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Invited paper

Identifying primary stressors impacting macroinvertebrates in the Salinas River (California, USA): Relative effects of pesticides and suspended particles

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Pesticides are the primary stressor impacting macroinvertebrates in sections of the lower Salinas River.

Abstract

Laboratory dose–response experiments with organophosphate and pyrethroid pesticides, and dose–response experiments with increasing particle loads were used to determine which of these stressors were likely responsible for the toxicity and macroinvertebrate impacts previously observed in the Salinas River. Experiments were conducted with the amphipod *Hyalella azteca*, the baetid mayfly *Procloeon* sp., and the midge *Chironomus dilutus* (Shobanov, formerly *Chironomus tentans*). The results indicate the primary stressor impacting *H. azteca* was pesticides, including chlorpyrifos and permethrin. The mayfly *Procloeon* sp. was sensitive to chlorpyrifos and permethrin within the range of concentrations of these pesticides measured in the river. *Chironomus dilutus* were sensitive to chlorpyrifos within the ranges of concentrations measured in the river. None of the species tested were affected by turbidity as high as 1000 NTUs. The current study shows that pesticides are more important acute stressors of macroinvertebrates than suspended sediments in the Salinas River.

Keywords: Pesticides; Macroinvertebrates; Toxicity; Suspended particles

1. Introduction

The Salinas River is the largest of the three coastal rivers flowing into the Monterey Bay National Marine Sanctuary in central California. Large areas in this watershed are cultivated year-round, primarily in row crops such as lettuce, strawberries, artichokes, and crucifer crops. Studies have shown that ambient water samples from the river and specific tributaries are toxic to standard test species in laboratory tests (Hunt et al., 2003; Anderson et al., 2003a,b). Using laboratory and in situ toxicity tests, chemical analyses and toxicity identification evaluations (TIEs), we recently showed that organophosphate pesticides associated with agriculture drainwater were responsible for sediment and water column toxicity in samples collected in the Salinas River downstream of one of these tributaries (Anderson et al., 2003b; Phillips et al., 2004). TIEs suggested that pyrethroid pesticides might also be partially responsible for sediment toxicity to the amphipod *Hyalella azteca*, but these results were inconclusive due to a lack of pyrethroid analyses in river samples and limited dose–response information for selected pyrethroids using *Hyalella* (Anderson et al., 2003b; Phillips et al., 2004). Our previous study also demonstrated drainwater impacts on a number of macroinvertebrate community metrics. There were significant negative correlations between the number of Ephemeroptera taxa, taxonomic

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richness and the percentage Chironomidae and concentrations of diazinon and chlorpyrifos in the river. We also found significant negative correlations between these metrics and suspended particles (measured as turbidity) in the river. Thus, our results indicated that resident species might be impacted by co-occurring stressors (i.e., multiple pesticides, and possibly, suspended particles) associated with agriculture drainwater (Anderson et al., 2003b). Our ability to resolve the relative influences of these stressors on macroinvertebrates was constrained by a lack of dose–response information for pesticides and suspended particle effects on key resident species.

The current study provides dose–response information for the organophosphate pesticides diazinon and chlorpyrifos, and for suspended particles, using resident and surrogate macroinvertebrates. We also provide dose–response data for the pyrethroid pesticides bifenthrin and permethrin. In terms of pounds per acre applied, diazinon, chlorpyrifos, and permethrin comprise the most commonly applied pesticides in the Salinas Valley. Bifenthrin is also among the more commonly used pyrethroid pesticides in the Salinas Valley. Experiments were conducted using three species: the amphipod *H. azteca*, the baetid mayfly *Procloeon* sp., and the midge *Chironomus dilutus*. These species were from genera or families that were shown to be impacted by agriculture drainwater in previous studies, but for which pesticide sensitivities or sensitivities to suspended particles are unknown. We compared LC50s from these experiments to the ranges and mean concentrations of the pesticides and suspended particles previously measured in the Salinas River. Current results were combined with previous results in a weight-of-evidence to determine which are the most important stressors impacting macroinvertebrates in this system.

