

IN SITU WATER AND SEDIMENT TOXICITY IN AN AGRICULTURAL WATERSHED

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Abstract—The Salinas River receives inputs from extensive farmlands before flowing into the Salinas River National Wildlife Refuge and the Monterey Bay National Marine Sanctuary (CA, USA). Previous monitoring using laboratory toxicity tests and chemical analyses identified toxic agricultural drain-water inputs in this system. Using caged daphnids (*Ceriodaphnia dubia*) and amphipods (*Hyaella azteca*), we investigated in situ toxicity at stations downstream from an agricultural drain relative to a reference station. A flow sensor indicated highly variable inputs from irrigation, and daily synoptic chemical analyses using enzyme-linked immunosorbent assay techniques demonstrated fluctuating concentrations of organophosphate pesticides. Test organism mortality in the field coincided with contaminant concentrations that exceeded chemical effect thresholds for the test species. Laboratory toxicity tests using *C. dubia* were comparable to results from field exposures, but tests with *H. azteca* were not. Laboratory exposures can be reasonable surrogates for field evaluations in this system, but they were less effective for assessing short-term temporal variability. Results from the field toxicity studies corroborated results of bioassessment surveys conducted as part of a concurrent study. Toxicity identification evaluations indicated that organophosphate pesticides caused toxicity to daphnids and that effects of suspended solids were negligible.

Keywords—In Situ *Ceriodaphnia dubia* *Hyaella azteca* Organophosphates Toxicity identification evaluation

INTRODUCTION

As part of the California State Water Board monitoring programs designed to assess ambient water quality in state surface waters, ongoing studies have been conducted in the lower Salinas River of central California (USA). The initial phase of the Salinas River assessment included monitoring of eight stations for 15 months and subsequent identification of a consistently contaminated agricultural drainage creek [1]. Water from this drainage caused complete mortality in laboratory exposures of *Ceriodaphnia dubia* in every survey, and toxicity identification evaluations (TIEs) determined the cause of laboratory toxicity to be the organophosphate pesticides chlorpyrifos and diazinon [1]. The second phase of the assessment was designed to investigate effects of the drainage creek on the river ecosystem. A weight-of-evidence approach, including benthic macroinvertebrate surveys at stations downstream from this drainage, coupled with laboratory toxicity tests and TIEs determined that pesticides entering the river from the drainage impacted resident macroinvertebrates [2]. This study also found correlations between macroinvertebrate declines and suspended particles (measured as turbidity) associated with drain water. The present study is the most recent phase of the Salinas River assessment, and it includes laboratory and in situ exposures of daphnids and amphipods to assess spatial and short-term temporal variability of toxicity and the interactive effects of contaminants and environmental factors.

Exposure of test organisms to ambient samples in the lab-

oratory provides valuable information regarding toxic effects, including potential risks to resident biota and ecosystems [3,4]. Although laboratory exposures often are useful predictors of instream effects, they are limited in their ability to integrate the effects of environmental variables and contaminants. To examine these issues, researchers have compared standard in vivo exposures to protocols that have been adapted for the field [5–8]. Previous studies have found variations in results between laboratory and in situ exposures: Laboratory exposures, even those using composite samples, tend to measure the toxicity of single events, whereas field exposures integrate the spatial and temporal effects of contaminant inputs [6]. Exposure of standard toxicity testing organisms in situ has been used extensively in river environments to assess water and sediment toxicity, and this method is recognized as a useful tool for assessing non-point source contaminants [6,9,10]. In an agricultural setting, organisms caged in the field can be exposed to pulses of single or multiple contaminants that fluctuate based on chemical application and irrigation events. Field exposures can produce greater effects than laboratory exposures and, thus, may produce more ecologically relevant results [6]. Field exposures of standard test organisms can also detect contaminant effects while maintaining control of factors such as the source, health, and age of the exposed organisms [8].

In the present study, the cladoceran *C. dubia* and the amphipod *Hyaella azteca* were exposed in the laboratory and in situ to determine the spatial and short-term temporal extent of toxicity and if laboratory results were comparable to those in the field. Chemical and physical causes of toxicity were then investigated using TIEs on water and sediment from in situ containers.

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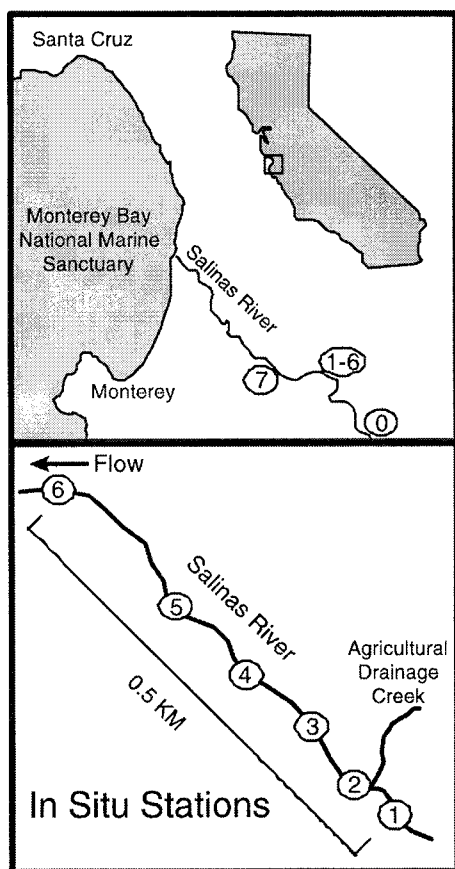


Fig. 1. Map of central California (USA) showing in situ study area with locations of in situ exposure stations.

