Stormwater-related transport of the insecticides bifenthrin, fipronil, imidacloprid, and chlorpyrifos into a tidal wetland, San Francisco Bay, California

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HIGHLIGHTS

• Suisun Marsh, in California, provides habitat to several imperiled fish species.
• Pesticides were sampled in creek waters flowing to the marsh after a winter storm.
• Urban creeks were toxic to invertebrates due to bifenthrin and fipronil.
• No toxicity was seen in agriculture-affected creeks, at least during the winter.
• Fipronil was measurable in the marsh, but not toxic due in part to dilution.

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ABSTRACT

Suisun Marsh, in northern San Francisco Bay, is the largest brackish marsh in California, and provides critical habitat for many fish species. Storm runoff enters the marsh through many creeks that drain agricultural uplands and the urban areas of Fairfield and Suisun City. Five creeks were sampled throughout a major storm event in February 2014, and analyzed for representatives of several major insecticide classes. Concentrations were greatest in creeks with urban influence, though sampling was done outside of the primary season for agricultural pesticide use. Urban creek waters reached maximum concentrations of 9.9 ng/l bifenthrin, 27.4 ng/l fipronil, 11.9 ng/l fipronil sulfone, 1462 ng/l imidacloprid, and 4.0 ng/l chlorpyrifos. Water samples were tested for toxicity to Hyalella azteca and Chironomus dilutus, and while few samples caused mortality, 70% of the urban creek samples caused paralysis of either or both species. Toxic unit analysis indicated that bifenthrin was likely responsible for effects to H. azteca, and fipronil and its sulfone degradate were responsible for effects to C. dilutus. These results demonstrate the potential for co-occurrence of multiple insecticides in urban runoff, each with the potential for toxicity to particular species, and the value of toxicity monitoring using multiple species. In the channels of Suisun Marsh farther downstream, insecticide concentrations and toxicity diminished as creek waters mixed with brackish waters entering from San Francisco Bay. Only fipronil and its degradates remained measurable at 1–10 ng/l. These concentrations are not known to present a risk based on existing data, but toxicity data for estuarine and marine invertebrates, particularly for fipronil’s degradates, are extremely limited.

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1. Introduction

Insecticide residues entering aquatic habitats via runoff have been shown to have effects ranging from selection for pesticide-resistant genotypes (Weston et al., 2013), to mortality of indicator species (Bailey et al., 2009), to changes in community composition (Schulz and Liess, 1999). Organophosphate insecticides, such as diazinon and chlorpyrifos, have long been associated with aquatic toxicity following rain-related transport of residues into waterways (Bailey et al., 2009; Kuivila and Foe, 1995). Pyrethroids have been an increasingly important insecticide class for the past decade as organophosphate use has declined. They have been shown to enter creeks at toxic concentrations after rain events, even traveling downstream more than 20 km from their source while retaining their toxicity to aquatic life (Weston et al., 2014). In recent years, use of phenylpyrazole insecticides, especially fipronil, has become more common, and it is now commonly detected in urban creeks at concentrations acutely toxic to a variety of invertebrates (Weston and Lydy, 2014). Neonicotinoids, such as imidacloprid, are an
emerging class of insecticides with potential for aquatic toxicity (Smit et al., 2015), though the research focus has largely been on their toxicity to pollinators (Cresswell, 2011).

The present study examined the potential for insecticide-related aquatic toxicity in agriculture and urban-influenced creeks flowing into Suisun Marsh, and in the sloughs of the marsh itself. Suisun Marsh is the largest brackish marsh in California, and is located near the confluence of the Sacramento and San Joaquin Rivers in northern San Francisco Bay. Freshwater enters the marsh through several upland creeks that flow through agricultural or urban lands before becoming sloughs as they enter the marsh, where they broaden and their flow becomes tidally influenced. Thus, there is the potential for these creeks to transport a variety of agricultural and/or urban contaminants into Suisun Marsh.

Intensive sampling was conducted during the largest storm event of the 2013/2014 winter rainy season. Sampling was done to quantify water concentrations of representatives from several insecticide classes (the organophosphate chlorpyrifos, the phenylpyrazole fipronil and its degradates, the neonicotinoid imidacloprid, and eight pyrethroids). The compounds were selected based on high use, prior linkage with aquatic toxicity in the region, or emerging use with little previous monitoring (imidacloprid). Since insecticide effects on fish within the marsh could be indirect through toxicity to their invertebrate prey, toxicity testing of water samples was conducted with the amphipod, Hyallela azteca, and the chironomid, Chironomus dilutus. While the study was focused on the Suisun Marsh watershed, findings should be internationally relevant, as many estuarine areas receive runoff from mixed-use urban and agricultural watersheds, and the insecticides investigated are used worldwide.