2. Materials and method

The pyrethroid pesticides bifenthrin and permethrin were tested with *H. azteca*, *C. dilutus*, and *Procloeon* sp., and the organophosphate pesticides diazinon and chlorpyrifos were tested with *Procloeon* sp. Dose–response data from the literature were used to determine the sensitivities of *H. azteca* and *C. dilutus* to chlorpyrifos and diazinon. Stock solutions of permethrin and bifenthrin (100 mg/L in methanol) were obtained from Accustandard (New Haven, CT, 100% purity). These were used to prepare nominal concentrations of 0, 500, 1000, 5000, 10,000, and 20,000 ng/L for tests with *C. dilutus*. Secondary stocks (100 µg/L in methanol) were prepared from the primary stocks to give final nominal concentrations of 0, 5.6, 10, 18, 32, and 56 ng/L for tests with *Hyalella* and 18, 32, 56, 100, 180, 320, and 560 ng/L for tests with *Procloeon*. Stock solutions of 250 mg/L chlorpyrifos and diazinon in methanol were prepared from pesticides obtained from ChemService (Westchester, PA, 99% purity). Secondary (2000 and 500 µg/L diazinon and chlorpyrifos, respectively) and tertiary (20 and 5 µg/L diazinon and chlorpyrifos, respectively) stocks were prepared from these to give final test concentrations of 0.5, 1.0, 2.5, and 5.0 µg/L for diazinon, and 0.0625, 0.125, 0.25, and 0.50 µg/L for chlorpyrifos. Responses of all animals to all pesticides were compared to both well water and methanol controls (1% methanol in well water).

2.1. *H. azteca* and *C. dilutus* 96-h survival tests

Water-only dose–response experiments with *H. azteca* (provided by Aquatic Biosystems, Fort Collins, CO) and *C. dilutus* (provided by Chesapeake Culture, Hayes, VA) were 96-h static exposures conducted in 20 ml glass vials containing 15 ml solution. *H. azteca* treatments were replicated three times, with each replicate containing five animals (7–14 days old), and *C. dilutus* treatments were replicated 10 times with one organism per replicate (3rd instar). Survival was monitored daily. Dissolved oxygen (mg/L), pH, temperature (°C) were measured at the beginning and end of all experiments using meters and probes. These instruments were calibrated in the laboratory as per manufacturer's recommendations. Alkalinity (total as $CaCO_3$) and hardness (calcium as $CaCO_3$) were measured in well water controls. The test temperature was 23 °C. Containers were not aerated.

2.2. *Procloeon* sp. 48-h toxicity tests

Mayfly larvae were collected from a reference station in the Salinas River in May 2000 for diazinon and chlorpyrifos tests, and again in March 2004 for bifenthrin and permethrin tests. This reference station has been used in our previous studies, is 11 km upstream of our current study area, and has been demonstrated to have uniformly low pesticide concentrations in sediment and water (Hunt et al., 2003; Phillips et al., 2004). *Procloeon* sp. taxonomy was verified by a qualified taxonomist. Larvae were transported to the lab in river water then transferred to 20 L plastic trays where they were held until testing. Culture tray water was renewed daily with well water, but mayflies were not fed. Minor larval mortality was observed during the holding period. Testing commenced within 4 days of the collection date. Larvae of unknown age were used in these experiments. The approximate range of lengths of *Proloeon* sp. larvae tested was 0.5–1 cm (head to base of cerci). Water-only dose–response experiments with the organophosphate pesticides were 48 h exposures, renewed at 24 h. Tests with pyrethroid pesticides were 48 h static exposures. Tests were conducted in 200 ml glass bowls containing 100 ml test solution. Each concentration was replicated 3–5 times, with each replicate containing five animals. Survival was monitored daily. Water quality and temperature were measured as described above. The animals were not fed and the containers were not aerated.