MATERIALS AND METHODS

Study site

The present study focused on a highly modified creek channel that drains furrow runoff from agricultural fields into the Salinas River (Fig. 1). Creek flow fluctuates daily depending on irrigation and rain events, and numerous sources of drain water contribute varying concentrations of organophosphate pesticides. The predominant crop in the watershed is lettuce. Flow was monitored at a culvert approximately 0.5 km upstream of the confluence with the river for six weeks as part of the previous river assessments and for 4 d during one of the in situ experiments. During an initial monitoring period (June–July 2000), the flow ranged from 0 to 2,748 L/s. Average daily flow in the drainage contributed from 2 to 63% of the flow in the main river. During the *C. dubia* in situ experiment (June 2001), the flow ranged from 0 to 288 L/s, with average daily flows contributing 3 to 6% of the river flow. During past phases of the assessment, turbidity measured in the river ranged from 10 to greater than 1,000 nephelometric turbidity units (NTU).

The first laboratory/in situ experiment exposed caged *C. dubia* at an uninfluenced upstream station (station 1) and at four downstream stations (stations 3–6) on June 4–8, 2001. In a separate experiment conducted July 26–August 2, 2002, *H. azteca* were exposed at stations 2 and 4, at a station that was approximately 10 km upstream (station 0), and at a station that was approximately 10 km downstream from the input (station 7). Station 0 was chosen for the *H. azteca* exposures because beaver activity in the river had significantly altered

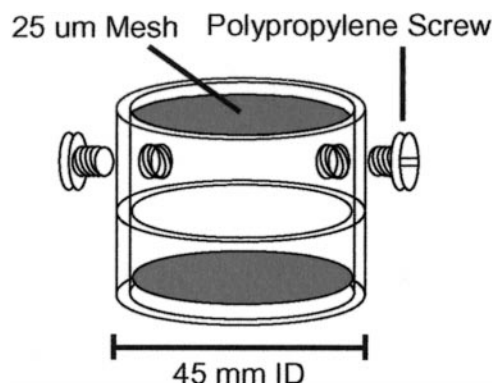


Fig. 2. Schematic of *Ceriodaphnia dubia* exposure chamber. ID = inner diameter.

the river flow, thus flooding station 1 and causing it to be impacted by the agricultural drainage. Hunt et al. [1] demonstrated that station 0 was a suitable upstream reference station. Station 7 was added further downstream to ensure that it was below the influence of the drainage.

Toxicity testing

Both test organisms were exposed to sediment and water. *Ceriodaphnia dubia* were used primarily for water-column exposures, but containers were placed on the sediment. Therefore, sediment may have influenced the results. *Hyalella azteca* were intended for sediment exposures, but because of their epibenthic behavior and an exposure chamber that allowed river water to circulate through it, physical and chemical components of the river could have influenced the results. *Ceriodaphnia dubia* have been used in previous in situ studies and are appropriate surrogates for the resident daphnids. Amphipods from the genus *Hyalella* reside in the Salinas River system.

Acute 96-h *C. dubia* exposures followed the method of the U.S. Environmental Protection Agency (U.S. EPA) [11]. Two 25°C exposure regimes were used in the laboratory experiment. Water samples were collected on day 0 for a static laboratory exposure, and additional samples were collected daily for a renewed laboratory exposure. Amber glass bottles were immersed just below the water surface to collect surface water but to avoid entrainment of the surface microlayer. Field exposure chambers were constructed of polycarbonate tubing (inner diameter, 45 mm) and 25-µm mesh (Fig. 2). Five *C. dubia* neonates were loaded into the chambers at the laboratory by submerging the chambers in water, transferring the neonates through the threaded port, and closing the port with a nylon screw. With both screws in place, the chambers hold water as long as they are handled gently. Chambers were then transported to the field in polypropylene mesh bags that were immersed in a bucket of laboratory water. The mesh bags were staked to the sediment, leaving the chambers haphazardly arranged. Twenty chambers were loaded for each site, with five chambers to be sacrificed daily for mortality counts, water-quality measurements, and organophosphate pesticide measurements. Physical and chemical parameters were measured in a composite sample from five chambers. Water-quality measurements included dissolved oxygen, pH, ammonia, conductivity, alkalinity, and hardness. Temperature was measured continuously with Optic StowAway temperature recorders (Onset Computer, Pocasset, MA, USA). Turbidity in the river