2. Material and methods

2.1. Description of study area

Suisun Marsh contains 470 km² of marsh, much of it diked and seasonally flooded to support waterfowl hunting. Among the diked wetlands is a network of tidal sloughs, with salinities temporally varying from 0 to 17 psu depending on the volume of river flow entering the Bay (Meng et al., 1994). Given that most of the wetlands surrounding San Francisco Bay have been lost to agriculture or urban development, the wetlands of Suisun Marsh are considered critical spawning and rearing habitat for a diverse assemblage of native and introduced fish species (Meng et al., 1994; Meng and Matern, 2001; O’Rea and Moore, 2014). Of particular significance is the use of the marsh by several native species whose numbers have dramatically declined throughout the estuary in recent decades (Sommer et al., 2007), such as Sacramento splittail (Pogonichthys macrolepidotus), longfin smelt (Spirinchus thaleichthys), and delta smelt (Hypomesus transpacificus). The marsh and adjacent Suisun Bay provides summer and fall habitat for subadult and adult delta smelt (Sommer and Mejia, 2013), and there is evidence that it provides spawning habitat for delta smelt in winter and spring months as well (Bennett, 2005; Murphy and Hamilton, 2013).

2.2. Field sampling

Sampling addressed impacts of runoff following winter storms, as these events have been shown to result in both urban and agricultural pesticide inputs to estuarine waters in environments similar to the study site (Weston et al., 2014). We recognize that this focus does not address conditions during the summer growing season, when most agricultural pesticide application occurs. However, pesticide inputs at that time would be inherently unpredictable, as they depend on application and irrigation practices of individual growers, and the volume of pesticide-contaminated runoff from irrigation return flows is likely to be much smaller than the volume of runoff accompanying storm events. All sampling was conducted in response to a single major rain event, with light rain beginning 6 February 2014, and heavier rains from the night of 7 February until 9 February. Rainfall accumulations at Cordelia, California (gauge location = 38.172, –122.129) were 1.4 cm on the 6th, 2.7 cm on the 7th, 4.8 cm on the 8th, and 3.1 cm on the 9th. In this region of California, most rainfall occurs from November through March, but the 2013/2014 wet season had exceptionally little rainfall, and accumulation never exceeded 1 cm in any day of the entire wet season up until the February storm sampled for the present study. Thus, the sampled rain event can be considered a “first flush”, the first major rain event of the season, often accompanied by high suspended sediment loads entering the San Francisco Bay estuary and with pesticides associated with those particles (Goodwin and Denton, 1991; Bergamaschi et al., 2001).

There were two types of sampling sites: creek samples and slough samples (Fig. 1). The former were collected from most of the major creeks that flow to Suisun Marsh, at the last vehicle-accessible location prior to their entry to the marsh. Creek sites were sampled in both morning and afternoon of 8 February, and in the morning of 9 February. Suisun Creek was the only sampled creek for which pesticide sources in the watershed were primarily agricultural (87% of developed land agricultural, 5% urban or residential; Fairfield-Suisun Sewer District, 2004). Laurel Creek and McCoy Creek watersheds were largely urban (3% agricultural, 95% urban/residential, and 14% agricultural, 68% urban/residential, respectively). Green Valley and Ledgewood Creek watersheds had mixed land uses (50% agricultural, 45% urban/residential, and 74% agricultural, 24% urban/residential, respectively). All the urban areas in the study area are served by storm drain systems that divert untreated runoff from the streets to nearby creeks. The region’s municipal wastewater treatment plant discharges into Suisun Marsh, approximately midway between sites LLC and SSV, thus the discharge would not affect water quality at the creek sites, but could influence some of the slough sites, especially SSV and SSO.

Flow at the Laurel Creek sampling site (LLC) was weak compared to the other waterways because it only received runoff from urban storm drains in the immediate vicinity. The main flow from the upper reaches of Laurel Creek is diverted eastward, joining with flow from McCoy Creek, and their combined flow was characterized at site MCC.

As the creeks enter Suisun Marsh, velocities decrease, conductivity increases, and tidal action becomes significant. Five slough sites were sampled within the marsh daily from 8 to 10 February, and a sixth site (site SSO) was sampled 10 February near the outfall of the slough system into Grizzly Bay, an embayment of northern San Francisco Bay. In order to best represent freshwater flowing seaward from the sloughs, rather than tidally-driven brackish waters flowing into the marsh from Grizzly Bay, slough sites were sampled within the 3-h period preceding the lowest tide each day. Nightfall prevented sampling at low tide or shortly thereafter.

Water samples were collected just below the surface either from the bank or using a stainless steel bailer from bridges, depending on access at each site. The only exception was site SSO, which required boat access. Samples were collected in glassware certified clean for pesticide analysis (1-Chem 200 series, Fisher Scientific, Waltham, MA), using 4-1 bottles for toxicity testing samples and 1-1 bottles for chemistry samples. Hexane (10 ml) was added as a keeper solvent to samples intended for pyrethroid, chlorpyrifos, and fipronil analysis. Samples were kept at 4 °C, with toxicity testing done within 48 h and pesticide extractions done within 96 h. Total suspended solid (TSS) samples were collected in 250-mL glass bottles.

