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Environmental fate of fungicides and other current-use pesticides in a central California estuary

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ABSTRACT

The current study documents the fate of current-use pesticides in an agriculturally-dominated central California coastal estuary by focusing on the occurrence in water, sediment and tissue of resident aquatic organisms. Three fungicides (azoxystrobin, boscalid, and pyraclostrobin), one herbicide (propyzamide) and two organophosphate insecticides (chlorpyrifos and diazinon) were detected frequently. Dissolved pesticide concentrations in the estuary corresponded to the timing of application while bed sediment pesticide concentrations correlated with the distance from potential sources. Fungicides and insecticides were detected frequently in fish and invertebrates collected near the mouth of the estuary and the contaminant profiles differed from the sediment and water collected. This is the first study to document the occurrence of many current-use pesticides, including fungicides, in tissue. Limited information is available on the uptake, accumulation and effects of current-use pesticides on non-target organisms. Additional data are needed to understand the impacts of pesticides, especially in small agriculturally-dominated estuaries.

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1. Introduction

Coastal estuaries are among the most ecologically important and critically threatened habitats in California and worldwide (Sheehan and Tasto, 2001; Barbier et al., 2011). Less than 20% of California's coastal wetlands remain, and these are impacted by serious water-quality degradation (Dahl, 1990; Sheehan and Tasto, 2001; California Natural Resources Agency, 2010). Rain and irrigation water that drain from agricultural and urban areas transports pesticides and other contaminants into surface waters and aquatic habitats (De Vlaming et al., 2000). Several previous studies have documented the occurrence and biological effects of current-use pesticides in three major central coast rivers/estuaries (Hunt et al., 1999; Anderson et al., 2003, 2006). The Santa Maria watershed along the central California coast contains year-round, intensively-cultivated agricultural land that supports an approximately $500 million/year industry, producing much of the Nation's lettuce, berries and crucifer crops (California Department of Pesticide Regulation, 2013). This estuary and lagoon provide critical nursery and foraging habitat for numerous marine and estuarine fish and invertebrate species, including the threatened tidewater goby. Pesticide-specific monitoring in the Santa Maria estuary has been limited; however, several studies have begun to address the fate and subsequent toxicity of current-use pesticides in the estuary (Anderson et al., 2006, 2010; Phillips et al., 2006, 2010).

Many studies in agricultural areas throughout California have documented the occurrence of a wide variety of current-use pesticides (CUPs) in water and sediment (Sapozhnikova et al., 2004; Kuivila and Hladik, 2008; Hladik et al., 2009) but only a few studies have assessed the uptake and accumulation of CUPs in aquatic organisms. For example, studies have documented the environmental occurrence of CUPs in clams (Pereira et al., 1996), in crabs and crab embryos (Mortimer, 2000; Dugan et al., 2005; Smalling et al., 2010) and fish (Sapozhnikova et al., 2004; Hoai et al., 2011). Limited information on the occurrence of CUPs in aquatic organisms is available because CUPs are considered less environmentally persistent and less bioaccumulative compared to legacy organochlorine pesticides such as *p,p′*-DDT. However, due to high use in agricultural watersheds, there is concern over the wide application of CUPs and their possible impacts on aquatic ecosystems.

Little is known about the effects of some CUPs on non-target organisms. Fish, invertebrates, and other non-target organisms in agricultural watersheds are exposed to a wide variety of pesticides

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throughout their life cycles. Fungicide application typically occurs throughout the growing season, which increases the likelihood of chronic exposure to low concentrations potentially impacting macroinvertebrate communities (Schäfer et al., 2011). The effects of the fungicide, azoxystrobin, on larval salmon have also been documented (Olsvik et al., 2010). Herbicides may directly impact phytoplankton communities due to their similarity in structure to target plant species. For example, the sub-lethal effects of herbicides on phytoplankton growth and cell density were more pronounced at increased salinities (DeLorenzo et al., 2011). Insecticides such as pyrethroids and organophosphates (OPs) are highly toxic to marine and freshwater fish and invertebrates (US Environmental Protection Agency, 2013) and have the potential to alter benthic community structure in agricultural estuaries (Anderson et al., 2010). Pyrethroid and OP insecticides may also directly affect salmon and other fish species through disruption of olfactory sensory neurons necessary in predator avoidance behaviors (Scholz et al., 2000; Moore and Waring, 2001; Sandahl et al., 2004). Although non-target organisms are exposed to complex mixtures of pesticides in most agricultural watersheds, little information is currently available on the effects of these mixtures particularly those containing fungicides. Tierney et al. (2008) noted that mixtures of both herbicides and insecticides elicited a greater response in salmon than individual compounds.

The overall objective of the study was to document the concentrations of pesticides in water, bed sediment, fish and sand crabs in the Santa Maria estuary. The occurrence of pesticides was evaluated relative to toxicity and benthic macroinvertebrate community impacts in a separate report describing results of a larger study (Anderson et al., 2010). This information on pesticide concentrations and exposure of biota to bioavailable contaminants is intended to provide a baseline for future evaluations of watershed-scale effectiveness of agricultural management practices that are slated for implementation.

2. Materials and methods

2.1. Study area and sample collection

The Santa Maria River watershed comprises approximately 486,840 hectares along California's central coast. Orcutt Creek drains approximately 20,230 hectares of land southeast of the Santa Maria River estuary (Smalling and Orlando, 2011) (Fig. 1). Inputs to the Santa Maria estuary are dominated by Orcutt Creek and a much smaller drainage ditch that enters the river near the entrance to the Rancho Guadalupe Dunes Preserve. Together, the flows from these two sources comprise 92% of the total input of water into the estuary (Anderson et al., 2010). River flows are frequently blocked by a littoral berm, and water collects behind the berm to form a large semicircular lagoon (approximate area of 15 hectares) which fills until the water level overtops the beach dune barrier. Flow then erodes the beach dune barrier and the estuary drains over a period of hours to a few days. This filling/draining cycle occurred repeatedly during the course of the study (Smalling and Orlando, 2011). Water samples were collected from Orcutt Creek and at two sites in the Santa Maria Estuary (Fig. 1). The upper estuary sampling site (Santa Maria upper) was located one kilometer downstream of the confluence of Orcutt Creek, but the precise location varied slightly with changes in estuary size during the study. The lower estuary sampling site (Santa Maria lower) was located as close to the beach dune barrier as possible but also varied slightly in location with changes in estuary size. The upper estuary site was selected to represent direct inputs into the estuary from Orcutt Creek while the lower site was selected as an integrator of the entire estuary.