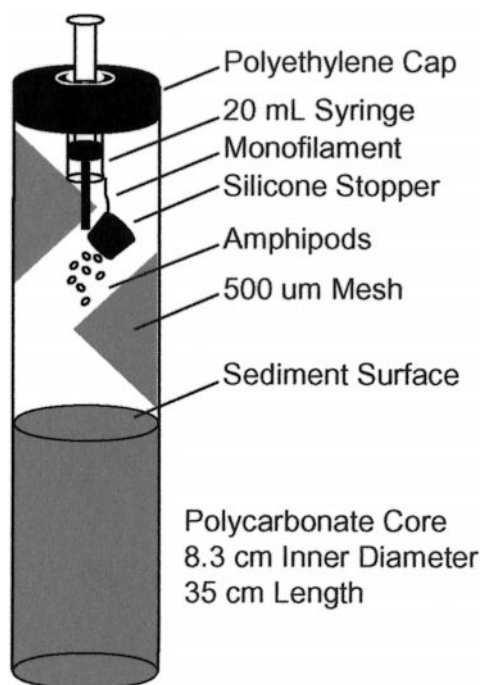


Fig. 3. Schematic of *Hyalella azteca* exposure tube.

was measured at deployment and during daily counts. Samples were transported on ice and stored in the dark at $4 \pm 3^\circ\text{C}$. Laboratory exposures were conducted in 15-ml volumes replicated five times in 50-ml glass beakers.

Solid-phase laboratory *H. azteca* tests followed the method of the U.S. EPA [12] and were conducted at 23°C . Laboratory exposures were initiated on the same day as field exposures, with 5-cm surficial sediment samples collected 2 d earlier. Samples were collected with a polycarbonate tube (inner diameter, 7.5 cm). Five-centimeter sections of surficial sediment were composited in a 10-L polycarbonate tub. Samples were transported on ice and stored in the dark at $4 \pm 3^\circ\text{C}$. Laboratory tests were conducted by adding 100 ml of sediment to eight 300-ml screened beakers. Overlying water was renewed twice daily. *Hyalella azteca* in situ exposure chambers (tubes) were constructed of polycarbonate tubing (inner diameter, 8.25 cm) and 500- μm mesh (Fig. 3). Tubes were inserted into the substrate, and amphipods were introduced to the sediment–water interface via syringe. Syringes were loaded with 10 amphipods and inserted through the tube cap before tube insertion. Exposures were terminated by capping the bottom of the tube, removing it from the substrate, and sieving its contents through a 400- μm screen. Ten exposure tubes were placed at each site. Five tubes at each site were collected on day 4, and mortality was quantified. The remaining five tubes at each site were collected on day 10 for final mortality counts. Water-quality parameters and concentrations of chlorpyrifos and diazinon were measured in overlying and pore water collected from additional tubes at days 0, 4, and 10. Temperature was measured continuously, and turbidity was measured on days 0, 4, and 10. Initial grain size ([13]; <http://www.astm.org>) and total organic carbon (TOC) [14] measurements were taken on day 0, and final measurements were taken on Day 10 from both inside and outside an exposure tube.

Organophosphate pesticide measurements

Two replicates of each water-quality sample were analyzed for chlorpyrifos and diazinon with enzyme-linked immuno-

sorbent assay (ELISA). Samples from *C. dubia* exposures consisted of water collected from inside the chambers. Samples from *H. azteca* exposures consisted of overlying water collected from inside the chambers and interstitial water extracted from sediment within the chambers. All replicate measurements had coefficients of variation less than 15%. Measurements were compared to a five-point standard curve. The lowest detectable dose was calculated from analysis of laboratory standards according to the manufacturer's methodology (Strategic Diagnostic, Newark, DE, USA) as the amount of the pesticide required to achieve a ratio of 85% between the mean absorbance of the standard and the mean absorbance of a negative control [15]. Absorbance is inversely proportional to concentration. The lowest detectable dose was 0.03 $\mu\text{g/L}$ for diazinon and 0.05 $\mu\text{g/L}$ for chlorpyrifos. External standards and sample duplicates were measured with each batch of samples. External standards were 19% and 13% accurate for chlorpyrifos and diazinon, respectively, and duplicates had coefficients of variation less than 20%.

Toxicity identification evaluations

A composite water sample was collected from additional *C. dubia* chambers placed at station 3 for a phase 1 TIE [16], including additional procedures designed to determine the effects of suspended solids on *C. dubia* survival. The sample was collected by removing both nylon screws and carefully draining the chamber into an amber glass bottle. Treatments included a baseline to determine the level of sample toxicity, centrifugation to remove solids and reduce total suspended solids, centrifugation (4 C 3200 g) combined with solid-phase extraction of nonpolar organic compounds on a C8 column, and subsequent elution of extracted compounds using methanol. The methanol eluate was added back to clean dilution water for testing. Suspended solids that were removed via centrifugation were vortexed, added back to C8 column rinsate, and tested with *C. dubia* to investigate whether particles contributed to mortality. Particles were kept in suspension in the test containers by inverting the containers twice daily. An additional treatment with piperonyl butoxide (PBO) was used to block the metabolic activation of acetylcholinesterase-inhibiting organophosphate pesticides [17]. Reduction of toxicity with PBO addition indicates that organophosphate pesticides caused toxicity, yet increased toxicity can indicate the presence of a pyrethroid pesticide. All treatments were conducted on 0, 10, 50, and 100% samples. The 0% sample was laboratory dilution water that served as a blank for each treatment by undergoing the same treatment as the other samples. Chlorpyrifos and diazinon were measured using ELISA, and TIE exposures were conducted for 96 h.

