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MONITORING ACUTE AND CHRONIC WATER COLUMN TOXICITY IN THE NORTHERN SACRAMENTO–SAN JOAQUIN ESTUARY, CALIFORNIA, USA, USING THE EURYHALINE AMPHIPOD, *HYALELLA AZTECA*: 2006 TO 2007

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Abstract—After the significant population decline of several pelagic fish species in the Northern Sacramento–San Joaquin (SSJ) Estuary (CA, USA) in 2002, a study was performed to monitor water column toxicity using the amphipod *Hyalella azteca*. From January 1, 2006 to December 31, 2007, water samples were collected biweekly from 15 to 16 sites located in large delta channels and main-stem rivers, selected based on prevalent distribution patterns of fish species of concern. Ten-day laboratory tests with *H. azteca* survival and relative growth as toxicity endpoints were conducted. The enzyme inhibitor piperonyl butoxide ([PBO], 25 μg/L) was added to synergize or antagonize pyrethroid or organophosphate (OP) insecticide toxicity, respectively. Significant amphipod mortality was observed in 5.6% of ambient samples. Addition of PBO significantly changed survival or growth in 1.1% and 10.1% of ambient samples, respectively. Sites in the Lower Sacramento River had the largest number of acutely toxic samples, high occurrence of PBO effects on amphipod growth (along with sites in the South Delta), and the highest total ammonia/ammonium concentrations (0.28 ± 0.15 mg/L). Ammonia/ammonium, or contaminants occurring in mixture with these, likely contributed to the observed toxicity. Pyrethroid insecticides were detected at potentially toxic concentrations. Overall, results of this study identified specific areas and contaminants of concern and showed that water in the Northern SSJ Estuary was at times acutely toxic to sensitive invertebrates. Environ. Toxicol. Chem. 2010;29:2190–2199. © 2010 SETAC

Keywords—Ambient toxicity Pesticides Ammonia Invertebrates

INTRODUCTION

Contaminants are considered to be one of several factors acting individually or in concert with other stressors to negatively affect populations of pelagic fish species in the Northern Sacramento–San Joaquin (SSJ) Estuary, California, USA. Since 2002, abundance indices of numerous fish species have shown marked declines and record lows, among them the endemic delta smelt (*Hypomesus transpacificus*), age-0 striped bass (*Morone saxatilis*), longfin smelt (*Spirinchus thaleichthys*), and threadfin shad (*Dorosoma petenense;* [1]). Although several of these have shown evidence of a steady decline in abundance since 1967, a precipitous step-change to very low abundance during the period 2002 to 2004 appears to have occurred.

Agricultural, industrial, urban, and mining sources release a wide array of contaminants into the Estuary and its tributaries. The Sacramento and San Joaquin rivers, in particular, carry contaminants from urban and agricultural runoff ([2]; http://escholarship.org/uc/item/06n8b36k; [3]), metals from historic mining activities [4,5], selenium [6], and discharge from municipal wastewater treatment plants [7]. In addition, herbicides and insecticides are applied directly to the water surface for aquatic plant and mosquito abatement. Water quality criteria for the protection of freshwater aquatic life have in the past been exceeded primarily because of insecticides ([8]; http://www.cuwa.org/; [9]); however, the knowledge base needed for extrapolating such information to population level effects on resident species does not yet exist.

The toxic effects of contaminants on aquatic ecosystems are often subtle and can be difficult to detect and quantify, especially in large water bodies such as the SSJ Estuary. Contaminants can negatively affect ecological fitness, and consequently survival, of individual species at different trophic levels through sublethal physiological, behavioral, or immunological effects [10–12], potentially leading to changes in food web and ecosystem dynamics. To detect such sublethal effects in field studies is challenging, at best, especially for nonmodel species, for whom biomarker tools or toxicity testing protocols are not readily available. Alternative tools for toxicity screening include laboratory testing of ambient water samples with sensitive model species and using endpoints that can be easily interpreted, such as survival and growth. For some species, toxicity identification evaluation methods (TIEs) are available to identify the causative group of chemicals when toxicity is detected [13]. Although these conventional tools are generally less sensitive than biochemical or histological endpoints, few biomarkers are currently understood well enough to provide conclusive evidence of contaminant impacts on aquatic species in field monitoring. Moreover, extrapolating effects seen at the biomarker level to individual or population-level toxicity continues to be a challenge.

The 2006 to 2007 monitoring study presented here was designed to provide information on the occurrence and distribution of toxicity in the larger channels and rivers of the Northern SSJ Estuary. The test organism, the epibenthic amphipod species *Hyalella azteca*, was chosen based on its high

All Supplemental Data may be found in the online version of this article.

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sensitivity to contaminants ([14]; http://www.epa.gov/ecotox/), its relatively large salinity tolerance range (0–15 ppt), its being a resident of the Northern SSJ Estuary, and its routine use in toxicity testing [15]. The wide range of salinities of ambient water samples required the addition of multiple controls, and additional work is still needed to complete TIE protocols for this species. For the current study, TIE methods used were based on existing protocols for *Ceriodaphnia dubia* [13], and effect concentrations for piperonyl butoxide (PBO) were determined. Because of concerns about potential loss of pesticide-associated toxicity during sample transport and storage, concurrent partial TIE tests (addition of PBO) were conducted routinely to increase test sensitivity for two classes of current-use insecticides, organophosphates (OPs), and pyrethroids, and to guide subsequent chemical analyses.

METHODS

Sampling and water quality

From January 1, 2006 to December 31, 2007, a total of 693 water samples were collected biweekly by boat or from shore from 15 to 16 sampling sites (Table 1, Fig. 1) in accordance with prevalent distribution patterns of pelagic fish species of concern [16]. Site Hood was sampled as of September 5, 2007. Water was pumped from a depth of 0.5 m using a submersible bilge pump (Model 02, ITT Industries Rule) into 3.8-L amber low-density polyethylene cubitainers (Fisher Scientific), and additional water samples for chemical analyses were collected in precleaned 1-L wide-mouth amber glass bottles (I-Chem certified 300 series). Sampling containers were rinsed three times with ambient water before filling. All samples were immediately packed in wet ice for transport to the laboratory, where they were stored in the dark at 0 to 6°C until test initiation.

Water quality parameters were measured at each sampling site at the time of collection, including pH, specific conductivity (SC), dissolved oxygen, and temperature. Dissolved oxygen and conductivity were measured using a YSI85 and a YSI30 meter (YSI), respectively; temperature and pH were measured with a Beckman 255 pH meter (Beckman Coulter). All meters were calibrated according to the manufacturer's instructions on each sampling day. Turbidity and total ammonia-nitrogen (ammonia/ammonium) were measured within 24 h of sample receipt in the laboratory using a Hach 2100P turbidimeter and a Hach DR/890 colorimeter with the low-range (0–2.5 mg/L N; manufacturer's estimated detection limit, 0.08 mg/L) Hach AmVer Ammonia Test'N Tube Reagent Set (Hach Company). Field un-ionized ammonia concentration was calculated for each sample based on total ammonia/ammonium concentration measured in the laboratory, and water temperature, SC, and pH measured in the field at the time of sampling. For analysis of ammonia/ammonium effects on amphipod survival and growth, un-ionized ammonia was calculated using the water temperature, pH, and SC measured in the laboratory at test initiation.