2.3. Analytical procedure

For those samples intended for analysis of pyrethroids, chlorpyrifos, fipronil, or fipronil degradates, the analytical surrogates 4,4′-dibromooctfluorobiphenyl (DBOFB) and decachlorobiphenyl (DCBP) (Supelco, Bellefonte, PA) were added to the samples, and approximately 850 ml of water was liquid-liquid extracted using U.S. Environmental Protection Agency Method 3510C (USEPA, 2013). Three sequential
extractions were performed with 60 ml dichloromethane (all solvents from Fisher Scientific), with one aliquot also used to extract the empty sample bottle. All dichloromethane extracts were combined and reduced in volume to 5 to 10 ml under a stream of nitrogen for overnight shipment to the analytical laboratory at Southern Illinois University.

After arrival at the laboratory, extracts were solvent exchanged to hexane, concentrated to 1 ml, and eluted through a dual layer solid phase extraction cartridge (SPE) containing 300 mg of graphitized black carbon, 600 mg of primary/secondary amine and capped with anhydrous Na₂SO₄ (Wang et al., 2009). The SPE was primed with 3 ml of hexane prior to the introduction of the extract. The target pesticides were eluted with 10 ml of 1:1 hexane:acetone (v/v) solution, and solvent exchanged to 0.1% acetic acid in hexane with a final volume of 1 ml. The extracts were analyzed using an Agilent 6850 gas chromatograph 5975 XL mass spectrometer (GC–MS; Agilent Technologies, Palo Alto, CA) with negative-ion chemical ionization and selected-ion monitoring. Inlet, ion source, and quadrupole temperatures were 260, 150, and 150 °C, respectively. A HP-5 MS column (30 m × 0.25 mm × 0.25 μm film thickness) was used for separation of the analytes using helium as a carrier gas with the flow rate set at 1.8 ml/min. A 2 μl sample was injected into the gas chromatograph using pulsed splitless mode. The oven was set at 50 °C for 1 min, heated to 200 °C at 20 °C/min, then to 295 °C and held at 205 °C for 5 min. Quantification was performed using internal standard calibration. Calibration curves were based on area using concentrations of 2, 5, 10, 50, 100, 250, and 500 ng/ml of each pesticide and surrogate, while the concentrations of the internal standards were 20 ng/ml for each standard. Analytes included chlorpyrifos, bifenthrin, cyfluthrin, cyhalothrin, cypermethrin, deltamethrin, esfenvalerate, permethrin, tefluthrin, fipronil, fipronil desulfanyl, fipronil sulfide, and fipronil sulfone. Data were reported down to a concentration of 1 ng/l. Quality assurance samples included a blank, lab control spike, matrix spike, matrix spike duplicate, and field duplicate, all run with every batch of 20 samples. Recovery in matrix spikes ranged from 36 to 110%, and averaged 77%.
For those samples intended for analysis of imidacloprid, the surrogates thiacloprid and acetamiprid (ChemService, West Chester, PA) were added to each sample of approximately 850 ml, as well as 35 g sodium chloride, and the samples were liquid-liquid extracted as detailed above. Cleanup methods were similar to those described above with the following exceptions: the SPE was primed with 3 ml of a 75:25 hexane:acetone solution; unwanted interference was washed from the column with 7 ml of 90:10 hexane:chloromethane (v/v) solution; and the target pesticide was eluted from the SPE with 3.5 ml of 1:1 acetone:nitrite:chloromethane (v/v) solution. The eluent was evaporated to near dryness and reconstituted to 0.5 ml in 80:20 high performance liquid chromatography water:acetoni trile acidified by 0.1% of trifluoroacetic acid.

Quantification of imidacloprid was done using an Agilent 1260 HPLC interfaced with a 3200 Q Trap triple quadrupole/linear ion trap mass spectrometer (AB Sciex; Toronto, Canada). The HPLC system was equipped with a Waters Xterra® phenyl column (2.1 mm × 100 mm, 3.5 μm particle size) and the column temperature was maintained at 30 °C. The mobile phase consisted of water and methanol, both spiked with 0.1% formic acid (v/v). The mobile phase flow rate was 0.2 ml/min and the following gradient was employed: 10% methanol ramped to 70% methanol in 7 min (linear) and then ramped to 80% methanol in 6 min (linear), followed by a linear increase to 90% methanol in 2 min (held for 1 min) and then a change to 10% methanol in 1 min (held for 4 min). The mass spectrometry system was equipped with a Turbo Ion Spray® electrospray ionization probe operated in multiple reaction monitoring mode and in positive mode for quantitative determination. The ion pairs monitored were m/z 256.0 → 209.0 and 256.0 → 175.0. Quality control samples were as described above, and imidacloprid concentrations were reported down to 10 ng/l. Recovery in matrix spikes averaged 88%.

The TSS was quantified as the dried mass retained on a Whatman 934-AH filter (Whatman, Florham Park, NJ).