Water samples for the analysis of dissolved pesticides were collected from the upper and lower estuary sites approximately monthly between February and October 2008 (1 storm and 7 dry season events). At each site, a grab sample was collected at a single depth in 1-L pre-cleaned amber bottles. Water samples from Orcutt Creek were collected three times (1 storm and 2 dry season events) in 2008 using a depth-integrating, isokinetic sampler following standard USGS sampling protocols (Wilde et al., 1998).

Bed sediments were collected from eight randomly selected sites throughout the estuary during October 2008 using a small, solvent rinsed hand core or a petite ponar sampler (Anderson et al., 2010). Bed sediment samples were also collected from active depositional areas at the Orcutt Creek site in October 2008 in clean, amber glass jars using a solvent rinsed, stainless steel spoon. All sediment samples were sieved through a 2 mm (stainless steel) sieve in the field and shipped on ice to the laboratory where they were stored frozen at −20 °C prior to extraction and analysis.

Fish were collected in the lower estuary in October of 2008 using a 100-foot beach seine. Targeted species included the starry flounder (*Platichthys stellatus*) and staghorn sculpin (*Leptocottus armatus*). The species and size of each fish (Table 1) were recorded after collection, and all samples were wrapped in solvent rinsed aluminum foil, packed on ice and transported to the laboratory for processing, extraction and analysis. Four individual flounder were dissected in the laboratory and homogenized muscle tissue was placed in 250 mL pre-cleaned clear glass jars and stored at −20 °C. Muscle tissue from two of the samples (fish ID # 20 and 21) were mistakenly composited and homogenized so the final number of flounder samples analyzed was three. Due to their small size, whole-body sculpin samples were analyzed. Three whole body samples were cut into smaller pieces using solvent-rinsed, stainless steel shears, and then homogenized in a solvent-rinsed stainless steel blender. The single whole-body homogenate was placed in a 250 mL pre-cleaned clear glass jar and stored at −20 °C.

Approximately 50 sand crabs (*Emerita analoga*) were collected in the surf zone at three sites in August 2008 using dip nets and gloved hands and placed in 500 mL pre-cleaned, clear glass jars and transported on ice to the laboratory. Both males and females were collected. Due to the timing of collection, many of the females were gravid. Sand crab samples were collected from the mouth of the estuary (mouth), 50 m north of the mouth (north), and 50 m south of the mouth (south), similar to the methods described in Dugan et al. (2005). Samples from each site were homogenized crudely in a clean, stainless steel blender in the laboratory and the homogenates were stored frozen at −20 °C prior to extraction and analysis.

2.2. Sample extraction

2.2.1. Water and bed sediment

Filtered water samples were analyzed for a suite of 68 pesticides by extracting one liter of sample water onto an Oasis HLB solid-phase extraction (SPE) cartridges (Smalling and Orlando, 2011). Prior to extraction, all water samples were filtered using either a continuous-flow centrifuge (Smalling and Orlando, 2011) or a 0.7 μm glass fiber filter and were spiked with ^{13}C-atrazine, and diethyl d_{10}-diazinon as recovery surrogates. Following extraction, the SPE cartridges were dried under carbon dioxide, eluted with ethyl acetate, reduced and deuterated internal standards were added (Hladik et al., 2008).

Bed sediment samples were extracted for 34 fungicides and 57 other currently-used pesticides based on methods described previously (Smalling and Kuivila, 2008; Smalling et al., 2013). Briefly, sediment samples were extracted three times using a Dionex 200 Accelerated Solvent Extractor (ASE) with dichloromethane at 100 °C and 1500 psi. Sulfur was removed using a gel-permeation/