A TIE with *H. azteca* was also conducted using pore water extracted from station 2 sediment obtained from additional cores placed at the site. Ten-day sediment and pore-water tests conducted as part of the previous surveys demonstrated similar toxicity. We conducted TIEs with pore water because the aqueous fraction allowed use of a greater variety of TIE manipulations. Pore water was extracted via refrigerated centrifugation at 4 C 3200 g. Single amphipods were placed in 10 replicate scintillation vials containing 10 ml of pore water. Survival was measured daily, and water-quality parameters were measured on days 0, 4, and 10. Chlorpyrifos and diazinon concentrations were measured on day 0. Physical and chemical parameters were measured in a single replicate. Although sediment toxicity was observed in the field at station 2, pore water

Table 1. Mortality (Mort.) and combined toxic units (TU) of chlorpyrifos and diazinon from *Ceriodaphnia dubia* in situ and laboratory exposures

		Station 1		Station 3		Station 4		Station 5		Station 6	
		Mort. (%)	TU	Mort. (%)	TU	Mort. (%)	TU	Mort. (%)	TU	Mort. (%)	TU
In situ	Day 1	0	0	100 ^a	3.59	80 ^a	1.35	0	1.34	7	0.13
	Day 2	0	0.19			100 ^a	1.68	33 ^a	1.41	20 ^a	0.25
	Day 3	0	0.30					100 ^a	5.35	80 ^a	3.20
	Day 4	7	1.24							100 ^a	2.34
Laboratory renewed	Day 0		0.13		5.85	47 ^a	1.63		1.42		1.09
	Day 1	0	0	100 ^a		100 ^a	0.12	40	1.13	13	0.11
	Day 2	7	0.13					100 ^a		100 ^a	
	Day 3	13	0.23								
Laboratory static	Day 4	13									
	Day 0		0.13		5.85		1.63		1.42		1.09
	Day 1	0		100 ^a		7		0		0	
	Day 2	0				100 ^a		100 ^a		67 ^a	
	Day 3	0								87 ^a	
	Day 4	0								100 ^a	

^a Significant mortality compared to station 1 for in situ exposures and compared to laboratory controls for laboratory exposures. ($p < 0.05$)

extracted from the sediment did not cause mortality to *H. azteca* in the initial laboratory test. We surmised that physical factors in the field, including temperature that spanned a wide range, contributed to toxicity. Because lower temperatures can increase the toxicity of some pesticides, such as Type I pyrethroids and DDT [18], we investigated the effect of temperature on the toxicity of the pore water. *Hyalella azteca* were exposed at 15°C as well as at the standard temperature of 23°C. Addition of PBO was included to investigate the role of nonmetabolically activated pesticides in causing toxicity. Piperonyl butoxide often is used as a synergist for pyrethroids, and the addition of PBO to a sample containing pyrethroids can potentiate toxicity. Piperonyl butoxide was added to the pore water, and amphipods were exposed at 23°C. The TIE exposures were conducted with a single amphipod in 10 replicate scintillation vials containing 10 ml of pore water.

Data analysis

Toxicity data were analyzed for significant mortality using analysis of variance and Tukey tests. Significant differences from the control ($\alpha < 0.05$) are reported for laboratory data, whereas significant differences from a reference station are reported for field data. Toxic unit contributions from the organophosphate pesticides were calculated and summed based on lethal concentration to 50% of the organisms (LC50) for the test organisms. One toxic unit was equal to the concentration of the chemical divided by the LC50. The 96-h *C. dubia* LC50 values of 0.054 µg/L for chlorpyrifos and 0.335 µg/L for diazinon [19] and the 10-d *H. azteca* LC50 values of 0.086 µg/L for chlorpyrifos and 6.51 µg/L for diazinon [20] were used. The TIE mortality results were compared using toxic

units calculated from each dilution series (100 divided by the calculated LC50). Correlations between *C. dubia* and *H. azteca* mortality and physical and chemical measurements were determined using the Spearman rank procedure (Systat 7.0.1; SPSS, Chicago, IL, USA).

RESULTS

Ceriodaphnia dubia toxicity tests

Samples from station 1 demonstrated acceptable (>90%) survival in the in situ exposures and in the static and renewed laboratory tests, but complete mortality was observed at all stations downstream of the input for all in situ and laboratory exposures (Table 1). Significant mortality coincided with concentrations of chlorpyrifos and diazinon above the tolerance limit of *C. dubia*. Samples collected from downstream stations contained from 0.13 to 3.59 combined toxic units, whereas laboratory exposures contained 0.11 to 5.85 combined toxic units. Both static and renewed laboratory exposures produced results similar to those of the field exposures, with the exception that in the laboratory, test organisms survived longer in water collected from station 6. Chlorpyrifos concentrations contributed 72% of the combined toxic unit values at stations below the input, and toxic units had a significantly positive correlation with mortality ($p < 0.001$) (Table 2). Mortality and toxic units had a less significant relationship with turbidity ($p < 0.01$). Dissolved oxygen, pH, and conductivity were within acceptable limits for the test organism, and temperature ranged from 12.7 to 31.0°C (Table 2).