2.4. Toxicity testing of water samples

Samples were tested with H. azteca, using animals maintained in culture at the University of California Berkeley. Ten individuals, 7 to 10 d in age, were placed in 100-ml beakers containing 80 ml water, with five replicates per sample. A 1-cm² nylon screen was placed in each beaker to provide a substrate to which the animals cling, and its size was kept to a minimum to avoid adsorption of toxicants. Since pyrethroid toxicity is temperature dependent (Weston et al., 2009), tests were done at 13 °C to approximate the temperature at the sample sites, with the test animals acclimated to the test temperature by gradual decrease over three days. Testing was done under a 16-h light:8-h dark photoperiod. A yeast/cereophyll/trout food solution (1 ml per beaker) was provided on the second day, and after a 6-h feeding period, approximately 80% of the water was replaced with fresh sample. Renewal water was held in the dark at 4 °C after collection, but brought to test temperature prior to use. Conductivity, alkalinity, hardness and pH were measured at test initiation and termination; temperature and dissolved oxygen were measured at 0, 48 and 96 h. Tests were terminated at 96 h. Pyrethroids cause varying degrees of paralysis in H. azteca, ranging from animals that are motionless except for occasional twitching to others that attempt to swim but are unable to do so. Tests were scored for the number of dead amphipods and those that were alive but showing paralysis. All tests were accompanied by a control using moderately hard water (Smith et al., 1997), prepared by adding salts to Milli-Q purified deionized water. When testing slough samples, a high conductivity control (14,000 μS/cm) was also prepared by adding Instant Ocean (United Pet Group, Blacksburg, VA) to deionized water. A single sample was also tested by addition of piperonyl butoxide (PBO), known to increase toxicity if due to pyrethroids (Amweg et al., 2006). Piperonyl butoxide at 50 μg/l was added to test waters in a methanol carrier, with methanol concentration at 12.5 μl/l. The PBO was renewed with the water change on the second day. A treatment control (laboratory water with PBO) was also included. The PBO test was performed at 17 °C, since nearly a week had elapsed by the time the sample was established as toxic by the initial testing, and we wished to minimize further delay that would have been necessary to temperature-acclimate additional H. azteca to the 13 °C of the previous tests.

Creek samples were also tested with C. dilutus, though the higher conductivity in the sloughs precluded their testing with this species. Test water (600 ml) was added to 1-l beakers, with five replicates per sample. A thin layer of washed sand (Fisher Scientific) was placed in each beaker to allow tube building. Ten 3rd-instar individuals were added to each beaker from cultures maintained at University of California Berkeley. Test temperature, light regime, feeding, and water change were as described above except that the second-day feeding consisted of 0.5 ml of a Tetrafen fish food slurry (United Pet Group). After 96 h, survivors were recorded, as well as those still alive but unable to perform typical thrashing movements when gently prodded (also referred to as unable to perform figure-8 movement; Pape-Lindstrom and Lydy, 1997).

Samples were compared to concurrent controls using CETIS software (Tidepool Scientific Software, McKinleyville, CA) by t-test if parametric assumptions were met, or by Wilcoxon Rank Sum if they were not.

3. Results and discussion

3.1. Relationship of sampling to rainfall patterns

The creek sites were repeatedly sampled as stormwater runoff entered and their flows increased. Water depth in Suisun Creek is monitored by the California Department of Water Resources, and was 0.4 m at the gauging station prior to the storm event. Samples were collected at stages of 0.5, 1.2 and 1.3 m on the rising limb of the hydrograph, with the stage peaking at 1.7 m. The other sampled creeks are not gauged, but stages at the times of sampling relative to peak stage would be comparable. Conductivity in the creeks ranged from 89 to 553 μs/cm, with temperatures of 11 to 13 °C (Table 1).

Within the sloughs of the marsh, conductivity rises and falls throughout each tidal cycle, depending on the relative proportions of freshwater from the creeks and brackish water from Grizzly Bay. In any given sample within the marsh sloughs, conductivity during the storm ranged from 854 to 10,302 μS/cm (Table 1). Prior to the rain event, conductivity at the mouth of Suisun Slough (near site S50) fluctuated from 14,000 to 16,000 μS/cm throughout the tidal cycle, or approximately 8 to 9 psu. As runoff from the storm moved downstream into the marsh, the low conductivity excursions at low tides became more pronounced, eventually reaching a minimum of 5561 μS/cm at the mouth of Suisun Slough on 10 February (California Department of Water Resources, 2014). As sampling of slough sites occurred over three days from 8 to 10 February, we successfully captured the period of greatest runoff influence, and therefore our sampling should reflect the period of highest pesticide concentration during the runoff event.