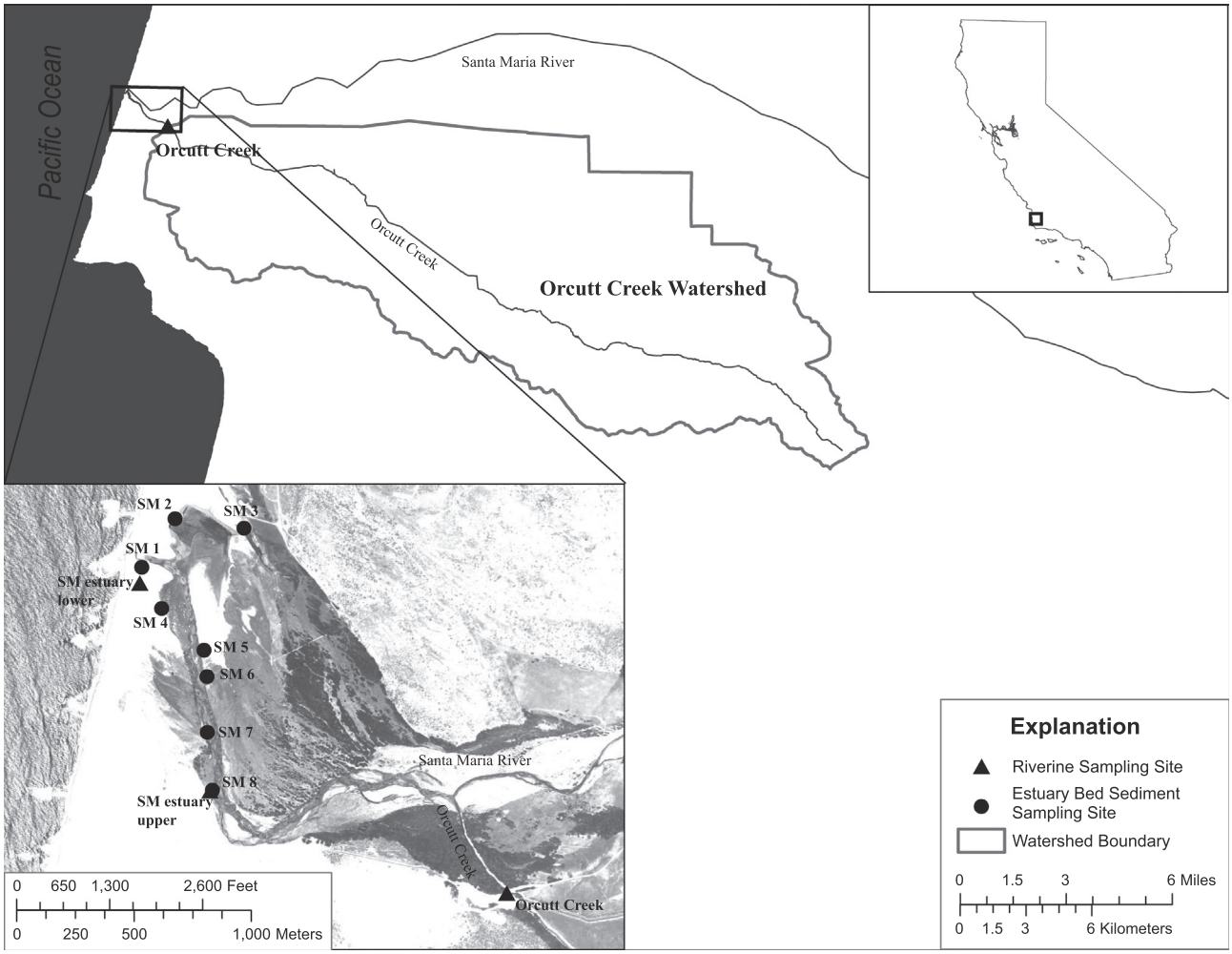

Fig. 1. Location map of the study area in coastal California and the sampling sites in the Santa Maria estuary and Orcutt Creek.

Table 1
Species, sex, and length of fish collected near the mouth of the Santa Maria estuary located in central California in October, 2008.

Field ID #	Date collected	Species	Tissue	Sex	Length (cm)
20	10/9/2008	Starry flounder[a]	Muscle	M	16.6
21	10/9/2008	Starry flounder[a]	Muscle	M	25.0
22	10/9/2008	Starry flounder	Muscle	M	23.7
26	10/9/2008	Starry flounder	Muscle	F	18.6
23	10/9/2008	Staghorn sculpin[b]	Whole body	M	10.4
24	10/9/2008	Staghorn sculpin[b]	Whole body	F	12.3
25	10/9/2008	Staghorn Sculpin[b]	Whole body	F	12.4

[a] Muscle tissue composite.
[b] Whole body composite.

high-pressure liquid chromatography system (GPC/HPLC). Finally, sample matrix was removed using either stacked pre-packed Carbon/Alumina SPE cartridges or deactivated Florisil depending on the compounds of interest. In addition, moisture content, percent organic carbon, and percent nitrogen were measured for each sediment sample (Smalling and Orlando, 2011).

2.2.2. Fish and sand crabs

Fish and sand crab samples were extracted and analyzed for 98 pesticides (Table S1) based on minor modifications to methods described previously (Smalling et al., 2010). Tissue samples were thawed and homogenized with sodium sulfate using a clean, solvent rinsed mortar and pestle. Samples were spiked with trifluralin-d_{10}, ring-^{13}C-*p,p′*-DDE and phenoxy-^{13}C-*cis*-permethrin as recovery surrogates and extracted three times with dichloromethane using a Dionex 200 Accelerated Solvent Extractor (ASE) (Sunnyvale, CA, USA) at 100 °C and 1500 psi. Following extraction, sample extracts were dried over sodium sulfate and reduced to 1 mL. Ten percent by volume of each raw extract was allowed to evaporate to a constant weight in a fume hood for gravimetric lipid determination to the nearest 0.001 g using a microbalance. A majority of the lipid was removed using gel permeation chromatography followed by 6% deactivated Florisil previously activated at 550 °C for 16 h. All tissue data was normalized to percent lipid to decrease species variability in pesticide concentrations and to compare results with previous studies in California.

2.3. Instrumental analysis

A Varian Saturn 2000 gas chromatograph mass spectrometer operating in ion trap mode (GC-ITMS) was used for the analysis of fungicides, herbicides and insecticides in water samples (Hladik et al., 2008; Smalling and Orlando, 2011) and for the analysis of herbicides and insecticides in bed sediment samples (Smalling and Kuivila, 2008; Smalling and Orlando, 2011). GC-ITMS data was collected in full scan and selective ion storage (SIS) modes (Hladik et al., 2008; Smalling and Kuivila, 2008; Smalling and Orlando, 2011).

An Agilent 7890/5975 gas chromatograph mass spectrometer operating in electron ionization mode (GC-EIMS) was used for the analysis of fungicides in bed sediment samples (Smalling et al., 2013) and for the analysis of fungicides, herbicides and insecticides in tissue samples (Table S1). GC-EIMS data for all pesticides was collected in selective ion monitoring mode (SIM) with each compound having one quantifier ion and 1–2 qualifier ions (Smalling et al., 2013). For more information on the compounds analyzed in tissue please see Table S1 of the Supplementary material.

2.4. Quality assurance/quality control

All sample glassware was hand washed and rinsed with tap water followed by acetone and hexane prior to use. All solvents and other reagents were ACS grade or better (Thermo Fisher Scientific, Waltham, MA, USA). Pesticide standard materials were purchased from Chem Service (West Chester, PA, USA), Riedel-de Haën (Seelze, Germany), Supelco (Bellefonte, PA, USA) and Ultra Scientific (North Kingstown, RI, USA) or were donated by the EPA National Pesticide Repository (Ft. Meade, MD, USA). Purities ranged from 95–99%. Internal standards ([$^{2}H_{10}$] acenaphthene) and surrogates ($^{13}C_3$-atrazine, diethyl-d_{10}-diazinon, trifluralin-d_{10}, ring-^{13}C-*p,p′*-DDE and phenoxy-^{13}C-*cis*-permethrin) were purchased from Cambridge Isotope Laboratories (Andover, MA, USA). Neat pesticides were dissolved in acetone or methanol for an initial concentration of 1 mg/mL.

