accounted for 0.728 TUs, and this is sufficient to explain the low amphipod survival observed in this sample (Table 14). The pyrethroid pesticide permethrin and the organophosphate pesticide chlorpyrifos were detected in sediments from Davis Road and the Blanco Drain in October 2009 (Table 15). Bifenthrin was also detected at Blanco Drain in October 2009. The combination of bifenthrin, permethrin, and chlorpyrifos in the Davis Road sample amounted to 0.630 TUs (Table 14). This could partially explain the low amphipod survival in this sample (34% survival). Permethrin was also detected in sediments from the Salinas River estuary stations 4 and 5 in May 2008, and stations 2 and 6, in October 2009. All permethrin detections were below toxicity thresholds for *H. azteca.*

Table 14. Mean percent survival (standard deviation) and organic carbon-corrected toxic unit (TU) sums for Salinas River sediment tests. The chemicals driving the sum TU are listed for sum TU > 0.1. Bif = bifenthrin, Chl = chlorpyrifos, Cyh = cyhalothrin, Cyp, = cypermethrin. Shading indicates significant toxicity or sum TU values > 0.5.

	Jun-08			Oct-08			Oct-09		
Station	Survival Mean	OC-Corrected Sum TU	Sum TU Chemicals	Survival Mean	OC-Corrected Sum TU	Sum TU Chemicals	Survival Mean	OC-Corrected um TU	Sum TU Chemicals
Salinas 1	95 (11)			89 (10)			79 (33)		
Salinas 2	88 (10)	0.002		98 (5)			98 (5)	0.078	
Salinas 3	91 (4)			95 (5)	0.001		89 (14)		
Salinas 4	79 (11)	0.010		96 (5)			81 (16)		
Salinas 5	91 (11)	0.038		98 (5)			86 (15)		
Salinas 6	73 (20)	0.001		95 (5)			90 (8)	0.003	
Salinas 7	63 (19)	0.001		83 (16)			85 (23)		
Salinas 8	94 (7)	0.002		91 (6)			76 (23)		
Blanco	89 (6)	0.001		29 (36)	0.724	Bif, Cyh, Cyp	35 (34)	0.210	Bif
Davis	96 (7)	0.028		94 (5)			34 (18)	0.630	Chl

Table 15. Concentrations of total organic carbon (%) and detected organic chemicals (ng/g) in Salinas River estuary and tributary sediments.

June 2008	**TOC (%)**	Chlorpyrifos	Malathion	Bifenthrin	Cyhalothrin	Cypermethrin	Permethrin	p p' DDD	p p' DDE	p p' DDT	Fipronil Sulfide	Dacthal	3,5 DCA	Trifluralin	Oxyfluorfen	Napropamide
Salinas 1	0.1															
Salinas 2	0.87							1.9	3.9			0.4				
Salinas 3	1.55								2.5			0.7				
Salinas 4	0.43						0.3		1.1		0.2	0.3		0.12		
Salinas 5	0.71						2.9		1.5							
Salinas 6	0.42								1.0							
Salinas 7	0.67								1.8	0.7		0.3				
Salinas 8	0.34								2.1			0.4				
Blanco	2.77							47.7	205	30.0		2.3				
Davis	2.27								5.1	1.5						
October 2008																
Salinas 1	0.32															
Salinas 2	1.96								2.1							
Salinas 3	1.05		8.4						3.9				3.2			
Salinas 4	2.74								4.4			0.7				
Salinas 5	0.59															
Salinas 6	1.49															1.4
Salinas 7	0.46															2.0
Salinas 8	0.51							4.2	8.1	0.4						
Blanco	3.2	1.5		4.7	1.7	3.3		57.8	216	27.7		2.2			13.5	
Davis	1.35								1.5							
October 2009																
Salinas 1	0.14															
Salinas 2	1.11	1.2					1.8	1.7	7.6	0.9		0.7				
Salinas 3	1.21															
Salinas 4	2.74								1.3							
Salinas 5	2.58								3.0							
Salinas 6	0.87						0.3		2.8							
Salinas 7	1.06								2.0							
Salinas 8	0.96							4.9	8.7	6.4		0.2				
Blanco	3.85	5.0		2.1			7.1	33.9	137	31.1		4.2			15.7	
Davis	0.46	4.3					4.8	1.5	12.1	6.8		4.3				

Pesticides in Sand Crab Tissue

Of the fungicides and pesticides analyzed in sand crabs from the Salinas River estuary mouth, only DDTs were detected in these samples (Table 16). As with the Pajaro River estuary sand crabs, the total DDT concentrations in these tissues are accounted for by the primary degradate p'p DDE. Total DDT concentrations in Salinas River estuary sand crabs were comparable to concentrations measured in sand crabs from the Santa Maria estuary mouth, and higher than those from the Pajaro River estuary.

Table 16. Concentrations (in ng/g lipid weight) of selected pesticides detected in sand crabs collected in August 2008 from the Salinas River estuary mouth. All sand crab samples were homogenates of 50 gravid and non-gravis female sand crabs collected from 3 stations. The north and south stations were 50m in either direction from the mouth of the estuary.

Location	% Lipid	Azoxystrobin	Bifenthrin	Boscalid	Chlorpyrifos	Cyfluthrin	Diazinon	Pyraclostrobin	Σ DDTs
Mouth	4.42	ND	ND	ND	ND	ND	ND	ND	413
North	2.34	ND	ND	ND	ND	ND	ND	ND	964
South	4.69	ND	ND	ND	ND	ND	ND	ND	121

Pesticides in Fish Tissue

In addition to DDT, two fungicides, azoxystrobin and pyraclostrobin, were the most frequently detected current-use pesticides detected in fish from the Salinas River estuary (Table 17.). Concentrations of pyraclostrobin ranged from non-detect to 453 ng/g lipid weight. As was observed in fish from the Pajaro River estuary, no organophosphate or pyrethroid pesticides were detected in fish from the Salinas River estuary.

Table 17. Concentrations (in ng/g lipid weight) of selected pesticides detected in fish collected in October 2008 from the Salinas River estuary.

Species	ID #	% lipid	Azoxystrobin	Bifenthrin	Boscalid	Chlorpyrifos	Cyfluthrin	Diazinon	Pyraclostrobin	Σ DDTs
Croaker	27-31	3.13	ND	ND	ND	ND	ND	ND	ND	803
Sculpin	32-36	1.05	83.4	ND	197	ND	ND	ND	329	989
Starry flounder	37	0.53	216	ND	ND	ND	ND	ND	434	7412
Starry flounder	38	1.21	ND	ND	ND	ND	ND	ND	ND	5390
Starry flounder	39	1.13	277	ND	49.0	ND	ND	ND	453	5644

Benthic Community Characterization

All five Salinas River estuary stations sampled for benthic community structure were classified as severely affected (RBI category 4) in both the May and November sampling periods (Table 18). The number of species found ranged from 5 to 9, and 3 to 9, in the May and November samples, respectively. While there were relatively few species in these samples, there were higher abundances of animals than were found in the Pajaro River estuary samples, particularly in the May samples where the densities ranged from 343 to 3,989 individuals. Fewer animals were found in the November samples (range = 20 to 795 individuals). Only negative indicator species were found in these samples (*Capitella sp.* and oligochaetes), no positive indicators present. The dominant species present during both sampling periods were amphipods such as *Americorophium sp.* and *Eogammarus confervicolus*. These are species whose sensitivity to pollution is unknown, but as described above, are known to occur at unimpacted stations in the San Francisco estuary. As development and validation of benthic indices proceed with analysis of more coastal wetland and estuarine habitats (Habitat E in Ranasinghe et al., in press), determination of the specific stressor tolerances of species common to these habitats should be evaluated. Based on the relative abundances of the amphipods *Americorophium stimpsoni, A. spinicorne* and *Eogammarus confervicolus,* it would be useful to determine which, if any, of these amphipods should be categorized as indicator species for Habitat E assemblages. Thompson et al. (2010) noted wide disagreements among benthic ecologists on appropriate indicator taxa in mesohaline and tidal freshwater habitats, but listed list the two *Americorophium* species found in the Salinas River estuary as sensitive indicator species.

Table 18. Benthic community indices for five Salinas River estuary stations monitored in May and November 2008. Relative Benthic Index (RBI) is scaled from 0 (most impacted) to 1 (least impacted).

Station	Number Taxa	Abundance	Number Mollusc Taxa	Number Crustacea Taxa	Number Crustacea	Number Amphipod Taxa	Number *Capitella*	Number Oligochaeta	Number *Tellina*	Number *Grandifoxus*	Number *Eohaustorius*	RBI Score
May 2008												
Salinas 1	5	343	0	3	338	3	0	3	0	0	0	0.03
Salinas 2	12	3989	0	3	3925	3	1	13	0	0	0	0.04
Salinas 3	8	758	3	3	745	3	0	1	0	0	0	0.12
Salinas 4	9	3719	0	5	3680	4	0	20	0	0	0	0.09
Salinas 5	7	1574	0	5	1541	3	0	7	0	0	0	0.07
November 2008												
Salinas 1	6	795	0	4	787	3	0	0	0	0	0	0.08
Salinas 2	3	20	0	3	20	3	0	0	0	0	0	0.04
Salinas 3	7	437	0	6	431	3	0	0	0	0	0	0.11
Salinas 4	4	42	0	2	37	2	0	0	0	0	0	0.03
Salinas 5	9	610	0	5	591	3	0	4	0	0	0	0.08

Instantaneous Pesticide Loading

Similar to the trend observed in the Pajaro River, the highest dissolved pesticide loads occurred during the storm events, particularly in the larger tributaries when the flows were higher (Appendix, Table A4). In the Salinas River the highest loads of both chlorpyrifos and diazinon were detected in the winter of 2008 during the first two storms. During the remainder of the year, particularly during the irrigation season, organophosphate pesticide loads to the estuary were low and less than 0.5 g/d (Table A4). The October 2009 storm was not large enough to increase the flow in the Salinas River; however it did increase flow in Blanco Drain. During this storm a large pulse of malathion and myclobutanil moved through the system (Table A4) where dissolved loads of these chemicals went from non-detect to 39 g/d and 49 g/d, respectively.

Suspended sediment pesticide loads in the Salinas River estuary were lower than those of the Pajaro River estuary during all storm events even though the suspended sediment concentrations were similar. Of the current-use pesticides detected, chlorpyrifos had the highest loads compared to all other compounds including the pyrethroids. Overall, suspended sediment pesticide loads in the Salinas River were low during all storm events indicating very little input of pesticides associated with suspended sediments to the estuary even during high flow events.

Santa Maria estuary and Orcutt Creek Tributary

Water Toxicity Testing

A high incidence of water toxicity was observed in the upper and lower Santa Maria River estuary stations (Table 19). At the upper estuary station, nine of the eleven (82%) irrigation season water samples, and two of the four storm season water samples were toxic to *H. azteca*. In addition, 36% of the irrigation season water samples from the lower estuary station, and 50% of the storm season samples were toxic to *H. azteca*. Toxicity to *H. azteca* was also observed in two of the three Orcutt Creek water samples, and 95% of the water samples tested with *C. dubia* were significantly toxic (Table 19).

Elevated chlorpyrifos concentrations accounted for water toxicity to *C. dubia* and *H. azteca* in the majority of samples in Orcutt Creek and in the Santa Maria River estuary (Table 20). The LC50s for chlorpyrifos toxicity to *H. azteca* and *C. dubia* are 86 ng/L and 53 ng/L, respectively (Phipps et al., 1995; Bailey et al., 1997). Many of the samples had very high concentrations of diazinon, which contributed to toxicity to *C. dubia* (LC50 = 320 ng/L; Bailey et al. 1997). The diazinon concentrations in these water samples likely had a negligible contribution to observed mortality of *H. azteca* (LC50 = 6,510 ng/L; (Ankley and Collyard, 1995)). Summed chemical TUs in Orcutt Creek ranged from 3 to 12.6 in samples toxic to *H. azteca*, and were always greater than 0.47 TUs. Sum TUs in the upper estuary ranged from 0.56 to 6.44 in the samples toxic to *H. azteca*. Sum TUs in the lower estuary ranged from 0.55 to 4.51 in the samples toxic to *H. azteca* (Table 20).

In no cases were there sufficient concentrations of pyrethroids in water to account for *H. azteca* mortality. In a concurrent study in 2008 and 2009, five pyrethroid pesticides were detected in Orcutt Creek water samples and in samples from the lower Santa Maria River (Station 312SMA; (Phillips et al., 2010). Station 312SMA is approximately 100 meters east of the upper estuary station sampled in the current study. Concentrations of two pyrethroids, cyhalothrin and cypermethrin, were at or above water toxicity thresholds for *H. azteca* in the concurrent study (Phillips et al., 2010).

While the majority of samples from Orcutt Creek and the Santa Maria River estuary had sufficient chlorpyrifos to account for the observed *C. dubia* mortality, many of the samples also had toxic concentrations of diazinon (Table 20). Sum TUs in Orcutt Creek ranged from 0.45 to 7.9 in the samples toxic to *C. dubia*. Sum TUs ranged as high as 5.40 and 4.77, respectively, in the upper and lower estuary samples toxic to *C. dubia*.

Table 19. Mean percent survival (standard deviation) of amphipods *H. azteca* and cladocerans *C. dubia* in Santa Maria River upper and lower estuary and tributary samples. Grey shading indicates survival significantly lower than the controls.