3.2. Pesticide concentrations

Bifenthrin was detected in nearly every creek sample, and it was the only pyrethroid measurable in the creeks with the exception of two samples containing cyfluthrin just above the reporting limit (Table 2). Bifenthrin concentrations ranged from 3.2 to 9.9 ng/l in those creeks with the greatest urban influence, and the compound has consistently been the dominant pyrethroid in urban creeks throughout the U.S. (Weston et al., 2011; Kuivila et al., 2012). It was detected in only one of three samples in the creek draining only agricultural lands (1.5 ng/l; Suisun Creek), though most agricultural use would have occurred in the summer, about six months earlier. The magnitude of agricultural bifenthrin use is comparable to its non-agricultural use in Solano County,
where the study area is located (590 kg/yr versus 523 kg/yr, respectively; California Department of Pesticide Regulation, 2015).

Fipronil and its environmental degradates were found in highest concentrations in those creeks with greatest urban influence (McCoy and Laurel Creeks), at lower concentrations in creeks with mixed urban and agricultural contributions, and were nearly always undetectable in samples from agricultural waterways. These results are consistent with fipronil use patterns, for while there is 227 kg/yr of fipronil used for non-agricultural purposes in Solano County, there are no approved agricultural uses of fipronil in California. In the predominantly urban waterways, fipronil concentrations were 14.5 to 27.4 ng/l, followed by fipronil sulfone at 8.3 to 11.9 ng/l, fipronil desulfynil at 4.8 to 7.1 ng/l, and fipronil sulfide at 1.7 to 8.4 ng/l; values very similar to concentrations observed after rain in many urban waterbodies throughout northern California (Weston and Lydy, 2014).

Imidacloprid is primarily an agricultural-use insecticide with some urban applications (235 kg/yr versus 54 kg/yr, respectively, in Solano County). It was detected in particularly high concentrations (889 to 122.0124 ng/l) in Laurel Creek. The other predominantly urban waterway, McCoy Creek, contained much lower concentrations (26.5 to 33.2 ng/l). It is unclear why imidacloprid concentrations were dramatically higher in McCoy Creek, but at the point Laurel Creek was sampled, it only serves a very small watershed of residences and businesses within Suisun City, so it is possible that pesticide use at a very small number of homes could have led to dramatic differences in creek quality.

Chlorpyrifos was absent from the agricultural Suisun Creek, and found in urban-influenced creeks at <4 ng/l. Though nearly all urban-use products containing the compound were removed from retail stores over a decade ago, low concentrations such as those seen remain common in urban runoff in the region (Weston and Lydy, 2010a).

Within the sloughs of Suisun Marsh, bifenthrin was always undetectable, as was chlorpyrifos except for a single sample at the reporting limit. Fipronil or a degrade was found in almost every slough sample, though at low concentrations. Median fipronil concentration in the sloughs was 1.4 ng/l (versus 11.7 ng/l median in the creeks), and the maximum concentration was 5.1 ng/l. The low to immeasurable insecticide concentrations in the sloughs can be attributed in large part to dilution. Water entering the slough system from San Francisco Bay on rising tides had a conductivity of approximately 15,000 μS/cm, whereas freshwater entering via the creeks was typically near 300 μS/cm (range = 89 to 553 μS/cm). The conductivity of the slough samples ranged from 854 to 10,302 μS/cm, with a median of 6327 μS/cm. Thus, on the basis of salt content, it can be estimated that the slough samples were on average 60% freshwater from the creeks (range 30–95%). Dilution with San Francisco Bay water would have reduced insecticide concentrations by nearly one half (assuming no insecticides in Bay water), and hydrophobic pesticide concentrations would have been further reduced by processes such as particle deposition and adsorption of pesticides to plant material and other substrates (Moore et al., 2009). Concentrations of fipronil and its degradates remained measurable.

### Table 1

<table>
<thead>
<tr>
<th>Site</th>
<th>Waterway</th>
<th>Coordinates</th>
<th>Dates of sampling within Feb. 2014</th>
<th>Temp. range of samples (°C)</th>
<th>Conductivity range of samples (μS/cm)</th>
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</thead>
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<td><strong>Creek sites</strong></td>
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<td>GVC</td>
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<td>11.3–13.4</td>
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<td>11.1–13.3</td>
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<td>M2S</td>
<td>Montezuma Slough</td>
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<td>11.4–12.4</td>
<td>8370–8464</td>
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<td>SSO</td>
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<td>38.1354, -122.0805</td>
<td>10</td>
<td>13.2</td>
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### Table 2

<table>
<thead>
<tr>
<th>Pesticide concentrations (ng/l) in water from the creeks flowing to Suisun Marsh and in the sloughs of the marsh. Data are presented in the order samples were collected.</th>
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</thead>
<tbody>
<tr>
<td><strong>Chlorpyrifos</strong></td>
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<td><strong>Urban creeks</strong></td>
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<tr>
<td>McCoy Creek</td>
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<tr>
<td>Laurel Creek</td>
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<td><strong>Mixed urban and agricultural creeks</strong></td>
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<td>Green Valley Creek</td>
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<td><strong>Agricultural creek</strong></td>
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<td>Suisun Creek</td>
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<td><strong>Suisun Marsh Sloughs</strong></td>
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<tr>
<td>Montezuma Slough</td>
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<tr>
<td>Suisun Sl. (outlet)</td>
</tr>
</tbody>
</table>