All information regarding water and sediment quality assurance and quality control (QA/QC) information is published elsewhere (Smalling and Orlando, 2011). Water samples were held for no longer than 48 h at 4 °C prior to extraction. Sediment and tissue were stored frozen at −20 °C and held for no longer than 2 years prior to extraction.

For the tissue analysis, performance-based (QA/QC) protocols included the parallel analysis of procedural blanks, matrix spikes, and replicates. Laboratory blanks consisting of approximately 5 g baked sodium sulfate carried through the extraction and cleanup steps did not contain any detectable levels of pesticides. The final method recoveries in laboratory spiked fish and crab tissue ranged from 78% to 95%. Mean recoveries of surrogates in the environmental samples were 91 ± 13%, 96 ± 12% and 91 ± 11% for trifluralin-d^{10}, ring-$^{13}C_{12}$-*p,p′* DDE and phenoxy-$^{13}C_6$-*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%.

Method detection limits (MDLs) for all compounds in water, sediment and tissue ranged from 0.9 to 12 ng/L (Hladik et al., 2008; Smalling and Orlando, 2011), 0.6–3.8 μg/kg dry weight (Smalling and Orlando, 2011; Smalling et al., 2013) and 0.5–3.1 μg/kg wet weight (Table S1), respectively. Analytes can be identified at concentrations less than the MDL with lower confidence in the actual value and are reported as estimates. Limits of detection (LOD) for all pesticides measured were also calculated and can be defined as the concentration of the analyte in the spiked sample that produced a signal greater than three times the background signal. Information on detection limits for all compounds measured in tissue can be found in Table S1 of the Supplementary material.

3. Results and discussion

3.1. Pesticides in water and bed sediment

Twenty-four pesticides were detected in water samples collected from the upper and lower estuary sites and Orcutt Creek, including 6 fungicides, 8 herbicides, 5 insecticides and 5 pesticide degradates. The range of maximum concentrations was 1.8–14,000 ng/L. The fungicides, azoxystrobin and boscalid, and the herbicide, propyzamide (used on leafy greens) were detected frequently in water throughout the year (84%, 100%, and 100% of the samples, respectively; Table 2). Maximum fungicide concentrations were an order of magnitude lower than the laboratory derived aquatic life benchmarks for fish and invertebrates (Elskus, 2012; US Environmental Protection Agency, 2013). Similar to other studies in California agricultural estuaries (Hunt et al., 1999, 2003; Anderson et al., 2006), two organophosphate insecticides (OPs), chlorpyrifos and diazinon were frequently detected (100% and 89% respectively; Table 2) with concentrations sometimes exceeding the LC_{50} values for the test organisms, *Hyalella azteca* and *Ceriodaphnia dubia* (Anderson et al., 2006, 2010).

The timing of maximum surface water pesticide concentrations typically coincided with pesticide use in the surrounding watershed beginning in the late spring. For example, both boscalid use and water concentrations from the two estuary sites increased rapidly in May, peaked in July, and began to decrease in August (Fig. 2). Similarly, the highest concentrations for many of the frequently detected pesticides were observed in July during peak application (Smalling and Orlando, 2011; California Department of Pesticide Regulation, 2013). In agricultural areas in California a significant correlation was often observed between pesticide application rates and in-stream pesticide concentrations, especially for OPs (Hunt et al., 2006).

Twenty-two pesticides were detected in bed sediment collected in October 2008 and included 4 fungicides, 7 herbicides, 7 insecticides and 4 pesticide degradates. Two fungicides (boscalid and pyraclostrobin), one herbicide (dacthal) and three insecticides (chlorpyrifos, diazinon and permethrin) were detected frequently in bed sediments (Table 2). The range of maximum concentrations detected in sediment was 0.7–88.8 μg/kg dry weight (Table 3) and pyraclostrobin was the only current-use pesticide detected at all sites sampled. This is one of the first studies to analyze a number of fungicides in California and is the first study to document the frequent detection of pyraclostrobin in a California watershed. In a recent study, pyraclostrobin was the most frequently detected pesticide in bed and suspended sediment collected from potato growing regions throughout the United States (Smalling et al., 2013). However, most studies to date in California have focused on the occurrence and toxicity of OPs and pyrethroids (Anderson et al., 2006, 2010; Phillips et al., 2006). Chlorpyrifos (an OP) was detected frequently in bed sediment samples (89%) with concentrations ranging from 43.7 μg/kg (Orcutt Creek) to 2.2 μg/kg at the SM 1 site near the mouth of the estuary (similar to the lower site for water sampling). In addition, bifenthrin and permethrin (two pyrethroids) were detected less frequently (22% and 67%, respectively) and at much lower concentrations compared to chlorpyrifos (Tables 2 and 3). The legacy organochlorine pesticide, *p,p′*-DDT and its primary breakdown products *p,p′*-DDD and *p,p′*-DDE were detected in 7 of the 8 samples collected from the estuary as well as Orcutt Creek (Table 2).

Table 2
2008 Pesticide use data[a] in the Orcutt Creek watershed and pesticide detection frequency in water, sediment, fish and sand crabs collected in 2008. Water was collected eight times between February and October from the 2 sites in the estuary (upper and lower) and three times (February, April and July) from Orcutt Creek. Bed sediment was collected in October from 9 sites (8 estuary sites and Orcutt Creek). Fish (starry flounder and staghorn sculpin) were collected in October and sand crabs were collected from 3 sites (mouth, north south) in August.