Additional chambers without daphnids were deployed at station 3 to collect water for the TIE. These chambers were

Table 2. Mean and range of physicochemical parameters from *Ceriodaphnia dubia* in situ and laboratory exposures^a

Station	Diss. oxygen (mg/L)			pH			Cond. (µS/cm)			Chlorpyrifos (µg/L)			Diazinon (µg/L)		
	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.
1	8.5	7.54	9.34	8.57	8.35	9.05	549	515	565	0.006	0.000	0.049	0.06	0	0.11
3	8.63	8.54	8.72	8.75	8.6	8.89	735	728	742	0.227	0.180	0.306	0.1	0.05	0.19
4	8.73	7.5	9.93	8.67	8.34	9.01	588	569	608	0.057	0.000	0.082	0.05	0.04	0.07
5	8.48	7.34	9.15	8.69	8.38	9.04	582	565	602	0.092	0.055	0.207	0.15	0.04	0.51
6	8.68	7.09	9.61	8.69	8.46	9.03	616	596	634	0.051	0.000	0.124	0.09	0	0.3

^a Diss. = dissolved; Cond. = conductivity.

Table 3. *Ceriodaphnia dubia* toxicity identification evaluation results^a

Treatment	% Mortality				TU from dilution	TU from OP	Turbidity (NTU)
	0% Sample	10% Sample	50% Sample	100% Sample			
Baseline	0	0	100	100	4.5	3.7	437
Centrifuged sample	0	0	13	100	1.7	2.4	10
C8 column rinsate	7	0	0	0	<1		
C8 column eluate	0	0	0	100	1.4	1.1	
Turbidity add-back	0	0	0	0	<1		482
Piperonyl butoxide	0	0	0	13	<1	4.3	

^a Mortality (%) at four sample concentrations, toxic units (TU) calculated from the dilution series, toxic units calculated from organophosphate pesticides (OP), and turbidity. NTU = nephelometric turbidity units.

removed from station 3 on day 1 when complete mortality occurred. In this sample (Table 3), 2.4 toxic units of combined chlorpyrifos and diazinon were measured. We attempted to determine the effects of pesticides and suspended particles by creating treatments both with and without particles and organophosphates. Although the turbidity of the surrounding river was 42 NTU, the particles inside the chamber created a turbidity of 437 NTU when resuspended. Turbidity in the river did not exceed 75 NTU during the *C. dubia* experiment, so the in situ chambers likely reduced water flow and allowed particles to settle inside them. The sample was centrifuged, and the turbidity was reduced from 437 to 10 NTU. A reduction in chlorpyrifos and diazinon concentrations from 3.7 to 2.4 toxic units occurred, and toxicity was reduced from 4.5 to 1.7 toxic units. Passing centrifuged sample through a C8 solid-phase extraction column completely removed chlorpyrifos, diazinon, and toxicity. Although the column eluate did not completely return the organophosphates to pre-extraction concentrations, complete mortality occurred at the highest concentration of this treatment (Table 3). By reconstituting the previously removed particles in a sample of postcolumn rinsate, a treatment was created that contained particles but no measurable chlorpyrifos and diazinon. Although the turbidity was 482 NTU, no mortality occurred in this treatment. Addition of the metabolic inhibitor PBO also completely mitigated toxicity in the original sample (Table 3).

Hyalella azteca toxicity tests

We observed greater mortality in the in situ exposures than in the laboratory exposures at all stations. No significant mortality was observed in laboratory exposures in any of the samples after 10 d (Table 4). Amphipod survival in the in situ exposures varied by station. Minimal mortality occurred at station 0 on day 4. Although station 0 was intended as an uninfluenced reference station, mortality increased to 32% at station 0 by day 10 (Table 4). Complete mortality was observed on day 4 at station 2, which was immediately downstream of the input. Mortality was 54% at station 4 on day 4 and increased to 62% by day 10. No significant mortality was observed at station 7 after 10 d. Subsequent statistical comparisons were made between impacted stations and station 7. Mortality at two stations coincided with concentrations of chlorpyrifos greater than the 10-d tolerance limit of *H. azteca*. The maximum combined toxic units from the *H. azteca* exposures were lower than those measured in the *C. dubia* exposures. Overlying water from station 2 contained 1.41 toxic units, and the pore water contained 1.28 toxic units. When chlorpyrifos was detected, the concentrations accounted for 98% of the combined toxic units (Table 5). Concentrations of chlorpyrifos

and diazinon did not exceed *H. azteca* LC50 values in the laboratory exposures. Overlying toxic unit values were significantly correlated with amphipod mortality in the in situ exposures ($p < 0.01$), and mortality had a significant positive relationship with turbidity and overlying chlorpyrifos concentrations ($p < 0.005$). Dissolved oxygen, pH, and conductivity were within acceptable limits for the test organism, and temperature in the field ranged from 13.2 to 25.0°C (Table 5).