2.3. Suspended sediment experiments

Experiments to assess the effects of suspended sediments associated with agriculture drainwater were conducted using soil from a certified organic farm. Approximately 400 L of topsoil was obtained from Blue Heron Farms in Watsonville, California. This farm has been a certified organic operation since 1974 (personal communication Tim Voss, Blue Heron Farms); no pesticides have been applied to soil on this farm for 30 years. The soil was sun dried and dry sieved to 500 µm. The soil was then suspended in well water and sequentially wet-sieved to a final sieve size of 64 µm. For these experiments, the wet-sieved soil was dissolved in well water in four 114 L plastic bins to approximate four nominal suspended sediment concentrations that spanned the range reported in Anderson et al. (2003b). Nominal suspended sediment concentrations, measured as turbidity, were 0, 250, 500 and 1000 NTUs. Sediments were kept in suspension by vigorously aerating each bin as their solutions were continuously pumped with a submersible pump (pump rate = 60 L per min) to each of four 13 L head tanks that were also heavily aerated to keep the particles in suspension. Turbid water from the head tanks flowed by gravity to a manifold fitted with five valves, each supplying water to one of five replicate exposure chambers.

Exposure chambers were 1.7 L polyethylene plastic food dishes fitted on both ends with 2.5 cm diameter 500 µm mesh screens. Each of the replicates contained 10 animals (amphipods, chironomids, or mayflies). The water flowed through the exposure containers and into a catch basin that returned it to the bin supplying that suspended sediment treatment. Approximately 300 ml of medium fine-grained sand was added to the exposure chambers for the *Procloeon* and *C. dilutus* experiments to provide substrate for these species. No substrate was used in the *Hyalella* experiments. The control water for these experiments was well water and controls for the *Procloeon* and *C. dilutus* exposure contained a sand substrate as described above. Turbidity was monitored at the beginning and end of each day. To account for settlement of suspended sediment during the course of the experiments, additional suspended sediment was added to each treatment after turbidity measurements to return the concentration to the target nominal concentration. Turbidity was monitored with a model 2100 turbidimeter (Hach, Loveland, CO). Total suspended solids were measured during one exposure in each nominal turbidity treatment for comparison using EPA procedures (US EPA, 1983). Suspended sediment exposures were conducted for 96 h, at which time the contents of the chambers were sieved through a 500 μm mesh screen and survival was recorded.

2.4. Chemical analyses

Selected concentrations of 1 L samples of bifenthrin and permethrin were measured for comparison to nominal concentrations. Samples for pyrethroid analyses were prepared by mixing ten 100-ml samples (1 L total), each prepared from respective stock solutions of bifenthrin and permethrin. Each sample was mixed at the time of test initiation in 100 ml Erlenmeyer flasks following the same procedures used for filling the exposure chambers. Pyrethroid pesticides were measured using Gas Chromatography–Mass Spectroscopy (GC–MS; U.S. EPA method 1660) ECD-MS detector following methods developed by the California Department of Fish and Game Water Pollution Control Laboratory (reporting limit bifenthrin and permethrin = 0.01 μg/L and 0.02 μg/L, respectively). Chlorpyrifos and diazinon were measured using two methods, Enzyme Linked Immunosorbent Assays (ELISAs – analyses described below), and GC–MS (U.S. EPA methods 8140 and 8141A). Standard quality assurance procedures including measurement of standard reference materials and quantification of surrogate recoveries and matrix spikes were used in all analyses. All chemical analyses met prescribed quality assurance guidelines.

2.5. ELISA tests

All chlorpyrifos and diazinon concentrations were measured using Enzyme Linked Immunosorbent Assays (ELISAs) following procedures recommended by Sullivan and Goh (2000). ELISA readings were compared to a five-point standard curve, using standards provided by the manufacturer. After analysis of a group of samples, accuracy of the ELISA method was determined by measuring an external chlorpyrifos or diazinon standard. All standard measurements were within ±20% of nominal. Precision of the ELISA method was determined with duplicate measures of one sample by calculating the coefficient of variation. CVs were always less than 20. The lowest detectable dose was 30 ng/L for diazinon and 50 ng/L for chlorpyrifos.