Compound	Type	Pesticide use (kg)	Detection frequency (%)			
			Water (N = 19)	Sediment (N = 9)	Fish (N = 4)	Sand crabs (N = 3)
3,4-Dichloroaniline	D	–	11	nd	nd	nd
3,5-Dichloroaniline	D	–	16	11	25	nd
Azoxystrobin	F	343	84	33	100	100
Bifenthrin	I	192	nd	22	100	100
Boscalid	F	1432	100	89	100	100
Chlorothalonil	F	1228	11	nd	50	nd
Chlorpyrifos	I	4427	100	89	100	100
Cyfluthrin	I	6.4	nd	nd	25	100
Dacthal	H	357	89	78	25	nd
Diazinon	I	1131	89	67	100	100
Ethalfluralin	H	7.5	5	nd	nd	nd
Iprodione	F	89	nd	nd	100	nd
Fipronil	I	<1	5	11	nd	nd
Fipronil sulfide	D	–	5	11	nd	nd
Fipronil sulfone	D	–	5	nd	nd	nd
Malathion	I	12,230	58	22	25	nd
Myclobutanil	F	194	37	nd	nd	nd
Napropamide	H	1246	74	67	100	nd
Oxyfluorfen	H	2218	89	67	nd	nd
Pendimethalin	H	194	5	nd	nd	nd
Permethrin	I	895	5	67	nd	nd
Phosmet	H	<1	nd	11	nd	nd
Prometryn	H	604	95	33	nd	nd
Propiconazole	F	172	21	22	nd	nd
Propyzamide	H	2013	100	33	nd	nd
Pyraclostrobin	F	696	53	100	100	100
Trifluralin	H	554	58	33	nd	nd
p,p'-DDD	D	–	nd	89	100	100
p,p'-DDE	D	–	84	89	100	100
p,p'-DDT	I	–	nd	89	100	100

D, Degradate; F, fungicide; H, herbicide, I, insecticide; nd, not detected; –, not applied.
[a] California Department of Pesticide Regulation (2013).

Although p,p'-DDT has been banned in the United States since 1972, its primary degradate, p,p'-DDE, continues to persist in the environment particularly in agricultural areas where DDT was used intensively in the past (Pereira et al., 1996). Many of the sediment samples collected throughout the estuary were considered moderately to highly toxic to *H. azteca* and sediment toxicity was potentially caused by mixtures of chlorpyrifos and pyrethroids (Anderson et al., 2010). Estuarine *in situ* benthic community structure was also impacted and it has been suggested that these impacts were due to the proximity of this system to Orcutt Creek (Anderson et al., 2006, 2010).

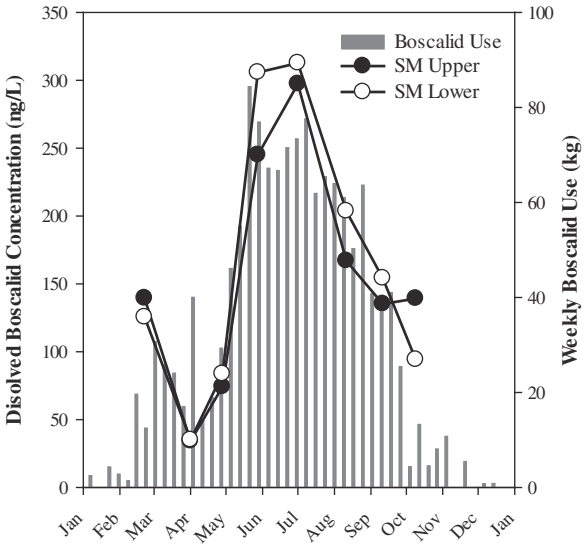

Fig. 2. Concentration of the fungicide, boscalid, in water at the upper (●) and lower (○) estuary sites collected between February and October 2008 compared to weekly boscalid use (gray bars) in the Orcutt Creek watershed.

No relationship between organic carbon content and the distance from the source (Orcutt Creek) was observed so all sediment data was carbon normalized to directly compare sites. A significant correlation was observed between carbon-normalized pesticide concentrations at the eight estuary sites and the linear distance from Orcutt Creek. The total CUPs (sum of all pesticides detected in the samples) and total DDTs were higher in samples collected near the source compared to those collected closest to the mouth (Fig. 3). This significant relationship also holds true when comparing concentrations of other frequently detected pesticides including chlorpyrifos (R^2 = 0.873) and pyraclostrobin (R^2 = 0.721). Although, pesticide concentrations decreased closer to the mouth, they were still detected frequently and have the potential to impact non-target organisms.

3.2. Pesticides in tissue

Thirteen current-use pesticides and p,p'-DDT and its two major degradation products, p,p'-DDE and p,p'-DDD, were detected in fish collected in October 2008 (Table 2). Total percent lipid in the fish ranged from 1.2% to 2.7% depending on species, sex and size of the fish (Table 4). Due to the limited number of fish collected, the types of samples analyzed (whole body versus muscle tissue) and the fact that two flounder were composited (N = 4), no

Table 3
Pesticide concentrations (μg/kg dry weight) and total organic carbon (%) in bed sediment samples collected from Orcutt Creek and eight sites (SM 1–8) within the estuary. Samples were collected in October, 2008. SM[a] 8 is located in the upper estuary closest to Orcutt Creek and SM 1 is located near the mouth of the estuary.

