# URBAN AND AGRICULTURAL PESTICIDE INPUTS TO A CRITICAL HABITAT FOR THE THREATENED DELTA SMELT (*HYPOMESUS TRANSPACIFICUS*)

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**Abstract:** The Cache Slough complex is an area of tidal sloughs in the Sacramento–San Joaquin River Delta of California (USA), and is surrounding by irrigated agricultural lands. Among the species of concern in the area is the delta smelt (*Hypomesus transpacificus*), a federally listed threatened species. Releases of the organophosphate insecticide chlorpyrifos and pyrethroid insecticides were examined to determine whether they represented a threat to the copepods on which delta smelt feed (*Eurytemora affinis* and *Pseudodiaptomus forbesi*) and to aquatic life in general, represented by the standard testing organism, *Hyalella azteca*. There was a single incident of toxicity to *H. azteca* as a result of discharge of agricultural irrigation water containing chlorpyrifos. Pyrethroids were not found in samples collected during the dry season. Following rain events, however, the waters of western Cache Slough repeatedly became toxic to *H. azteca* because of the pyrethroids bifenthrin and cyhalothrin. The 96-h median lethal concentrations (LC50s) for *E. affinis* and *P. forbesi* for the pyrethroids bifenthrin and cyhalothrin were 16.7 ng/L to 19.4 ng/L when tested at 20 °C. However, their LC50s may be 5 mg/L to 10 ng/L at in situ temperatures of the Cache Slough, comparable to the peak bifenthrin concentration observed. The dominant pyrethroid source appeared to be urban runoff entering a creek 21 km upstream of Cache Slough. Pyrethroids of urban origin were supplemented by agricultural inputs of pyrethroids and chlorpyrifos as the creek flowed toward Cache Slough. *Environ Toxicol Chem* 2014;33:920–929. © 2014 SETAC

**Keywords:** Pyrethroids    Bifenthrin    Urban runoff    Delta smelt    *Hyalella azteca*

## INTRODUCTION

The Sacramento–San Joaquin Delta, at the head of the San Francisco Bay Estuary, has undergone substantial ecological change as a result of the agricultural development and urbanization of California (USA). Of particular concern has been population declines of pelagic fish species, including the delta smelt, *Hypomesus transpacificus*, which has shown more than an order of magnitude decline in abundance in the past 10 yr [1]. The delta smelt is a federally listed threatened species endemic to the low salinity and freshwater portions of the estuary. One of the more critical habitats for delta smelt is an area of the Delta known as the Cache Slough region. Delta smelt gather in the area to spawn beginning in February, with most departing by June, although some remain throughout the year [2,3].

The causes for the smelt's decline have not been established, although stressors include water diversions, wetland loss, introduced species, and contaminants. Pesticides are among those contaminants that have been suggested as a contributing factor [4]. While acute pesticide-induced mortality to fish is rare, of greater concern are sublethal effects, including endocrine disruption and impaired immune function, or indirect effects, such as toxicity to key prey species [5,6]. Toxicity to the amphipod *Hyalella azteca* has been observed in Cache Slough, with pyrethroid pesticides suspected as contributors [7]. Urban tributaries to Cache Slough contain pyrethroids at concentrations 5 times the *H. azteca* 96-h median effective concentration (EC50), although the samples were taken over 20 km upstream of Cache Slough [8].

The present study was intended to establish whether chlopyrifos and pyrethroid pesticides were present in the Cache Slough region when adult delta smelt were spawning and juveniles were present, determine the sources of the pesticides, and establish whether their presence was a threat to aquatic life. Water samples for pesticide analysis were collected during periods with and without rainfall. The samples were tested with *H. azteca* as a general measure of environmental quality, but of particular concern was pyrethroid toxicity to copepods on which delta smelt feed. Historically, the dominant prey has been the calanoids *Eurytemora affinis* and *Pseudodiaptomus forbesi* [9,10]. The potential for toxicity to copepods was inferred by laboratory exposures of these species to pyrethroid-spiked waters to determine concentrations causing mortality, and then comparison with concentrations observed in Cache Slough.

## MATERIALS AND METHODS

### Study area

Cache Slough (Figure 1) is located 30 km southwest of Sacramento, California (USA). It is tidal freshwater, with depths up to 10 m. Flood control, land reclamation, and conveyance projects in the last century have channelized waters within levees, with land behind the levees developed for agriculture. Liberty Island, in the eastern Cache Slough complex, was reclaimed farmland until 1997, when the levees breached, creating a shallow lake. Many tributaries carry irrigation and stormwater runoff from surrounding farmlands to the Cache Slough complex, with some waterways also carrying urban runoff from Vacaville (via Ulatis Creek) or West Sacramento (via the Deep Water Ship Channel).
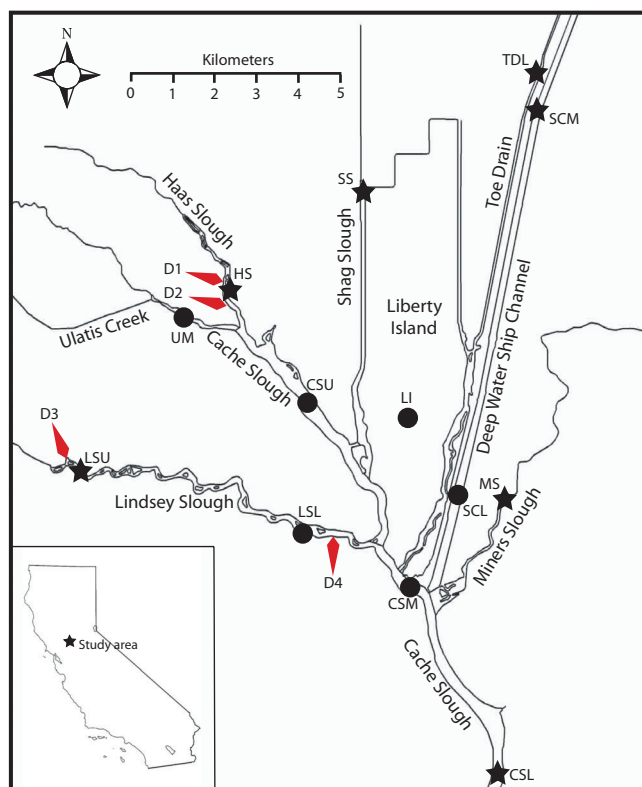
Figure 1. Map of the Cache Slough complex. The 6 core sites are indicated by filled circles; the sites used for source sampling are indicated by star symbols. Approximately 20 local drains from adjacent farm fields are located along the banks, and 4 that were sampled (D1–D4) are indicated by elongated arrows. Source sites TDU and SCU are not shown, but are off the map to the north, 20 km farther up the Toe Drain and 14 km farther up the Deep Water Ship Channel, respectively. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