2.6. Data analyses

Median effect concentrations (LC50s) were calculated from mean measured organophosphate concentrations and nominal pyrethroid concentrations using (ToxCalc™ Statistical Software, Tidepool Software, McKinleyville, CA), using the trimmed Spearman–Karber method (Hamilton et al., 1977). The dose–response information resulting from the pesticide and suspended particle exposures was compared to data from our Salinas River study to determine whether effect thresholds were within the range of pesticide or suspended sediment concentrations measured in the river. For this comparison, we used the mean and range of diazinon and chlorpyrifos concentrations measured at the station with the highest pesticide concentrations in our previous study. These concentrations were measured at Station #2, which was located at the confluence of the agricultural drainage creek and the Salinas River (Anderson et al., 2003b). We also compared the turbidities from Station #2 in that study to those in the current dose–response experiments. Pyrethroid pesticides were not measured in our previous study, so to investigate the likelihood of exposure to these pesticides, we relied on pyrethroid concentrations reported as part of a California Department of Pesticide Regulation study (Kelley and Starner, 2004). These authors reported concentrations of pyrethroids in water and sediments sampled weekly over 16 weeks from June through September, 2003 from a station in the agriculture drainage creek 0.5 km upstream of our Station #2 from Anderson et al. (2003b). The mean and range of these measures were used to estimate the mean and range of concentrations of permethrin in the Salinas River at Station #2 (these authors did not detect any bifenthrin in their study).

3. Results

3.1. Chemical confirmation

Measured concentrations of bifenthrin and permethrin were considerably lower than nominal concentrations in all cases. For bifenthrin experiments with *H. azteca*, the bifenthrin recovery ranged from 19% to 56%, and average measured concentrations of bifenthrin were 37% of nominal concentrations. For bifenthrin experiments with *Procoleon* sp., recovery ranged from 55% to 77%, and average measured concentrations of bifenthrin were 65% of nominal concentrations. For bifenthrin experiments with *C. dilutus*, recovery ranged from 36% to 65%, and average measured concentrations of bifenthrin were 54% of nominal concentrations. For permethrin experiments with *H. azteca*, recovery ranged from 0% to 61%, and average measured concentrations of permethrin were 54% of nominal concentrations (Table 1). For permethrin experiments with *Procoleon* sp., recovery ranged from 32% to 61%, and average measured concentrations of permethrin were 43% of nominal concentrations (Table 1). For permethrin experiments with *C. dilutus*, recovery ranged from 61% to 75%, and average measured concentrations of permethrin were 68% of nominal concentrations (Table 1). Percent recoveries of laboratory control spikes of bifenthrin and permethrin in water during chemical analyses exceeded quality assurance

Table 1
Nominal and measured concentrations (ng/L) of diazinon, chlorpyrifos, permethrin and bifenthrin in water-only dose–response experiments with *Procloeon* sp., *H. azteca*, and *C. dilutus*

Procloeon expts.					
Diazinon nominal conc.	Mean ELISA conc.	% of nominal	Chlorpyrifos nominal conc.	Mean ELISA conc.	% of nominal
0 – Control	0	0	0 – Control	0	0
0 – Methanol	0	0	0 – Methanol	0	0
500	590	118	63	57	91
1000	1030	103	125	99	79
2500	3180	127	250	251	100
5000	5270	105	500	592	118
Bifenthrin nominal	Bifenthrin measured	% of nominal	Permethrin nominal	Permethrin measured	% of nominal
56, 56	31, 43	55, 77	56, 56	18, 23	32, 41
320, 320	206, 202	64, 63	180	110	61
			320	127	40
Hyalella expts.					
Bifenthrin nominal	Bifenthrin measured	% of nominal	Permethrin nominal	Permethrin measured	% of nominal
10, 10	2, 5	22, 50	18, 18	ND, 11	0, 61
32, 32	6, 18	19, 56	32	16	50
			56	29	52
Chironomus expts.					
Bifenthrin nominal	Bifenthrin measured	% of nominal	Permethrin nominal	Permethrin measured	% of nominal
560, 560	200, 364	36, 65	1000, 1000	752, 654	75, 65
1800, 1800	964, 1110	54, 62	3200, 3200	1960, 2250	61, 70

thresholds. The range of percent recoveries of bifenthrin was 89–116% (mean percent recovery = 102%). The range of percent recoveries of permethrin was 69–115% (mean percent recovery = 89.5%).