U indicates undetected at <1 ng/l for chlorpyrifos, bifenthrin, fipronil, and its degradates, and <10 ng/l for imidacloprid.

a Creek sites were sampled 8 Feb. morning, 8 Feb. afternoon, 9 Feb. morning, except imidacloprid was only analyzed in the two morning samples. Slough sites were sampled 8 Feb. afternoon, 9 Feb. afternoon and 10 Feb afternoon, except Suisun Slough Outlet was only sampled on 10 Feb. afternoon.

b Laurel Creek was the only site containing a pyrethroid other than bifenthrin. Cyfluthrin was found at U, 1.2, 1.7 ng/l.
throughout the sloughs both because of greater water solubility, and because initial concentration in the creeks were the highest of all analytes.

Total suspended solid concentrations ranged from 3 to 1476 mg/l in the creeks (median 79 mg/l) and 41–641 mg/l in the marsh (median 82 mg/l). It is recognized that the presence of suspended solids in the water can influence the toxicity of hydrophobic pesticides (particularly pyrethroids in the present study) with adsorption leading to less toxicity than expected based on pesticide concentration. However, in the six samples with H. azteca or C. dilutus toxicity (discussed in Section 3.3) and measurable bifenthrin concentrations, total suspended solid concentrations were relatively low at 12 to 59 mg/l, and would not have had an appreciable influence in most instances (Yang et al., 2006). Samples with measurable bifenthrin, but lacking toxicity, tended to have higher TSS concentrations (though only two samples, with 106 and 1476 mg/l).

### 3.3. Toxicity testing

Creek samples were tested in 96-h exposures using both H. azteca and C. dilutus. Only one sample caused H. azteca mortality significantly greater than that of the controls (a modest 14 ± 9% mortality in the 8 Feb. Laurel Creek sample), but impaired movement was often observed. H. azteca showed significant paralysis in 4 of 10 creek samples (Table 3), including both urban creeks (McCoy and Laurel Creeks) and Ledgewood Creek which carries both urban and agricultural runoff. Statistically significant mortality to C. dilutus was limited to a single sample (McCoy Creek, 9 Feb. = 64 ± 31% mortality), but inability to perform the typical thrashing motion when disturbed was observed in 6 of the 10 samples. The effects were greatest in the urban McCoy and Laurel Creeks, in which none or nearly none of the individuals were able to move normally. Lesser but still significant effects were seen in Ledgewood Creek.

To help identify the cause of toxicity, PBO was added to Laurel Creek water (a composite of two samples from the site, since sample volume was insufficient to test either sample alone). H. azteca were unaffected in the laboratory water control, either with or without PBO (only 6 and 0% dead or paralyzed, respectively). However, every individual exposed to Laurel Creek water in the presence of PBO was dead or paralyzed, a significant difference as compared to 84 ± 15% without PBO (p = 0.05; Kolmogorov–Smirnov two-sample test). This difference was consistent with pyrethroids as the cause, as it has been shown their toxicity is increased by PBO (Anweg et al., 2006).

Further evidence supporting bifenthrin as the cause of H. azteca toxicity is evident in the relationship between the proportion of individuals affected and the toxic units (TU) of bifenthrin present in the samples, where TU equals the measured concentration divided by the 96-h EC50 (3.3 ng/l; Weston and Jackson, 2009) (Fig. 2). Not only was there a significant correlation between effect and bifenthrin TU among the samples (r = 0.920; p < 0.01), but >50% effect would be expected in samples exceeding 1 TU if bifenthrin were the cause of toxicity, similar to the results seen.

Fipronil was unlikely to have contributed to the observed H. azteca toxicity. The species is extremely insensitive to fipronil and its degradates, with 96-h EC50s of 728, 458, and 213 ng/l for fipronil, the sulfone and the sulfide, respectively (Weston and Lydy, 2014). Even in the worst-case creek site (Laurel Creek, 8 Feb. morning sample) there was still <0.1 TU for H. azteca when summed for fipronil and its degradates.

The converse is true for C. dilutus with effect levels for bifenthrin at least 25 times greater than concentrations seen in the creeks (the maximum concentration observed of 9.9 ng/l compared to 96-h EC50 > 253 ng/l; Weston et al., 2015). However, C. dilutus toxicity would be expected at the observed concentrations of fipronil, fipronil sulfone and fipronil sulfide (96-h C. dilutus EC50 = 32.5, 7.7, and 9.9 ng/l, respectively; Weston and Lydy, 2014). A TU analysis suggests

### Table 3

Results of toxicity tests using H. azteca or C. dilutus exposed to creek waters for 96 h. Asterisk indicates effect significantly greater than control. All sites were sampled twice (morning of 8 Feb. and morning of 9 Feb.; toxicity testing not done with afternoon 9 Feb. samples).