Toxicity testing

Hyalella azteca were purchased from Aquatic Research Organisms. On receipt, amphipods were moved to 10-L aquaria, fed, and acclimated to laboratory test conditions for 48 h. The 10-d testing procedure used in this study were based on protocols described in the Quality Assurance Management Plan for the State of California's Surface Water Ambient Monitoring Program ([17] http://www.waterboards.ca.gov/water_issues/programs/swamp/qamp.shtml#appendixf). At test initiation, water samples were shaken vigorously in original sampling containers, and sub-samples were filtered through a 53-μm screen to remove debris and larger organisms. Water was then warmed to test temperature (23 ± 1 °C) in 600-ml beakers, using a water bath maintained at 25 ± 2°C, and aerated at a rate of 100 bubbles per minute until dissolved oxygen concentration was 4.9 to 8.9 mg/L. Deionized water amended to U.S. Environmental Protection Agency (U.S. EPA) moderately hard specifications (hardness: 90–100 mg/L $CaCO_3$, alkalinity: 50–70 mg/L $CaCO_3$, electrical conductivity (EC): 330–360 μS/cm; pH, 7.8–8.2 [18]) was used for controls. One or multiple high-conductivity controls were added when the SC of an ambient sample was greater than 10,000 μS/cm. A low conductivity control was added when the SC of an ambient sample was less than 100 μS/cm. Filtered (1 μm A/E glass fiber filter) Pacific Ocean seawater from Bodega Bay Marine Laboratory or deionized water was used to increase or decrease the conductivity of control water.

Tests were initiated with 9- to 14-d-old *H. azteca*. Each of four replicate 250-ml glass beakers contained 100 ml water, a small piece of nitex screen (approximately 6 cm^2) for use as substrate for *H. azteca*, and 10 organisms. Animals were fed a mixture of yeast, organic alfalfa, and trout chow (1 ml/replicate) at test initiation and on days 2, 4, 5, 6, and 8. Tests were conducted at 23 ± 2 °C with a 16:8-h light:day photoperiod. Mortality was recorded daily, and 80% of water was renewed on day 5. On day 10, the surviving *H. azteca* were dried to

Table 1. Sampling locations and global positioning system (GPS) coordinates in the Northern Sacramento–San Joaquin Estuary (CA, USA) from 2006 to 2007

Sampling site	Location	Latitude	Longitude
323	San Pablo Bay near Rodeo Flats	38-02′-53.9″N	122-16′-58.1″W
340	Napa River along Vallejo seawall	38-05′-51″N	122-15′-43.9″W
405	Carquinez Straight west of Benicia army dock	38-02′-22.9″N	122-09′-01.8″W
504	Suisun Bay east of Middle Point	38-03′-16.2″N	121-59′-22.2″W
508	Suisun Bay off Chipps Island	38-02′-43.8″N	121-55′-07.7″W
602	Grizzly Bay northeast of Suisun Slough	38-06′-50.4″N	122-02′-46.3″W
609	Montezuma Slough at Nurse Slough	38-10′-01.9″N	121-56′-16.8″W
704	Sacramento River across from Sherman Lake	38-04′-09″N	121-46′-31″W
711	Sacramento.River near tip of Grand Island	38-10′-43.7″N	121-39′-55.1″W
804	Middle of Broad Slough	38-01′-05.5″N	121-47′-49.2″W
812	San Joaquin River west of Oulton Point	38-05′-25.1″N	121-38′-25.8″W
902	Old River at mouth of Holland Cut	38-01′-09.1″N	121-34′-55.9″W
910	San Joaquin River between Hog and Turner Cut	38-0′-06.5″N	121-26′-55.3″W
915	Old River—Western arm	37-56′-33″N	121-33′-48.6″W
Hood	DWR Water Quality Monitoring Station	38-22′-03.6″N	121-31′-13.6″W
Light 55	Sacramento River Deep Water Channel at Light 55	38-16′-26.5″N	121-39′-42.9″W