Enzyme Linked Immunosorbent Assays (ELISAs) of diazinon and chlorpyrifos in the *Procloeon* sp. experiments showed that nominal and measured concentrations were comparable (Table 1). Measured concentrations of diazinon in the experiments with *Procloeon* sp. ranged from 118% to 127% of the nominal concentrations. Measured concentrations of chlorpyrifos in the experiments with *Procloeon* sp. ranged from 79% to 118% of the nominal concentrations (Table 1).

The LC50s for all diazinon and chlorpyrifos tests with *C. dilutus* are based on measured concentrations. The LC50s for all pyrethroid pesticides used in these experiments are presented as nominal concentrations. For the experiments with diazinon and chlorpyrifos we have shown that the nominal concentrations were reasonable approximations of the measured concentrations. The actual LC50s and LOECs for bifenthrin and permethrin for *Hyalella*, *Procloeon*, and *C. dilutus* are probably considerably lower than those reported here. Based on the fact that recoveries of spiked pyrethroids measured in the laboratory analyses were acceptable, it is likely that lower recoveries in samples collected at the initiation of the tests were the result of loss of pyrethroids to the sides of the Erlenmeyer mixing flasks (Wheelock et al., 2005).

3.2. Pesticide dose–response experiments

The mayfly *Procloeon* was sensitive to chlorpyrifos within the range of chlorpyrifos concentrations measured in the Salinas River in our previous study. The mean LC50 for chlorpyrifos toxicity to *Procloeon* was 81 ng/L. The mean concentration of chlorpyrifos measured at the most contaminated station in the Salinas River in our previous study was 183 ng/L (Anderson et al., 2003b). The range of chlorpyrifos concentrations previously measured in the river was 48–515 ng/L (Table 2). *Procloeon* was less sensitive to diazinon. The mean LC50 for diazinon was 1930 ng/L, which is greater than the concentrations of diazinon we previously measured in the river. The mean concentration of diazinon measured in the Salinas River in our previous study was 460 ng/L, and the range of concentrations measured was 190–790 ng/L.

Procloeon sp. were relatively sensitive to the pyrethroid pesticides bifenthrin and permethrin. The mean LC50s for bifenthrin and permethrin toxicity to *Procloeon* sp. were 84.3 and 89.6 ng/L, respectively (Table 2). The permethrin LC50s were within the mean and range of permethrin concentrations in water in this system reported by Kelley and Starner (2004). The mean and range of permethrin concentration reported by these authors were 104.8, and 71.2–162 ng/L, respectively (Table 2). These authors did not detect any bifenthrin in their study.

H. azteca was more sensitive than *Procloeon* sp. to both bifenthrin and permethrin. The mean LC50s for bifenthrin and permethrin toxicity to *H. azteca* were 9.3 and 21.1 ng/L, respectively (Table 3). The permethrin LC50 was also within the mean and range of permethrin concentrations in water in this system reported by Kelley and Starner (2004).

The midge *C. dilutus* was relatively insensitive to bifenthrin and permethrin. The mean LC50s for bifenthrin and permethrin toxicity to *C. dilutus* were 26,150 and 10,450 ng/L, respectively (Table 4). The permethrin LC50 was well above the mean (104.8 ng/L) and range (71.2–162 ng/L) of permethrin concentrations in this system reported by Kelley and Starner (2004).

Published dose–response data for diazinon and chlorpyrifos toxicity to *C. dilutus* were used to assess risk of these pesticides to chironomids in the Salinas River. The LC50 for chlorpyrifos toxicity to *Chironomus tentans* (*C. dilutus*) reported by Phipps et al. (1995) was 70 ng/L, lower than the range of chlorpyrifos concentrations we measured in our study (48–515 ng/L; Table 4). The 96-h LC50 for diazinon toxicity to *C. tentans* (*C. dilutus*) reported by Belden and Lydy (2000) was 30,000 ng/L, much higher than the range of diazinon concentrations we measured in our previous study (190–790 ng/L; Table 4).