Total organic carbon (%)	Orcutt Creek 1.3	SM 8 2.7	SM 7 1.6	SM 6 2.4	SM 5 1.9	SM 4 2.0	SM 3 3.3	SM 2 2.2	SM 1 1.1
3,5-Dichloroaniline	nd	3.1	nd	nd	nd	nd	nd	nd	nd
Azoxystrobin	2.3	2.2	nd	nd	nd	2.1	nd	nd	nd
Bifenthrin	(0.2)	nd	nd	nd	nd	nd	nd	1.5	nd
Boscalid	6.2	9.3	5.0	9.4	6.9	8.9	nd	6.6	3.2
Chlorpyrifos	43.7	23.1	10.8	10.1	12.7	9.1	nd	5.0	2.2
Dacthal	(0.8)	(1.1)	nd	(0.5)	(0.6)	(0.6)	nd	(1.1)	(0.2)
Diazinon	(1.0)	nd	(1.1)	(1.1)	(1.4)	(1.3)	nd	(1.7)	nd
Fipronil	nd	nd	nd	nd	nd	nd	nd	(0.8)	nd
Fipronil sulfide	nd	nd	nd	nd	nd	nd	nd	(1.4)	nd
Malathion	nd	nd	nd	nd	2.4	nd	4.1	nd	nd
Napropamide	4.2	nd	3.5	4.6	6.4	3.1	15.3	nd	nd
Oxyfluorfen	60.2	29.0	2.7	11.5	14.4	nd	3.5	nd	nd
Permethrin	1.5	nd	(0.9)	2.0	2.4	2.0	nd	2.4	nd
Phosmet	nd	nd	nd	nd	nd	nd	(0.2)	nd	nd
Prometryn	nd	nd	(0.8)	nd	nd	4.6	nd	nd	(1.2)
Propyzamide	nd	(1.3)	nd	4.1	nd	nd	nd	9.0	nd
Propiconazole	2.4	1.3	nd	nd	nd	nd	nd	nd	nd
Pyraclostrobin	6.9	8.2	5.3	8.7	5.8	7.8	5.7	2.9	1.8
Trifluralin	(0.4)	(0.2)	nd	nd	nd	nd	nd	(0.7)	nd
ΣCUPs	*130*	*78.8*	*30.2*	*51.9*	*53.0*	*39.5*	*28.9*	*33.0*	*8.6*
p,p′-DDD	17.3	19.8	6.9	8.3	10.2	8.3	nd	4.0	1.4
p,p′-DDE	80.8	88.8	41.1	40.6	54.8	47.3	nd	12.0	7.6
p,p′-DDT	32.7	23.1	6.5	10.9	11.7	10.1	nd	3.4	1.2
ΣDDTs	*131*	*132*	*54.5*	*59.7*	*76.7*	*65.7*	*nd*	*19.4*	*10.2*

Results in parentheses are less than the method detection limit and are estimates.
[a] SM; Santa Maria estuary.

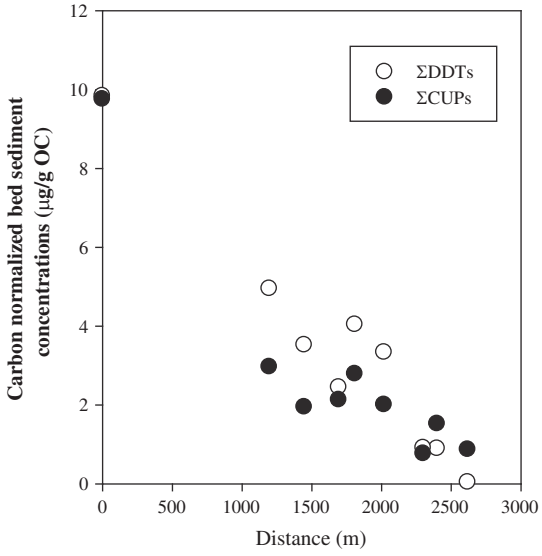

Fig. 3. Change in the organic carbon normalized bed sediment concentrations of total current use pesticides (∑CUPs; ●) and total DDTs (ΣDDTs; ○) in the estuary with distance from the source (Orcutt Creek). R^2 values for ΣCUPs and ΣDDTs were 0.836 and 0.941, respectively.

comparisons could be made between species and all data were combined for discussion purposes. Maximum concentrations of CUPs ranged from 4.1 to 1045 μg/kg lipid weight (Table 4). Pyraclostrobin, was detected in the four fish samples and at the highest average concentration (417 μg/kg lipid weight) compared to the other CUPs (Fig. 4). Three other fungicides, azoxystrobin, boscalid, and iprodione were also detected in all fish samples (Table 2). Chlorothalonil was observed in two of the four samples (Table 4) and to date is the only fungicide that has been previously reported in fish tissue (Sapozhnikova et al., 2004). Several insecticides, including bifenthrin (pyrethroid) and chlorpyrifos and diazinon (OPs) were also detected in all samples collected. Only a limited number of studies have included current-use pesticides in their analysis of tissue samples, including fish (Sapozhnikova et al., 2004), crab embryos (Smalling et al., 2010) and amphibians (Fellers et al., 2004). Specifically, no studies to date have reported the occurrence and accumulation of the fungicides azoxystrobin, boscalid, iprodione and pyraclostrobin in field-collected fish.

Legacy contaminants were also detected frequently and total DDT concentrations were an order of magnitude higher than individual CUPs in fish tissue. The observed concentrations of total DDTs were consistent with data collected over three decades in coastal California (Rassmussen, 1993). These results are not surprising since DDE and to a lesser extent DDD and DDT, are known to biomagnify in the environment (Fisk et al., 1998) and have been detected in fish worldwide (Albaiges et al., 1987; Bindelman et al., 1990). In contrast, there are very little data on the bioaccumulation and biotransformation of CUPs despite decades of use. Current-use pesticides are less hydrophobic and more readily metabolized compared to their legacy counterparts. For example, biomagnification factors of less than 1 were calculated for several triazole fungicides (Konwick et al., 2006) indicating that these pesticides will not biomagnify in aquatic food webs. In addition, rapid transformation of these fungicides occurred with laboratory derived half-lives of less than 5 days (Konwick et al., 2006). It is also important to consider the readily formed *in vivo* metabolites of CUPs, which may cause greater harm to aquatic biota than the parent compound (Sinclair and Boxall, 2003; Brander et al., 2012). Thus, for accurate environmental assessment, there is a need to characterize the persistence and accumulation of CUPs and their compound-specific metabolites in aquatic biota.

Ten pesticides, including 3 fungicides, 4 insecticides and DDT, DDD and DDE were detected in sand crab tissues (Table 2). The

Table 4
Lipid normalized pesticide concentrations (μg/kg lipid wt) in fish and invertebrates collected near the mouth of the Santa Maria estuary in central California in 2008. Included is also the ratio of total current-use pesticides (CUPs) to total DDTs for each sample. Field ID's and percent lipid are also presented.