Silt and clay (<0.074 mm particle size) accumulated in the amphipod in situ exposure chambers. During the 10-d exposure, percentages of silt and clay increased up to 33-fold, and percentages of TOC increased up to eightfold relative to adjacent sediments outside the chambers (Fig. 4). Percentage TOC correlated with percentage silt and clay in the in situ samples ($p < 0.0025$), and overlying water toxic units in the chambers correlated with both percentage TOC ($p < 0.05$) and percentage silt and clay ($p < 0.1$). Amphipod mortality was positively correlated with percentage TOC and percentage silt and clay ($p < 0.005$ and 0.01, respectively).

As with the daphnid experiment, additional chambers without amphipods were deployed to collect sediment pore water for a TIE. These were removed from station 2 on day 4 after 100% mortality was observed in the in situ chambers. Sediment was brought to the laboratory, and pore water was extracted via centrifugation. An initial test was conducted with *H. azteca* to determine the magnitude of toxicity. This test showed 0% mortality in the pore water. The ELISA analyses were also performed to determine concentrations of chlorpyrifos and diazinon. The concentrations of organophosphates were low (0.7 combined toxic units).

We assumed that because all the in situ amphipods exposed to station 2 sediment were dead by day 4, the sample from this station would be acutely toxic when tested in the laboratory. Other than differences in contaminant concentrations between the laboratory and the field, one possible explanation was that temperature in the field affected survival. *Hyalella azteca* was maintained at 23°C in the laboratory, yet temperatures in the field ranged from 13.2 to 25.0°C. Because increased toxicity at lower temperatures is a characteristic of some pesticides (e.g., Type I pyrethroids and DDT [18,21–23]), we investigated the effects of low temperature on pore-water toxicity. *Hyalella azteca* were exposed to pore water at 23°C, both with and without PBO, and at 15°C for 96 h. The baseline treatment tested at 23°C had no mortality, but 70% and 89% mortality occurred in the 15°C and PBO treatments, respectively. Control survival in all these treatments was 100%.

Table 4. Mortality (Mort.) and combined toxic units (TU) of chlorpyrifos and diazinon from pore water (PTU) and overlying water (OTU) from *Hyalella azteca* in situ and laboratory exposures

	Station 0			Station 2			Station 4			Station 7		
	Mort. (%)	PTU	OTU	Mort. (%)	PTU	OTU	Mort. (%)	PTU	OTU	Mort. (%)	PTU	OTU
In Situ												
Day 0	0	0	0	0	0.04	0.05	0	0.05	0.04	0	0.01	0.63
Day 4	10	0	0	100 ^a	1.28	1.41	54 ^a	0.01	1.14	0	0.01	0.01
Day 10	32	0	0.90	100 ^a	0.72	0.90	62 ^a	0.01	0.77	12	0.01	1.13
Laboratory												
Day 0	0	0	0	0	1.11	0	0	0.02	0	0	0.53	0
Day 4	4	—	0.60	6	—	0.67	8	—	0	8	—	0
Day 10	10	—	0	2	—	0	10	—	0	8	—	0

^a Significant mortality compared to station 7 for in situ exposures and compared to laboratory controls for laboratory exposures. ($P < 0.05$.)

DISCUSSION

When designed correctly, in situ toxicity tests allow investigation of the interaction of multiple chemical and physical stressors on resident and surrogate species [8,24,25]. Field exposures are also particularly useful for assessing contaminant effects in systems where inputs are temporally variable and in situations where spatial patterns of contamination are not obvious [25]. In our comparison of laboratory and in situ exposures using *C. dubia*, we found little difference between the two methods. This may have been because pesticide contamination in the Salinas River from agricultural drain water was a consistent phenomenon during this experiment. Both exposure methods in the *C. dubia* experiment showed complete mortality at all downstream stations within 96 h. Slightly greater mortality was found with the station 1 sample in the laboratory renewal experiment, and daily mortality differed somewhat between the different methods depending on the pesticide mixtures in the respective exposure chambers. Although differences in the concentrations of pesticides in the laboratory and in situ exposure chambers were observed, sufficient concentrations of chlorpyrifos and diazinon were found to account for the observed effects in both (Table 1).

Our analyses of drain-water flow into the Salinas River show a consistent pattern of discharge from the creek. Flow increases daily with the onset of irrigation in the adjacent upstream fields and slowly subsides in the evening. *Ceriodaphnia dubia* died at all stations downstream of the input. Stations were arrayed downstream of the input to attempt to define the spatial extent of toxicity. That station 6 was toxic demonstrates toxicity extends at least 0.5 km below the input. Given that 7-d survival and reproduction tested with *C. dubia* is more sensitive than the 96-h acute test to chlorpyrifos and diazinon, it is probable that more pervasive toxicity would be measured in this system using the chronic test [26,27].