Ulatis and Alamo Creeks originate west of Vacaville and have shown no detectable pyrethroids prior to entering the city [8]. Within the city, the creeks receive urban runoff, and Alamo Creek is diverted to a constructed channel known as New Alamo Creek (Figure 2). Old Alamo Creek begins within the city limits, and carries secondary-treated municipal wastewater to New Alamo Creek. New Alamo Creek then flows into Ulatis Creek, and their combined flow enters Cache Slough. From Vacaville to Cache Slough, the creek flows for 21 km through irrigated agricultural lands.

*Sample collection*

Water samples were collected from 6 locations in Cache Slough and adjacent waterways, referred to hereafter as core sites (Figure 1), with each site sampled 11 times to 16 times. Sampling began in February 2011, corresponding to the time when delta smelt move into Cache Slough to spawn. Much of the sampling was triggered by rain events, including 16 to 19 February 2011 (4.6 cm total precipitation), 13 to 15 March 2011 (2.4 cm), 18 to 20 March 2011 (4.7 cm), 23 to 26 March 2011 (3.4 cm), 4 to 5 June 2011 (1.7 cm), and 13 to 14 March 2012 (3.4 cm). (All precipitation totals were based on the Liberty Island gauge, except for the June event, which used the Vacaville airport gauge.) In May and June 2011, when rain events are rare but agricultural irrigation increases, core site sampling was approximately weekly. Samples were collected by boat using a pump suspended 1 m below the surface. Glass bottles certified clean for pesticides (I-Chem 200 series; Fisher Scientific) were filled for chemical analysis, toxicity testing, and total suspended solids (TSS). Temperature and conductivity usually showed no difference throughout the water column, indicating little stratification. On 2 occasions (February and June 2011), sediment samples were collected using a Petite Ponar grab (Wildco), and the upper 2 cm of sediment was used for pesticide analysis.

Water was also collected from tributaries to the Cache Slough complex to determine where pesticides originated. These locations are referred to as source sites (Figures 1 and 2). Source sampling was done during 3 rain events and 2 nonrain occasions. Source sampling also included local drains along the banks of the Cache Slough complex that discharge runoff from adjacent croplands. Flow from these drains depends on pumping by individual growers, so sampling could take place only if the drains were flowing while we were in the area. There are approximately 20 such drains, but only 4 were flowing at a time that permitted sampling. All source sites were sampled for pesticides and TSS.

A third group of sites, known as the Ulatis transect, was sampled during 3 winter rain events. This transect was sampled in a Lagrangian fashion, tracking a parcel of water as it moved down Ulatis Creek toward Cache Slough. The creek was divided into 6 segments of 0.8 km to 4.5 km in length, delimited by road crossings. Flow rates were estimated at both ends of each segment based on timing the movement of surface debris in the creek, and the average of values from both ends of each segment was assumed to apply to the entire segment. Sampling began at the Vacaville city limit and proceeded to downstream sites in succession as that original water parcel was estimated to have reached the next sampling site (sequentially ULT, UBY, UF, UH, U113, and UB; Figure 2). Major discharges to Ulatis Creek, such as tributary streams or large agricultural drains, were also sampled (Fox Drain, Gibson Creek, Sweany Creek, Hawkins Drain, Highway 113 Drain, New Alamo Creek). The Ulatis transect sites were sampled from the bank, filling bottles for pesticide and TSS analysis.

We attempted to sample the source sites and Ulatis transect sites on the first day of each rain event, because flows rise rapidly in response to runoff. Core sites in the Cache Slough region were typically sampled on the second day of the rain event to allow time for runoff to reach the complex.

*Zooplankton sampling*

Plankton were sampled in upper Cache Slough (site CSU) concurrently with core site sampling to characterize the copepods that were available as delta smelt prey and potentially at risk for pesticide toxicity. Zooplankton collection was done with a 63-$\mu$m net with a 0.5-m mouth and 2-m length. Duplicate samples were collected by 5-min oblique tows. However, on 24 May 2011 and thereafter, loss of equipment made it possible only to make vertical tows from just above the bottom to the water surface. The organisms were narcotized with soda water, and preserved with sucrose formalin solution. With the use of a Folsom plankton splitter (Wildco), half the sample was analyzed without further manipulation, and the other half was sieved on a 125-$\mu$m screen to retain copepods of interest. Both splits were subsampled by identifying organisms using a Bogorov counting cell (Wildco). Copepods were identified to species when possible, and all other organisms to major taxon.

*Toxicity testing*

Testing was done with *H. azteca* as has typically been done when pyrethroids are of concern [7,8,11], following protocols
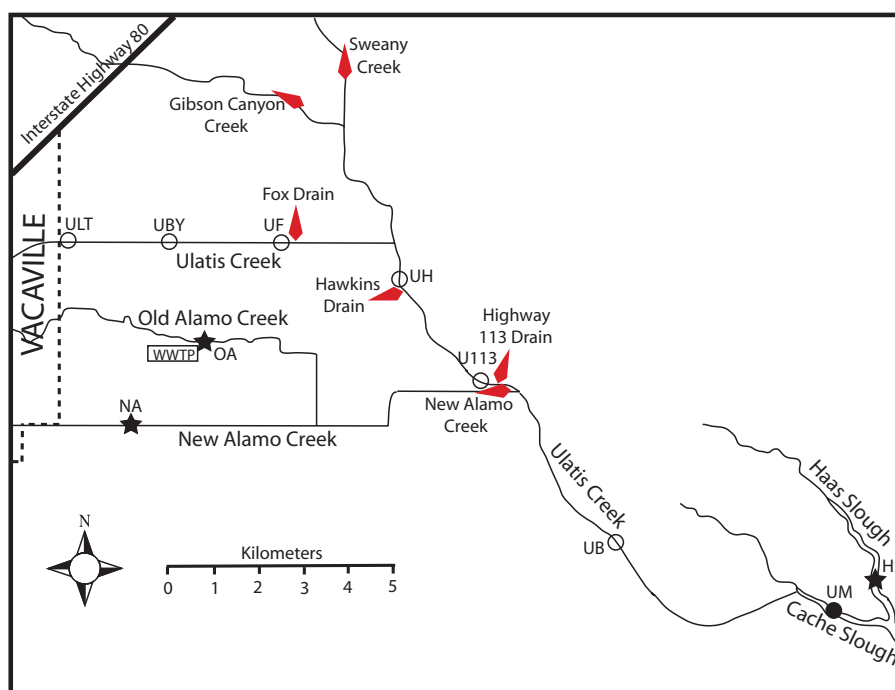
Figure 2. Map of Ulatis Creek, extending from the urban portion of Vacaville (west of the dotted line) to upper Cache Slough. Sites used for the Ulatis transect are shown by open circles, some of which were also used for source sampling. Other sampled source locations are indicated by star symbols. Samples were also collected from the major creeks and drains discharging to Ulatis Creek at locations indicated by the elongated arrows. The location of the municipal wastewater treatment plant (WWTP) is shown, as are core sites UM and HS to provide overlap with Figure 1. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