3.3. Suspended particle experiments

There were no apparent effects of suspended particles on mayflies, amphipods, or midges in our experiments. Mean survival of *Procloeon* at the highest turbidity tested, 1000 NTUs (nominal), was 86.7%, while mean survival in the controls was 73.3% (Table 5). Mean survival of *Hyalella* at 1000 NTUs was 98%, while mean control survival was 90.3%. Mean survival of *C. dilutus* at 1000 NTUs was 94.3%, while mean control survival was 94.7%. Survival of all species tested at all lower turbidities was equal to or greater than their survival in the controls. Turbidities measured throughout the experiments

Table 2
Sensitivity of the baetid mayfly *Procloeon* sp. to selected pesticides relative to concentrations measured in the Salinas River in ng/L

	LC50	Mean concentration measured (river)[a]	Range concentration measured (river)[a]
Permethrin	89.6	104.8	71.2–162
Bifenthrin	84.3	ND	ND
Diazinon	1940	460	190–790
Chlorpyrifos	81	180	50–520

[a] Concentrations measured at the most impacted station, see text for explanation.

Table 3
Sensitivity of the amphipod *Hyalella azteca* to selected pesticides relative to concentrations measured in the Salinas River in ng/L

	LC50	Mean concentration measured (river)[a]	Range concentration measured (river)[a]
Permethrin	21.1	104.8	71.2–162
Bifenthrin	9.3	ND	ND
Diazinon	6500[b]	460	190–790
Chlorpyrifos	86[b]	180	50–520

[a] Concentrations measured at the most impacted station, see text for explanation.
[b] From Phipps et al. (1995).

Table 4
Sensitivity of the midge *Chironomus dilutus* to selected pesticides relative to concentrations measured in the Salinas River in ng/L

	LC50	Mean concentration measured (river)[a]	Range concentration measured (river)[a]
Permethrin	10,450	104.8	71.2–162
Bifenthrin	26,150	ND	ND
Diazinon	30,000[b]	460	190–790
Chlorpyrifos	70[c]	180	50–520

[a] Concentrations measured at the most impacted station, see text for explanation.
[b] From Belden and Lydy (2000).
[c] From Phipps et al. (1995).

were somewhat lower than the nominal values, due to settlement of particles throughout the night (Table 5). For example, measured turbidities in the 1000 NTU treatment ranged between 755.9 and 909.8. The mean measured turbidities in tests with *Procloeon* sp., *H. azteca* and *C. dilutus* in the 1000 NTU treatment were 840, 836 and 824 NTUs, respectively. Despite the variability of suspended sediment in these experiments, the range of turbidities was comparable to the range we previously measured in the Salinas River. The mean turbidity measured in the river in the previous study was 521.4 NTUs, and the range was 163.1–955.1 NTUs (Anderson et al., 2003b). Mean total suspended solid (TSS) concentrations corresponded to the measured turbidities in these experiments. The mean TSS measured in the 250 NTU treatment was 297.7 mg/L. The mean TSS in the 500 NTU treatment was 483 mg/L, and the mean TSS in the 1000 NTU treatment was 848.7 mg/L (Table 5).

4. Discussion

Our approach to identifying the primary stressors impacting macroinvertebrate communities in the Salinas River followed an iterative process involving an initial toxicity assessment that described water column toxicity in selected agriculture drainage creeks (Hunt et al., 2003). This was followed by more detailed studies that demonstrated effects in the river downstream of two agriculture drains (Anderson et al., 2003a,b; Phillips et al., 2004). In the latter studies, we found that *Ceriodaphnia* survival in toxicity tests and macroinvertebrate densities were negatively correlated with the organophosphate pesticides diazinon and chlorpyrifos, and with suspended sediment concentrations (as turbidity). When combined with previous measures of pesticide concentrations, TIEs, and dose–response information from the literature, results from the current study help resolve the relative contributions of these stressors in this system.