	Starry flounder			Staghorn sculpin	Sand crabs		
Field ID	20–21	22	26	23–25	Mouth	North	South
Lipid (%)	2.7	1.7	2.7	1.2	3.5	6.2	5.0
3,5-Dichloroaniline	nd	26.1	nd	nd	nd	nd	nd
Azoxystrobin	512	404	418	120	37.5	13.1	15.9
Bifenthrin	26.7	41.1	25.9	12.2	18.9	8.5	8.1
Boscalid	58.9	46.7	450	35.7	34.1	31.4	41.2
Chlorothalonil	(0.8)	4.1	nd	nd	nd	nd	nd
Chlorpyrifos	147	248	205	22.2	314	302	437
Cyfluthrin	32.1	nd	nd	nd	198	128	218
Dacthal	nd	nd	24.6	nd	nd	nd	nd
Diazinon	119	121	75.1	42.2	242	199	162
Iprodione	201	295	245	14.8	nd	nd	nd
Malathion	73.5	nd	nd	nd	nd	nd	nd
Napropamide	467	572	480	100	nd	nd	nd
Pyraclostrobin	118	176	1045	330	2258	1351	2251
ΣCUPs	*1754*	*1933*	*2968*	*677*	*2698*	*1678*	*2631*
p,p′-DDE	7784	10,738	10,664	1082	504	898	1754
p,p′-DDD	1178	1.792	1668	525	39.1	21.2	29.5
p,p′-DDT	708	1438	980	852	29.7	13.3	16.2
ΣDDTs	*9760*	*13,928*	*13,311*	*2459*	*572*	*932*	*1800*
ΣCUP/SDDT[a]	*0.2*	*0.1*	*0.2*	*0.3*	*4.7*	*1.8*	*1.5*

Results in parentheses are less than the MDL and are considered estimated.
nd, not detected.
[a] Ratio of ΣCUPs to ΣDDTs.

total percent lipid in the sand crab composites ranged from 3.5% to 6.2% (Table 4). Maximum pesticide concentrations ranged from 18.9 to 2258 μg/kg lipid weight (Table 4), which is similar to what has been previously reported for legacy pesticides in this estuary (Dugan et al., 2005).

Pyraclostrobin, was detected in all three sand crab samples and at the highest concentration (2258 μg/kg lipid weight) compared to the other pesticides including total DDTs (Fig. 4). Two other fungicides (azoxystrobin and boscalid) were also detected in the three samples but at concentrations 50 times lower than pyraclostrobin. The pyrethroids, bifenthrin and cyfluthrin were detected in all sand crab samples (Table 2) with maximum concentrations of 18.9 and 218 μg/kg lipid weight, respectively (Table 4). Although frequently detected, lipid-normalized pyrethroids concentrations (Table 4) in the current study were almost an order of magnitude lower than concentrations observed in crab embryos collected from gravid crabs residing in urban/suburban salt marshes in Northern California (Smalling et al., 2010). Reported agricultural pyrethroid use in the Orcutt Creek watershed is relatively low (California Department of Pesticide Regulation, 2013), while sites in the previous study (Smalling et al., 2010) received input from a golf course and an urban area where pyrethroid use is increasing. Two OPs, diazinon and chlorpyrifos, were detected in every sand crab sample with maximum concentrations of 242 and 347 μg/kg lipid weight, respectively (Table 4). Several studies have reported the occurrence of OPs in crabs collected from areas adjacent to agricultural (Dugan et al., 2005) and urban (Mortimer, 2000; Smalling et al., 2010) watersheds.

The concentrations of DDTs observed in sand crabs from these two areas in the summer of 2000 (Dugan et al., 2005) and 2008 (current study) are similar to those reported by Burnett (1971) for samples collected in these locations in November 1970 and February 1971. These results suggest that comparable amounts of DDTs persist and are biologically available over 40 years after the use of this pesticide was banned in the USA. In 2000, Dugan et al. (2005) examined the relationship of total DDT concentration in sand crabs and distance from the mouth of the estuary. The study examined samples collected 0–900 m from the mouth and found a significant negative correlation between total DDT concentration and the distance from the river mouth, suggesting an exposure gradient in the vicinity of the Santa Maria River. In the current study, sand crabs were collected only 50 m north, 50 m south and at the mouth and total DDT concentrations were similar among the three sites.

Many of the most frequently detected compounds in fish and sand crabs were typically observed in water and sediment samples collected throughout the study (Table 2) with the exception of the pyrethroids. Bed sediment toxicity in the Santa Maria River watershed has been frequently attributed to pyrethroids (Anderson et al., 2006, 2010; Phillips et al., 2006) but in the current study bifenthrin was detected only once in sediment samples above the MDL and although, permethrin was detected frequently, observed concentrations were below reported LC_{50} values for sediment. Conversely, bifenthrin was detected in 100% of the fish and crab samples and cyfluthrin (not detected in sediment) was observed in 25% of the fish and 100% of the crab samples. In the case of pyrethroids, the sediment and/or the water are not good indicators of exposures in fish or crabs potentially due to method limitations. Average ΣDDT concentrations in fish were nearly an order of magnitude higher compared to the sand crabs. Conversely, average pyraclostrobin concentrations in sand crabs were approximately 5 times higher than those concentrations observed in fish. Thus, the concentration of total CUPs in crabs was higher than total DDTs and the ratio of CUPs to DDT ranged from 1.5 to 4.7 (Table 4). Routes of exposure as well as life histories (including feeding) may explain the differences in pesticide profiles observed in the field. Flounder and sculpin are bottom dwellers that live/forage in the sediment within the estuary whereas sand crabs are filter feeders residing in the surf zone. Alternatively, food web position (ΣDDTs) and organismal specific metabolic rates (ΣCUPs) could explain the observed differences between crabs and fish; however, limited information is available on food web dynamics and trophic position in California coastal estuaries such as the Santa Maria.