comparable to acute tests with a variety of other freshwater species [12]. Toxicity tests commenced 24 h to 48 h after water collection. Each of 5 replicate 100-mL beakers received 80 mL of test water. A 1-cm$^2$ nylon screen served as a substrate to which the amphipods clung. Then, 10 *H. azteca*, 7 d to 10 d old, were taken from cultures maintained at the University of California, Berkeley, and added to each beaker. Because pyrethroid toxicity is highly temperature dependent [13], tests were conducted at temperatures prevailing in Cache Slough at the time of sample collection. Water temperatures in the slough ranged from 10 °C to 23 °C over the course of the study, although the lowest test temperature used in laboratory tests was 13 °C, as we had not verified *H. azteca* control performance at lower temperatures. Amphipods, cultured at 23 °C, were acclimated to the intended test temperature (e.g., if to be tested at 13 °C, the temperature was dropped to 17 °C the first day, and to 13 °C the second day, and the animals were used on the third day). Testing was done under a 16:8-h light:dark photoperiod. Feeding with 1 mL per beaker yeast/cerophyll/trout food solution occurred on the second day. 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 (ranges observed: 127–800 µS/cm, 56–240 mg/L, 60–280 mg/L, and 7.2–8.4, respectively). Temperature and dissolved oxygen were measured at 0 h, 48 h, and 96 h. Tests terminated at 96 h. Pyrethroids are neurotoxins and cause paralysis in *H. azteca*, ranging from animals that are motionless except for twitching of an appendage to others that attempt to swim but are unable to do so. Tests were scored by recording the numbers of dead amphipods and of those that were alive but showed paralysis. All tests of field samples were accompanied by a control using moderately hard [12] laboratory water (range of survival: 86%–100%), and a field duplicate was included every 20 samples.

Several samples were retested by addition of piperonyl butoxide (PBO), a synergist expected to increase toxicity to *H. azteca* if a pyrethroid is present [14,15]. It was added to test waters at 50 µg/L in a methanol carrier, with the methanol concentration kept below 12.5 µl/L. The PBO was renewed at the 48-h water exchange. Treatment controls (laboratory water with PBO) were always included and never showed any effect.

Statistical analysis was done using CETIS (Tidepool Scientific Software). Comparisons between field samples and controls, or between the treatments with and without PBO, were made using *t* tests.

Laboratory exposures were done with the copepods *E. affinis* and *P. forbesi*, to determine sensitivity to bifenthrin and lambda-cyhalothrin, so these data could be compared with pyrethroid concentrations in Cache Slough. Tests were conducted in 600-mL beakers containing moderately hard water [12] prepared to 2 psu (3700 µS/cm) using Instant Ocean (Spectrum Brands). Pyrethroids from ChemService were added in a methanol carrier, with solvent controls incorporated in all tests. Four replicate beakers were used at each of 6 test concentrations. Twenty copepods, 16 ± 2 d in age, were added to each beaker, and held at 20 °C under 16:8-h light:dark photoperiod. Water was changed on the second day. Copepods were fed daily with 400 µg C/L/d to 500 µg C/L/d of Instant Algae (equal volumes of *Nannochloropsis* and *Pavlova* from Reed Mariculture). After 96 h, surviving copepods were enumerated. Median lethal concentrations (LC50s) were determined by the trimmed Spearman–Karber method. A composite of water samples collected at test initiation and 48-h water replacement, from a concentration near the middle of the range, was used to report results as actual rather than nominal concentrations.

*Analytical chemistry*

Water samples were preserved on the day of collection by addition of 10 mL hexane, and held at 4 °C for <72 h prior to

extraction. The analytical surrogates 4,4'-dibromooctafluorobi-phenyl (DBOFB) and decachlorobiphenyl (DCBP) were added to the samples, and the water was liquid:liquid–extracted using US Environmental Protection Agency method 3510C [16]. Three sequential extractions were performed with 60 mL dichloromethane, with 1 aliquot also used to extract the empty sample bottle. The combined extracts were concentrated to 1 mL in hexane and analyzed following Wang et al. [17]. The extract was added to a dual-layer graphitized black carbon and primary/secondary amine column (Supelclean ENVI™-Carb II/Supelclean™ primary/secondary amine column, 300 mg/600 mg, 6.0 mL; Sigma-Aldrich/Supelco). The cartridge was conditioned with 3 mL hexane, loaded with the extract, and then eluted with 7 mL of 30% dichloromethane in hexane. Samples were concentrated, transferred to gas chromatography vials, reduced in volume to near dryness, and reconstituted to 1 mL in 0.1% acetic acid in hexane to avoid pyrethroid isomerization [18].

Sediment samples from the field sites were processed following methods detailed in You et al. [19]. Frozen sediment was freeze-dried at $-80\,°C$ for 24 h. Approximately 5 g of dry sediment were mixed with 1 g of silica and 2 g of copper powder, and surrogate standards (DBOFB and DCBP) were added. Sediment samples were extracted with a matrix-dispersive accelerated solvent extraction method using a Dionex 200 instrument (33-mL stainless steel cells) with 1:1 dichloromethane:acetone (v/v) at $100\,°C$ and 1500 pound-force per square inch for 2 5-min static cycles. The extract was collected in 60-mL glass collection vials, and cleanup was done as for the water extracts.