	Run 1 Storm 1 1/7/08	Run 2 Storm 2 2/26/08	Run 3 Irrigation 4/4/08	Run 4 Irrigation 4/30/08	Run 5 Irrigation 5/30/08	Run 6 Irrigation 7/3/08	Run 7 Irrigation 8/14/08	Run 8 Irrigation 9/12/08	Run 9 Irrigation 10/10/08	Run 10 Storm 3 2/9/09	Run 11 Irrigation 9/10/09	Run 12 Irrigation 9/24/09	Run 13 Irrigation 10/16/09	Run 14 Irrigation 10/23/09	Run 15 Storm 4 10/30/09
H. azteca	Percent Survival (Standard Deviation)														
Santa Maria Up	96 (6)	68 (18)	88 (8)	58 (11)	82 (8)	5 (12)	0 (0)	2 (5)	26 (15)	0 (0)	16 (6)	0 (0)	0 (0)	0 (0)	0 (0)
Santa Maria Low	88 (5)	88 (11)	88 (5)	64 (6)	96 (9)	84 (5)	76 (6)	11 (16)	92 (8)	0 (0)	98 (4)	100 (0)	0 (0)	8 (8)	0 (0)
Orcutt				88 (5)						0 (0)			0 (0)		
C. dubia															
Santa Maria Up	96 (9)	12 (18)		0 (0)		0 (0)		0 (0)			0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Santa Maria Low				0 (0)								0 (0)			0 (0)
Orcutt	0 (0)	0 (0)		0 (0)		0 (0)							0 (0)	0 (0)	0 (0)

Table 20. Relationships between water toxicity to *H. azteca* and *C. dubia* and major detected chemicals in samples from the Santa Maria River estuary and its tributaries. Toxicity is mean percent survival (standard deviation). Shading indicates toxic sample, TUs > 0.5, and chemicals that exceed either the *H. azteca* or *C. dubia* LC50. Sum TUs for *H. azteca* were calculated by adding individual TUs from chlorpyrifos, diazinon, bifenthrin, permethrin, and the DDT metabolites. Sum TUs for *C. dubia* were calculated by adding individual TUs from chlorpyrifos, diazinon, malathion, and permethrin.

Station	Run	*H. azteca* % Surv.	Toxic Units	*C. dubia* % Surv	Toxic Units	Chlorpyrifos	Diazinon	Malathion	Permethrin	Fipronil	Fipronil Sulfide	Fipronil Sulfone	p p' DDE	p p' DDD	p p' DDT
SM Upper	1	96 (6)	0	96 (9)	0										
SM Lower	1	88 (5)	0												
Orcutt	1			0 (0)	1.58	57.4	145	84.0					7.20		
SM Upper	2	68 (18)	0.23	12 (18)	0.45	18.8	30.6			3.2	5.4	8.2	5.6		
SM Lower	2	88 (11)	0.12			9.6	8.20						3.4		
Orcutt	2			0 (0)	0.63	24.4	53.8						1.8		
SM Upper	3	88 (8)	0.20			10.0		32.4					110		
SM Lower	3	88 (5)	0.32			20.0		75.6					122		

Station	Run	*H. azteca* % Surv.	Toxic Units	*C. dubia* % Surv	Toxic Units	Chlorpyrifos	Diazinon	Malathion	Permethrin	Fipronil	Fipronil Sulfide	Fipronil Sulfone	p p' DDE	p p' DDD	p p' DDT
SM Upper	4	58 (11)	0.60	0 (0)	2.16	42.2	432	16.0	0.8				2.8		
SM Lower	4	64 (6)	0.55	0 (0)	2.93	38.1	691	101					1.9		
Orcutt	4	88 (5)	0.47	0 (0)	2.08	34.2	458	10.2					2.6		
SM Upper	5	82 (8)	0.42			29.4	524	168					2.8		
SM Lower	5	96 (9)	0.21			11.2	500								
SM Upper	6	5 (12)	0.56	0 (0)	2.67	39.0	584	223					3.6	1.8	
SM Lower	6	84 (5)	0.24			16.4	321	110					1.8		
Orcutt	6			0 (0)	3.05	26.8	616	1308					1.6		
SM Upper	7	0 (0)	2.54			218	56.8						4.4		
SM Lower	7	76 (6)	0.65			53.4	189								
SM Upper	8	2 (5)	1.76	0 (0)	3.18	150	110	13.0					3.2		
SM Lower	8	11 (16)	1.03			87.4	99						3.2		
SM Upper	9	26 (15)	0.78			66.6	22.6	22.0					3.2		
SM Lower	9	92 (8)	0.27			23.0	43.9								
SM Upper	10	0 (0)	6.44			552	163	232					6.0		
SM Lower	10	0 (0)	4.51			386	112	47.6					6.4		
Orcutt	10	0 (0)	12.63			1082	282	401					8.0		
SM Upper	11	16 (6)	0.87	0 (0)	1.42	75.0							2.7		
SM Lower	11	98 (4)	0.08			6.6									
SM Upper	12	0 (0)	1.54	0 (0)	2.54	132	15.6						7.5		
SM Lower	12	100 (0)	0.26			21.7	55.6								
Orcutt	12			0 (0)	2.85	149	15.4						14.0		3.4
SM Upper	13	0 (0)	2.15	0 (0)	3.33	168	34.3	118					43.9	6.7	8.8
SM Lower	13	0 (0)	1.36			101	32.7	120					32.2		11.4
Orcutt	13	0 (0)	3.07	0 (0)	4.04	206	31.8	88.0	4.9				82.0	14.7	20.8
SM Upper	14	0 (0)	1.54	0 (0)	2.49	131	5.7	11.7					26.9		
SM Lower	14	8	1.11			95.1	0.0	42.7							
Orcutt	14			0 (0)	2.82	148	5.3	20.8					18.0		4.7
SM Upper	15	0	3.02	0	5.40	235	304	40.2					47.2		14.4
SM Lower	15	0	2.33	0	4.77	195.6	337	50.6					4.2		
Orcutt	15			0	7.90	276	858	32.3					20.2		

Santa Maria Water TIEs

Two TIEs were conducted on water samples from the Santa Maria estuary, one from the upper estuary, and one from the lower estuary. Both were tested with *H. azteca* in February 2009 (Table 21). The upper estuary was significantly toxic at all dilutions tested. There was 0% survival in the 100% sample, and 47% survival in the 10% sample dilution. This sample contained 10.7 TUs. Toxicity was reduced to 3.7 TUs with the addition of Amberlite resin, and there was 0% survival in the resin eluate treatment. These results suggest toxicity due to an organic chemical. Toxicity was also partially reduced with centrifugation (5.1 TUs) and was completely removed with HLB extraction (<1 TU). The HLB eluate was also toxic (2.8 TUs). These results corroborate the Amberlite results and also suggest an organic toxicant. Toxicity of the upper estuary water was reduced with addition of the carboxylesterase enzyme (3.7 TUs), but less so with the addition of BSA (6.3 TUs). These results suggest toxicity was partly caused by a pyrethroid pesticide. Toxicity of this sample was greatly increased with addition of the metabolic inhibitor PBO, which also suggests toxicity due to a pyrethroid. No pyrethroids were detected in this water sample, or in the Amberlite eluate treatment.

The lower estuary sample was less toxic than the upper estuary sample, but was significantly toxic at all but the 10% dilution (Table 21). There was 0% survival in the 100% sample, and 27% survival in the 25% sample dilution. This sample contained 5.1 TUs. Toxicity was reduced to 2.6 TUs with Amberlite treatment. These results suggest toxicity due to an organic chemical. Toxicity was also partially reduced with centrifugation (2.1 TUs) and was completely removed with HLB extraction (<1 TU). The HLB eluate was moderately toxic (1.3 TUs). These results also suggest an organic toxicant. Toxicity of the lower estuary water was reduced with addition of the carboxylesterase enzyme (2.4 TUs), and less so with the addition of BSA (4.0 TUs). These results suggest toxicity was partly caused by a pyrethroid pesticide. However, toxicity of this sample was not increased with addition of PBO. No pyrethroids were detected in this water sample. Reasons for the lack of detections of pyrethroids in water from the Santa Maria estuary and the Orcutt Creek tributary likely include sample processing procedures (sample filtration and solid-phase extraction rather than liquid-liquid extraction) and detection limits above toxicity thresholds for pyrethroids. Detection limits for pyrethroids have been shown to be lower using GC/MS/MS, instead of GC/MS (Hladik et al., 2008).

Table 21. Mean percent survival (standard deviation) of *H. azteca* from a TIE using water collected from the Upper Santa Maria River estuary (a) and the Lower Santa Maria River estuary (b). Toxic units are based on the LC50 of the treatment dilution series. Detected chemicals were measured by GC/MS.

(a) Upper estuary	Mean Percent (SD) Survival					Toxic	ELISA Chlor.	Other Detected	Concentration	LC50
Treatment	Control	10%	25%	50%	100%	Units	ng/L	Chemicals	ng/L	ng/L
Baseline	100 (0)	47 (42)	0 (0)	0 (0)	0 (0)	10.7	934	Chlorpyrifos	552	86
Amberlite	100 (0)	93 (12)	67 (12)	0 (0)	0 (0)	3.7	NA	Diazinon	153	6510
Centrifuge	100 (0)	93 (12)	33 (23)	0 (0)	0 (0)	5.1	569	Malathion	232	
HLB	93 (12)	93 (12)	93 (12)	100 (0)	93 (12)	<1	ND			
HLB Eluate	93 (12)	80 (20)	80 (0)	13 (23)	0 (0)	2.8	526			
Enzyme	94 (10)	88 (11)	67 (31)	0 (0)	0 (0)	3.7	826			
BSA	93 (12)	100 (0)	0 (0)	0 (0)	0 (0)	6.3	863			
PBO	93 (12)	20 (35)	0 (0)	0 (0)	0 (0)	15.6	934			

(b) Lower estuary	Mean Percent (SD) Survival					Toxic	ELISA Chlor.	Other Detected	Concentration	LC50
Treatment	Control	10%	25%	50%	100%	Units	ng/L	Chemicals	ng/L	ng/L
Baseline	93 (12)	100 (0)	27 (12)	0 (0)	0 (0)	5.1	597	Chlorpyrifos	385	86
Amberlite	100 (0)	87 (12)	100 (0)	20 (20)	0 (0)	2.6	NA	Diazinon	112	6510
Centrifuge	87 (12)	100 (0)	100 (0)	40 (35)	0 (0)	2.1	326	Malathion	47.6	
HLB	93 (12)	93 (12)	100 (0)	100 (0)	87 (12)	<1	ND			
HLB Eluate	93 (12)	100 (0)	100 (0)	93 (12)	27 (23)	1.3	156			
Enzyme	87 (23)	100 (0)	100 (0)	20 (0)	0 (0)	2.4	646			
BSA	100 (0)	87 (12)	60 (40)	0 (0)	0 (0)	4.0	668			
PBO	80 (20)	80 (20)	27 (46)	7 (12)	0 (0)	4.7	692			

Sediment Toxicity

As was observed with water toxicity testing, a relatively high frequency of sediment toxicity was observed in samples from the Santa Maria estuary during this study. Eleven of 24 sediment samples collected from June 2008 to October 2009 were toxic to amphipods *H. azteca* (46%, Table 22). The highest magnitude of toxicity was observed in samples from the upper estuary stations, reflecting the proximity of these stations to the Orcutt Creek confluence. Sediments from all of the Orcutt Creek samples were highly toxic to amphipods. Moderate toxicity was also observed in October 2009 in samples from Stations 1 and 2, the two stations nearest the mouth if the estuary. Amphipod growth data is presented in Table A1.

Analysis of the sediment showed that they were contaminated by complex mixtures of metal and organic chemicals. No metals were detected at concentrations exceeding established toxicity

thresholds (Table A2). A number of organic chemicals were detected, including herbicides, fungicides, and organochlorine, organophosphate, pyrethroid and carbamate pesticides (Table 23). Of the eleven estuary samples that were toxic to *H. azteca*, five had sum TU greater than 0.5 and four had sum TU greater than 0.1. The sum TU values were driven by concentrations of chlorpyrifos and several pyrethroids, including bifenthrin, cyhalothrin, and cypermethrin. There were no toxic concentrations of chemicals in the two lower estuary samples that exhibited moderate toxicity. The Orcutt Creek samples had sum TU concentrations ranging from approximately 0.8 to 3.1. These values were driven by concentrations of chlorpyrifos, bifenthrin, cyhalothrin, cypermethrin, and esfenvalerate.

Table 22. Mean percent survival (standard deviation) and organic carbon-corrected toxic unit (TU) sums for Santa Maria River sediment tests. The chemicals driving the sum TU are listed for sum TU > 0.1. Chl = chlorpyrifos, Cyp. = cypermethrin, Cyh = cyhalothrin, Esf = esfenvalerate, Bif = bifenthrin. Shading indicates significant toxicity or sum TU values > 0.5.

	Jun-08			Oct-08			Oct-09		
Station	Survival Mean	OC-Corrected Sum TU	Sum TU Chemicals	Survival Mean	OC-Corrected Sum TU	Sum TU Chemicals	Survival Mean	OC-Corrected um TU	Sum TU Chemicals
Santa Maria 1	93 (9)			89 (9)	0.115	Chl	60 (20)		
Santa Maria 2	100 (0)			85 (6)	0.283	Chl, Bif	66 (21)		
Santa Maria 3	95 (8)	0.001		83 (15)			94 (7)		
Santa Maria 4	86 (9)	0.084		89 (11)	0.279	Chl	100 (0)	0.016	
Santa Maria 5	99 (4)	0.086		54 (32)	0.399	Chl	48 (21)	0.528	Chl
Santa Maria 6	76 (15)	0.798	Chl	21 (17)	0.249	Chl	100 (0)	0.142	Chl
Santa Maria 7	94 (12)	0.305	Chl	11 (21)	0.410	Chl	64 (25)	0.150	Chl
Santa Maria 8	41 (19)	1.387	Chl, Cyp	14 (23)	1.575	Chl, Cyh, Cyp	0 (0)	2.581	Chl, Bif, Cyh
Orcutt Creek	4 (5)	0.816	Chl, Cyh	0 (0)	3.141	Chl, Cyh, Cyp, Esf	0 (0)	1.943	Chl, Cyh

Table 23. Concentrations of total organic carbon (%) and detected organic chemicals (ng/g) in Santa Maria River estuary and tributary sediments.