Our previous data showed significant toxicity to the amphipod *H. azteca* at stations downstream of an agriculture drain where high concentrations of organophosphate pesticides were measured, and where amphipod field densities were impacted. Toxicity identification evaluations showed that sediment toxicity was due to mixtures of the organophosphate pesticide chlorpyrifos, and some other non-metabolically activated pesticide (Anderson et al., 2003b; Phillips et al., 2004). TIE evidence in these studies included increased mortality of amphipods with the addition of the metabolic inhibitor piperonyl butoxide, which may suggest toxicity due to pyrethroid pesticides (Kakko et al., 2000). Conclusive evidence of pyrethroid toxicity was constrained by a lack of dose–response information with *H. azteca* for selected pyrethroids, and absence of pyrethroid measurements in field sediments in our study area (Anderson et al., 2003b; Phillips et al., 2004). In the present study, we found that *H. azteca* is sensitive to the pyrethroid pesticide permethrin within the range of permethrin concentrations measured in this system. The mean LC50 for permethrin toxicity to *H. azteca* in water is 21.1 ng/L (Table 3). California Department of Pesticide Regulation recently reported mean water concentrations of permethrin in this system in 16 weekly samples of 104.8 ng/L (Kelley and Starner, 2004). Permethrin concentrations were measured in the agriculture drainage creek approximately 0.5 km upstream of Salinas River Station #2, the station where our previous TIE work was conducted. While we found strong negative correlations between turbidity and macroinvertebrate densities (Anderson et al., 2003b), our current data show that *H. azteca* tolerate concentrations of suspended particles as high as those measured in the river (1000 NTUs; Table 5). When data from these studies are considered as a weight-of-evidence, they confirm that one of the primary stressors impacting *H. azteca* in our study area on the Salinas River was the organophosphate pesticide chlorpyrifos, and indicate that the pyrethroid pesticide permethrin is also a likely important stressor to amphipods in this system.

Our results suggest that chlorpyrifos and permethrin also impact the baetid mayfly *Procloeon* sp. in this system. *Procloeon* sp. were sensitive to chlorpyrifos within the mean

Table 5
Effects of suspended particles on survival of *Procloeon* sp., *H. azteca*, and *C. dilutus* in 96-h experiments (results from three experiments for each species)

Nominal turbidity (NTUs)	Average measured turbidity (NTUs)	Mean TSS (mg/L)	Mean *Procloeon* survival (%)	Mean *Hyalella* survival (%)	Mean *Chironomus* survival (%)
0	1, 4, 3		73.3	90.3	94.7
250	195, 191, 188	298	73.3	92.7	95.3
500	419, 405, 409	483	74.3	92.7	96.3
1000	824, 836, 840	849	86.7	98.0	94.3
Mean concentration measured in the river[a]	521				
Range concentrations measured in the river[a]	163–955				

TSS = total suspended solids.
[a] Concentrations measured at the most impacted station, see text for explanation.

and range of concentrations measured at Station #2 (Table 2). *Procloeon* sp. was also sensitive to permethrin within the mean and range of permethrin concentrations reported by Kelley and Starner (2004). As with *H. azteca*, *Procloeon* sp. tolerated turbidities as high as those measured in our previous study, suggesting this is a less important acute stressor than pesticides in this system (Table 5).