Extracts were analyzed on an Agilent 6890 gas chromatograph with a microelectron capture detector. The detector temperature was $320\,°C$. Extracts were injected using an Agilent 7683 autosampler in pulsed splitless mode, and the qualification of pesticides was confirmed by dual columns (RTX 1614 and DB-608). Calibration was performed using the external standard method, and the calibration curve was linear within the concentration range, with linear regression coefficients $r^2 > 0.995$. Samples were analyzed for the organophosphate chlorpyrifos and 8 pyrethroids (bifenthrin, cyfluthrin, cyhalothrin, cypermethrin, deltamethrin, esfenvalerate, fenpropathrin, and permethrin). Quality control measures included blanks, lab control spikes, matrix spikes, matrix spike duplicates, and field duplicates, all done with every batch of 20 samples. The reporting limit was 1 ng/L (1 ng/g for sediments), recovery from matrix spikes ranged from 45% to 142%, and relative difference percentages between matrix spike duplicates never exceeded 24%. Total suspended solids were quantified as the dried mass retained on a Whatman 934-AH filter.

## RESULTS AND DISCUSSION

### Zooplankton characterization

The most abundant groups were copepod nauplii (44% of total individuals) and rotifers (25%). The identifiable copepod genera (calanoids *Eurytemora*, *Pseudodiaptomus*, and *Sinocalanus*; cyclopoids *Acanthocyclops* and *Limnoithona*) comprised 9% of the total zooplankton numbers as collected by the 63-μm net (Figure 3) and included the taxa considered to be the primary smelt prey [9,10]. *Sinocalanus doerrii* was the dominant



Figure 3. Zooplankton composition at core site CSU throughout the study period. The inset pie chart indicates the average composition of the entire zooplankton community from February to June, represented by the material collected in the 63-μm net. The bars indicate the densities of 5 dominant copepod species, based on the mean of 2 replicates of the >125-μm fraction.

calanoid taxa on most occasions. *Eurytemora affinis* was present starting in mid-March and remained in Cache Slough for most sampling events, although always in relatively low abundance. It made up 18% of copepods at peak relative abundance in early May. *Pseudodiaptomus forbesi* became more abundant as the spring progressed, achieving dominant status by late June. *Limnoithona tetraspina* was present in most sampling events, but typically comprised <3% of identified copepods. *Acanthocyclops vernalis* comprised 68% of copepods in early May but was rare or absent at other times. The abundance of copepod adults and copepodids generally increased from February to June, as is typical for the San Francisco Bay and Delta [20].

*Copepod pyrethroid sensitivity*

When copepods were tested with pyrethroid-spiked laboratory water, control survival was 92% to 98%. The bifenthrin 96-h LC50 for *E. affinis* was 16.7 ng/L (95% confidence interval 15.4–18.0). Lambda-cyhalothrin LC50s for *E. affinis* and *P. forbesi* were 19.4 ng/L (17.8–21.2 ng/L) and 16.8 ng/L (13.8–20.5 ng/L), respectively. These values may underestimate the toxicity of pyrethroids in Cache Slough if temperature is taken into account. Copepod LC50 values were derived at 20 °C, but temperatures in Cache Slough during February and March, when storm runoff transports pyrethroids to the Cache Slough, are typically 10 °C to 13 °C. Pyrethroid toxicity has been commonly found to increase as temperature decreases in taxa ranging from insects [21], to fish [22], to reptiles [23]. We do not have data specifically for these copepod species, but temperature dependence of pyrethroid toxicity to *H. azteca* has been characterized. A decrease in temperature from 23 °C to 13 °C approximately triples toxicity [13]. Under in situ winter conditions, the pyrethroid LC50s for the copepods may be in the range of 5 ng/L to 10 ng/L if they respond to temperature comparably to *H. azteca*, although copepod-specific data are needed.

For comparison, the *H. azteca* 96-h LC50 at 23 °C for bifenthrin is 7.7 ng/L, with an EC50 for death or paralysis at 3.3 ng/L (median value) [24]. The *H. azteca* 96-h EC50 for lambda-cyhalothrin is 2.3 ng/L [25]. At in situ winter temperatures of Cache Slough, the *H. azteca* EC50s for both pyrethroids would be approximately one-third of these values [13] and at or below the 1 ng/L reporting limit.

*Core sites within the Cache Slough complex*

During the period of interest, February to June, electrical conductivity in upper Cache Slough is typically 600 µS/cm to 800 µS/cm. Rain events and the associated freshwater inflow decrease conductivity to near 200 µS/cm, with these low-conductivity excursions typically lasting 1 d to 4 d [26]. Excluding the June rain event when rainfall was light and scattered, and had little effect on conductivity of Cache Slough waters, all other rain-triggered sampling events captured these periods of freshwater inflow, with conductivities in upper Cache Slough (site UM) ranging from 250 µS/cm to 330 µS/cm at the time of sampling.

The TSS was measured (Table 1) because pyrethroids are strongly hydrophobic and thus in part associated with suspended sediments. The median TSS among the core site samples was 37 mg/L. Only 3 of 75 samples exceeded 100 mg/L. Because our analytical method quantified total pyrethroids, both dissolved and particle-associated, bioavailability and toxicity of a given pyrethroid concentration could be TSS dependent. In a study with a variety of pyrethroids and particle types, 25 mg/L TSS rarely affected toxicity to *Ceriodaphnia dubia* [27]. A

concentration of 50 mg/L reduced toxicity approximately 60% of the time, and 100 mg/L nearly always reduced it. Therefore, we expected that TSS would not significantly influence bioavailability in approximately half of our samples but could be important in those with higher TSS concentrations.

The eastern Cache Slough complex (sites LI, SCL, and CSM) never showed toxicity, rarely contained pyrethroids (only permethrin, in 1 of 38 samples), and never exceeded 18 ng/L chlorpyrifos (Supplemental Data, Table S1). In the western complex (sites UM, CSU, and LSL), upper Cache Slough and its tributary, Lindsey Slough similarly showed no toxicity or measurable pyrethroids during the 6 sampling periods without rain. However, toxicity and potentially toxic concentrations of pesticides were common following rain events (Table 1; Supplemental Data, Table S1). In 5 of 6 rain events, 1 or more of the 3 sampling sites in this region showed toxicity to *H. azteca*. When data from all rain events were combined, approximately half the samples from the western area showed toxicity, and the proportion of dead or paralyzed individuals ranged from 24% to 88%.