<table>
<thead>
<tr>
<th>Sample site</th>
<th>% H. azteca affected* (±standard deviation)</th>
<th>% C. dilutus affected* (±standard deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory controls</td>
<td>0 ± 0</td>
<td>8 ± 4</td>
</tr>
<tr>
<td></td>
<td>8 ± 13</td>
<td>16 ± 15</td>
</tr>
<tr>
<td>Urban creeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>McCoy Creek</td>
<td>26 ± 21*</td>
<td>94 ± 9*</td>
</tr>
<tr>
<td></td>
<td>16 ± 15</td>
<td>100 ± 0*</td>
</tr>
<tr>
<td>Laurel Creek</td>
<td>94 ± 9*</td>
<td>98 ± 4*</td>
</tr>
<tr>
<td></td>
<td>94 ± 5*</td>
<td>100 ± 0*</td>
</tr>
<tr>
<td>Mixed urban and agricultural creeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ledgewood Creek</td>
<td>34 ± 18*</td>
<td>66 ± 13*</td>
</tr>
<tr>
<td></td>
<td>8 ± 8</td>
<td>74 ± 17*</td>
</tr>
<tr>
<td>Green Valley Creek</td>
<td>7 ± 8</td>
<td>20 ± 19</td>
</tr>
<tr>
<td></td>
<td>6 ± 5</td>
<td>30 ± 7</td>
</tr>
<tr>
<td>Agricultural creek</td>
<td>2 ± 4</td>
<td>10 ± 17</td>
</tr>
<tr>
<td>Suisun Creek</td>
<td>0 ± 0</td>
<td>42 ± 28</td>
</tr>
</tbody>
</table>

* Individuals not moving normally; either dead or showing paralysis.
fipronil and its degradates were responsible for the observed *C. dilutus* toxicity (Fig. 2). Not only did the proportion of individuals affected correlate with the sum TU of fipronil and its degradates (r = 0.883; p < 0.01), but >50% effects generally became evident at about 1 TU, precisely where they would be expected if these compounds were responsible.

Chlorpyrifos concentrations were below levels of concern with respect to both *H. azteca* and *C. dilutus* (96-h EC50s of 96 and 510 to 750 ng/l, respectively; *Weston and Lydy*, 2010a; *Pape-Lindstrom and Lydy*, 1997). Similarly, imidacloprid concentrations at creek sites (typically <70 ng/l; maximum 1462 ng/l) were below reported toxicity thresholds for the two species tested and were unlikely to have played a role in observed effects. *C. dilutus* is a relatively sensitive species, with an imidacloprid 96-h LC50 of 5750 ng/l, with concentrations about half of that reported to inhibit growth (*Stoughton et al.*, 2008; though those tests were done with a commercial formulation, making comparisons to our data difficult). The reported *H. azteca* imidacloprid 96-h LC50s are 65,430 and 526,000 ng/l (*Stoughton et al.*, 2008; *England and Bucksath*, 1991 as reported by *Stoughton et al.*), with a 96-h EC50 (immobilization) of 55,000 ng/l, and growth effects with as little as 2220 ng/l (*Stoughton et al.*, 2008; using a specific commercial formulation).

While the urban streams flowing to Suisun Marsh demonstrated acute toxicity when tested, and contained bifenthrin and the fipronil compounds at concentrations expected to cause toxicity, no toxicity was seen in the sloughs of Suisun Marsh. In tests of slough waters with *H. azteca*, the proportion of animals dead or paralyzed ranged from 0 to 12%, and were not significantly different from the control (6%) or the high conductivity control (7%). Given that bifenthrin appeared responsible for the *H. azteca* toxicity observed in the creeks, and the compound was undetectable in the slough samples, the lack of toxicity is not surprising.

We were not able to test the slough waters with *C. dilutus* because while *H. azteca* can be used for testing both fresh and estuarine waters, *C. dilutus* cannot (*Munns et al.*, 2002). Mortality of *C. dilutus* would be expected simply due to the salt content of the slough samples (*Sargent*, 1978).

### 3.4. Risks to resident species

The bifenthrin concentrations observed in Laurel, McCoy, and Ledgewood Creeks were found to be toxic to *H. azteca*, as would be expected given the species EC50 (3.3 ng/l; *Weston and Jackson*, 2009), but they would also be on the threshold of toxicity to several other benthic invertebrates. *Weston et al.* (2015) determined bifenthrin 96-h EC50s for 12 benthic macroinvertebrates, most from northern California. Bifenthrin concentrations seen in the urban creeks flowing to Suisun Marsh were one-third to one-half the 48 to 96-h EC50 of two mayflies, a stonefly, and a caddisfly. Bifenthrin effects to fish within these creeks could occur through the food web, though sublethal effects on fish themselves tend to be at higher concentrations than those seen (30 to 140 ng/l for impairment of swimming, or 70 ng/l for gene transcription effects in fathead minnows; *Beggel et al.*, 2010, 2011).