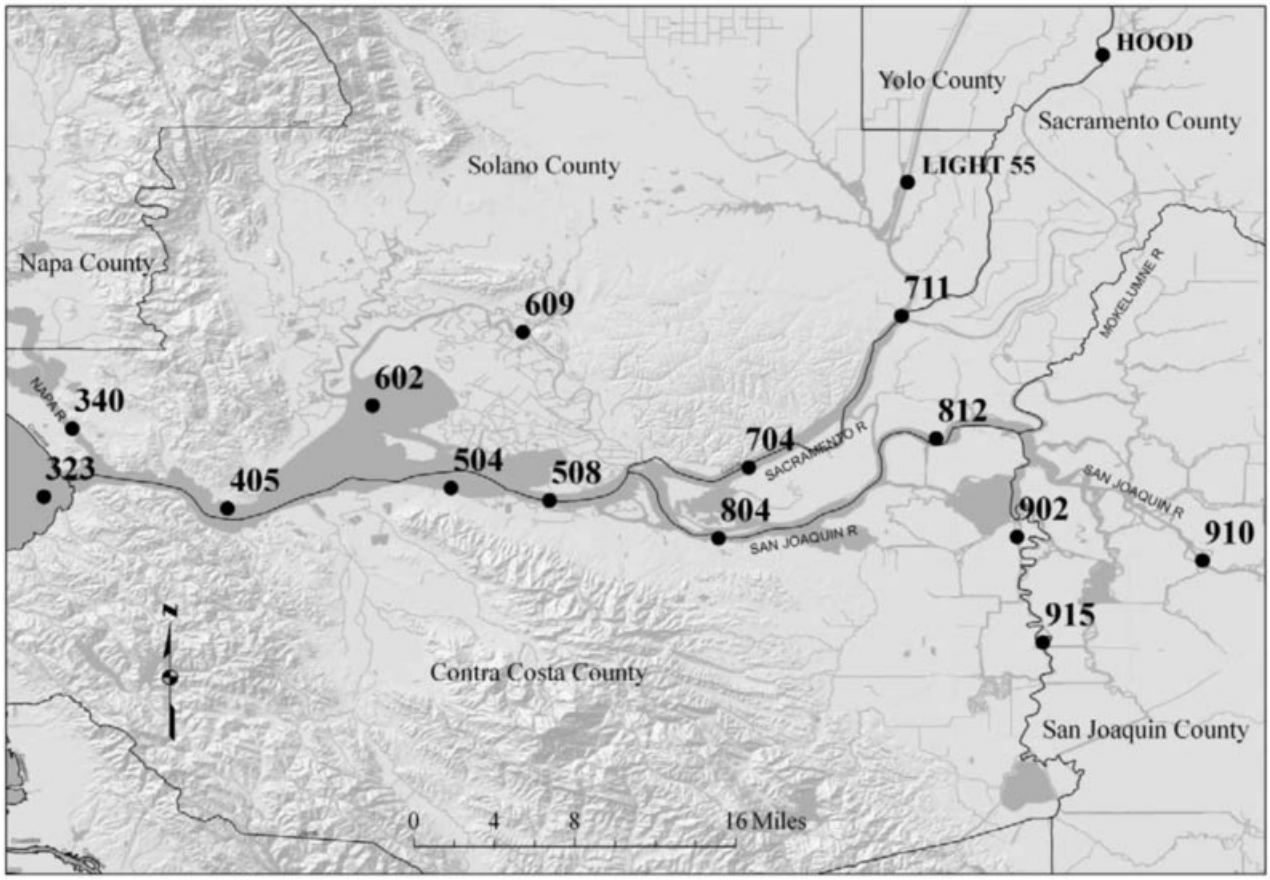

Fig. 1. Map of sampling locations in the Northern Sacramento–San Joaquin Estuary (CA, USA).

constant weight at 103 to 105°C and weighed using a Mettler AE 163 balance.

Addition of PBO

All tests were conducted with and without PBO to synergize [19] or antagonize [20] toxicity of pyrethroid or OP insecticides, respectively, because of concerns that toxicity may be lost during sampling, transport, and storage, and to guide subsequent chemical analyses. Tests were initially conducted with 100 μg/L PBO, reduced to 50 μg/L as of May 2006. These concentrations do not affect amphipod survival; however, a test with 5 to 100 μg/L PBO showed that the effect concentration (EC25) for growth (as final dry weight/individual relative to control) is 42.4 μg/L (Supplemental Data, Table S1). The PBO test concentration was subsequently reduced to 25 μg/L as of July 27, 2006. Pairwise analysis of control treatments to identify potential effects of PBO on growth showed that 100 μg/L, but not 50 μg/L, PBO significantly reduced growth in samples tested earlier, and consequently, those data were excluded from further analysis.

Test development for chronic toxicity endpoint (growth)

Amphipod growth in laboratory control water was generally lower than in ambient samples because of the lack of particulate organic matter (POM) naturally present in ambient water, which *H. azteca* used as an additional food source. To correct for this and increase the sensitivity of the growth endpoint, natural POM was added as supplemental food to control treatments as of January 4, 2007. Particulate organic matter was concentrated to $\times 100$ by centrifugation of nontoxic water samples (SC $\leq$ 1,500 μS/cm) in a continuous flow centrifuge (2,000 *g*, IEC Chemical Centrifuge, International Equipment) then added to control treatments for a final concentration of 1% to match the concentration in ambient water. An additional control without POM was included in each test as of May 1, 2007. Results of the current study show that final weight of control animals increased significantly when natural POM was added (Supplemental Data, Table S2).

Toxicity identification evaluation

Phase I TIEs involve procedures to either remove or inactivate specific classes of chemicals [13]. In this study, phase I TIEs were conducted on samples that caused at least 50% mortality within 7 d. Samples that met these criteria were collected at site 323 on July 12, 2006; site 711 on April 12, 2007; and at Hood on October 2, 2007. Toxicity identification evaluation treatments included air stripping (aeration at 150 bubbles/min for 2 h) to reduce or remove toxicity caused by volatile chemicals such as surfactants, chlorine, and ammonia; low test temperature (15°C) to increase toxicity caused by pyrethroid insecticides; addition of ethylenediaminetetraacetic acid (3 concentrations) to chelate metals, making them unavailable to biota; addition of PBO (25 μg/L, see above); removal of nonpolar organic chemicals by solid-phase extraction columns (Varian Bond Elut C8, Varian). Appropriate control and method blank treatments were included for all TIE manipulations. Improved organism performance after TIE manipulation

is defined as the absence or a delay of mortality by 24 h or longer.

Statistical analysis

All statistical analyses were performed using JMP 5.0.1 (SAS Institute; 1989–2003). Survival and final weight data obtained for ambient samples were compared with their PBO-containing counterpart, and with controls using the U.S. EPA standard statistical procedures for single concentration static renewal toxicity tests [18]. Shapiro-Wilk's test and Bartlett's test were used to examine normality of distributions and homogeneity of variances ($\alpha = 0.01$). Each sample was compared individually with the appropriate electrical conductivity (EC) control. A one-tailed Wilcoxon (Mann-Whitney) test was used ($\alpha = 0.05$) when data distribution was nonnormal in either treatment. When distributions were normal, a homoschedastic or heteroschedastic one-tailed *t* test was performed ($\alpha = 0.05$), depending on the presence or absence of homogeneity of variance. Comparisons between sample and sample with PBO used the same approach with two-tailed tests ($\alpha = 0.05$).

Salinity tolerance and effects of PBO on toxicity endpoints. Amphipod survival and growth data from control treatments were analyzed using linear and quadratic regressions to determine salinity-dependent effects on test endpoints to better interpret test results and determine the need for additional control treatments. Only data from controls and nontoxic ambient samples without PBO were considered, to avoid bias by contaminated samples. Electrical conductivity measured at test initiation was log-transformed, and weight was transformed to percentage of method control to remove between-test variation in amphipod size. To examine the effects of PBO on *H. azteca* survival and final weight per individual at high EC, organism performance was examined in high-conductivity control treatments (10–30 mS/cm) with or without 25 μg/L PBO. *Hyalella azteca* responses were examined as paired response variables in a multiple response regression model with EC as the predictor variable.

Amphipod survival and final weight were highest at EC of 300 to 3,000 μS/cm, and decreased significantly at 15,950 μS/cm or greater (9.7 ppt at 23°C), and 11,300 μS/cm (6.7 ppt at 23°C), respectively (Supplemental Data, Fig. S1, Table S3). Piperonyl butoxide (25 μg/L) did not alter the effect of high EC on survival or final weight (survival: PBO effect: $F_{1,84} = 0.0001$, $p = 0.925$, PBO·EC Interaction: $F_{1,84} = 0.0005$, $p = 0.8419$; Weight: PBO effect: $F_{1,53} = 0.0038$, $p = 0.6551$, PBO·EC Interaction: $F_{1,53} = 0.0031$, $p = 0.6861$).

Field ammonia/ammonium analysis and effects. Because *H. azteca* is relatively sensitive to ammonia/ammonium [21], data of measured total ammonia/ammonium and un-ionized ammonia concentrations were analyzed in more detail. Total ammonia/ammonium as well as un-ionized ammonia field concentrations (calculated using pH, SC, and temperature measured in the field) were compared between sampling sites using ANOVA with Tukey's multiple comparison procedure ($p \leq 0.05$). The relationships between ammonia/ammonium in ambient water samples (calculated using pH, EC, and temperature measured at test initiation) and *H. azteca* survival and final weight were examined using multivariate regression models with linear and quadratic terms for EC effects, and ammonia/ammonium concentration as a linear effect. Models were examined using data from each site individually as well as from all sites combined.