Excluding the 5 June event (discussed separately below), there were 8 toxic samples. Five of these had pyrethroid concentrations expected to cause toxicity because concentrations were near or above reported *H. azteca* EC50 values. While 96-h EC50s are the only benchmark available against which to compare field data, we recognize they are an imperfect comparison. They may be overly protective because we do not have data to show how long elevated pyrethroid concentrations persisted and because they do not account for reduction in bioavailability as a result of the suspended sediment in the samples. Conversely, they may be underprotective because they were derived at 23 °C and pyrethroids are more toxic at the 10 °C to 13 °C prevailing in Cache Slough during the winter [13]. Despite these limitations, a comparison of field pyrethroid concentrations with known EC50s or LC50s (Table 1) suggests that bifenthrin may have contributed to toxicity on 2 occasions (19 February and 19 March), cyhalothrin on 1 occasion (19 March), and cypermethrin on 1 occasion (19 February). Permethrin was found twice, once at about half its *H. azteca* 96-h LC50 of 21 ng/L [26]. There was 1 occasion (UM, 25 March) when both bifenthrin and cyhalothrin concentrations were high enough to expect toxicity, although no toxicity was found. This sample became toxic when PBO was added, however, suggesting that pyrethroid concentrations were slightly below toxic thresholds in the original sample.

Three of the 8 toxic samples (again excluding 5 June) lacked detectable pyrethroids (UM, 19 February; UM duplicate, 15 March; CSU, 25 March). The thresholds of toxicity for several pyrethroids are near the analytical reporting limit and are below reporting limits if increased pyrethroid toxicity at cold, in situ temperatures is considered. The potential for additive toxicity of multiple pyrethroids [28], all of which may be below reporting limits, only compounds this difficulty. There is evidence from the toxicity identification evaluation manipulations that pyrethroids were indeed responsible for the toxicity even though they were not analytically quantifiable. Two of the 3 toxic samples lacking measurable pyrethroids were tested with the addition of PBO, and the proportion of affected animals increased more than 2-fold (CSU) and nearly 9-fold (UM).

The cause of toxicity on 5 June was unique, as suggested by no effect on toxicity after addition of PBO. Chlorpyrifos, rather than pyrethroids, was likely the toxicant of concern. Its concentration was 79 ng/L to 81 ng/L at LSL, compared with an *H. azteca* 96-h EC50 of 96 ng/L [11]. If chlorpyrifos was the

Table 1. Toxicity to *Hyallela azteca* in 96-h exposures, effect of piperonyl butoxide (PBO) on toxicity, total suspended solids (TSS), and pesticide concentration at core sites in upper Cache and lower Lindsey Sloughs following rain events

| Date and site | Rainfall (cm)[a] | % dead/paralyzed[b] (mean ± SD) | Effect of PBO on toxicity[c] | TSS (mg/L) | Pesticide concentration (ng/L)[d,e] | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Chlor (EC50 = 96 ng/L) | Bifen (EC50 = 3.3 ng/L) | Cyper (EC50 = 1.7 ng/L) | Cyhal (EC50 = 2.3 ng/L) | Perm (LC50 = 21 ng/L) |
| 19 February 2011 | 1.5 | | | | | | | | |
| UM | | 66 ± 28* | Increase | 77.8 | 13.8 | U | U | U | U |
| CSU | | 24 ± 21* | | 79.0 | 10.2 | 6.6 | 4.7 | U | 12.5 |
| LSL | | 6 ± 9 | | 18.7 | U | U | U | U | 4 |
| 15 March 2011 | 1.9 | | | | | | | | |
| UM | | 34 ± 23 | | 38.0 | 4.4 | U | U | U | U |
| UM duplicate | | 38 ± 13* | | 36.4 | 3.8 | U | U | U | U |
| CSU | | 4 ± 5 | | 12.4 | 3.0 | U | U | U | U |
| LSL | | 0 ± 0 | | 19.6 | 2.4 | U | U | U | U |
| 19 March 2011 | 3.1 | | | | | | | | |
| UM | | 44 ± 25* | | 97.3 | 3.2 | 2.1 | U | U | U |
| CSU | | 76 ± 11* | | 80.3 | 2.8 | U | U | 2.3 | U |
| CSU duplicate | | 88 ± 16* | | No data | 2.9 | U | U | 2.2 | U |
| LSL | | 76 ± 21* | | 13.5 | 2 | U | U | 1.7 | U |
| 25 March 2011 | 2.1 | | | | | | | | |
| UM | | 10 ± 14 | Increase | 98.0 | 7.1 | 2.5 | U | 1.4 | U |
| CSU | | 34 ± 22* | Increase | 119.2 | U | U | U | U | U |
| 5 June 2011 | 0 | | | | | | | | |
| UM | | 0 ± 0 | | 56.2 | 11.4 | U | U | U | U |
| CSU | | 2 ± 4 | | 23.9 | 43.2 | U | U | U | U |
| LSL | | 32 ± 19* | No effect | 16.2 | 81.3 | U | U | U | U |
| LSL duplicate | | No data | | No data | 79.1 | U | U | U | U |
| 15 March 2012 | 2.2 | | | | | | | | |
| UM | | 8 ± 13 | | 97.9 | 16.0 | U | U | U | U |
| CSU | | 2 ± 4 | | 18.3 | 6.2 | U | U | U | U |
| LSL | | 6 ± 5 | | 16.0 | U | U | U | U | U |

[a] Accumulated precipitation as reported at Liberty Island over 48 h (day before and day of sampling). No precipitation was reported at Liberty Island during the 5 June event, but rainfall was scattered and accumulations of 0.53 cm to 2.44 cm seen at other gauges in the surrounding area.

[b] Tests were done at temperatures of 13 °C to 14 °C to approximate Cache Slough waters, except June samples at 17 °C.

[c] TIE tests on UM from 19 February 2011 were done about 5 d after the original test, with 10% dead/paralyzed without PBO and 86% affected with PBO. TIE tests on other occasions were done concurrently with the original sample, and affected animals increased from 10% to 38% for UM on 25 March 2011, and from 34% to 80% for CSU on 25 March 2011. LSL showed no statistically significant effect, increasing only from 14% to 18%.