Previous studies have shown that this drainage is contaminated with toxic mixtures of chlorpyrifos, diazinon, and other

pesticides and that these contaminants impact water-column and benthic species in laboratory toxicity tests [1,2]. As in the current study, Anderson et al. [2] found that concentrations of chlorpyrifos and diazinon were correlated with turbidity and that both contaminant concentrations and turbidity were highly correlated with *C. dubia* mortality. Both factors were also highly correlated with impacts on a number of key macroinvertebrate metrics (e.g., species richness and number of ephemeroptera taxa). Because the particles were allowed to settle out and the samples decanted before use, those authors concluded that particles did not cause the toxicity observed in their laboratory toxicity tests. The current study was designed, in part, to use in situ exposures and TIEs to compare results of laboratory and field exposures in the Salinas River and to explore the relationship between contaminant effects and suspended particles, measured as turbidity.

Turbidity correlated with toxicity in the field exposures, but results of the TIE with *C. dubia* suggest that toxicity in the in situ chambers was caused by chlorpyrifos, not by turbidity. Toxicity was removed when a sample from the in situ chambers was passed through a C8 column. A 100% mortality rate was observed for *C. dubia* in the C8 column eluate, and sufficient chlorpyrifos eluted from the column to account for the observed mortality. Although diazinon was measured in this sample, the concentration was considerably lower than the LC50 for this chemical. Because diazinon and chlorpyrifos toxicity are additive [19], it is possible that the small amount of diazinon in this sample contributed to toxicity. Evidence that toxicity was caused by chlorpyrifos was confirmed with addition of the metabolic-inhibitor PBO, which reduced mortality to 13%. When turbidity was removed via centrifugation and centrifuged particles were resuspended in the pesticide-free column rinsate, no mortality was observed (Table 2).

Turbidity in the river during our *C. dubia* exposures did not exceed 75 NTU, yet turbidity in the sample from the in situ chambers used in the TIE was 437 NTU. The in situ

Table 5. Mean and range of physicochemical parameters from *Hyalella azteca* in situ and laboratory exposures^a

Station	Diss. oxygen (mg/L)			pH			Cond. (μS/cm)			Chlorpyrifos (μg/L)			Diazinon (μg/L)		
	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.
0	6.09	4.19	8.9	8.33	8.09	8.7	619	421	866	0.013	ND	0.077	ND	ND	ND
2	5.51	3.51	8.66	7.99	7.77	8.19	644	540	736	0.051	ND	0.120	0.177	ND	0.428
4	5.63	4.51	8.61	8.12	7.9	8.45	592	432	738	0.016	ND	0.097	0.093	ND	0.311
7	6.04	4.09	8.07	8.09	7.88	8.35	505	471	731	0.019	ND	0.095	0.062	ND	0.157

^a Diss. = dissolved; Cond. = conductivity; ND = not determined.

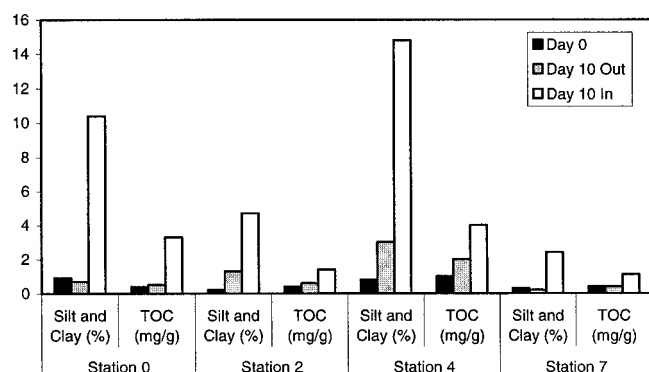


Fig. 4. Grain size (%) and total organic carbon (TOC; %) results from *Hyalella azteca* in situ exposures. Day 0 measurements were taken before tube deployment, and Day 10 measurements were taken both outside and inside tubes at the termination of the exposure.

chambers apparently increased the deposition of particles by reducing the flow of water, thus allowing settlement within the chamber. Others have identified this as a potential problem associated with using mesh-lined chambers [24], but in this case, the increased particle deposition may have had more to do with chamber placement than with chamber design. The chambers were held in polypropylene bags staked to the river bottom. Bags were not suspended above the bottom, both because the stations were relatively shallow (<0.5 m) and because we wanted to make sure the chambers remained submerged during the exposure. As a result of this placement, there might have been less particle flow through the screens than would have occurred if the chambers had been suspended, thus allowing more particles to settle in the chambers.

Our comparison between laboratory and in situ exposures using *H. azteca* demonstrated that laboratory exposures significantly underestimated field toxicity. None of the laboratory exposures were toxic, whereas three of the field exposures were significantly toxic. Significant amphipod mortality was measured at the confluence of the input and the river (station 2) and also approximately 100 m downstream (station 4). As many as 1.4 toxic units of chlorpyrifos were found in the overlying water from station 2, which is enough to cause the observed mortality. Chlorpyrifos concentrations measured in the station 4 overlying water (0.097 $\mu\text{g/L}$, or 1.13 toxic units) were also sufficient to account for the observed mortality. Overlying water from station 7 contained 1.1 toxic units of chlorpyrifos and only demonstrated 12% mortality. It is possible that the pesticide measured in the station 7 in situ containers accumulated there near the end of the exposure and insufficient time was allowed for mortality to occur. No chlorpyrifos or diazinon was detected at this station on day 4. Station 0 had significant mortality but lower organophosphate concentrations. In samples from this station on day 10, 0.9 toxic units of chlorpyrifos were found, but none was detected on day 4. Toxicity at this station could have been caused by unmeasured contaminants or by pulses of contaminants in the system between days 4 and 10.