Analytical chemistry

Samples that showed significant differences in survival or growth between PBO-treated and ambient samples based on U.S. EPA standard statistics and one-way ANOVA and Tukey's multiple comparison procedure (27 samples) were submitted to the California Department of Fish and Game Water Pollution Laboratory, Rancho Cordova, CA, USA, for chemical analysis. Whole sample extracts were analyzed for either pyrethroids (bifenthrin, cyfluthrin, cypermethrin, deltamethrin, esfenvalerate/fenvalerate, fenpropathrin, lambda-cyhalothrin, *cis/trans* permethrin) or 41 OP insecticides (Supplemental Data, Table S4) by gas chromatography (Agilent 6890 plus, Agilent Technologies) with dual columns (DB5 and DB17) and dual flame photometric detectors in phosphorous mode (OP insecticides), or dual microelectron capture detectors (pyrethroid insecticides). Pyrethroids were confirmed using GC-MS or GC-MSMS. Detection limits of analytes are listed in the Supplemental Data, Table S4. As of June 20, 2007, samples for organic chemical analysis were preserved by addition of 10 ml/L dichloromethylene immediately on receipt at the laboratory because of concerns that labile organic chemicals could break down during storage.

Quality assurance/quality control

Test acceptability criteria for *H. azteca* toxicity tests required 90% control survival [17]. To evaluate whether organism sensitivity was consistent throughout the project period, positive control reference toxicant tests were performed once a month using NaCl as the toxicant. If an effect concentration, LC50 or EC25, was outside the 95% confidence interval, test organism sensitivity was considered atypical, and results of tests conducted during that month were considered suspect. To assess laboratory testing precision, 39 duplicate ambient water samples were collected and tested. In addition, 16 bottle blanks and 13 trip blanks were tested to ascertain the cleanliness of the sampling container and detect potential contamination of water samples during collection and transport. Any deviations from test protocols were recorded.

RESULTS

Field water quality

Water quality in the Sacramento–San Joaquin Delta and Estuary is characterized by large geographic and seasonal variation. Temperature (5.8–28.6°C), SC (86–30,260 μS/cm), hardness (16–3,720 mg/L $CaCO_3$), alkalinity (10–280 mg/L $CaCO_3$), and turbidity (1.4–219.7 nephelometric turbidity units) varied widely between sampling sites because of the extent of tidal influence, river flows, and air temperature (Table 2). Dissolved oxygen concentrations ranged from 5.1 to13.9 mg/L and pH from 6.1 to 8.7. Total ammonia/ammonium concentrations at stations Hood and 711, both sites on the lower Sacramento River, were significantly higher than at all other sampling sites (Table 3). Other sites with elevated total ammonia/um concentrations were Light 55, 405, 609, and 910. Un-ionized ammonia concentrations were highest at site 711, followed by Light 55.

10-Day acute toxicity

Control survival (with and without POM) of *H. azteca* in 2006 to 2007 was 96.5 ± 4.3 (mean ± SD; $n = 125$), and survival in controls with PBO was 95.6 ± 7.7 ($n = 125$). No significant differences between controls with and without POM

Table 2. Water quality parameters measured in the field at sampling locations from 2006 to 2007

		Temperature (°C)		DO (mg/L)		pH		SC (μS/cm)	
Site	*n*	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
323	14	15.1 ± 3.9	10.7 –1.9	8.9 ± 1.3	6.7 –10.5	7.6 ± 0.2	7.3 –7.8	13,184 ± 8,311	174 –30260
340	38	16.4 ± 5.1	6.5 –25.2	9.2 ± 2.1	5.8 –13.5	7.7 ± 0.3	7.1 –8.6	10,940 ± 8,464	217 –25760
405	47	16 ± 4.1	7.9 –23.1	9.3 ± 1.3	7 –12	7.7 ± 0.3	6.7 –8.2	14,572 ± 8,762	163 –28200
504	50	15.9 ± 4.4	7.2 –24.6	9.6 ± 1.3	7 –12.5	7.7 ± 0.3	6.3 –8.1	6,829 ± 5,350	123 –17540
508	50	16 ± 4.6	7.2 –24.7	9.6 ± 1.5	7.3 –13	7.6 ± 0.4	6.3 –8.1	3,863 ± 3,449	100 –12250
602	49	15.9 ± 4.3	7.4 –22.7	9.6 ± 1.2	7.5 –12.5	7.7 ± 0.3	6.8 –8.1	9,780 ± 6,664	145 –18860
609	49	16.3 ± 4.9	6.7 –26.3	8.8 ± 1.4	5.1 –12.4	7.5 ± 0.3	6.1 –7.9	6,072 ± 4,954	188 –15130
704	50	16.2 ± 4.6	7.2 –25.3	9.6 ± 1.5	6.8 –13.5	7.6 ± 0.3	6.6 –8.2	1,319 ± 1,541	107 –5540
711	50	16 ± 5	6.1 –25	9.6 ± 1.7	6.8 –13.9	7.5 ± 0.3	6.6 –8.3	176 ± 89	95 –695
804	50	16.4 ± 4.9	7.2 –26.5	9.5 ± 1.5	6.5 –12.9	7.7 ± 0.4	6.6 –8.5	1,381 ± 1,562	114 –5550
812	48	16.7 ± 5	6.7 –26.3	9.5 ± 1.7	6.5 –13.6	7.6 ± 0.3	6.9 –8.4	340 ± 224	94 –832
902	50	16.7 ± 5.4	5.8 –27.2	9.5 ± 1.5	7.1 –12.9	7.7 ± 0.5	6.3 –8.7	389 ± 199	132 –830
910	50	17.2 ± 5.5	6.6 –28.6	8.7 ± 2	5.3 –12.9	7.5 ± 0.3	6.6 –8.3	404 ± 163	115 –702
915	50	17 ± 5.6	6.6 –28	9.3 ± 1.7	6.4 –13.3	7.6 ± 0.4	6.2 –8.7	369 ± 173	86 –721
Hood	14	19.1 ± 3.8	10.8 –23.7	8.7 ± 1.2	7 –11.4	7.3 ± 0.2	7 –7.6	205 ± 64	124 –328
Light 55	48	17 ± 5.2	6.4 –28.6	9.5 ± 1.7	6.6 –13.9	7.8 ± 0.3	6.8 –8.3	302 ± 93	96 –534