[d] Analytes detected included chlorpyrifos (Chlor), bifenthrin (Bifen), cypermethrin (Cyper), cyhalothrin (Cyhal), and permethrin (Perm). The other analytes (cyfluthrin, deltamethrin, esfenvalerate, and fenpropathrin) were never detected. U indicates undetected at 1 ng/L.

[e] To aid interpretation of the chemistry data, reported *H. azteca* 96-h EC50s and LC50s are shown for each pesticide [11,24,25,35]. However, these values were derived at 23 °C and, for the pyrethroids, are likely to be about one-third the values shown at 13 °C to 14 °C used for most of the toxicity tests [13].

* Toxicity significantly greater than control.

SD = standard deviation; EC50 = median effective concentration; LC50 = median lethal concentration; TIE = toxicity identification evaluation; U = undetected at 1 ng/L.

toxic agent, PBO would have been expected to decrease toxicity [15]. While it is possible that an unmeasured toxicant may be involved, chlorpyrifos could still be the cause if a PBO-induced decreased toxicity was counteracted by pyrethroids present at less than the detection limit [15]. The chlorpyrifos of 5 June can be attributed to a specific local agricultural drain, as discussed below. Because rainfall was light and scattered on this occasion, it is suspected that the chlorpyrifos input was from irrigation return flow, and was merely coincidental with the rain event.

The potential for copepod toxicity was inferred by determining their LC50s and comparing them with concentrations found in Cache Slough. Observed bifenthrin concentrations of 2.1 ng/L to 6.6 ng/L following winter storms are below the *E. affinis* 96-h LC50 of 16.7 ng/L at 20 °C but could be a toxicity concern if in situ temperatures are taken into consideration (estimated in situ temperature-adjusted LC50 of 5–10 ng/L if the copepod's pyrethroid toxicity to temperature relationship is similar to that of *H. azteca*, as discussed earlier). Cyhalothrin concentrations in Cache Slough were about 2 ng/L, well below the measured *E. affinis* LC50 of 19.4 ng/L at 20 °C but potentially on the threshold of causing toxicity if temperature adjustments are considered. Therefore, there is potential for acute lethal toxicity to *E. affinis* in western Cache Slough following winter rains. *Pseudodiaptomus forbesi* is no less sensitive to pyrethroids but is found in the spring and summer when both pyrethroid concentrations and the influence of cold temperatures are diminished.

In February 2011, in the midst of the rainy season, sediment samples were collected at all core sites. None contained pyrethroids above the 1 ng/g detection limit, and chlorpyrifos concentrations reached only 2 ng/g. Three sites in the western study area where toxicity had often been observed (UM, CSU, and LSL) were resampled in June 2011 with the same negative results. These findings indicate that there is not a reservoir of sediment-sorbed pesticide to provide continuous exposure to resident biota in between runoff-related events.

*Pesticide sources to Cache Slough complex*

The various creeks and sloughs discharging to the Cache Slough complex were sampled to identify those that regularly contained pesticides at or above concentrations in Cache Slough, and therefore could be significant sources (Table 2; Supplemental Data, Table S2). The median TSS of all source samples was 24 mg/L, with 7 of 75 samples containing >100 mg/L. Lower Cache Slough (site CSL) never contained detectable pyrethroids, suggesting that water entering the Cache Slough complex from the Sacramento River to the south is not a significant pyrethroid source, nor does the Slough export measurable pyrethroids to the Delta. The absence of pyrethroids in upper Lindsey Slough also suggests it was not a significant pyrethroid source.

Many of the other waterways contained pyrethroids on 1 of the 4 to 7 times they were sampled (Deep Water Ship Channel, Toe Drain, Shag Slough, Haas Slough, Miners Slough). Bifenthrin, cyfluthrin, cyhalothrin, and cypermethrin all were observed at concentrations near or above *H. azteca* 96-h EC50s. Pyrethroids could be responsible for toxicity to sensitive aquatic species within any of these waterways. However, these waterways were unlikely to be responsible for the pyrethroids and associated toxicity observed in the western portion of the Cache Slough complex. They did not contain pyrethroids with the regularity seen in Cache Slough and, with the exception of Haas Slough, they discharge to the eastern portion of the complex where pyrethroids were seldom measurable.

The Ulatis/Alamo creek system, however, was noteworthy with respect to both the frequency and relative magnitude of pyrethroid detections. It contained the highest concentrations found for bifenthrin, cyhalothrin, cypermethrin, and permethrin.

Table 2. Sources of pesticides to the Cache Slough complex, showing range of total suspended solids (TSS), the maximum pesticide concentration observed, and the number of detections as a proportion of the total sampling events at the site (data in parentheses)

| Site | Map label | Pesticide sources[a] | TSS range (mg/L) | Pesticide concentration (ng/L)[b,c] | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Chlor (EC50 = 96 ng/L) | Bifen (EC50 = 3.3 ng/L) | Cyflu (EC50 = 1.9 ng/L) | Cyper (EC50 = 1.7 ng/L) | Cyhal (EC50 = 2.3 ng/L) | Perm (LC50 = 21 ng/L) |
| Upper Lindsey Slough | LSU | A | 7.6–20.4 | 24.2 (5/6) | U | U | U | U | U |
| Upper Ulatis Creek[d] | ULT–U113 | U, A | 0.3–652 | 72.2 (7/7) | 19.4 (6/7) | U | U | 1.8 (4/7) | 17.3 (2/7) |
| Old Alamo Creek | OA | M | 1.9–28.0 | U | 8.0 (4/6) | 6.3 (1/6) | 12.1 (1/6) | 4.9 (1/6) | 17.9 (3/6) |
| New Alamo Creek | NA | U, A | 1.5–136 | 3.8 (4/7) | 13.9 (3/7) | U | 5.1 (1/7) | U | U |
| Lower Ulatis Creek[e] | UB | U, A | 8.2–422 | 45.6 (5/7) | 8.0 (4/7) | 2.6 (1/7) | 3.0 (1/7) | 27.0 (1/7) | 10.3 (1/7) |
| Haas Slough | HS | A | 13.2–56.8 | 36.7 (6/7) | 3.9 (1/7) | 6.6 (1/7) | 9.6 (1/7) | U | U |
| Shag Slough | SS | A | 11.9–41.5 | 6.0 (4/6) | 6.4 (1/6) | U | U | U | U |
| Toe Drain (upper and lower) | TDU/TDL | A | 23.9–88.3 | 15.0 (3/4) | 3.3 (1/4) | U | U | 1.6 (2/4) | U |
| Deep Water Ship Channel (upper and middle) | SCU/SCM | U, A | 5.8–640 | 6.9 (4/7) | 1.5 (1/7) | 3.2 (1/7) | U | U | U |
| Lower Cache Slough | CSL | S | 13.0–86.7 | 10.3 (4/7) | U | U | U | U | U |
| Miners Slough | MS | A | 5.1–31.7 | 1.2 (1/5) | U | U | 3.6 (1/5) | U | U |
| Local drain 1 | D1 | A | No data | 3.9 (1/2) | U | U | U | U | U |
| Local drain 2 | D2 | A | No data | 1.9 (1/1) | U | U | U | U | U |
| Local drain 3 | D3 | A | No data | U | U | U | U | U | U |
| Local drain 4 | D4 | A | No data | 79.0 (1/2) | U | U | U | 6.7 (1/2) | U |