Laurel and McCoy Creeks are also a concern due to the presence of fipronil and its degradation products. *Weston and Lydy* (2014) determined EC50s of fipronil and its degradates for 14 macroinvertebrate species. The concentrations of fipronil and/or its sulfone degradate found in these two creeks were approximately half the EC50s for one-third of the species. As for bifenthrin, any fipronil effects on fish within the creeks are more likely to be manifested through the food web, as endocrine effects, gene transcription, and swimming performance in fathead minnows (*Beggel et al.*, 2010, 2012; *Bencic et al.*, 2013), as well as developmental defects in zebrafish (*Steinh et al.*, 2006), have all been seen at concentrations at least three orders of magnitude higher than those we observed.

Though the creeks flowing into Suisun Marsh contain both bifenthrin and fipronil from urban runoff at concentrations representing a threat to a variety of benthic taxa, the threat is not unique to Fairfield and Suisun City, and is comparable to that seen in urban streams in many other northern California communities (*Weston and Lydy*, 2010b, 2012, 2014; *Weston et al.*, 2014). Conditions elsewhere in the U.S. have not been well documented, though there are indications that they may be similar (*Kuivila et al.*, 2012). It is noteworthy that regulatory monitoring of Fairfield-Suisun City urban creek waters, as done by the municipal stormwater utility, requires testing with *H. azteca*, *Selenastrum capricornutum*, *Ceriodaphnia dubia*, and *Pimephales promelas*. Excluding *H. azteca*, the utility’s monitoring program is unlikely to show any insecticide toxicity to the other species, since their bifenthrin and fipronil LC50s far exceed the maximum concentrations observed (*Konwick et al.*, 2005; *Yang et al.*, 2006; *USEPA*, 2007; *Werner and Moran*, 2008; *Baird et al.*, 2013). The utility’s *H. azteca* monitoring only scores survival, an endpoint on which the present study found little effect, and is less than half as sensitive as the paralysis endpoint of the present study (*Weston and Jackson*, 2009). Their program also tests *H. azteca* at 23 °C, at which pyrethroids are one-third as toxic as at the in situ 13 °C used in the present study (*Weston et al.*, 2009).

Excluding Laurel Creek (889–1462 ng/l), imidacloprid concentrations < 70 ng/l at the other sites are not known to represent a threat to resident macroinvertebrates. The most sensitive species known are mayflies, with 96-h LC50s of 650–1770 ng/l (*Alexander*, 2006; *Roessink et al.*, 2013). For the protection of aquatic life in general, concentrations less than 8.3 and 200 ng/l have been recommended for chronic and acute exposure, respectively (*Smit et al.*, 2015). The acute threshold is the more relevant to stormwater runoff events, and thus only Laurel Creek would exceed this benchmark.

Dilution and the other factors that reduce insecticide concentrations as creek waters move into Suisun Marsh substantially mitigate risks to estuarine species within the marsh habitat. Few data exist by which to compare fipronil concentrations found in the marsh to the tolerance of estuarine and marine species. A compilation of data by the U.S. Environmental Protection Agency (2007) suggests that no acute toxicity would be likely, though only five estuarine or marine species have been tested (most sensitive being *Americanamysis bahia* with 96-h fipronil LC50 of 140 ng/l).

### 4. Conclusions

The present study documented considerable risk of insecticide toxicity to invertebrates within the urban creeks. Bifenthrin was likely responsible for toxicity to *H. azteca*, fipronil and its sulfone degradate likely responsible for toxicity to *C. dilutus*, and both compounds could pose a risk to multiple other macroinvertebrate species. The same compounds investigated in the present study are widely used in many other countries, and this work illustrates the co-occurrence and toxicity of multiple insecticides in urban runoff. Each compound has the potential for toxicity to a unique subset of species within receiving waters, thus toxicity testing with multiple species provides the best means to assess these risks. The co-occurrence of bifenthrin, fipronil, chlorpyrifos, and imidacloprid in the creeks, as well as many other urban runoff contaminants not measured in the present study, could also pose a risk due to additive or synergistic effects that are largely unknown given the current state of knowledge.

The present study failed to show risk once creek waters were diluted within the marsh, however it would be premature to entirely dismiss this potential. First, our study focused solely on winter rains as a transport mechanism for insecticide entry into the marsh. Agricultural sources are likely to be more significant during periods of peak pesticide use during the growing season, with irrigation runoff providing a mechanism for off-site movement of residues. Entry of agricultural insecticides into the marsh through this route is likely to be unpredictable and highly episodic, and therefore difficult to monitor.
Second, fenitrothion and its degradates were commonly found throughout the marsh following the rain event. While the concentrations measured are not known to be acutely lethal to the marine and estuarine invertebrates that have been tested, they are on the threshold of sublethal toxicity to the most sensitive freshwater invertebrate that has been tested (C. dilutus). Given the few estuarine/marine species tested with fenitrothion, and that estuarine/marine testing of fenitrothion degradates has been done with only a single species (A. bahia; USEPA, 2007), we cannot rule out possible adverse effects even at the < 10 ng/L concentrations seen for fenitrothion and its degradates throughout the marsh.

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References