		Turbidity (NTU)		Hardness (mg/L as $CaCO_3$)		Alkalinity (mg/L as $CaCO_3$)	
Site	*n*	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
323	14	89.4 ± 61.4	19.8 –219.7	1,566 ± 939	60 –3450	95 ± 56	62-250
340	38	24.7 ± 23	4.9 –89.5	1,207 ± 1,114	80 –3720	126 ± 60	57- 80
405	47	33.2 ± 38.9	6.1 –205.7	1,713 ± 1,096	58 –3600	98 ± 29	49-180
504	50	15.1 ± 13	1.4 –83.8	758 ± 598	46 –1940	80 ± 26	30-190
508	50	14.6 ± 12.1	4.2 –83.4	432 ± 389	44 –1400	71 ± 13	46-100
602	49	34.8 ± 39.1	4.8 –200.7	1,052 ± 825	52 –3240	91 ± 23	48-140
609	49	36.6 ± 23.9	8.6 –109.2	687 ± 594	60 –1880	87 ± 20	52- 50
704	50	18.7 ± 18.8	4.6 –128.6	170 ± 151	46 –618	69 ± 12	48-114
711	50	10.4 ± 10.9	2.3 –60.8	60 ± 19	44 –180	61 ± 10	42-82
804	50	10.3 ± 4.6	4.4 –29	213 ± 271	38 –1680	64 ± 16	10-88
812	48	6.9 ± 2.4	3 –13.8	75 ± 24	16 –124	61 ± 11	36- 82
902	50	5.4 ± 2.5	2.2 –13.2	83 ± 35	40 –272	61 ± 12	34 –78
910	50	7.6 ± 2.8	3 –13	93 ± 33	38 –156	71 ± 19	30 –104
915	50	5.1 ± 2.1	2 –10.9	80 ± 24	32 –160	61 ± 12	34 –79
Hood	14	7.7 ± 4	2.8 –14.1	66 ± 11	52 –88	68 ± 10	50 –86
Light 55	48	23.4 ± 11.7	9.5 –68.9	100 ± 52	60 –412	88 ± 18	60 –140

DO = dissolved oxygen; SC = specific conductivity; NTU = nephelometric turbidity units; SD = standard deviation.

addition to food, or between controls with and without PBO, were found. Overall, 35 (5.6%) of 623 ambient water samples significantly reduced *H. azteca* survival, but mean survival was below 80% in only 14 (2.3%) samples (Supplemental Data, Table S5). The percentage of acutely toxic samples was far higher in 2007, a relatively dry year, than in 2006, a year with unusually high river flows (Fig. 2). In 2006, only 1.7% of 353 samples tested caused acute toxicity, whereas 8.5% of

Table 3. Total ammonia/ammonium and un-ionized ammonia concentrations at sampling sites, 2006 to 2007[a]

		Total ammonia nitrogen (mg/L)				Un-ionized ammonia (mg/L)			
Site	*N*	Mean	SD	Range	Rank	Mean	SD	Range	Rank
323	14	0.11	0.04	(0.06 –0.2)	BC	0.001	0.001	(0 –0.003)	ABCD
340	39	0.08	0.07	(0 –0.33)	C	0.001	0.001	(0 –0.002)	D
405	47	0.13	0.08	(0 –0.49)	B	0.002	0.001	(0 –0.006)	ABC
504	50	0.1	0.06	(0 –0.26)	BC	0.001	0.001	(0 –0.005)	CD
508	50	0.1	0.06	(0 –0.24)	BC	0.001	0.001	(0 –0.006)	CD
602	49	0.11	0.07	(0 –0.27)	BC	0.001	0.001	(0 –0.005)	ABCD
609	50	0.12	0.08	(0 –0.27)	B	0.001	0.001	(0 –0.003)	CD
704	50	0.11	0.07	(0 –0.3)	BC	0.001	0.001	(0 –0.005)	BCD
711	50	0.21	0.11	(0.06 –0.54)	A	0.003	0.003	(0 –0.013)	A
804	50	0.09	0.06	(0 –0.29)	C	0.002	0.002	(0 –0.008)	BCD
812	48	0.09	0.06	(0 –0.29)	C	0.001	0.001	(0 –0.005)	CD
902	50	0.06	0.05	(0 –0.24)	C	0.001	0.002	(0 –0.01)	CD
910	50	0.15	0.1	(0 –0.44)	B	0.002	0.002	(0 –0.007)	ABCD
915	50	0.07	0.07	(0 –0.38)	C	0.001	0.001	(0 –0.006)	CD
Hood	14	0.28	0.15	(0 –0.51)	A	0.002	0.001	(0 –0.004)	ABCD
Light 55	48	0.12	0.08	(0 –0.29)	B	0.003	0.003	(0 –0.012)	AB

[a] Un-ionized ammonia concentrations were calculated using water temperature, specific conductivity (SC), and pH measured at the time of sampling. Sites are ranked with different letters to indicate statistical differences ($p \leq 0.05$), where A represents sites with highest and D sites with lowest concentrations. SD = standard deviation.

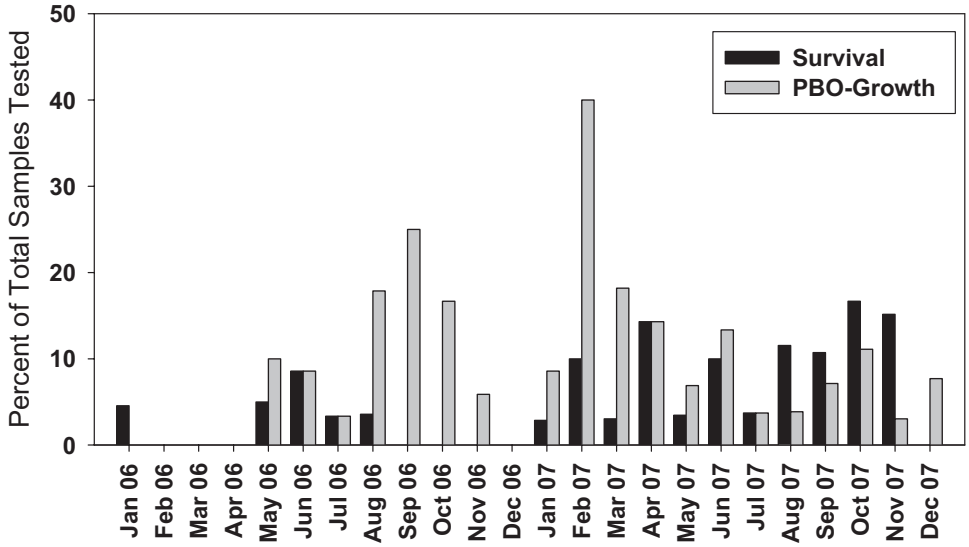

Fig. 2. Percentage of total water samples tested causing significant *Hyalella azteca* mortality (dark bars) and piperonyl butoxide (PBO) effects on growth (light bars) by month, January 1, 2006 to December 31, 2007. Growth data for January 1 to May 14, 2006 were excluded because PBO concentration was above the effect concentration for growth.