[a]Sources: agriculture (A), urban (U), municipal wastewater (M), and Sacramento River (S).
[b]Analytes detected included chlorpyrifos (Chlor), bifenthrin (Bifen), cyfluthrin (Cyflu), cypermethrin (Cyper), cyhalothrin (Cyhal), and permethrin (Perm). The other analytes (deltamethrin, esfenvalerate, and fenpropathrin) were never detected. U indicates undetected at 1 ng/L.
[c]To aid in interpretation of the chemistry data, reported *Hyallela azteca* 96-h median effective concentrations (EC50s) or median lethal concentrations (LC50s) are shown for each pesticide [11,24,25,35]. However, these values were derived at 23 °C and, for the pyrethroids, are likely to be about one-third the values shown at in situ winter temperatures [13].
[d]Combining data from three sites along Ulatis Creek upstream of the confluence with New Alamo Creek (ULT, UH, U113).
[e]Site UB on lower Ulatis Creek reflects the combined flow of Upper Ulatis, Old Alamo, and New Alamo.
EC50 = median effective concentration; LC50 = median lethal concentration.

Maximum concentrations of these pyrethroids were far above *H. azteca* EC50 values for all but permethrin. The most downstream location on Ulatis Creek (site UB), only 5 km from Cache Slough, contained bifenthrin in most rain events, with measurable concentrations ranging from 5.4 ng/L to 8.0 ng/L.

Old and New Alamo Creeks, as well as Ulatis Creek (site ULT), were all sampled near the eastern city limits of Vacaville, prior to passing through the extensive downstream agricultural lands. The presence of pyrethroids at these sites indicates they are of urban origin, either runoff or municipal wastewater in the case of Old Alamo Creek. Effluent from Vacaville's wastewater treatment plant on Old Alamo Creek has been shown to contain bifenthrin, cyhalothrin, and permethrin [8]. The city's urban runoff contains up to 30 ng/L bifenthrin, as well as cyfluthrin, cyhalothrin, cypermethrin, and permethrin [8].

Six samples were collected from 4 local agricultural drains that discharge directly into the Cache Slough complex. Only 1 of these samples contained analytes reaching concentrations of concern. Drain D4 was releasing chlorpyrifos at 79 ng/L on 5 June 2011, a concentration near the *H. azteca* EC50 of 96 ng/L [11]. This drain may have been responsible for the toxicity and 79 ng/L to 81 ng/L chlorpyrifos previously discussed on the same date at the LSL core site, only 850 m away.

*Intensive sampling along Ulatis Creek*

Given the apparent significance of Ulatis Creek as a pesticide source, much of the 21 km of the creek from Vacaville to Cache Slough was sampled following 3 rain events, using a Lagrangian approach in which each site along the creek was sampled as a given parcel of water reached that point. The intent of this approach was to determine whether the urban pesticides leaving Vacaville remained in creek waters when they reached Cache Slough, and the importance of additional downstream agricultural pesticide inputs.

On 18 March 2011, Ulatis Creek contained 19.4 ng/L bifenthrin as it left Vacaville (Table 3; Supplemental Data Table, S3). After traveling 12 km and reaching site U113 4 h later, creek waters contained 79% of the initial concentration (15.4 ng/L). The 4 major agricultural discharges that had been sampled in this reach contributed no additional bifenthrin. Downstream of U113, the Highway 113 Drain provided

bifenthrin of agricultural origin to the creek (15.8 ng/L) and New Alamo Creek provided bifenthrin (9.3 ng/L) that could have been either of agricultural origin or from the city of Vacaville. At the last accessible downstream site (UB), creek waters still contained 8 ng/L, 16 km and 5.5 h from the point of initial sampling in Vacaville. The bifenthrin in Ulatis Creek was likely responsible for the 2 ng/L bifenthrin and *H. azteca* toxicity observed in sampling of upper Cache Slough core sites the following day (19 March core site data previously discussed).

Concurrent with the 18 March bifenthrin release from the Highway 113 Drain, agricultural runoff in the drain contained 1235 ng/L cyhalothrin. Ulatis Creek had contained only 1.2 ng/L cyhalothrin prior to reaching Highway 113, but this single drain was sufficient to raise the concentration in creek waters to 27 ng/L, nearly 12 times the *H. azteca* 96-h EC50 [25]. Cyhalothrin was found infrequently in the Cache Slough complex during the present study, but the following day upper Cache Slough and lower Lindsey Slough contained approximately 2 ng/L cyhalothrin (approximately the *H. azteca* EC50) and were acutely toxic. It is likely that the discharge from Highway 113 Drain was responsible for the appearance and toxicity of cyhalothrin in these areas, up to 19 km from the point of release.

A year later, on 13 March 2012, Highway 113 Drain was releasing runoff containing 453 ng/L chlorpyrifos, nearly 5 times the *H. azteca* EC50 (Table 3). Again, the discharge was sufficient to substantially change water quality in the creek, raising chlorpyrifos concentrations in Ulatis Creek from 13.9 ng/L to 28.3 ng/L.