June 2008	TOC (%)	Chlorpyrifos	Diazinon	Malathion	Phosmet	Bifenthrin	Cyfluthrin	Cyhalothrin	Cypermethrin	Danitol	Esfenvalerate	Fenvalerate	Permethrin	p p' DDD	p p' DDE	p p' DDT	Fipronil	Fipronil Sulfide	Dacthal	Oxyfluorfen	Napropamide	Prometryn	Propyzamide	Tau-Fluvalinate	Trifluralin	3,5 DCA
Santa Maria 1	0.17														0.2											
Santa Maria 2	0.08																									
Santa Maria 3	1.13													0.5	2.6								3.7			
Santa Maria 4	0.33	0.5													0.7											
Santa Maria 5	1.00	1.4												2.0	10.5	3.5			0.4	2.5						
Santa Maria 6	1.37	18.7											2.5	8.0	31.0	7.3			1.4	9.1						
Santa Maria 7	1.39	7.2											1.0	3.8	14.6	6.0			1.7	11.0						
Santa Maria 8	1.74	23.2	1.2						3.8				8.4	18.8	79.3	36.5			1.6	61.9			14.2			
Orcutt Creek	1.47	14.5	0.9					1.3					7.4	15.0	62.1	28.5			0.7	38.2	7.2	2.1	12.2	1.4	0.2	
October 2008																										
Santa Maria 1	1.12	2.2												1.4	7.6	1.2			0.2			1.2				
Santa Maria 2	2.17	5.0	1.7			1.5							2.4	4.0	12.0	3.4	0.8	1.4	1.1				9.0		0.7	
Santa Maria 3	3.32			4.1	0.2									1.3		0.0				3.5	15.3					
Santa Maria 4	1.97	9.1	1.3										2.0	8.3	47.3	10.1			0.6		3.1	4.6				
Santa Maria 5	1.9	12.7	1.4	2.4									2.4	10.2	54.8	11.7			0.6	14.4	6.4					
Santa Maria 6	2.44	10.1	1.1										2.0	8.3	40.6	10.9			0.5	11.5	4.6		4.1			
Santa Maria 7	1.55	10.8	1.1										0.9	6.9	41.1	6.5				2.7	3.5	0.8				
Santa Maria 8	2.66	23.1						4	6.3		3.7	1.4	6.8	19.8	88.8	23.1			1.1	29.0			1.3		0.2	3.1
Orcutt Creek	1.33	43.7	1.0					2	3.9		3.1	0.9		17.3	80.8	32.7			0.8	60.2	4.2				0.4	
October 2009																										
Santa Maria 1	0.29														0.3											
Santa Maria 2	0.26														0.3											
Santa Maria 3	3.33														1.2	0.5										
Santa Maria 4	1.36	0.4													3.4	1.1						0.4	1.8			
Santa Maria 5	0.16	1.5													0.8											
Santa Maria 6	1.68	4.0												5.5	29.9	4.5			0.6				3.0		0.1	
Santa Maria 7	2.93	7.6												5.2	25.2	6.0			0.8				1.6		0.2	
Santa Maria 8	2.49	27.3				1.3	1.7	18.6	0.7	3.2	1.3	0.6	6.2	10.2	50.8	10.8			1.68	22.8					0.8	
Orcutt Creek	1.76	51.8						2.0					2.1	15.2	68.0	21.1			0.9	43.8				1.9	2.5	

Santa Maria Sediment TIE

A whole-sediment TIE was conducted on composite samples from the upper Santa Maria estuary in December 2008, using samples collected in October 2008. Samples from Stations 6, 7, and 8 were combined to provide sufficient sediment for the TIE. The baseline (untreated) sediment was moderately toxic; amphipod survival was 50% in this sample (Table 24). Survival increased to 96% with the addition of Amberlite. However, survival also increased with the addition of 10% reference sediment (dilution control = 86% survival), so the results do not allow differentiation between dilution of toxicity with the 10% Amberlite from reduction of toxicity through binding of organic chemicals. Amphipod survival increased to 96% with the addition of carboxylesterase enzyme to the sediment overlying water. Addition of BSA also partly reduced sample toxicity (72% survival). The enzyme and BSA results were not significantly different, and do not allow differentiation between reductions of toxicity due to ester hydrolysis by the enzyme, from toxicity reduction due to sorption of pesticides to the protein base of the enzyme. Toxicity of this sample was greatly increased with the addition of PBO to the sediment overlying water. This suggests toxicity was partly caused by a pyrethroid pesticide. This was confirmed by chemical analysis of sediment from Station 8. This sediment contained five pyrethroids and the concentration of cypermethrin exceeded the *H. azteca* LC50 values (Tables 22 and 23). There were 1.575 sum TUs in the Station 8 sediment, and this value was accounted for by a combination of chlorpyrifos, cyhalothrin and cypermethrin. Because the TIE was conducted on a mixture of sediments from Stations 6, 7, and 8, the concentrations of pyrethroids in the mixture used in the TIE likely differed from that in the Station 8 sample. No pyrethroids other than permethrin were detected in sediments from stations 6 and 7, and the concentrations of permethrin were well below its toxicity threshold. Only chlorpyrifos was detected at concentrations that could account for toxicity in the station 6 and 7 samples.

Chemical analysis of Orcutt Creek and Santa Maria estuary sediments support evidence from the TIE and suggest that toxicity was caused by a combination of chlorpyrifos and several pyrethroid pesticides. Based on a comparison of chemicals driving the toxic unit calculations, the majority of toxic sediments contained toxic concentrations of chlorpyrifos, pyrethroids, or mixtures of the two classes of pesticides (Tables 22 and 23). The two pyrethroids accounting for most of the toxicity were cyhalothrin and cypermethrin. These results are consistent with previous studies in

the lower Santa Maria River watershed that have indicated sediment toxicity is caused by these same pesticides (Anderson et al., 2006b; Phillips et al., 2006; Phillips et al., 2010).

Table 24. Mean percent survival (standard deviation) of *H. azteca* in a TIE using sediments composited from Santa Maria estuary stations 6, 7, and 8.

Treatment	Mean Survival (SD)
Sample Baseline	50 (24)
10% Amberlite	96 (5)
Control (10% Amberlite)	96 (5)
Enzyme	96 (9)
Control (Enzyme)	98 (4)
Bovine Serum Albumin (BSA)	90 (8)
Control (BSA)	100 (0)
Piperonyl Butoxide (PBO)	2 (4)
Control (PBO)	96 (5)
Dilution Control	86 (13)
Control	98 (4)

Pesticides in Sand Crab Tissue

Of the three estuaries monitored in this study, the highest concentration of total DDTs were detected in sand crabs collected from the Santa Maria estuary. Concentrations in sand crabs from the Santa Maria estuary mouth ranged from 570 to 1800 ng/g lipid weight, which is similar to what has been detected previously in sand crabs from this estuary (Table 25; Dugan et al., 2005). In 2000, Dugan et al. (2005) examined the relationship of total DDT concentration and distance from the mouth of the estuary. The study examined samples collected 0-900m from the mouth and found a significant negative correlation between total DDT concentration and the distance from the river mouth which suggests a persistent exposure gradient for sand crabs living in the vicinity of the Santa Maria River. In the present study, sand crabs were collected 50m north and south of the mouth and concentrations of total DDTs were similar at the three sites in each estuary. This indicates that sand crabs in the general vicinity of the river mouth are exposed to a relatively constant amount of total DDTs.

Few studies have measured a variety of different current-use pesticides in tissue, particularly sand crabs. In the present study, seven current-use pesticides including four fungicides, two pyrethroids and two organophosphate insecticides were detected in sand crabs collected from the Santa Maria estuary. Many of the pesticides detected in the sand crabs were detected in water and sediment samples collected from the estuary throughout the study. In samples from the Santa Maria the total (summed) amount of current-use pesticides measured was higher than total DDTs (Table 25). Current-use pesticides were only detected in sand crabs collected from the Santa Maria estuary sites. Pyraclostrobin, a strobilurin fungicide used on leafy greens and berries, was detected in all three samples and at the highest concentration (1351 to 2258 ng/g lipid weight) compared to the other pesticides detected including total DDTs. Two other fungicides (azoxystrobin and boscalid) were also detected in the samples at much lower concentrations but were also detected frequently in water samples collected during the study. This is the first report that documents the presence of fungicides in aquatic organisms exposed to a continuous pulse of agricultural chemicals.

Bifenthrin and cyfluthrin were detected in the three sand crab composite samples collected from the Santa Maria estuary. Average concentrations of bifenthrin and cyfluthrin were 12 and 181 ng/g lipid weight, respectively. Pyrethroid concentrations were almost and order of magnitude lower than concentrations measured in crab embryos collected from gravid crabs living in the upper salt marsh in urban/suburban watersheds (Smalling et al., 2010). The use of pyrethroids in agricultural watersheds, particularly those sampled in this study, are relatively low compared to urban and suburban areas (DPR, http://www.cdpr.ca.gov/docs/pur/purmain.htm). However, pyrethroids were detected in bed sediment collected from within the estuary and the detection in the crabs was not surprising indicating the potential for transport of these pesticides out of the estuary.

The organophosphate insecticides, diazinon and chlorpyrifos, were detected in every sand crab sample from the Santa Maria estuary and average concentrations were 201 and 351 ng/g lipid weight. Diazinon was also detected in adult and megalop sand crab samples collected in May 2000 (Dugan et al., 2005), although concentrations in the present study were slightly lower. The use of organophosphates has declined over the last decade which might explain the differences in

concentrations in sand crabs from the Santa Maria estuary. Although, concentrations were lower in 2008 compared to 2000, the continued presence of OP insecticides in crab embryos suggests a fairly constant supply of these pesticides may be available on the beaches near the Santa Maria River.

Table 25. Concentrations (in ng/g lipid weight) of pesticides detected in sand crabs collected in August 2008 from the Santa Maria River estuary mouth. All sand crab samples were homogenates of 50 gravid and non-gravid female sand crabs collected from 3 stations. The north and south stations were 50m in either direction from the mouth of the estuary.

Location	% Lipid	Azoxystrobin	Bifenthrin	Boscalid	Chlorpyrifos	Cyfluthrin	Diazinon	Pyraclostrobin	Σ DDTs
Mouth	3.46	37.5	18.9	34.1	314	198	242	2258	573
North	6.18	13.1	8.5	31.4	302	128	199	1351	932
South	4.95	16.0	8.1	41.2	437	218	162	2251	1800

Pesticides in Fish Tissue

Five to ten fish were collected in each estuary in depending on their size in October of 2008 and an effort was made to collect the same species in each estuary for comparative purposes. Targeted species were starry flounder (*Platichthys stellatus*), staghorn sculpin (*Leptocottus armatus*), and topsmelt (*Atherinops affinis*). In addition to these species, white croaker (*Genyonemus lineatus*) was collected in the Salinas River estuary. Total percent lipid in the fish varied and ranged from 0.5 to 7.7 %. All data was normalized to percent lipid in order to decrease species variability in pesticide concentrations and to compare results with previous studies in California.

Thirteen current-use pesticides as well as DDT and its two major degradation products, DDE and DDD, were detected in fish collected from the three estuaries. DDE was the most frequently detected pesticides and was detected in every sample with concentrations ranging from 400 to 11,000 ng/g lipid weight. Total DDT concentrations were highest in fish collected from the Santa Maria estuary (Table 26) followed by the Salinas and samples from the Pajaro had the lowest average DDE concentrations. These results are similar to previous data collected over the last three decades in the three watersheds (TSMP data). On average fish collected from the Santa Maria estuary had higher concentrations of total DDTs compared to the Salinas and the Pajaro. These results are not surprising because on a land-use and estuary size the Santa Maria is

the smallest watershed and has some of the highest pesticide use. Total DDT concentrations were similar between species and on average the starry flounder had the highest concentrations compared to the other species in the three estuaries. These results are not surprising based on the biomagnification potential of DDT and its degradation products. DDE and to a lesser extent DDD and DDT are known to biomagnify in the environment. The starry flounder are considered to be at a higher trophic position compared to croaker, sculpin and smelt meaning lipid normalized concentrations of hydrophobic contaminants will increase with trophic position. Even though DDT has been banned since 1970 it is ubiquitous contaminant and has been detected in fish worldwide.

In fish monitoring studies very few current-use pesticides are analyzed so there is limited data for comparison particularly in these three estuaries. A study conducted in the Salton Sea in 2001 analyzed several predominant fish species for a limited suite of current-use pesticides (Sapozhnikova et al., 2004). This is one of the first studies to measure these pesticides in different fish tissues particularly in California. Concentrations of current-use pesticides were an order of magnitude lower than many of the legacy pesticides detected in the two different types of fish collected. However, current-use pesticides were detected, have the potential to accumulate in fish tissue and should be included in local/regional monitoring studies. In the present study a variety of different current-use pesticides including five fungicides, two herbicides, five insecticides and one pesticide degradate were detected in whole fish samples. Two fungicides, azoxystrobin and pyraclostrobin, were the most frequently detected current-use pesticides (Table 26) and concentrations of pyraclostrobin ranged from 29 to 1045 ng/g lipid weight. Fungicides are a relatively understudied group of pesticides, particularly in the U.S.; therefore this is the first study that measured a variety of these newer registered fungicides in tissue.

Fish collected from the Santa Maria estuary had the highest number of pesticides detected and concentrations were typically higher than those detected in fish from the Salinas and Pajaro Rivers. The herbicide, DCPA was the only pesticide detected more frequently and at higher concentrations in fish collected from the Salinas compared to the other two estuaries. The organophosphate insecticides, chlorpyrifos and diazinon were detected in all samples collected

from the Santa Maria estuary with concentrations ranging from 22-248 and 45-121 ng/g lipid weight, respectively. The concentrations of the organophosphates detected in the Santa Maria estuary were slightly higher than those detected in fish collected from the Salton Sea (Sapozhnikova et al., 2004). Bifenthrin was the most frequently detected pyrethroid and occurred in all samples collected from the Santa Maria estuary with concentrations ranging from 12 to 41 ng/g lipid weight. In these agricultural watersheds, the pesticides detected most frequently in water and sediment from these estuaries were the ones detected in many of the samples and at some of the highest concentrations. For this reason, particularly in agriculturally dominated watersheds with high pesticide use, certain current-use pesticides should be included in tissue analysis. To date, there is limited information on the effects of these pesticides on higher level aquatic organisms.

This is one of the first data sets to report concentrations of a wide variety of current-use pesticides in fish and sand crabs. Thirteen current-use pesticides as well as DDT and its two degradation products were detected frequently in fish, and seven current-use pesticides and the DDTs were detected in sand crabs. Total DDT concentrations in both fish and sand crab samples were higher than total current-use pesticide concentrations. Contaminant concentrations varied by species and estuary indicating differences in the amount of pesticides available for uptake between the three estuaries. The Santa Maria estuary was the most impacted by elevated pesticide concentrations in water and sediment and fish and sand crab samples corroborated these results.

Table 26. Concentrations (in ng/g lipid weight) of selected pesticides detected in fish collected in October 2008 from the Santa Maria River estuary.