340 samples tested in 2007 were toxic. Most toxic samples (60%) were collected from sites in the lower Sacramento River (Hood, 711, 704) and the Deep Water Shipping Channel (Light 55). Hood had the highest percentage (75%) of toxic samples (Table 4, and Supplemental Data Table S5).

Synergistic/antagonistic effects of PBO on H. azteca *survival*

Significant changes in survival caused by PBO addition were detected in seven water samples (1.1% of total). Piperonyl butoxide reduced acute toxicity in samples collected from Hood on September 7 and October 2 and 31, 2007, and from Light 55 on October 31, 2007, indicating the potential presence of OP insecticides; however, none were detected. Piperonyl butoxide increased acute toxicity in samples collected on January 25, 2006 from site 323, on August 22, 2006 from site 711, and on July 25, 2007 from site 340, indicating the potential presence of pyrethroid insecticides. The pyrethroids cyfluthrin (3 ng/L) and esfenvalerate (16 ng/L) were detected in the sample from site 340, but none were detected at site 711 (Table 5, and Supplemental Data, Table S5).

Effects on H. azteca *growth*

Amphipod growth relative to controls was not a sensitive indicator of toxicity, partially because of the variable size of the organisms, and—more importantly—the variability in food content of ambient water samples from different sites. Final dry weight of *H. azteca* exposed to laboratory control water was generally lower than in ambient samples. In-house tests showed that this was attributable to the lack of POM naturally present in ambient water samples, which *H. azteca* used as a supplemental food source. Addition of natural POM increased mean final dry weight of control animals by 37% (Supplemental Data, Table S2).

Table 4. Number of samples causing significant differences in survival or growth of *Hyalella azteca* by sampling site

Site	Total samples tested	Reduced survival[a]	PBO effect on survival	Synergistic PBO effect on growth	Antagonistic PBO effect on growth
	[*n*]	[*n*] (% of Total)	[*n*]	[*n*]	[*n*]
323	14	1 (7.1%)	1[b]	0	0
340	38	0 (0%)	1[b]	3	0
405	47	3 (6.4%)	0	2	2
504	50	1 (2%)	0	4	1
508	50	1 (2%)	0	4	0
602	49	3 (6.1%)	0	2	3
609	49	0 (0%)	0	4	1
704	50	2 (4%)	0	2	0
711	50	8 (16%)	1[b]	6	1
804	50	1 (2%)	0	1	1
812	48	1 (2.1%)	0	2	2
902	50	0 (0%)	0	7	1
910	50	1 (2%)	0	4	3
915	50	2 (4%)	0	6	1
Hood	8	6 (75%)	3[c]	1	0
Light 55	48	5 (10.4%)	1[c]	4	2

[a] Ambient samples (with or without piperonyl butoxide [PBO]) significantly different from appropriate control.
[b] Synergistic effect of PBO.
[c] Antagonistic effect of PBO.

Table 5. Detected concentrations of insecticides in water samples in which addition of piperonyl butoxide (PBO) caused significant changes in *Hyalella azteca* survival or growth[a]

Site	Sampling date	Ambient	Ambient with PBO	Ambient with PBO as % of ambient	Analytical results
			10-d Survival ± SE (%)		
340	Jul 25, 2007	67 ± 10.4	44 ± 8.7[b]	66	3 ng/L cyfluthrin[c] 16 ng/L esfenvalerate[c]
405	Oct 4, 2007	76 ± 5.0[c]	77 ± 4.6[d]	101	5 ng/L permethrin[c,e]
Light 55	Feb 1 2007	77 ± 9.4[d]	95 ± 2.8	123	6 ng/L diazinon[e]
			Final dry weight ± SE (μg/individual)		
340	Feb 13 2007	98 ± 11	63 ± 6[b]	65	63 ng/L cyfluthrin[c]
508	Mar 1 2007	101 ± 11	61 ± 6[b]	60	3 ng/L λ-cyhalothrin[c]
902	Aug 22 2006	124 ± 16	59 ± 7[b]	48	5 ng/L cyfluthrin[c] 24 ng/L permethrin[c]
915	Feb 28 2007	116 ± 10	65 ± 5[b]	56	2 ng/L λ-cyhalothrin[c]

[a] SE = standard error.
[b] Significant reduction or increase in final weight due to PBO ($p \leq 0.05$).
[c] Pyrethroid insecticide scan.
[d] Significantly different from appropriate control ($p \leq 0.05$).
[e] Organophosphate insecticide scan.

Synergistic/antagonistic effects of PBO on H. azteca *growth*

Addition of PBO resulted in significantly different growth relative to the corresponding ambient treatment in 70 water samples (10.1% of samples tested; Supplemental Data, Table S6). Piperonyl butoxide addition increased amphipod weight in 21 (30%), and decreased it in 49 (70%). Of these, 28 samples were subject to chemical analyses. Sites in the South-Eastern Delta (902, 910, 915) and the Lower Sacramento River (711) had the highest number of samples exhibiting such PBO effects on growth (Table 4). Patterns of several neighboring sites sampled on the same date, showing similar organism responses, were seen repeatedly (Supplemental Data, Tables S5 and S6). For example, PBO addition resulted in an increase in growth in samples collected from three neighboring sites (902, 910, and 812) on June 6, 2007. Distinct seasonal patterns were not apparent (Fig. 2).

Toxicity identification evaluations

Results of TIEs are summarized in the Supplemental Data, Table S5. Few samples caused reduced survival to the extent required for TIE procedures to be successful. In all three samples tested, toxicity was lost by the time TIE procedures were performed.

Analytical chemistry

Seven of 35 water samples (20%) analyzed during the reporting period contained detectable concentrations of insecticides (Table 5). A sample from site 340 (collected July 25, 2007), which caused a significant reduction in *H. azteca* survival after PBO addition, contained 3 ng/L cyfluthrin and 16 ng/L esfenvalerate. One sample from site 405, collected October 4, 2007, contained 5 ng/L permethrin. A sample collected on February 1, 2007, from Light 55 contained 6 ng/L diazinon. In addition, several water samples that caused a significant negative PBO effect on *H. azteca* growth contained detectable amounts of pyrethroid pesticides: Five nanograms per liter cyfluthrin and 24 ng/L permethrin were detected at site 902 (August 22, 2006), 63 ng/L cyfluthrin at site 340 (February 13, 2007), and 2 and 3 ng/L lambda-cyhalothrin at sites 915 (February 28, 2007) and 508 (March 1, 2007), respectively, were found.

In-house studies demonstrated that adsorption and degradation of pyrethroids can occur within the time our samples were typically stored before solvent extraction (≈14 d); thus, actual pyrethroid concentrations were underestimated. Only 38% of permethrin spiked into laboratory control water was detected after a mock sampling procedure and 14-d storage at 4°C in the dark (I. Werner, unpublished data). A water sample collected on October 4, 2007, from site 405 was stored with and without the solvent dichloromethane. Analysis of the sample preserved with dichloromethane resulted in the detection of 3 ng/L esfenvalerate, and esfenvalerate was below detection limits in the sample stored without dichloromethane.