The Ulatis transect was repeated following rain in June 2011 and March 2012. Bifenthrin concentrations leaving Vacaville were 6.6 ng/L to 6.9 ng/L, well below the 19.4 ng/L observed on the first sampling occasion. The bifenthrin travelled 5 km to 12 km downstream, but did not reach Cache Slough at measurable concentrations. The primary difference between the first event and the 2 subsequent events was the intensity of rainfall. All sampling was initiated after approximately 2 cm of precipitation had fallen. However, it had fallen in the 2 h preceding the first event, but over an 8-h to 10-h period preceding the other 2 events. The greater intensity of rain in the first event produced far higher TSS concentrations in the creek,

Table 3. Results of three Lagrangian sampling transects on Ulatis Creek with data on total suspended solids (TSS; mg/L) and those pesticides (ng/L) found associated with toxicity in the present study[a]

| Site | 18 March 2011 | | | | 4 June 2011 | | | | 13 March 2012 | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | TSS | Chlor | Bifen | Cyhal | TSS | Chlor | Bifen | Cyhal | TSS | Chlor | Bifen | Cyhal |
| ULT (21 km to Cache) | 355 | U | 19.4 | U | 17.4 | 2.9 | 6.6 | 1.8 | 26.3 | U | 6.9 | U |
| UBY (18 km to Cache) | 300 | U | 23.0 | U | 19.8 | 3.0 | 4.5 | 1.6 | 10.3 | U | 5.2 | U |
| UF (16 km to Cache) | 380 | 3.8 | 16.9 | 1.9 | 29.6 | 2.6 | 2.9 | U | 33.1 | 2.1 | 6.9 | U |
| Fox Drain | 23.7 | 1.1 | U | U | 86.9 | 5.4 | U | U | 95.5 | 7.6 | 2.3 | U |
| Gibson Creek | 59.0 | 3.0 | U | U | 11.3 | 3.3 | 3.5 | U | 8.5 | U | U | U |
| Sweany Creek | 17.9 | U | U | U | 8.4 | 10.0 | U | U | 15.7 | 11.5 | U | U |
| UH (13 km to Cache) | 454 | 7.1 | 12.6 | 1.2 | 30.3 | 4.7 | U | U | 41.3 | 13.1 | 3.1 | U |
| Hawkins Drain | 643 | U | U | U | 44.0 | 7.7 | U | 1.9 | 140 | 6.8 | U | U |
| U113 (9 km to Cache) | 338 | 2.9 | 15.4 | 1.2 | 11.8 | 12.2 | U | U | 74.0 | 13.9 | 1.6 | U |
| Highway 113 Drain | 561 | 13.6 | 15.8 | 1235 | 8.0 | 50.0 | U | U | 6.6 | 453 | U | 2.1 |
| New Alamo Creek | 443 | U | 9.3 | U | 33.1 | 2.0 | U | U | 111 | 11.1 | 2.0 | U |
| UB (5 km to Cache) | 422 | U | 8.0 | 27.0 | 21.4 | 7.7 | U | U | 18.5 | 28.3 | U | U |
| Cache Slough 24–48 h later | 80.3–97.3 | 2.8–3.2 | U–2.1 | U–2.3 | 23.9–56.2 | 11.4–43.2 | U | U | 18.3–97.9 | 6.2–16.0 | U | U |

[a]Sites along the mainstem of Ulatis Creek include the distance to the Ulatis Creek–Cache Slough confluence. The major creeks and agricultural drains discharging to Ulatis Creek are shown indented. Resulting effects on upper Cache Slough (sites UM and CSU) 24 h to 48 h later are shown. U indicates undetected at 1 ng/L. Chlor = chlorpyrifos; Bifen = bifenthrin; Cyhal = cyhalothrin.

flow rates approximately 40% greater (1.4 m/s vs 1 m/s at ULT), and 3-fold greater bifenthrin concentrations.

## CONCLUSIONS

There was no toxicity, or pesticide concentration likely to cause toxicity, in much of the Cache Slough complex. However, the western portion of the system, extending up to 10 km from the point of Ulatis Creek discharge, was often acutely toxic to *H. azteca* after winter rains. Pyrethroid insecticides were usually present at concentrations expected to cause toxicity, and PBO evidence supports their role in the observed effects.

It is more difficult to infer the potential for indirect food-mediated effects on the threatened delta smelt. Its diet is comprised predominantly of copepods, particularly *E. affinis* and *P. forbesi*, both of which were found in Cache Slough. With a 96-h bifenthrin LC50 to *E. affinis* of 16.7 ng/L, and potentially 5 ng/L to 10 ng/L if in situ temperatures are considered, the observed maximum bifenthrin concentration of nearly 7 ng/L at least indicates cause for concern. Potential acute toxicity to the fish itself cannot be adequately assessed because there are no delta smelt toxicity data available for bifenthrin and cyhalothrin. The 24-h esfenvalerate EC50 for swimming impairment of 10-d-old smelt is 40 ng/L [29]; however, esfenvalerate tends to be less toxic to aquatic life than many other pyrethroids [30].

Given that the Cache Slough complex is surrounded by croplands, it was unexpected that the pyrethroids appeared to be in large part of urban origin. Ulatis Creek and its tributaries Old and New Alamo Creeks provide a route for urban pesticide runoff to travel over 20 km to Cache Slough. Aided by tidal action, pyrethroids entering the Slough can then travel at least an additional 10 km. Although Vacaville runoff contains many pyrethroids, bifenthrin appears to be the compound of greatest concern, as noted in many other US localities [31–33].

Supplementing the urban pesticides, agricultural discharges contributed bifenthrin, cyhalothrin, and chlorpyrifos. In some instances, these inputs were to Ulatis Creek as it flowed toward Cache Slough, and in 1 instance it was directly into waters of Cache Slough from an adjacent property. The presence of pyrethroids in nearly all the tributaries sampled during the present study indicates that these agricultural inputs are common and widespread. Agricultural inputs are particularly difficult to detect and quantify because of their inherently intermittent nature, which is influenced by when individual growers choose to apply pesticides and irrigate. Our sampling of local drains and previous work [8] suggest that release of irrigation waters containing toxic concentrations of pesticides constitutes a small proportion of the total discharge events, further complicating efforts to detect pesticides representing a threat to water quality. The Cache Slough complex is a likely site for future habitat restoration efforts because of its importance to delta smelt and migrating salmon [34]. These restoration efforts will need to address contaminant inputs; and while it is unlikely that all entry of pesticides to the complex can be curtailed, given the diversity of sources, our data show that the Ulatis Creek system is a consistent source that should be a focus of any mitigation effort.

## SUPPLEMENTAL DATA

**Tables S1 through S3.** (126 KB PDF).

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