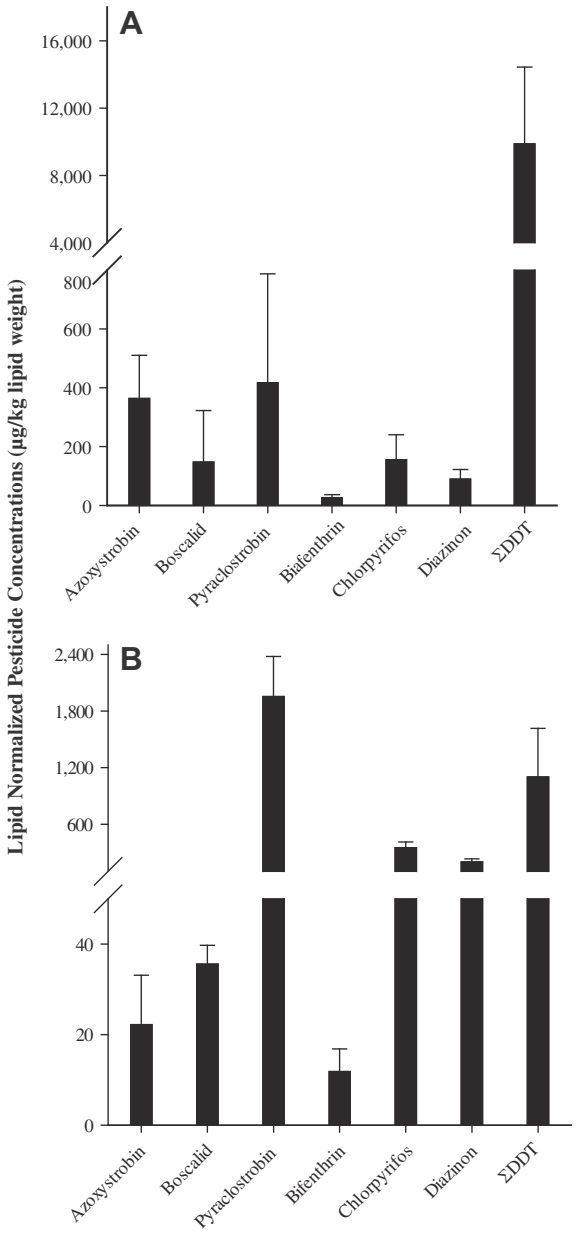

Fig. 4. Average (±standard deviation) lipid normalized fungicide and insecticide concentrations in (A) fish (staghorn sculpin, *Leptocottus armatus*; and starry flounder, *Platichthys stellatus*) collected in October 2008 and (B) sand crabs (*Emerita analoga*), collected in August 2008 near the mouth of the Santa Maria estuary. Graph only includes the pesticides detected in either 100 percent of the fish or sand crab samples. ΣDDTs represents the sum of *p,p′*-DDT, *p,p′*-DDD and *p,p′*-DDE.

3.3. Potential estuarine impacts

Many studies have focused on the potential effects of pesticides on migrating salmonids. Since most environmental concentrations are below acute toxicity levels, these studies addressed the likelihood of chronic toxicity to the fish such as sub-lethal effects (predator avoidance) and/or indirect effects such as the reduction of prey species, including benthic amphipods (Shreffler et al., 1992; Grimmaldo et al., 2009). A number of studies have demonstrated that concentrations of individual OP and pyrethroid insecticides had effects on olfactory response or behaviors associated with olfactory response in salmon (Scholz et al., 2000; Sandahl et al., 2004; Moore and Waring, 2001) at concentrations similar to what was detected in the current study. Another study with larval salmon demonstrated that the fungicide, azoxystrobin, may affect mitochondrial respiration and mechanisms controlling cell growth and proliferation (Olsvik et al., 2010). In addition, pesticide mixtures have been reported to have a greater effect on olfactory response in trout at environmentally relevant concentrations compared to individual compounds (Tierney et al., 2008). Although most of the previous studies emphasized potential effects on species that do not reside in the Santa Maria estuary, there is the potential that similar effects may be observed in steelhead trout, an ecologically important and endangered species that depends on central coast estuaries for key stages of its life cycle (Hayes et al., 2008). In addition, there is limited information on the effects of pesticide mixtures on year-around residents of the estuary such as flounder, staghorn sculpin and tidewater gobies.

Marine invertebrates have frequently been used as indicators of marine pollution (Beiras et al., 2003), because they are abundant in many near-shore environments and are relatively immobile compared to other organisms, such as fish (Smith et al., 1991). In the present study, sand crabs were chosen as a comparison to previous studies (Burnett, 1971; Dugan et al., 2005) and their role in near shore food webs for surf perch and other species makes their tissue burdens important to consider. This study adds to the limited information available on the occurrence, accumulation and effects of currently-used pesticides on sand crabs and other invertebrates residing in or near agriculturally dominated estuaries.

4. Conclusions

Elevated concentrations of pesticides were observed in water, sediment, fish and sand crab samples from the Santa Maria estuary, located at the outflow point of an agriculturally-dominated watershed. In water samples, a variety of CUPs were detected frequently throughout the year with maximum water concentrations generally following highest application rates in the late spring/early summer. CUPs were also detected frequently in bed sediments collected throughout the estuary with decreasing concentrations of most compounds with distance from the source (i.e. Orcutt Creek). Fish and sand crabs collected near the mouth of the Santa Maria estuary accumulated a number of current-use fungicides, herbicides, and insecticides. The fungicide, pyraclostrobin, was detected in 100% of the fish and sand crabs collected and this is the first study to report its accumulation in tissue. This is also one of the first data sets to report concentrations of a wide variety of CUPs in fish and sand crabs. This is particularly important given the dearth of information on the persistence, bioaccumulation, and biotransformation of CUPs and their compound-specific metabolites in aquatic biota. Total DDT concentrations were higher in fish compared to total CUPs, and contaminant concentration varied between species (fish and invertebrates) particularly for the legacy contaminants. In sand crab samples, total CUP concentrations were approximately 3 times higher than total DDTs and the fungicide, pyraclostrobin, was detected at the highest concentrations. This study provides data on the status of an ecologically important estuary on California's central coast, and includes a characterization of pesticide contamination in water, sediment and biota. As management practices are implemented in the watersheds influencing offsite transport, it is expected that pesticide loadings will decrease, and the percentage of occurrence and the accumulation in aquatic organisms should correspondingly decrease. The current study provides baseline information that will allow resource managers to track changes in ecosystem performance with changes in pesticide contamination in the estuaries and their key tributaries.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at PANGAEA. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.marpolbul.2013.05.028.

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