Effects of ammonia on H. azteca *survival and growth*

Amphipod growth was negatively correlated with total ammonia/ammonium ($p = 0.0021$) as well as un-ionized ammonia ($p \leq 0.001$). No significant correlation overall with 10-d survival (Supplemental Data, Table S7) was found. Analysis of data on a site-by-site basis showed, however, that at Light 55, survival was negatively correlated with total ammonia/ammonium ($p = 0.034$) and un-ionized ammonia ($p = 0.045$), and growth was negatively correlated with un-ionized ammonia ($p = 0.025$). At site 812, growth was negatively correlated with both total ammonia/ammonium and un-ionized ammonia ($p = 0.005$ and 0.009, respectively). Survival was positively correlated with total ammonia/ammonium and un-ionized ammonia at sites 504 ($p = 0.005$ and ≤ 0.001), 609 ($p = 0.001$ and 0.003), and 804 ($p = 0.011$ and 0.014).

Quality assurance/quality control

All tests conducted during the 2006 to 2007 testing period met test acceptability criteria. Monthly survival LC50 data obtained in reference toxicant tests consistently fell within the range specified by the U.S. EPA. Growth EC25 values were outside the 95% confidence interval in February and June 2007, with animals being less sensitive to NaCl than normal. Although the average EC25 was 4,395 μS/cm, the EC25 for February and June 2007 were 10,950 and 11,200 μS/cm, respectively. All survival and growth data obtained for field duplicates were in agreement with the respective ambient samples. No significant effects on survival were seen in either trip or bottle blanks, but final dry weight per organism was significantly lower than that of controls in tests initiated on April 19, 2006 (trip blank, 75% of control), July 28, 2006 (bottle blank, 63% of control), and February 15, 2007 (bottle blank, 76% of control). This did not affect results reported here,

because final dry weight was only compared between ambient and PBO-spiked ambient samples.

DISCUSSION

The results of this study raise concerns with regard to potential direct contaminant impacts on sensitive invertebrate species, as well as indirect or sublethal, direct effects on several fish species whose resident populations are in decline. Water in the upper Sacramento–San Joaquin Estuary was shown to be acutely toxic to *H. azteca* in 5.6% of samples tested. All samples were collected from large delta channels and main-stem rivers. Overall, this is relatively consistent with results of a 1993 to 1995 monitoring study [9], which detected toxicity to the waterflea *Ceriodaphnia dubia* in 6.9% of samples from main-stem rivers, and 4.1% of samples from main delta channels. The same study showed that ecologically important back sloughs and small upland drainages were far more frequently toxic (14.1–19.6%) than larger water bodies. Although small water bodies were not monitored in the study presented here, other studies conducted in 2005 to 2008 ([22]; http://www.waterboards.ca.gov/centralvalley/programs/ irrigated_lands/index.html) found widespread acute water column toxicity in sloughs, creeks, and agricultural drains of the Central, South, and North Delta.

Acute toxicity to *H. azteca* was most frequently detected during winter and early spring of 2007, a year with relatively little precipitation, in the lower Sacramento River near Hood and Rio Vista, and the nearby Deep Water Shipping Channel. This is the time of year when endangered delta smelt are spawning and rearing in this area, especially during years with low river flows [23]. Small zooplankton, most importantly copepod species, are their main food source [24]. In addition to acute toxicity observed in this area, amphipod growth was lowest overall in water samples from the lower Sacramento River. A significant negative correlation with ammonia/ammonium concentrations was found. Maximum ambient ammonia/ammonium concentrations measured at Hood and site 711 were 0.51 and 0.54 mg/L, respectively. These concentrations are well below known acute effect levels for *H. azteca* (10-d LC50: 72.9 mg/L total ammonia/ammonium; I. Werner, unpublished data); thus, unknown contaminants, whose concentrations co-varied with those of ammonia/ammonium, or mixture effects are more likely to be responsible for the observed effects. Indirect effects linked to food availability also could be a causative factor for reduced amphipod growth. Elevated ammonium concentrations (>0.072 mg/L) are associated with decreased chlorophyll-α production [25].

The largest known source of contaminants in the lower Sacramento River is effluent released from the area's largest municipal wastewater treatment plant, the Sacramento Regional Wastewater Treatment Plant. The Sacramento Regional Wastewater Treatment Plant currently discharges on average 536×10^6 L/d treated wastewater approximately 15 km upstream of our Hood sampling site. Six of eight samples collected from this site during fall and early winter of 2007 were acutely toxic to *H. azteca*, by far the highest percentage among our study sites. Effluents from municipal wastewater treatment plants with secondary treatment technology, such as Sacramento Regional Wastewater Treatment Plant, are significant sources of ammonia/ammonium [26], pyrethroid pesticides [3], and a large number of other chemicals ranging from flame retardants, pesticides, plasticizers, and water repellents to fragrances, pharmaceuticals, and personal care product ingredients [7,27].

In addition, winter storm runoff and irrigation return water from agricultural and urban areas, containing fertilizer, pesticides, and other chemicals, are known to be important sources of contaminantion and toxicity in the Delta [2,9]. Studies performed in the Delta and its tributaries from the mid-1990s until 2008 demonstrated that insecticides, as well as herbicides and cationic metals, were frequently present in the water column at concentrations acutely toxic to sensitive invertebrates and phytoplankton [2,9,22]. Pyrethroids at concentrations toxic to aquatic life were detected in water samples from Central Valley agricultural drains and creeks [28] and tributaries to San Francisco Bay [29]. In the current study, the OP diazinon was detected in only one water sample at very low concentration, but pyrethroids, specifically cyfluthrin, permethrin, lambda-cyhalothrin and esfenvalerate, were detected at potentially toxic concentrations in water samples collected from the Old River, the mouth of the Napa River, and the Carquinez Strait near Benicia.

Although data on the sensitivity of many resident invertebrate species are still lacking, the available information indicates that amphipods are among the most sensitive aquatic species with respect to pesticides and metals ([30]; http://www.pesticideinfo.org/), two contaminant groups most commonly associated with agricultural and urban runoff. For example, a review [31] of 18 microcosm and mesocosm studies with eight pyrethroids concluded that Amphipoda and Hydacarina were the most sensitive taxa, followed by Trichoptera, Copepoda, Ephemeroptera, and Hemiptera. Monitoring results obtained with *H. azteca* as test organisms are therefore likely to provide data on contaminant impact that are representative and protective of other estuarine crustaceans. However, extrapolating the results of acute toxicity tests to the field is difficult, and data presented here should primarily be used to direct and focus future research on the role of contaminants in the decline of multiple pelagic fish species.