Species	ID #	% lipid	Azoxystrobin	Bifenthrin	Boscalid	Chlorpyrifos	Cyfluthrin	Diazinon	Pyraclostrobin	Σ DDTs
Starry flounder	20-21	2.66	512	26.7	57.9	147	32.1	119	467	118
Starry flounder	22	1.74	404	41.0	46.7	248	ND	121	572	176
Starry flounder	26	2.66	418	25.9	450	205	ND	75.1	480	1045
Sculpin	23-25	1.15	120	12.2	35.7	22.2	ND	42.2	100	330

Benthic Community Characterization

As with benthic communities in the Pajaro and Salinas River estuaries, benthic assemblages in the Santa Maria estuary were classified as severely affected in the May and November 2008

sampling periods (Table 27). The May samples were dominated by oligochaetes and chironomids, likely due to the fact that the river lagoon was not open to the ocean during this time, and all stations had lower salinities. There were fewer species and individuals in these samples, and all stations were described as category 4 using the Relative Benthic Index. While there were negative indicators present in all of these samples, no positive indicator species were found.

A greater number of estuarine species were present in the November samples, reflecting the fact that the estuary was open to tidal influences. There was a greater range of species and relatively higher densities of animals present in these samples, relative to May. All samples except Station 5 contained mixtures of the amphipods *Americorophium sp*. and *Eogammarus confervicolus,* as well as oligochaetes and chironomids. Total amphipod numbers in the five estuary stations in November 2008 were 360, 83, 87, 130, and 1, at Stations 1-5, respectively (= total of *Americorophium sp.* + *Corophium sp.* + *Eogammarus confervicolus*). Amphipod abundance in the November 2008 samples corroborated amphipod mortality in the sediment toxicity tests conducted the previous month. For example, in the five lower estuary stations where sediment toxicity and benthic community structure were both characterized, the station with the lowest amphipod abundance in November 2008 (Station 5), also demonstrated the highest amphipod mortality in October 2008 sediment toxicity tests. As with all other benthic community samples in this study, only negative indicator species were found. The positive indicators *Tellina modesta, Grandifoxus grandis* and *Eohaustorius estuarius* were absent in all samples. As with the benthic community samples from the Pajaro and Salinas River estuaries, the pollution tolerances of the three amphipod species found in the Santa Maria River estuary are unknown, although they are listed as sensitive indicator taxa for mesohaline and tidal freshwater habitats in Thompson et al. (2010). Data on the relative sensitivities of *Americorophium stimpsoni, A. spinicorne* and *Eogammarus confervicolus* to pyrethroids and chlorpyrifos would allow determination whether these chemicals are affecting these species in the Santa Maria estuary.

Table 27. Benthic community indices for five Santa Maria River estuary stations monitored in May and November 2008. Relative Benthic Index (RBI) is scaled from 0 (most impacted) to 1 (least impacted).

Station	Number Taxa	Abundance	Number Mollusc Taxa	Number Crustacea Taxa	Number Crustacea	Number Amphipod Taxa	Number *Capitella*	Number Oligochaeta	Number *Tellina*	Number *Grandifoxus*	Number *Eohaustorius*	RBI Score
May 2008												
SM 1	3	9	0	0	0	0	0	7	0	0	0	-0.03
SM 2	1	348	0	0	0	0	1	348	0	0	0	-0.04
SM 3	6	302	0	2	3	1	0	1	0	0	0	0.02
SM 4	3	49	0	0	0	0	0	44	0	0	0	-0.03
SM 5	4	10	0	0	0	0	0	0	0	0	0	0.00
November 2008												
SM 1	5	707	0	3	388	2	0	1	0	0	0	0.03
SM 2	4	349	0	2	83	2	0	0	0	0	0	0.03
SM 3	8	211	0	3	88	2	0	43	0	0	0	0.04
SM 4	7	252	0	2	130	2	0	2	0	0	0	0.02
SM 5	6	226	0	2	2	2	0	163	0	0	0	0.02

Instantaneous Pesticide Loading

Orcutt Creek was the only tributary to the Santa Maria estuary sampled since it conveys approximately 90% of the freshwater to the upper estuary. Discharge typically ranged from 1.3 cfs during the dry season to 40 cfs during the first storm in 2008 (Appendix Table A5). Similar to tributaries of the Pajaro and Salinas River estuaries, the highest total dissolved pesticide load was observed during the first storm event in 2008, indicating that the first flush event was captured in this relatively small watershed. Fungicides were detected frequently in Orcutt Creek and loads were generally higher than the organophosphate pesticides, particularly during storm events (Table A5). During the dry season, pulses of pesticides were observed in Orcutt Creek. For example, the highest load of malathion was observed during an irrigation sampling event (12 g/d, Table A5) which indicates that application and irrigation practices also play a role in pesticide loads to the estuary. Even during the dry season, the highest pesticide loads were observed when the flows were the highest indicating off site transport of dissolved pesticides even during the dry season.

Suspended sediment pesticide loads were higher at Orcutt Creek compared to the smaller tributaries in the Pajaro and Salinas River watersheds. Total DDTs made up the majority of the total pesticide load to the upper estuary (Table A5). However, chlorpyrifos and permethrin were detected during every storm event over the 2 year sampling period with loads ranging from 0.02 to 1.8 and 0.02 to 0.13 g/d, respectively (Table A5).

Assessment of Endocrine Disruption

Because of chemical inputs to the estuary and grazing activity in the watershed, there is a potential for endocrine disruption in resident fish. Adult fathead minnows (*Pimephales promelas*) were used as a surrogate species and were exposed to sediment and water from Orcutt Creek and the Santa Maria River estuary to assess this potential. In addition to measurements of vitellogenin in fish in laboratory exposures, chlorpyrifos and diazinon were measured in the overlying water using ELISA. Chlorpyrifos was detected in the Orcutt Creek water (0.065 ug/L), and was detected in the Santa Maria estuary water sample, but below the reporting limit.

Diazinon was detected in both Orcutt Creek water (0.160 ug/L) and in Santa Maria estuary water (0.172 ug/L). Concentrations of chlorpyrifos and diazinon were below the toxicity thresholds for adult fathead minnows. Detection of these pesticides was consistent with measurements throughout the study, indicating that there are persistent inputs of pesticides into these waterways. All water quality parameters were within range tolerated by *P. promelas* during the course of these experiments (Table 28). Elevated levels of suspended sediments were present during sample collection because water was collected following a large rain event.

Table 28. Ranges of water quality parameters during the 6-day Fathead Minnow assay of Orcutt Creek and Santa Maria estuary.

Sample	Dissolved Oxygen (mg/L)	pH	Conductivity (uS/cm)	Total Ammonia (mg/L)	Hardness (mg/L)	Alkalinity (mg/L)
Control	6.69 - 8.31	8.05 - 8.31	4105 - 5620	ND - 5.8	173	129
Santa Maria	6.00 - 8.46	8.05 8.21	1693 - 1716	0.6 - 1.8	223	154
Orcutt Creek	5.72 - 7.51	8.01 - 8.21	1724 - 1759	0.5 - 1.2	357	151

Fish Survival

All aquaria were highly turbid during the exposure due to fish activity re-suspending sediment from the bottom of the aquaria. Four mortalities occurred during the exposure, including two males and one female in the control replicates, and one female from an Orcutt Creek replicate. Because of these mortalities, the test was terminated on Day 6. Mortality could potentially be caused by the suspended sediment, as all other test parameters were within the acceptable range of the organism.

Plasma Vitellogenin

Several difficulties were encountered when conducting the ELISAs for vitellogenin. Some of these issues might be related to the use of the Biosense kits, whereas others might be related to interfering factors in the samples. Although all appropriate procedures were followed, the data that were produced were highly variable and render quantitative data interpretation impossible. Vitellogenin induction in male fish provides evidence of exposure to EDCs. Test control fish that originated from uncontaminated environments, held in contaminant-free culture water, and

exposed in the same control water would be expected to have very low concentrations of vitellogenin. Although reported values in the literature vary among studies, there can be orders of magnitude differences between unexposed males and females. Vitellogenin concentrations in unexposed males range from < 10 ng/ml (Thorpe et al., 2007) to 20,000 ng/ml (Watanabe et al., 2007). Concentrations in unexposed females can range from approximately 400,000 ng/ml (Thorpe et al., 2007) to 17,400,000 ng/ml (Jensen et al., 2001, 2007; Watanabe et al., 2007). When this assay was recently conducted by the Los Angeles County Sanitation District (Carlita Barton, personal communication, LACSD, Los Angeles, CA), concentrations in control males averaged 300,000 ng/ml, which was higher than published normal values of 20,000 ng/ml or less. Of the six surviving males from the control, one had a vitellogenin concentration greater than 100,000 ng/ml and another control male had a vitellogenin concentration greater than 13,000,000 ng/mL. Unexpectedly high concentrations in male fish were also encountered in a similar study (Siegler et al., 2010). Consultation with U.S. EPA researchers and the kit manufacturer did not resolve this issue, but it was assumed that there was interference caused by the plasma sample interaction with the vitellogenin ELISA kit.

Although there was at least one extreme case of unexplained high vitellogenin in a control male, none of the males exposed to Santa Maria River water and sediment had elevated concentrations of vitellogenin, yet four males exposed to Orcutt Creek water and sediment had concentrations of vitellogenin greater than 11,000,000 ng/mL (Table 29). These concentrations were comparable to those of the female fish from the same exposures. Viewed qualitatively, these four elevated concentrations could be an indicator of endocrine disruption. There was no significant difference between the control and the Orcutt Creek results because of the high variability among and within the replicates.

Table 29. Individual male plasma vitellogenin concentrations.

Vitellogenin (ng/ml)					
Sample	Male	Replicate 1	Replicate 2	Replicate 3	Replicate 4
Control	1	2981	4378	Below Range	13449076
	2	100704	Dead	Dead	708
Santa Maria	1	448	1037	7827	165
	2	4882	32168	69461	8400
Orcutt	1	Over Range	46729	2277259	1227810
	2	11698542	19728	1677	769

Concentrations in unexposed females can range from approximately 400,000 ng/ml (Thorpe et al., 2007) to 17.4 million ng/ml (Jensen et al., 2001, 2007; Watanabe et al., 2007). All concentrations of vitellogenin in plasma from female fish were greater than the upper range of test detection (12.5 million ng/ml). Low concentrations of vitellogenin in females, which were not observed in this test, would have been an indication of endocrine disruption.

It is unclear why the vitellogenin concentrations were variable and randomly elevated, particularly in the control males. Discussions with the technical staff of Biosense, vitellogenin experts at U.S. EPA, and a number of researchers contacted at the national Society of Environmental Toxicology and Chemistry (SETAC) conference have suggested some possible causes. In an attempt to explain the unexpected results, we first confirmed the ELISA procedure step-by-step in consultation with Biosense. This confirmed that no mistakes were made in the preparation of standards and samples. The calibrators from all of the analysis events produced proportional absorbance values, so we determined that the preparations were accurate, and the plate reader was operating properly. It was assumed that the high concentrations, and therefore the variability, were the product of something in the plasma samples themselves. Biosense suggested interference could possibly occur from hemolysis, the rupturing of the blood cells during centrifugation, but U.S. EPA researchers have not experienced interference from hemolysis. Other possible interferences from the handling of the plasma samples could have been caused by not keeping the samples cold, or repeatedly thawing and re-freezing samples. Plasma samples in the current study were maintained on ice for no more than 5 minutes after centrifugation before they were cryo-frozen and placed in a -80 ºC freezer for storage. All other sample handling and analysis procedures followed standard or published methods obtained through the literature or personal communication.

The causes of (possible) interference and the resulting variability of the vitellogenin concentrations were not determined as part of this study, but advice for using the current method and suggestions for future analysis methods were gathered from fellow researchers consulted as part of this project. The use of the Biosense ELISA kits entail using microliter quantities of plasma in high dilutions (up to 1: 500,000), which could have a high potential for user error.

Researchers at the Los Angeles County Sanitation District indicated similar variability in vitellogenin results when using Biosense ELISA kits. Daniel Schlenk (UC Riverside, CA) suggested that using such small quantities of plasma in the ELISA may lend itself to user error that would result in the high level of variability seen in this test. His recommendations included either homogenizing plasma samples from a minimum of five fish or to conduct the analysis on liver tissue. Vitellogenin is produced in the liver before entering the blood stream, and as such, is potentially a more direct way of analyzing vitellogenin levels in fish. This alternative procedure is not discussed in the EPA protocol for fathead minnow exposures (USEPA, 2008), but is apparently used by researchers conducting this type of analysis. It has also been suggested that a subsample of fish be measured for vitellogenin upon arrival in order to determine if there are elevated concentrations of prior to testing.

Future studies of vitellogenin concentrations in fathead minnows exposed to effluent should incorporate higher replication, validation of vitellogenin levels in representative male fish by ELISA prior to testing, analysis of liver rather than plasma, and could incorporate homogenization of samples from replicate fish. Additionally, analysis of plasma sex steroid levels, which was not conducted in the current project, could provide further indication of whether endocrine disruption has occurred. Future studies could also incorporate the use of polymerase chain reaction (PCR), which has been used to evaluate gene expression linked to vitellogenin induction related to EDC exposure (Kolok et al., 2008). For example, in the present study, use of gene expression techniques could have provided additional evidence (or lack thereof) of endocrine disruption in the control male fish where elevated vitellogenin was measured using ELISA.

Santa Maria Estuary Summary

These results demonstrate that the Santa Maria River estuary is contaminated with toxic concentrations of organophosphate and pyrethroid pesticides and that this contamination is associated with ecological impacts in the estuary. Stations with the greatest contamination and toxicity also demonstrated severely impacted benthic macroinvertebrate communities. All stations had relatively depauperate macroinvertebrate assemblages, and species present were

primarily pollution tolerant groups such and chironomids and oligochaetes. This was particularly true during the May 2008 sampling period.

Of the three estuaries monitored for this study, the Santa Maria estuary was the most impacted by elevated pesticide concentrations. This likely reflects the proximity of agriculture discharge streams to the estuary. The upper estuary stations are approximately one km downstream of the confluence of Orcutt Creek and the river. Approximately 90% of the dry-weather flow observed in the lower Santa Maria River is comprised of discharge from the drainage ditch that enters the river near the Guadalupe Dunes Reserve entrance, combined with the flows of Solomon and Orcutt Creeks (SAIC, 2004). A number of previous studies have demonstrated that Orcutt Creek below its confluence with Solomon Creek (312ORC) is contaminated by toxic concentrations of chlorpyrifos, diazinon, and several pyrethroid pesticides (Anderson et al., 2006b; Phillips et al., 2006; Phillips et al., 2010). Studies have also found impacted macroinvertebrate communities in Orcutt Creek, and in the Santa Maria River downstream of its confluence with this creek (Anderson et al., 2006b). The current study demonstrates that these impacts extend into the estuary and are persistent over time.