Although complete mortality occurred at station 2 in the field exposures, pore water collected from additional exposure tubes contained low concentrations of chlorpyrifos and diazinon and was not toxic to *H. azteca* in the laboratory. Toxicity in the field could have been caused by a pulse of contaminants that dissipated by the time the remaining tubes were collected for the TIE. Our TIE results, however, suggest that lower field

temperatures could have exacerbated in situ amphipod mortality. Amphipod mortality increased from 0% in pore-water tests conducted at 23°C to 70% in pore-water tests conducted at 15°C. Increase in toxicity at lower temperature is a characteristic of some pesticides, such as DDT [16] and Type I pyrethroids [18,21–23]. These pesticides were not analyzed in these samples. The concentration of DDT from station 2 sediment collected in September 2000 was 4.96 $\mu\text{g DDT/g organic carbon}$ [2]. The 10-d LC50 for total DDT toxicity to *H. azteca* is 2,580 $\mu\text{g DDT/g organic carbon}$ [28], so it is unlikely that DDT concentrations at station 2 could account for the observed mortality. That mortality increased from 0 to 89% with the addition of PBO supports the hypothesis that a Type I pyrethroid pesticide was in this sample. By inhibiting cytochrome P450, PBO acts as a synergist in the presence of nonmetabolically activated pesticides, such as pyrethroids [29]. Results of our TIE were similar to the TIE results of Anderson et al. [2] using sediments from this station. Those authors concluded that toxicity to *H. azteca* was caused by a combination of chlorpyrifos and some nonmetabolically activated compound, such as a pyrethroid pesticide. Data from the California Department of Pesticide Regulation (Sacramento, CA, USA) show that pyrethroid pesticides are used widely in this part of the Salinas Valley (D. Paradies, Central Coast Regional Water Quality Control Board, San Luis Obispo, CA, personal communication).

Percentages of silt, clay, and organic carbon increased in the tubes during the exposure period. *Hyalella azteca* is an epibenthic organism that can tolerate a wide range of substrates, including sediments that are greater than 90% silt and clay [12]. Although mortality correlated with particle size and organic carbon, it is unlikely that grain size alone would have influenced toxicity in the present study. The tubes acted as sinks for silt and clay particles and, possibly, for the contaminants associated with these particles, such as organochlorines and pyrethroids. Chlorpyrifos, with a log K_{ow} value of 5.26, likely was transported into the tubes with the fine particles, whereas a log K_{ow} value of 3.70 for diazinon indicates that it is less likely to be absorptive. Anderson et al. [2] found that the greatest pesticide contamination and sediment toxicity occurred in samples with the highest TOC and finest grain sizes in this section of the Salinas River. If contaminants were transported into the tubes, the epibenthic *H. azteca* likely had significant interaction with them. Additional research is needed to separate the effects of total suspended solids and particle-borne contaminants on the toxicity of amphipods in the field.

In situ toxicity testing is a useful tool for evaluating spatial and temporal trends in toxicity and for investigating relationships between laboratory results and ecosystem observations [6,10,30]. Schulz and Liess [10] stated that the response of the bioassay must relate to the environmental stress as well as to the ecological responses in the field. Previous studies have shown high levels of concordance between toxicity to *C. dubia* and instream ecological impacts [4], and our current *C. dubia* in situ results show toxicity at stations where Anderson et al. [2] described impacted macroinvertebrate communities. Although several studies have reported the use of *H. azteca* in situ, we are unaware of any studies relating *H. azteca* toxicity results to instream effects. *Hyalella* is a resident genus in the Salinas River, and it was clearly affected by the agricultural input when exposed in situ. We noted significant reductions in the abundance of benthic invertebrates, including *Hyalella*

and daphnid species, at stations downstream from this drainage [2].

Our study shows how in situ toxicity testing with multiple species can be used in conjunction with laboratory tests, sediment and water chemistry, and TIEs to characterize effects of agricultural drain water on a river ecosystem. Results of the present and of previously conducted studies [1,2] demonstrate the impact of toxic concentrations of pesticides on resident organisms. Experiments with *C. dubia* showed that the laboratory exposures were predictive of in situ toxicity with this species. Experiments with *H. azteca* showed that the laboratory exposures underestimated in situ toxicity in the present study. The TIE evidence suggests that this may have been caused, in part, by characteristics of sediment-associated contaminants in these samples. Future work will emphasize TIEs and chemical analyses designed to confirm causes of in situ sediment toxicity in the Salinas River. These will include analyses of pyrethroid pesticides.

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