C. dilutus was used as a surrogate to assess the relative sensitivity of chironomids to pesticides and suspended sediments in this system. Previous results showed that chironomid densities declined downstream of the agriculture drain input and that their densities were negatively correlated with diazinon and chlorpyrifos concentrations and suspended sediment concentrations. Dose–response data from the literature indicate chironomids are very sensitive to chlorpyrifos and less sensitive to diazinon in laboratory studies. Phipps et al. (1995) reported a 10-day LC50 for chlorpyrifos toxicity to *C. tentans* (*dilutus*) of 70 ng/L. The mean chlorpyrifos concentration measured in the Salinas River in our previous study was 180 ng/L (Table 4; Anderson et al., 2003b). The 96-h LC50 for diazinon toxicity to *C. dilutus* reported in Belden and Lydy (2000) was 30,000 ng/L. The mean diazinon concentration previously measured in the river was 460 ng/L (Table 4). Assuming *C. dilutus* is representative of other chironomids in the Salinas River, these results suggest that chlorpyrifos is a potentially important stressor to midge larvae in this system. As with the other resident species we investigated, *C. dilutus* is not acutely sensitive to suspended particles. Thus, although we found strong negative correlations between macroinvertebrate densities and turbidity in our previous study, this likely resulted from the co-occurrence of suspended particles and pesticides, in particular chlorpyrifos. Our current results show that *C. dilutus* is relatively insensitive to permethrin and bifenthrin. The LC50s for permethrin was well above the mean and range of concentrations measured in this system, and no bifenthrin was detected (Table 4). We could find no published data on bifenthrin toxicity to this species, but data for permethrin suggest that *C. dilutus* (LC50 = 10,450 ng/L) is considerably less sensitive to this pesticide than other species in this genus. The LC50s for permethrin toxicity to the midges *C. plumulosus* and *C. salinarius* were 560 and 73 ng/L, respectively (Appendix 1 in Solomon et al., 2001).

Our current data using three representative macroinvertebrates suggest that suspended particles are less important stressors in our study area in the Salinas River, and that macroinvertebrate declines were caused by pesticides. These experiments underestimated chronic and interactive effects of pesticides and suspended sediments. In the case of diazinon and chlorpyrifos, previous reports have shown that toxicity of these pesticides are additive in mixtures (Bailey et al., 1997), and others have shown interactive effects of pyrethroid and organophosphate pesticides in mixtures (Lydy et al., 2004).

Our previous studies have shown that pesticide-contaminated agriculture drainwater enters the Salinas River on a daily basis (Hunt et al., 2003; Phillips et al., 2004), and although there are daily fluctuations in the concentrations of pesticides in the drainwater, the consistency of the input during the growing season constitutes chronic exposure. The acute LC50s used to determine risk of diazinon, chlorpyrifos and permethrin to resident macroinvertebrates in the current study likely underestimate chronic risk of these pesticides to *Hyalella*, *Procloeon*, and chironomids. In addition, pesticides in this system may influence macroinvertebrate community structure through behavioral or indirect mechanisms. These include sublethal influences on drift and predator avoidance behavior (Schulz and Liess, 2001; Schulz and Dabrowski, 2001). Acute exposures that only quantify mortality due to single chemicals may significantly underestimate sublethal risk due to mixtures of organophosphate and pyrethroid pesticides. The relative effects of suspended sediments on pesticide mixtures is beyond the scope of this study, but previous work has shown that in the case of more hydrophobic pesticides such as chlorpyrifos and pyrethroids, addition of sediment decreases toxicity of chlorpyrifos (Giesy et al., 1999) and cypermethrin (Maund et al., 2002) by reducing their bioavailability in water column toxicity tests. In addition, pesticide impacts on macroinvertebrate populations may be tempered by additional biotic factors such as development of chemical resistance after prolonged exposure to pesticides, influences of habitat refugia allowing for recolonization, and reduced bioavailability of pesticides through sorption to macrophytes.

Despite the limitations of the current study, the weight-of-evidence of our lab and field work in the Salinas River demonstrates that pesticides are important stressors of macroinvertebrates in parts of this system. Based on the current work, we conclude that suspended sediments associated with agriculture drainwater probably play a minor role in directly affecting key components of aquatic invertebrates communities in our study area, and that organophosphate and pyrethroid pesticides have a greater potential for impacting these communities.

There is growing awareness of environmental problems associated with agriculture runoff in Central California, and growers have organized to form watershed monitoring groups to better understand the extent and associated impacts of pesticide pollution in this region. In addition, through cooperative efforts of a number of stakeholders, on-farm practices to minimize pesticide runoff are being actively investigated. Ours and similar studies have been helpful in identifying the most important pesticides to target for reduction in drainwater runoff. Future monitoring will be used to determine which practices are most efficient at reducing these chemicals.

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