The potential for pesticide impacts on migrating salmonids and other fish in the Santa Maria estuary may be assessed based on likelihood of secondary impacts via the reduction of prey species and based on direct neurotoxic impacts on fish. Pesticides may impact fish health through reductions of key prey species, including gammaridian and corophiid amphipods. Amphipods were absent in the Santa Maria estuary during the May 2008 sampling events, but this may have been due to the fact that the lagoon was closed to the tidal influence and was dominated by freshwater species. As mentioned in the discussion of the benthic community data, the corophiid amphipods *Americorophium sp.* and the gammaridian species *Eogammarus confervicolus* were present in the November 2008 samples. Both groups have been shown to be important forage species for migrating salmon (Shreffler et al., 1992) and for littoral estuarine species (Grimmaldo et al., 2009). Numerous researchers have shown that estuarine benthic macrofauna are influenced by a variety of biotic and abiotic factors, including salinity, sediment grain size, total organic carbon, and temperature. Few researchers have assessed the interaction of these factors with pesticide inputs on estuarine benthic communities. While there is

insufficient data to directly link pesticides with impacts on resident amphipod species in the estuary, densities of these amphipods declined at the same upper Santa Maria estuary station where sediment toxicity was observed in laboratory tests with the amphipod *H. azteca*. Water and sediment samples further up the estuary and proximate to the Orcutt Creek confluence were consistently toxic to this amphipod. Previous bioassessments have shown declines in populations of *H. azteca* in the lower Santa Maria River and in Orcutt Creek (Anderson et al., 2006b). Since *H. azteca* is disproportionately important as a prey item for littoral fish species (Grimmaldo et al., 2009), impacts on this and other amphipod species in the Santa Maria River and its estuary are likely relevant to the health and survival of resident and migrating fish.

Pesticides may also directly affect salmon and other fish species through disruption of olfactory sensory neurons necessary for salmon homing and predator avoidance behaviors. A number of studies have demonstrated effects of single organophosphate and pyrethroid pesticides on olfactory response or behaviors associated with olfactory response in salmon. Scholz et al. (Scholz et al., 2000) showed that diazinon concentrations as low as 1,000 ng/L affected the olfactory-mediated alarm response in Chinook salmon. Diazinon concentrations in water from the lower estuary were as high as 691 ng/L, and were as high as 584 ng/L in the upper estuary. Diazinon in Orcutt Creek ranged as high as 858 ng/L. Sandahl et al. (Sandahl et al., 2004) showed that a chlorpyrifos concentration as low as 720 ng/L decreased olfactory response to the amino acid L-serine in Coho salmon. L-serine is associated with predator avoidance in some salmonids. Chlorpyrifos concentrations in water ranged as high as 552 ng/L at the upper estuary station during this study, and chlorpyrifos concentrations in Orcutt Creek were as high as 1,082 ng/L. Moore and Waring (Moore and Waring, 2001) showed that cypermethrin concentrations as low as 4 ng/L reduced olfactory response in Atlantic salmon, and a concentration of 1,000 ng/L reduced egg fertilization in this species. While few pyrethroids were detected in water in the current study, a concurrent study found cypermethrin concentrations as high as 3.5 ng/L in the Santa Maria River downstream of Orcutt Creek (Phillips et al., 2010). A recent study by Tierney et al. (Tierney et al., 2008) has demonstrated that pesticide mixtures containing organochlorine and organophosphate pesticides with triazine herbicides affect olfactory response in trout at environmentally relevant concentrations that were considerably lower than studies using single pesticides. Mixtures of the organophosphate pesticides diazinon, chlorpyrifos and

malathion in the Santa Maria River and estuary sometimes greatly exceeded the experimental concentrations described in the mixture experiments by Tierney et al. (2008). In addition to organophosphates, these water samples also contained complex mixtures of organochlorine pesticides (e.g., *p,p'* DDE) as well as herbicides (e.g., prometryn), amide herbicides (e.g., propyzamide), and various fungicides. While most of the previous studies emphasized olfactory effects on salmon and trout species that don't occur in the Santa Maria River, there is a high likelihood that steelhead which may occur in the river respond similarly to pesticides. The implication of these studies is that very low pesticide concentrations affect salmonid olfactory functions and therefore may impair predator avoidance and homing behaviors.

No studies have been published on direct or indirect effects of pesticides on shorebirds in the Santa Maria River estuary. Given the level of pesticide contamination in water, sediment, and resident fish and sand crabs, there is reason to be concerned about the potential for direct effects of pesticides on birds, either via contact with contaminated water or sediments, or via consumption of contaminated prey (e.g. (Sapozhnikova et al., 2004)). In addition, because many of the invertebrate species identified in this study are prey for wading shorebirds, declines in benthic macroinvertebrate abundances could have indirect impacts on foraging shorebirds. Assessment of the potential for pesticide impacts on shorebirds is beyond the scope of the current study but warrants attention in future work in this estuary.

Relationships between Sediment Chemistry and Toxicity in the Pajaro, Salinas and Santa Maria Estuaries and Tributaries

The relationship between sum toxic units and amphipod survival in all sediment samples from the Pajaro, Salinas and Santa Maria River estuaries is shown in Figure 9. These data indicate that except for one sample, all sediments contaminated by more than 0.4 sum TUs demonstrate significant amphipod mortality. In cases where significant mortality was observed in samples with lower TUs, toxicity may be due to chemicals other than those detected during analysis or present in mixtures at low concentrations. As discussed above, pyrethroid pesticides are particularly problematic in this regard because the method detection limits are within the range of toxicity. As was also discussed above, this is a possible explanation for the Pajaro River

estuary samples which demonstrated moderate toxicity and low concentrations of pesticides and metals.

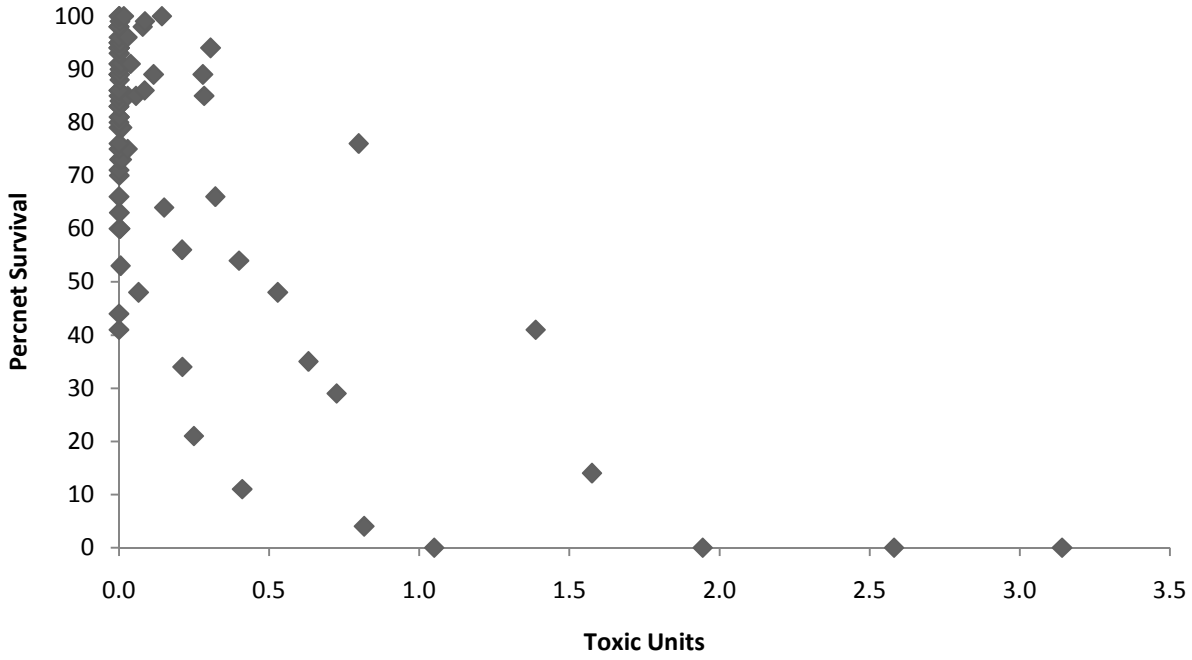


Figure 9. Relationship between sediment sum Toxic Units (TUs) and amphipod (*H. azteca*) survival in the Pajaro, Salinas and Santa Maria River estuaries and their tributaries. See text for a description of methods used to calculate sum TU.

Status of Management Practices affecting Pesticide Transport in the Project Watersheds

Overview

This section describes an attempt to characterize the status of management practice (MP) implementation and effectiveness throughout the Central Coast of California, particularly in the watersheds draining to the Pajaro, Salinas, and Santa Maria River estuaries. The goal is to link pesticide concentrations in the estuaries with the level of pesticide management activity in the watersheds at the time the surveys reported here were conducted (2008 – 2009). These relationships can then be re-assessed as part of future studies. It is possible that as management activity increases in scope and effectiveness, pesticide impacts in downstream critical habitats will decrease. It is also possible that management practice implementation may remain static or

decrease over the next five years because of concerns over food safety conflicts and a lack of information about conditions related to pathogen contamination.

Communications with agency staff, growers, and industry advisors indicate there is a substantial amount of pesticide-related management practice implementation occurring in these watersheds. Efforts to characterize the extent and effectiveness of these practices are currently not well standardized, though the Agriculture Water Quality Alliance (AWQA), the Resource Conservation Districts, and others, are working to coordinate these efforts. The information available to date is highly variable in terms of detail, format, and level of quantification. This section reviews the available information in an attempt to document a 2008-2009 baseline of management practice activity that can be used for comparison in future assessments.

Background

Pesticides used in residential, commercial, industrial, and agricultural applications can be transported to streams and other waterways as dissolved compounds or adsorbed onto particulates in surface water runoff. Impacts of pesticides on ambient water quality have been widely documented ((Anderson et al., 2003a; Anderson et al., 2003b; Hunt et al., 2003; Phillips et al., 2004; Phillips et al., 2006; Hunt et al., 2008) and data presented in this report). Of the activities designed to reduce pesticide transport from urban and agricultural sources, little is known about residential practices, information is becoming more available about municipal low impact development and stormwater practice effectiveness, and a greater amount of information is available about management practices used in agriculture. This section emphasizes activities of the agricultural community in these three watersheds.

On the California central coast, water quality short-courses, cooperative monitoring programs, and individual interactions between growers and specialists have been designed to increase the use of management practices that limit loading of contaminants to central coast streams. The definition of "management practice" is broad, however, and that diversity of approaches makes effectiveness evaluation difficult. Growers individually manage each crop on each property: reducing pesticide input during some cycles, changing irrigation practices where possible, changing cropping patterns, altering drainage systems, building retention basins, using reclaimed

runoff to irrigate cover crops, and switching to biological controls on some acres during some seasons, as well as installing discrete "practices" such as vegetated buffers, hedgerows, vegetated ditches and treatment amendments. This assessment of the status of practice implementation would be far simpler if all growers installed X number of X type of practice at X points draining X acres treating X liters of water with an efficiency of X% load reduction. This level of quantification is not possible, however, due to the range of techniques and the effort necessary to measure effectiveness.

As mentioned above, a number of entities are working to standardize the documentation of management practice implementation, particularly in agriculture but also in municipal stormwater systems. AWQA and its partners, including the Natural Resources Conservation Service (NRCS), the Resource Conservation Districts (RCDs), and the Regional Board, among others, are working to standardize reporting of the management practices implemented, the growers and acres affected, the effectiveness of different types of practices, and the potential reductions in loading to central coast streams. It is likely that AWQA partners will have such a standardized reporting system available for future assessments of the links between management practices and water quality improvement. The California Stormwater Quality Association (CASQA) has a number of programs supporting the evaluation of urban management practices, and central coast municipalities participate in this effort.

Conceptual model

A conceptual model of pesticide transport and biological effects provides a framework for considering the intent and function of the various management practices designed to mitigate pesticide impacts (Figure 10). Pesticides are applied on source areas (gardens, driveways, commercial buildings, farm fields), and their fate is affected by management of these areas. As they are transported off source areas (primarily by water in this model), they cross edges where they can be intercepted by vegetated buffers and other practices. Runoff is channeled through gutters, storm drains and ditches, where additional retention and breakdown processes can be promoted. Pesticide transport, and the associated biological impacts, in streams are affected by riparian condition and availability of wetland areas to retain and treat runoff. The river mouth estuaries are the last step in this model, because these are the habitats evaluated in this study.

Estuaries provide rare and critical habitat, and are thus considered here as areas for protection rather than mitigation.