Pyrethroid concentrations detected in this study most likely underestimate those present in the field (see earlier discussion). Measurement of these hydrophobic and relatively short-lived chemicals in water can present considerable challenges with respect to detection limits, adsorption to sampling equipment, and degradation during sample storage [32]. Analytical detection limits (1–3 ng/L in the current study) are in the range of biological effect concentrations for *H. azteca* [33]. This may explain the large number of analytical nondetections in samples that showed negative PBO effects on amphipod growth, especially because PBO enhances pyrethroid toxicity to *H. azteca* by a factor of approximately 2 to 4 [19,34]. This problem can be exacerbated when multiple pyrethroids, whose toxic effects are nearly additive [34], are present at concentrations below detection limits, or when mixtures of contaminants lead to synergistic effects [35].

Addition of the enzyme inhibitor PBO to ambient samples was intended to enhance our ability to detect the presence of bioavailable OP or pyrethroid insecticides in the water [19,20] and to address concerns over the potential loss of toxicity during sampling and testing [32]. Significant differences in amphipod survival were seen in just four samples from sites near the mouth of the Napa River (323, 340) and the lower Sacramento River (711, Hood), and chemical analysis confirmed the presence of two pyrethroids, cyfluthrin and esfenvalerate, in one sample (340). Concentrations detected were high enough to account for the observed effects on survival [33]. Although interpretation of these results remains difficult for the reasons outlined earlier, PBO effects on *H. azteca* survival are considered a reliable TIE tool [19]. Evaluating our results on growth

differences between ambient and PBO-treated ambient water samples is more difficult. In-house studies confirmed that addition of 25 μg/L PBO reduced the *H. azteca* growth EC25 of the pyrethroid bifenthrin threefold (from 2.18 to 0.77 ng/L), and increased the EC25 of the OP diazinon fourfold (from 1.27 to 4.7 μg/L; I. Werner, unpublished data), but more studies are needed. Our data on PBO growth effects should, at this point, be considered a single line of evidence rather than conclusive proof of the presence or absence of the two insecticide groups. In most (70%) of those ambient samples in which growth effects were detected, addition of PBO led to a reduction in amphipod growth. Pyrethroid insecticides were detected in four samples in which PBO increased toxicity; however, the presence of OP insecticides in samples in which PBO decreased toxicity was not confirmed. Despite the difficulty in confirming the PBO signal with analytical data, it appears that these effects are seen more frequently at certain sampling sites, indicating that they are not random. Piperonyl butoxide growth effects were seen most frequently at sites located in the Old River and San Joaquin River (902, 910, 915) and in the Sacramento River (711; Table 4); however, pyrethroids were detected in only two of these samples from the Old River (902, 915; Table 5). Pyrethroid concentrations detected in three samples, two from Napa River, and one from Old River, were above known lethal thresholds for *H. azteca* [34]; I. Werner, unpublished data, but amphipod survival was not significantly reduced in those samples. Phytoplankton or suspended sediment particles, and the relatively high test temperature ($23 \pm 1^{\circ}C$) likely reduced the chemicals' bioavailability [36] and toxicity [37].

Indirect and sublethal effects of contaminants may have severe effects on fish populations and aquatic communities, but measuring and evaluating these effects is a complex challenge. Like in many other estuaries, contaminant sources in the Sacramento–San Joaquin Estuary are diverse, ranging from industrial and urban point sources to nonpoint sources such as stormwater runoff and irrigation return water. Most of these contain mixtures of multiple chemicals whose combined toxic effects on aquatic species are still poorly understood [35,38]. Although the current study focused our attention on several specific contaminants, pyrethroid insecticides and ammonia/ammonium or associated contaminants, their measured concentrations are in a range in which direct toxicity to fish would not be expected [21,33]. However, ecological fitness may be impaired indirectly by the depletion of invertebrate prey, and directly by sublethal toxic effects on the reproductive system, behavior, or immune responses. In the wild, such changes can directly translate into increased vulnerability to predation or decreased food intake with possible consequences for growth and fecundity. For example, impairment of the olfactory function in salmon has been demonstrated after exposure to environmentally relevant concentrations of diazinon, an OP insecticide, and copper [39,40]. Copper concentrations of 2 μg/L, a concentration commonly detected in the Sacramento–San Joaquin Delta (I. Werner, unpublished data), completely eliminated the avoidance response of juvenile salmon to a predator cue. Male fathead minnows were less competitive in defending their nest and securing reproductive success after exposure to effluent from a municipal sewage plant [12], and pesticides, metals, in particular copper, and PCBs are among those contaminants identified to cause immunosuppressive effects in fish [33,41]. After considering such direct, indirect, and cumulative effects of chlorpyrifos, diazinon, and malathion on salmonid species, the U.S. National Oceanic Atmospheric Administration, National Marine Fisheries Services ([42]; http://www.nmfs.noaa.gov/pr/pdfs/pesticide_biop.pdf) concluded that reregistration of pesticide products containing these OP insecticides was likely to jeopardize the continued existence of 27 listed Pacific species. Furthermore, after a review of 216 studies on the effects of contaminants on diversity in coastal marine communities, no single contaminant was found to have greater impact than any other [43]. The authors concluded that anthropogenic contamination was consistently associated with reduced biodiversity, likely affecting the resilience of communities to other stresses.

Summary and conclusions

The results of this study demonstrated that, during 2006 to 2007, water in the Sacramento–San Joaquin Estuary was at times acutely toxic to the amphipod *H. azteca*. Areas most impacted were the Lower Sacramento River and the neighboring Deep Water Shipping Channel, followed by Carquinez Strait near Benicia and Suisun Bay. In general, the level of toxicity observed was not severe, with 10-d survival above 60% in most water samples. Amphipod growth was negatively correlated with total ammonia/ammonium and un-ionized ammonia. However, known ammonia effect concentrations for *H. azteca* far exceed those detected in ambient samples, and it is suggested that toxicants associated with ammonia/ammonium may have caused or contributed, directly or indirectly, to the observed effects. Pyrethroid insecticides were detected at potentially toxic concentrations. Mixture effects are likely, and detected concentrations may underestimate risk.

This study provided valuable information on the geographic distribution and potential causes of toxicity in the Northern SSJ Estuary. Numerous questions remain, however, regarding the sublethal, direct and indirect, impacts of toxicants on fish species whose resident populations are in decline. Toxicity detected in this study probably underestimates the extent of water column toxicity present in ecologically important back sloughs and small upland drainages. Recognizing that traditional bioassays cannot detect the chronic, sublethal effects of modern contaminants, for example, endocrine disrupting chemicals, immune suppressants, and others, whose effects can have far-reaching deleterious consequences for fish and invertebrate populations in the wild, is also important. Approaches involving sensitive and mechanism-based biomarkers of toxic effects therefore should be integrated into future monitoring programs to assess organism health and identify sublethal contaminant effects in fish species of concern.

SUPPLEMENTAL DATA

Fig. S1.
Tables S1–S7. (992 KB DOC).

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