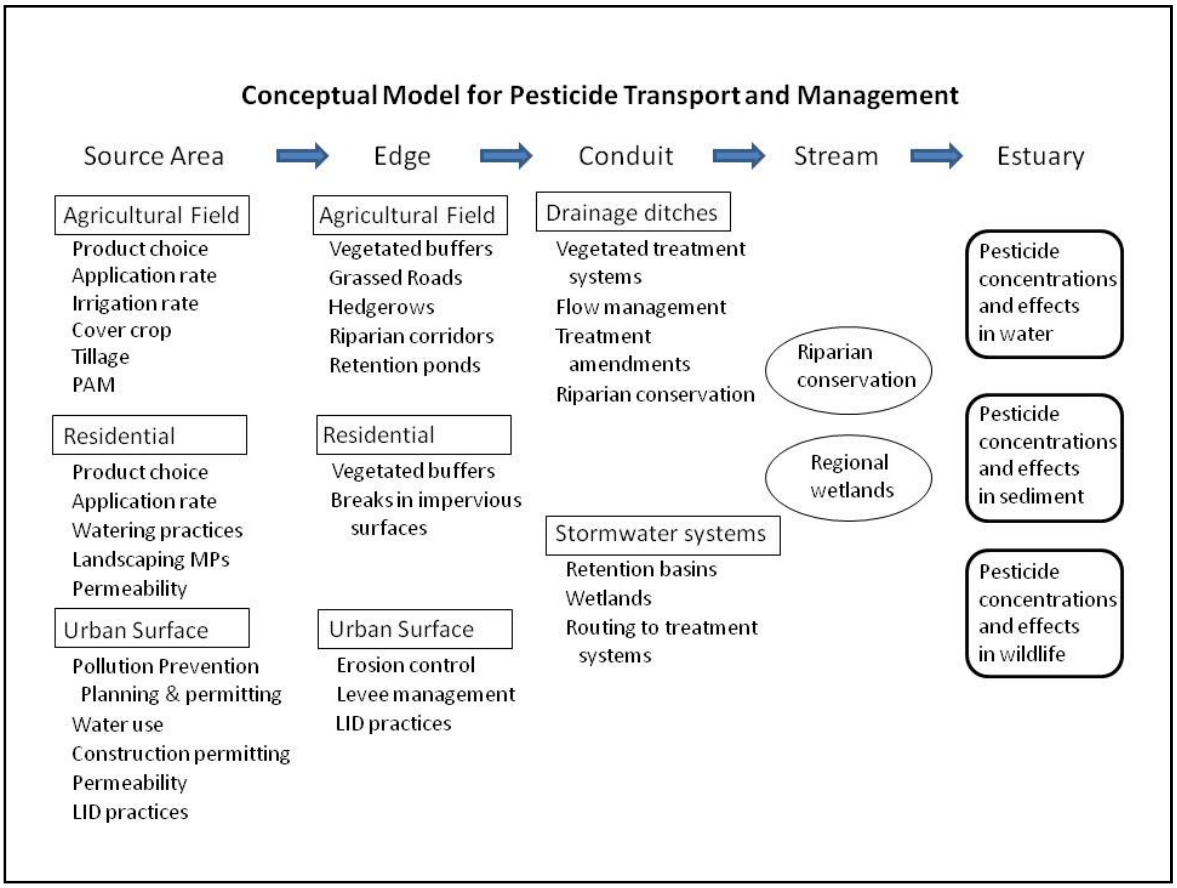


Figure 10. Conceptual model of pesticide transport and biological effects in central coast watersheds.

Regional Approaches to Management

In 2004 the Central Coast Regional Water Quality Control Board (Regional Board) adopted the Conditional Waiver of Waste Discharge Requirements for Discharges from Irrigated Lands (Waiver). Waiver conditions included enrollment in the program and development of Farm Water Quality Plans that identify management practices that are or will be implemented to address irrigation, pesticide and nutrient application, and erosion control. Data are not currently available to determine which practices have been implemented, the area or water volume affected, or the effectiveness of installed practices. As of June, 2008, however, the Regional Board reported 1735 operations were enrolled in the program, representing 396,030 acres. The

estimated number of non-enrollees was between 500 and 800 operations representing 40,000 acres. Practices specified in farm water quality plans checklists had been completed by 1419 operations.

In a 2007 grower survey, 91% (n = 165) of respondents reported adopting one or more conservation practices (Lowell et al., 2010). In the same survey, however, 21% (n = 38) of growers surveyed reported that they had removed or abandoned conservation practices in response to pressure from auditors, inspectors and other food safety professionals.

Industry advisors stated that the great majority of central coast growers are aware of the regulatory intentions of the Regional Board's program, are better informed, and are doing some sort of management designed to protect water quality. There was a consensus among advisors that there is currently no way to assess the effectiveness of these efforts on a watershed scale, and that few if any related changes are reflected in ambient monitoring data. Growers and advisors alike recommended strongly that education efforts continue, but not in the classroom. They recommended on-farm educational monitoring and outreach with individual growers to tailor management practice implementation with the unique conditions and operations of each farm. Advisors also indicated that the RCDs, as well as the Cooperative Monitoring Program, were the appropriate entities to conduct monitoring of implementation and effectiveness.

The effectiveness of the management practices implemented has either not been assessed or has been assessed using a variety of different methods, indicators, and data formats that do not allow synthesis into a comprehensive assessment of the type needed to document change at the watershed level.

The Waiver Cooperative Monitoring Program conducts proprietary educational monitoring to inform farm operators of the water quality in runoff and in adjacent waterways, and, where possible, of the effectiveness of management practices. These assessments are likely valuable to operators in optimizing the effectiveness of their practices, but are not available for assessments of the overall effectiveness of practices implemented within a given large watershed.

Changes in Pesticide Application

As indicated in the conceptual model (Figure 10), the choice of pesticides and application rates are primary source-control measures for urban and agricultural reduction of pesticide impacts on water quality. Pesticide selection is based on numerous factors, including crop type, crop rotation, soil type, season, pest populations and life cycles, location, cost, and environmental stewardship concerns, among others. Many pesticides are capable of causing adverse water quality impacts, even at low concentrations, and many newer compounds have not been well studied in terms of aquatic ecosystem impacts. However, numerous studies have identified pyrethroid pesticides (including bifenthrin, cypermethrin, and cyhalothrin) and organophosphate pesticides (particularly diazinon and chlorpyrifos) as being of greatest concern in central coast watersheds ((Anderson et al., 2003a; Anderson et al., 2003b; Hunt et al., 2003; Phillips et al., 2004; Phillips et al., 2006; Hunt et al., 2008) and data presented in this report).

Given that these pesticides have been prioritized for management, reducing their use on farms and in urban areas should be of particular value in reducing ambient water toxicity. As an example, chlorpyrifos has been implicated in both water and sediment toxicity in the study watersheds and elsewhere. Regional Board staff and industry advisors report that a primary source of chlorpyrifos is as a granular soil application to control root maggots in broccoli. Water that leaves a broccoli field recently treated with granular chlorpyrifos would likely be contaminated with the pesticide. Given the timing of applications and seasonal weather patterns, such transport would mainly occur in irrigation tailwater. Effective management must reduce or eliminate chlorpyrifos applications, retain water for extended periods (weeks) before allowing it to enter streams, or treat runoff with vegetated treatment systems or amendments such as Landguard®, a commercial enzyme that breaks down organophosphate pesticides, but is not yet a widely available option (http://csiro.au/solutions/pesticide bioremediation).

There are indications that some farm operators have changed their practices to reduce or eliminate chlorpyrifos. Large farm operations may have more options in this regard because of their ability to rotate crops on specific fields or to dedicate acreage to alternative practices. Smaller operations in the lower watershed areas may at times need to grow broccoli back to back to back in order to remain economically viable, and this likely creates pest conditions that limit

pesticide choices. When growers do switch from chlorpyrifos, industry advisors estimate that half the operators decrease their overall pesticide usage, while the other half switch to other pesticides, many of which are not analyzed for in current monitoring programs.

The Regional Board Total Maximum Daily Load (TMDL) unit staff has compiled pesticide use report information from the Department of Pesticide Regulation indicating that the pounds of chlorpyrifos applied to sub-watersheds in the Santa Maria watershed have decreased between 2006 and 2008 (Table 30). It is not clear whether this potential trend is replicated in the Salinas or Pajaro River watersheds, and the trend may be the result of exceptional efforts by one or a few large growers in the lower Santa Maria River. It is also not clear if similar reductions are occurring with pyrethroid pesticides.

Table 30. Pounds of chlorpyrifos applied to broccoli in Santa Maria sub-watersheds. Source: Regional Board TMDL staff and California Department of Pesticide Regulations, Pesticide Use Reporting Database. http://www.cdpr.ca.gov/docs/pur/purmain.htm

Sub-watershed	2006 (lbs.)	2008 (lbs.)
312GVS Green Valley Creek	2368	1383
312GVT Mid Orcutt Creek	548	465
Bradley Channel	1891	1512
312ORC Lower Orcutt Creek	6100	3736
Oso Flaco	2175	1920
Total	13084	9018

As with other implemented practices, there has not been sufficient coordination between the growers implementing the chlorpyrifos reductions and the scientists who monitor water quality to be able to determine the effectiveness of these changes in pesticide application in terms of water quality improvements.

Some information on practice effectiveness may exist in proprietary consultations and reports. Educational monitoring by the Waiver Cooperative Monitoring Program and by pesticide registrants working under the DPR re-evaluation process are likely producing information useful

in guiding the efforts of growers to reduce water quality impacts, but this information is not publicly available for the purpose of watershed-wide assessments.

Changes in Irrigation Practices and Pumping Rates

Contaminant transport to stream systems is a function of runoff volume and contaminant concentration. Loadings from agricultural land under irrigation can often be managed by increasing irrigation efficiency and decreasing tail water flow off the property. The Monterey County Water Resources Agency has been tracking irrigation practices in the Salinas Valley since 1993 (MCWRA, 2010). Their data show a steady increase in acres using drip irrigation and a steady decrease in acres using sprinkler and furrow methods. For example, vegetable crops using sprinkler and furrow irrigation decreased from 84,060 acres in 1993 to 27,828 in 2010, while vegetable acres on drip irrigation increased from 3,682 to 58,352 over the same period. The number of acres using other irrigation methods, such as hand-move, solid set and linear move sprinklers, as well as furrow-only methods, generally stayed unchanged, all at lower acreages than either sprinkler/furrow or drip.

A number of other agricultural management practices have been quantified by the MCWRA, most with gradual improvements since 2003. These include set asides, summer fallow, flow meter installation, pre-irrigation reduction, and micro-irrigation systems. Changes in irrigation management have been facilitated by advisors and extension specialists with the Central Coast Agricultural Water Quality Coalition, the University of California Cooperative Extension, and others. Comments by these advisors indicate that the changes in irrigation practices documented in the Salinas watershed by the MCWRA are likely occurring similarly in the lower Pajaro and Santa Maria River watersheds.

Urban water users have also implemented practices to reduce water use and, presumably, associated dry weather runoff volume. Activities that have been increasingly adopted since 2006 include conservation inserts with water bills, increased metering with new construction and retrofits, toilet replacement programs, incentive campaigns, public presentations, public service advertising, and increased enforcement (MCWRA, 2010).

Summary of other available information on management practice implementation and effectiveness

In order to coordinate future farming activities and water quality improvement, it is essential for the Regional Board and the agricultural community to accurately assess the extent of management practice implementation and the effectiveness of those efforts. It has been our intent throughout this project to quantify acreages, flow volumes, and related load reductions as they are affected by management activities. To gather this information, we targeted the following topics:

- Practices targeting pesticide fate and off-site transport
- Effectiveness evaluations for pesticide management practices
- Assessment of watershed-wide status of management practices
- Proportion of farms, acreage, and flows.
- Urban Management Practice Implementation, including low impact development
- Future changes in management practice implementation

We communicated with and received extensive responses from approximately 25 experts and practitioners involved with management practice implementation in California, we consulted the Natural Resource Projects Inventory, the DPR data base, websites from many agencies and non-profit organizations, and reviewed numerous reports and publications. The clear conclusion is that efforts are inadequate to identify management practices being implemented, the acreage or water volume affected, and their effectiveness at reducing pesticide loads. However, substantial efforts are underway to improve the situation, most noticeably by AWQA and its participants, including the RCDs and NRCS. Their efforts should be supported to the greatest extent possible, because without knowledge of implementation and effectiveness, regulation of these activities will be ineffective and potentially counter-productive.

Management Practice Implementation

Of the many groups contacted, the RCDs had the most quantifiable records of management practices. The RCD Monterey County tracking data included 35 projects addressing water quality in the 2006-2007 reporting period. Cumulatively, these practices were designed to

protect water quality on over 1000 acres agricultural land. Practices included gully stabilization (21 acres), sediment control basins (482 acres), sediment ponds (28 acres), critical area planting (102 acres), hedgerows (43 acres), livestock fencing (2500 linear feet), water diversions (3200 linear feet), structures for water control (148 acres), drip irrigation systems (156 acres), and plantings in vegetated treatment systems (60 acres). Total acreage treated per management practice ranged in size from 0.4 acres to 148 acres. Project watersheds included the Salinas Valley, Elkhorn Slough, and Gabilan watersheds. This description does not include all projects completed by the RCD, but is a summary of tracking data provided that is applicable to water quality management practices. For more information on these management practices, RCD Monterey County can be contacted at 831-424-1036.

The Santa Cruz County RCD tracking data included 22 projects that addressed water quality in the 2007-2008 reporting period for the Pajaro Watershed. Cumulatively these practices were estimated to reduce over 6000 tons of sediment annually from eroding off of agricultural lands. Practices included, critical area planting, stream bank stabilization, cover crops, grassed waterways, sediment basins, structures for water control, hedgerows, and planting in vegetated treatment systems. An additional report on six vegetated treatment systems established from 2007-2009 with RCD assistance estimated a sediment load reduction of 410 tons annually (Strong-Cvetich, 2009). This description does not include all projects completed by the RCD, but is a summary of tracking data provided that is applicable to water quality management practices. For more information on these management practices, Santa Cruz County RCD can be contacted at 831-464-2950.

The NRCS office in Hollister (Monterey County) reported that they have been working with agricultural producers on irrigation water management, pest management and nutrient management. Project tracking data for 2010 included hedgerows (4850 feet), cover crops (310 acres), and road work to prevent erosion (800 linear feet). This summary does not include all projects completed by the NRCS, but is a summary of tracking data provided that is applicable to water quality management practices. For more information on these management practices, NRCS Hollister can be contacted at 831-637-4360.

The NRCS Environmental Quality Incentives Program (EQIP) reported $18 million in Farm Bill funding obligated to contracts over a ten-year period (1999-2009) in central coast watersheds. Farmers have invested $15 million of their own money in this cost-sharing program as matching funds.

The Natural Resources Project Inventory (NRPI) database, managed by UC Davis, was searched for management practice implementation in the study watersheds. The following three project descriptions are summaries from this database, and are provided as examples of how data is presented and quantified:

1) The Central Coast Vineyard Team assisted growers in adopting practices to reduce the use of simazine and chlorpyrifos. Through replicated trials and on-farm demonstrations, growers adopted a variety of practices: ant-bait stations, vegetative insectaries, beneficial releases, use of reduced-risk materials, under vine vegetation, cultivation for weed control. Information was disseminated throughout the State. Applicable watersheds included the Salinas, Santa Maria, Santa Ynez, Estrella, and San Luis Rivers.

2) The Vegetative Conservation Practices for Water Quality and Habitat Diversity on Pajaro Valley Farms project of the Community Alliance with Family Farmers successfully combined ecological restoration and conservation activities with farmer outreach and education to protect and improve surface and ground water quality in the Pajaro River watershed. Project accomplishments include installation of over 16,000 linear feet of hedgerows and grassed waterways.

3) The Agriculture and Land-Based Training Association (ALBA) seeks support to match existing USDA Natural Resources Conservation Service conservation cost-share resources in order to further educate and assist farmers on water quality conservation. This project worked with 120 Spanish-speaking farmers to demonstrate pragmatic practices and help them to establish Farm Water Quality Plans and apply for cost-share resources. The overall goal is to reduce agricultural non-point source surface water pollutants through implementation,

demonstration and education activities promoting innovative landscape and crop management. Applicable watersheds included Elkhorn Slough and the Lower Salinas River.

Management Practice Effectiveness Studies

Research on management practice effectiveness has primarily focused on treatment and removal of pesticides in tailwater. Vegetated Treatment Systems include vegetated drainage ditches and constructed wetland ponds. Vegetation is effective at settling sediments and in removing pesticides (diazinon, pyrethroids) through infiltration into the root zone, sorption to vegetation, and reducing toxicity through dilution. Watanabe and Grismer (Watanabe and Grismer, 2001) evaluated diazinon removal by vegetated filter strips under controlled laboratory conditions and observed losses of diazinon up to 73% of total mass applied. Moore et al. (Moore et al., 2008)also used a simulated runoff event to evaluate removal of diazinon and permethrin in vegetated ditches in Yolo County, California. While these authors also described reductions in diazinon runoff using a V-shaped vegetated ditch, significant concentrations of diazinon remained in the system outflow after 5 hours. Moore et al. (Moore et al., 2009) found that vegetation was much more effective at removing the pyrethroid pesticide permethrin, due to its reduced solubility. Hunt et al. (Hunt et al., 2007) evaluated the effectiveness of vegetation (Pennywort) at removing diazinon and pyrethroids in a two-pond tailwater system in the Salinas Valley. Anderson et al. (in press) evaluated removal of pesticides in a vegetated ditch during actual irrigation events on a large scale commercial farm. The results demonstrated that the vegetated ditch was effective at significantly reducing pyrethroid concentrations in sediment between the input and output, but was less effective at removing diazinon in ditch outflow water. Residual diazinon was effectively removed using the enzyme amendment Landguard.

Recommendations

In order to effectively reduce pesticide loading and improve water quality in central coast waterways, information on management practice implementation and effectiveness must be gathered in a coordinated and efficient manner, using comparable measures and data formats to allow assessments at the watershed scale. The following are recommendations for steps to achieve this goal:

1. Support efforts by AWQA and its partners to standardize the reporting of management practice implementation. This may involve developing common terminology for practice types and features; a suite of standard indicators for area affected, water volume treated, and loads reduced; and a standardized reporting format that allows data integration and synthesis.

2. Develop and support a coordinated regional (if not statewide) approach to the monitoring and evaluation of practice effectiveness. This should include conducting intensive monitoring of a few practice types with high potential for load reduction; using the information from intensive monitoring to develop rapid assessment methods (RAMs) for each priority practice; and extensively applying the RAMs to do efficient, standardized effectiveness evaluations at a large number of operations throughout the region.

3. Greatly increase capabilities and support for on-farm educational monitoring and expert out-reach to assist growers with developing, implementing, and monitoring the effectiveness of specifically tailored management practices that address their unique situations and water quality threats.

4. Prioritize current and future grant funding (including funding targeted for implementation) to accomplish the three recommendations listed above. Large amounts of state and federal funds are currently targeted for implementation of management practices without a solid grasp of their potential effectiveness, and without sufficient large-scale monitoring support to know whether these efforts are improving water quality or how they could be better designed to achieve the Regional Board's water quality goals.

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Appendix 1

Table A1. Amphipod (*H. azteca)* percent survival and growth (mg/individual) in samples from all estuary stations and tributary stations. Shading indicates survival or growth significantly lower than the controls.

	June 2008				October 2008				October 2009			
	Survival		Growth		Survival		Growth		Survival		Growth	
Station	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Pajaro 1	86	20	0.243	0.044	98	5	0.147	0.019	94	9	0.344	0.151
Pajaro 2	91	11	0.112	0.015	95	5	0.113	0.016	93	5	0.271	0.194
Pajaro 3	44	29	0.097	0.036	48	30	0.091	0.047	71	15	0.317	0.084
Pajaro 4	80	17	0.123	0.028	73	15	0.07	0.031	81	11	0.354	0.072
Pajaro 5	81	14	0.155	0.021	75	15	0.081	0.029	56	14	0.218	0.06
Pajaro 6	94	7	0.165	0.02	60	20	0.085	0.03	66	14	0.209	0.057
Pajaro 7	85	25	0.128	0.047	53	21	0.088	0.011	60	21	0.384	0.152
Pajaro 8	99	4	0.195	0.025	100	0	0.13	0.021	89	10	0.273	0.065
MDD	75	32	0.149	0.032	84	7	0.12	0.011	0	0	NA	NA
Thurwachter	73	28	0.175	0.034	70	35	0.105	0.02	85	13	0.299	0.073
Watsonville	41	17	0.201	0.083	91	6	0.181	0.014	89	14	0.382	0.1
	June 2008				October 2008				October 2009			
	Survival		Growth		Survival		Growth		Survival		Growth	
Station	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Salinas 1	95	11	0.196	0.018	89	10	0.13	0.013	79	33	0.352	0.038
Salinas 2	88	10	0.199	0.032	98	5	0.117	0.024	98	5	0.251	0.028
Salinas 3	91	4	0.123	0.017	95	5	0.132	0.019	89	14	0.247	0.076
Salinas 4	79	11	0.199	0.027	96	5	0.153	0.018	81	16	0.226	0.054
Salinas 5	91	11	0.116	0.01	98	5	0.172	0.026	86	15	0.267	0.069
Salinas 6	73	20	0.146	0.037	95	5	0.271	0.017	90	8	0.254	0.019
Salinas 7	63	19	0.154	0.027	83	16	0.148	0.029	85	23	0.356	0.086
Salinas 8	94	7	0.171	0.018	91	6	0.164	0.026	76	23	0.36	0.08
Blanco	89	6	0.153	0.026	29	36	0.266	0.045	35	34	0.264	0.129
Davis	96	7	0.28	0.067	94	5	0.225	0.053	34	18	0.491	0.218
Santa Maria 1	93	9	0.242	0.022	89	9	0.135	0.018	60	20	0.407	0.115
Santa Maria 2	100	0	0.191	0.012	85	6	0.117	0.009	66	21	0.456	0.188
Santa Maria 3	95	8	0.235	0.014	83	15	0.133	0.021	94	7	0.325	0.079
Santa Maria 4	86	9	0.185	0.038	89	11	0.161	0.042	100	0	0.351	0.108
Santa Maria 5	99	4	0.193	0.017	54	32	0.101	0.03	48	21	0.312	0.086
Santa Maria 6	76	15	0.195	0.038	21	17	0.19	0.078	100	0	0.394	0.089
Santa Maria 7	94	12	0.133	0.012	11	21	0.132	0.011	64	25	0.396	0.114
Santa Maria 8	41	19	0.098	0.016	14	23	0.219	0.105	0	0	NA	NA
Orcutt	4	5	0.167	0.115	0	0	NA	NA	0	0	NA	NA

Table A2. Sediment Metal Concentrations (mg/kg dry wt.)

	Aluminum (Al)	Antimony (Sb)	Arsenic (As)	Barium (Ba)	Beryllium (Be)	Cadmium (Cd)	Chromium (Cr)	Cobalt (Co)	Copper (Cu)	Iron (Fe)	Lead (Pb)	Manganese (Mn)	Molybdenum (Mo)	Nickel (Ni)	Selenium (Se)	Silver (Ag)	Strontium (Sr)	Thallium (Tl)	Tin (Sn)	Titanium (Ti)	Vanadium (V)	Zinc (Zn)
June 2008																						
Pajaro 1	4241	0.069	3.091	32.75	0.129	**0.032**	30.57	4.074	3.377	12060	2.217	550.4	0.137	25.15	**0.031**	0.058	69.16	**0.025**	0.2	200.7	23.37	17.6
Pajaro 2	8479	0.142	4.249	72.74	0.245	0.122	56.11	7.63	10.75	18110	5.464	198.1	0.466	40.15	0.188	0.064	90.85	0.057	0.493	475	52.97	37.39
Pajaro 3	18480	0.529	5.401	109.1	0.535	0.419	76.49	14.19	30.76	28910	57.56	388.8	0.983	83.74	0.569	0.185	465.7	0.167	1.181	363.9	58.11	87.81
Pajaro 4	25390	0.236	5.967	102	0.634	0.396	94.12	17.19	39.54	38280	16.82	490.6	0.934	97.81	0.618	0.173	291.6	0.171	1.466	336.1	67.3	110.1
Pajaro 5	19890	0.298	7.37	177.5	0.545	0.415	74.03	14.58	33.39	29730	15.13	874.6	1.191	78.93	0.804	0.171	415	0.157	1.174	390.3	59.73	89.65
Pajaro 6	8828	0.153	3.719	137.5	0.251	0.082	59.17	9.411	12.34	17800	6.606	293.4	0.29	64.57	0.216	0.489	121.9	0.065	0.473	332	33.54	41.67
Pajaro 7	14610	0.326	6.339	141.7	0.427	0.412	65.76	13.07	28.26	24240	15.56	834.7	1.875	76.79	0.9	0.118	330	0.134	1.344	320.7	50.8	84.77
Pajaro 8	6207	0.118	3.605	69.33	0.174	0.149	57.87	7.146	9.191	11480	4.669	549.8	0.372	55.39	0.283	0.062	70.34	0.057	2.009	201.4	23.53	30.67
MDD	20600	0.227	7.45	261.5	0.569	0.333	93.47	17.24	38.83	30450	11.72	543	0.583	104.4	0.514	0.135	349	0.148	0.988	209.5	63.42	98.74
Thurwachter	9649	0.183	4.872	138.4	0.284	0.164	65.48	10.57	16.69	17900	7.957	671.7	0.583	68.81	0.359	0.081	136	0.073	0.575	275.7	39.89	51.95
Watsonville	24220	0.214	9.293	156.1	0.661	0.621	100.4	18.37	48.66	36960	17.8	636.7	1.406	105.4	0.785	0.224	268.2	0.177	1.852	291.7	73.82	136.9
October 2009																						
Pajaro 1	2311		2.583	11.08	0.085	**0.04**	13.03	2.608	2.444	5453	2.079	85.75	0.156	13.9	**0.041**	0.085	19.78	ND	0.113	71.04	10.88	12.65
Pajaro 2	9248	0.108	5.175	67.24	0.428	0.375	41.28	9.854	18.53	18750	11.49	267.7	1.461	51.58	0.29	0.08	45.75	0.119	0.622	125	37.99	63.82
Pajaro 3	12860	0.292	4.714	84.32	0.451	0.419	59.15	13.08	29.63	25250	60.1	260	1.647	73.31	0.365	0.139	471	0.161	0.988	153.1	46.5	78.89
Pajaro 4	13650	0.107	4.47	76.56	0.469	0.445	61.55	13.31	32.38	27340	14.18	394.2	1.268	75.01	0.378	0.128	325.1	0.17	0.936	135.4	42.65	86.07
Pajaro 5	9814	0.113	4.255	92.38	0.368	0.298	42.64	9.676	18.94	18840	10.06	344.1	1.145	48.27	0.33	0.121	291.3	0.124	0.666	136	35.64	57.21
Pajaro 6	10380	0.093	4.454	105.3	0.378	0.294	48.23	10.54	19.2	20350	11.62	346.4	1.267	55.5	0.602	0.085	189.9	0.123	0.927	115.5	36.91	59.75
Pajaro 7	11980	0.178	7.22	182.6	0.492	0.517	42.3	12.13	25.93	23620	18.47	918.2	1.871	54.27	0.762	0.15	394.6	0.169	1.156	125	41.67	85.97
Pajaro 8	7547	0.124	3.671	120.3	0.25	0.242	46.99	8.235	13.79	15530	9.508	8373	1.1	46.01	0.448	0.118	835.4	0.08	0.834	116.5	29.9	48
MDD	11540	0.127	4.337	157.8	0.287	0.227	54.2	11.47	23.43	19680	8.393	470.6	0.357	61.89	0.18	0.126	215.2	0.099	0.797	104.1	34.51	64.1
Thurwachter	16150	0.259	8.807	279.1	0.663	0.572	40.47	15.21	31.52	31370	21.58	1283	1.672	55.26	1.046	0.151	115.1	0.231	0.901	148	50.69	111.3
Watsonville	21730	0.205	6.463	115.3	0.51	0.477	84.22	16.95	37.24	36850	19.18	491.6	1.358	89.16	0.859	0.291	412.8	0.177	1.82	238.5	55.18	121

	Aluminum (Al)	Antimony (Sb)	Arsenic (As)	Barium (Ba)	Beryllium (Be)	Cadmium (Cd)	Chromium (Cr)	Cobalt (Co)	Copper (Cu)	Iron (Fe)	Lead (Pb)	Manganese (Mn)	Molybdenum (Mo)	Nickel (Ni)	Selenium (Se)	Silver (Ag)	Strontium (Sr)	Thallium (Tl)	Tin (Sn)	Titanium (Ti)	Vanadium (V)	Zinc (Zn)
June 2008																						
Salinas 1	2245	0.029	1.61	7.636	0.077	0.122	20.72	1.962	1.481	4139	1.362	49.11	0.122	13.3	ND	0.063	12.9	ND	0.13	158	7.991	8.479
Salinas 2	10570	0.136	3.268	106.7	0.34	0.373	56.09	9.713	13.21	17700	5.023	240.1	0.872	74.19	0.19	0.087	48.93	0.128	0.6	556.4	39.48	45.08
Salinas 3	17460	0.18	7.03	104.2	0.574	0.658	88.57	16.5	27.95	28420	7.518	455.1	1.294	136.3	0.484	0.133	81.24	0.184	0.856	373.5	55.93	74.79
Salinas 4	4980	0.067	1.795	35.33	0.165	0.193	33.17	5.053	5.089	8864	2.174	199.3	0.275	36.9	0.115	0.068	52.56	0.066	0.269	388.8	18.63	21.89
Salinas 5	7226	0.087	2.787	44.44	0.23	0.256	38.76	7.157	8.538	12300	3.087	393.4	0.552	51.74	0.216	80	0.089	0.377	370.3	24.81	31.75	**0.048**
Salinas 6	5598	0.078	1.923	34.37	0.184	0.198	32.44	5.346	5.645	9641	2.362	297.5	0.37	39.28	0.12	0.061	45.64	0.071	0.308	404.2	19.42	23.84
Salinas 7	7947	0.101	2.632	47.4	0.237	0.27	42.11	6.576	8.338	11420	3.073	439.9	0.531	47.06	0.24	159.3	0.083	0.424	420.9	25.98	30.66	**0.047**
Salinas 8	6980	0.106	2.429	44.41	0.242	0.401	35.92	6.305	8.084	11430	3.279	282.1	0.638	44.52	0.225	0.068	57.52	0.1	0.426	392.4	23.87	33.65
Blanco	13850	0.192	3.731	133.3	0.472	1.349	57.08	11.66	26.86	20150	8.642	1327	2.733	74.03	0.794	0.135	184.2	0.183	0.879	484.6	42.9	78.25
Davis	5065	0.148	2.437	95.95	0.167	0.498	28.05	4.375	7.255	7905	2.767	654.3	1.127	31.18	0.784	0.065	223	0.062	0.394	267.8	17.43	29.45
October 2009																						
Salinas 1	1348	ND	1.356	6.247	**0.048**	0.052	10.65	1.423	1.163	2779	1.009	55.77	0.135	8.615	**0.049**	0.076	48.99	ND	0.073	51.63	4.842	5.286
Salinas 2	12390	0.068	4.612	96.57	0.397	0.512	64.34	12.52	16.85	21560	5.872	377.1	0.63	94.1	0.214	0.138	92.68	0.155	0.635	283.8	39.59	55.34
Salinas 3	14170	0.06	4.141	95.85	0.414	0.451	68.85	13.2	17.9	23570	6.069	356.2	0.913	97.66	0.245	0.127	145.8	0.164	0.676	273.4	43.64	58.34
Salinas 4	17670	0.055	5.513	114.6	0.558	0.997	77.57	14.94	24.5	28520	8.991	511.4	2.69	104.4	0.534	0.162	195.8	0.227	0.96	341.1	54.96	76.9
Salinas 5	10380	**0.03**	3.946	51.84	0.309	0.366	50.79	9.397	12.99	17050	4.997	450.1	0.897	66.47	0.3	0.086	207.8	0.118	0.537	186.9	31.41	41.66
Salinas 6	5203	**0.047**	1.32	31.74	0.145	0.198	29.3	5.059	5.841	9673	2.85	170.7	0.335	35.22	0.145	0.095	171.9	0.067	0.272	214.7	17.48	24.64
Salinas 7	5449	**0.049**	1.805	35.31	0.155	0.237	29.42	5.119	6.407	9474	2.775	259.9	0.738	36.7	0.191	0.073	353.7	0.07	0.303	203.9	17.98	24.95
Salinas 8	5351	**0.042**	1.458	31.59	0.157	0.222	28.33	4.859	5.891	9390	455.1	177.2	0.815	32.37	0.122	0.066	164.3	0.077	0.392	229.2	17.54	25.21
Blanco	10040	0.104	2.873	106.4	0.336	0.977	42.99	9.568	21.31	16970	8.47	894.6	1.542	53.6	0.537	0.102	189.3	0.161	1.078	187	30.76	65.74
Davis	2958	0.072	0.955	26.62	0.074	0.18	16.53	3.026	7.435	5642	2.793	184.9	0.325	17.54	0.221	0.101	26.51	0.052	0.244	170.2	9.646	26.53
June 2008																						
Santa Maria 1	1056	0.058	2.196	3.839	0.07	**0.028**	2.414	1.041	1.136	3154	1.224	35.47	0.189	2.569	**0.049**	ND	8.804	ND	0.076	34.12	5.236	5.766

	Aluminum (Al)	Antimony (Sb)	Arsenic (As)	Barium (Ba)	Beryllium (Be)	Cadmium (Cd)	Chromium (Cr)	Cobalt (Co)	Copper (Cu)	Iron (Fe)	Lead (Pb)	Manganese (Mn)	Molybdenum (Mo)	Nickel (Ni)	Selenium (Se)	Silver (Ag)	Strontium (Sr)	Thallium (Tl)	Tin (Sn)	Titanium (Ti)	Vanadium (V)	Zinc (Zn)
Santa Maria 2	1074	0.074	3.111	4.04	0.074	0.069	2.745	1.04	1.164	3495	1.349	36.84	0.138	3.873	**0.034**	ND	7.262	ND	0.081	35.21	5.374	6.118
Santa Maria 3	1844	0.09	1.711	11.67	0.098	0.078	5.581	1.251	2.28	4065	2.216	56.63	0.239	3.985	0.185	ND	43.42	ND	0.142	57.72	7.903	10.06
Santa Maria 4	1624	0.113	3.405	5.663	0.101	0.05	3.611	1.137	1.456	4737	1.479	43.99	0.16	4.43	**0.037**	ND	10.84	ND	0.108	59.32	7.342	7.399
Santa Maria 5	2843	0.155	2.28	26.99	0.173	0.379	7.326	2.867	4.474	7141	2.415	131.1	0.535	8.25	0.161	**0.039**	33.26	**0.048**	0.226	83.02	12.67	18.54
Santa Maria 6	5048	0.231	3.819	49.62	0.304	0.373	12.07	4.115	11.47	11460	4.204	239.7	0.794	13.7	0.336	**0.049**	69.22	0.083	0.387	128.8	22.79	33.66
Santa Maria 7	7004	0.342	5.315	71.68	0.37	0.422	17.42	5.394	11.93	15250	5.465	225.9	1.107	19.49	0.388	0.066	37.6	0.123	0.527	200.7	32.11	43.5
Santa Maria 8	8592	0.192	5.379	74.23	0.476	0.602	19.52	6.103	15.92	16520	6.451	319.8	0.964	18.39	0.487	0.077	70.72	0.149	0.644	186.4	30.56	53.43
Orcutt Creek	8350	0.281	5.541	78.05	0.503	0.578	18.13	6.871	16.25	17490	7.384	308.3	1.035	18.17	0.401	0.127	55.97	0.159	0.708	213.2	31.27	55.99
October 2009																						
Santa Maria 1	775.5	ND	1.517	3.172	**0.04**	ND	1.642	0.631	0.762	2346	1.684	29.69	0.103	1.862	ND	0.085	12.94	ND	0.059	17.49	3.167	4.382
Santa Maria 2	827.7	ND	1.866	4.085	**0.041**	**0.035**	1.947	0.707	0.814	2469	1.19	30.92	0.083	2.068	**0.049**	0.083	23.03	ND	0.064	13.5	3.891	3.846
Santa Maria 3	2136	0.083	2.496	25.55	0.107	0.159	5.397	1.817	4.164	5394	2.641	129	0.542	5.798	0.575	0.083	173.7	**0.032**	0.166	53.11	11.08	13.3
Santa Maria 4	9766	0.286	4.499	81.13	0.344	0.295	21.54	5.11	9.473	16160	5.58	236.3	0.903	15.94	0.279	0.07	48.5	0.13	0.666	364.1	37.85	37.67
Santa Maria 5	1028	0.054	1.447	6.447	0.051	0.057	3.036	0.868	1.115	3217	1.58	47.39	0.187	2.735	ND	0.121	19.9	ND	0.075	28.64	5.049	4.889
Santa Maria 6	5102	0.141	3.563	46.3	0.251	0.396	12.09	4.209	8.703	11200	4.384	222.8	0.781	14.55	0.41	0.082	74.6	0.089	0.402	88.56	22.18	32.2
Santa Maria 7	5802	0.166	3.912	63.38	0.313	0.414	13.19	4.576	10.45	13610	5.919	293.4	0.663	14.02	0.389	0.093	136.3	0.117	0.471	103.7	24.15	40.74
Santa Maria 8	6296	0.152	3.363	66.08	0.327	0.524	13.79	4.825	11.4	13510	5.88	293.5	0.703	14.58	0.396	0.109	93.19	0.127	0.508	108.7	23.73	47.36
Orcutt Creek	7531	0.126	4.184	75.84	0.381	0.627	16.21	6.534	13.55	16660	7.127	339.4	0.699	17.59	0.232	0.086	57.87	0.155	0.559	110.5	28.64	52.94

Table A3. Pesticide loads (g/d) calculated from suspended sediment pesticide concentrations measured in tributaries of the Pajaro River estuary.

Station	Run	Sample Type	Discharge (cfs)	Azoxystrobin	Boscalid	Chlorpyrifos	DCPA	Diazinon	Malathion	Myclobutanil	Oxyfluorfen	SDDTs	Prometryn	Propyzamide	Pyraclostrobin	Total Load
MDD	1	storm	24	0.69	18.2	ND	0.46	0.97	ND	8.5	11.7	0.58	ND	0.33	ND	44
Thurwachter	1	storm	757	ND	44.8	ND	701	289	ND	47.4	270	ND	627	40.4	ND	2491
MDD	2	storm	30	0.75	11.7	0.51	0.35	3.96	ND	6.0	6.3	1.9	ND	0.25	ND	32
Thurwachter	2	storm	719	ND	39.4	ND	329	385	ND	27.1	256	3.9	171	64.0	ND	1330
MDD	4	dry	8.3	ND	0.56	ND	ND	0.27	ND	0.58	ND	0.15	ND	ND	ND	1.6
MDD	6	dry	1.4	ND	0.12	ND	ND	0.04	ND	N	ND	0.02	ND	ND	ND	0.18
MDD	10	storm	16	18.8	58.5	0.13	0.30	6.2	3.0	24.0	22.4	1.1	ND	1.9	18.6	163
Thurwachter	10	storm	200	101	200	0.88	5.2	16.0	ND	108	23.8	2.1	18.6	5.1	ND	536
MDD	12	dry	1	ND	0.07	ND	ND	ND	ND	ND	ND	0.01	ND	ND	ND	0.07
MDD	13	storm	26	23.9	2292	0.71	1.7	ND	128	168	8.5	1.6	ND	ND	452	3098
Thurwachter	13	storm	292	25.6	467	ND	ND	ND	365	207	ND	2.3	ND	ND	23.2	1089
MDD	14	dry	3.5	0.03	1.7	ND	0.01	ND	ND	0.55	0.11	0.06	ND	ND	0.49	3.0
MDD	15	dry	2	ND	0.64	ND	ND	ND	ND	0.17	0.03	0.02	ND	ND	0.21	1.1

Table A4. Pesticide loads (g/d) calculated from suspended sediment pesticide concentrations measured in tributaries of the Salinas River estuary.

Station	Run	Sample Type	Discharge (cfs)	Azoxystrobin	Boscalid	Chlorpyrifos	DCPA	Diazinon	Malathion	Myclobutanil	Oxyfluorfen	SDDTs	Prometryn	Propyzamide	Pyraclostrobin	Total Load
Blanco	1	storm	8.9	0.41	2.8	0.25	2.7	2.3	ND	ND	8.3	0.16	2.2	0.32	ND	19.4
Davis	1	storm	770	ND	36.9	15.4	566	48.2	ND	ND	43.7	2.3	ND	ND	ND	713
Blanco	2	storm	14	ND	1.5	0.50	1.8	4.2	ND	0.33	3.6	0.53	0.35	0.90	ND	13.9
Davis	2	storm	780	ND	42.0	53.4	273	114	ND	16.8	93.1	13.0	ND	54.2	ND	706
Blanco	4	dry	8.8	ND	0.39	ND	0.47	0.41	ND	0.09	ND	0.20	0.55	0.10	ND	2.3
Blanco	6	dry	5.3	ND	0.92	ND	0.09	1.1	ND	ND	0.52	0.07	0.12	0.17	ND	3.1
Blanco	10	storm	14	ND	1.5	0.50	1.8	4.2	ND	0.33	3.6	0.53	0.35	0.90	ND	13.9
Davis	10	storm	780	ND	42.0	53.4	273	114	ND	16.8	93.1	13.0	ND	54.2	ND	706
Blanco	12	dry	4	ND	0.26	0.01	0.11	0.08	ND	ND	ND	0.03	0.20	ND	ND	0.71
Blanco	13	storm	15	167	24.5	0.19	1.5	ND	39.1	49.3	0.81	0.59	2.5	0.96	1.7	288
Blanco	14	dry	2.6	ND	0.69	0.02	0.12	ND	ND	0.08	0.06	0.03	0.12	0.06	0.06	1.2
Blanco	15	dry	2.5	0.04	0.55	ND	0.12	ND	ND	0.05	0.03	0.01	0.13	0.06	ND	1.0

Table A5. Pesticide loads (g/d) calculated from suspended sediment pesticide concentrations measured in tributaries of the Santa Maria River estuary.

Station	Run	Sample Type	Discharge (cfs)	Azoxystrobin	Boscalid	Chlorpyrifos	DCPA	Diazinon	Malathion	Myclobutanil	Oxyfluorfen	SDDTs	Prometryn	Propyzamide	Pyraclostrobin	Total Load
Orcutt	1	storm	40	87.1	40.2	5.6	47.0	14.2	8.2	4.1	29.6	0.70	35.0	15.1	16.3	363
Orcutt	2	storm	24	7.5	11.3	1.4	2.3	3.2	ND	4.0	15.8	0.11	1.0	26.3	3.3	84.4
Orcutt	4	dry	1.3	0.17	0.42	0.11	0.05	1.5	0.03	0.03	0.34	0.01	2.1	0.70	0.23	5.7
Orcutt	6	dry	7.8	0.77	12.2	0.51	0.05	11.8	25.0	ND	1.1	0.03	7.8	110	ND	175
Orcutt	10	storm	12	24.4	45.0	31.8	13.5	8.3	11.8	11.9	11.3	0.23	15.3	25.5	40.7	292
Orcutt	12	dry	3.1	0.08	1.9	1.0	0.12	0.12	ND	ND	0.83	ND	0.37	1.5	ND	6.9
Orcutt	13	storm	11	1.3	11.8	4.8	1.2	0.85	2.4	ND	2.0	0.11	7.4	6.9	2.5	49.4
Orcutt	14	dry	8.6	0.41	5.2	3.0	0.44	0.11	0.44	ND	1.7	0.07	0.72	0.84	2.2	19.2
Orcutt	15	dry	2.2	0.13	1.2	1.3	0.05	4.6	0.17	0.45	0.26	0.02	0.25	0.09	1.6	10.5