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Integrated assessment of the impacts of agricultural drainwater in the Salinas River (California, USA)

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"Capsule": *Invertebrate mortality was correlated with levels of water and sediment contaminatioin in the Salinas River.*

Abstract

The Salinas River is the largest of the three rivers that drain into the Monterey Bay National Marine Sanctuary in central California. Large areas of this watershed are cultivated year-round in row crops and previous laboratory studies have demonstrated that acute toxicity of agricultural drainwater to *Ceriodaphnia dubia* is caused by the organophosphate (OP) pesticides chlorpyrifos and diazinon. In the current study, we used a combination of ecotoxicologic tools to investigate incidence of chemical contamination and toxicity in waters and sediments in the river downstream of a previously uncharacterized agricultural drainage creek system. Water column toxicity was investigated using a cladoceran *C. dubia* while sediment toxicity was investigated using an amphipod *Hyalella azteca*. Ecological impacts of drainwater were investigated using bioassessments of macroinvertebrate community structure. The results indicated that Salinas River water downstream of the agricultural drain is acutely toxic to *Ceriodaphnia*, and toxicity to this species was highly correlated with combined toxic units (TUs) of chlorpyrifos and diazinon. Laboratory tests were used to demonstrate that sediments in this system were acutely toxic to *H. azteca*, which is a resident genus. Macroinvertebrate community structure was moderately impacted downstream of the agricultural drain input. While the lowest macroinvertebrate abundances were measured at the station demonstrating the greatest water column and sediment toxicity and the highest concentrations of pesticides, macroinvertebrate metrics were more significantly correlated with bank vegetation cover than any other variable. Results of this study suggest that pesticide pollution is the likely cause of laboratory-measured toxicity in the Salinas River samples and that this factor may interact with other factors to impact the macroinvertebrate community in the system.

Keywords: Chlorpyrifos; Diazinon; Toxicity; Sediments; Macroinvertebrates

1. Introduction

The Salinas River is the largest of the three coastal rivers flowing into the Monterey Bay National Marine Sanctuary in central California. It provides significant freshwater habitat in this semi-arid region and the river is a primary migration corridor for endangered steelhead trout (*Onchorhynchus mykiss*, Busby et al., 1997). Large areas in this watershed are cultivated year-round. Recent studies have shown that samples from the river and some tributaries are toxic to standard test species in laboratory tests (Hunt et al., in press) and that agricultural drainwater impacts macroinvertebrate communties in the river (Anderson et al., in press). The current study was conducted on a previously uncharacterized agricultural drainage creek that is also the subject of nutrient, sedimentation, and pathogen monitoring studies conducted by local growers in the Salinas Valley.

In the current study, we investigated the impacts of agricultural drainwater flow in the Salinas River over an 18-month period using a combination of ecotoxicologic tools. Salinas River water and sediment toxicity were characterized using the cladoceran *Ceriodaphnia dubia* and the amphipod *Hyalella azteca*, respectively. Toxicity test results were compared to physical and water quality analyses, as well as selected pesticide measures in both water and sediment matrices. Ecological impacts were assessed by characterizing macroinvertebrate

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community structure upstream and downstream of the drainwater inputs. Possible causes of toxicity and impacts on macroinvertebrate community structure were investigated using a combination of chemical analyses, statistical correlations, and dose–response information from the literature, as well as habitat and physical factor assessments. The results of these investigations were combined in a weight-of-evidence evaluation of the impacts of agriculture drainwater on selected components of the river ecosystem, and were used to characterize chemicals potentially responsible for toxicity and associated ecological degradation.

2. Methods

2.1. Sampling sites

The study was conducted at the confluences of two agricultural drainages with the Salinas River. The drainages are hereafter referred to as the agricultural drainage creek (creek) and the agricultural drain (drain). These drainages enter the river approximately 60 km upstream (southeast) of the point where the river enters the Monterey Bay. The two streams are separated by a willow-covered earthen dike as they enter the river. Sediments deposited at the mouth of these two streams cause the creek to flow south, parallel to the Salinas River, but in the opposite direction of its flow. The creek enters the river approximately 50 m south of its divergence from drain (Fig. 1). The drain water flows parallel to the river in a generally northern direction until entering the river approximately 200 m downstream of the creek input. The four sampling stations were located so that Station No. 1 was upstream of both the creek and the drain. Station No. 2 was located approximately 60 m downstream of the creek input, but above the drain input. Station No. 3 was located approximately 25 m downstream of the drain input, therefore receiving direct input from drain water, combined with more diluted input from the creek. Station No. 4 was located approximately 50 m downstream of Station No. 3 (Fig. 1). Four water column toxicity tests were conducted to account for temporal variability: one each in April, May and September of 2000, and in May 2001. Macroinvertebrate community structure was

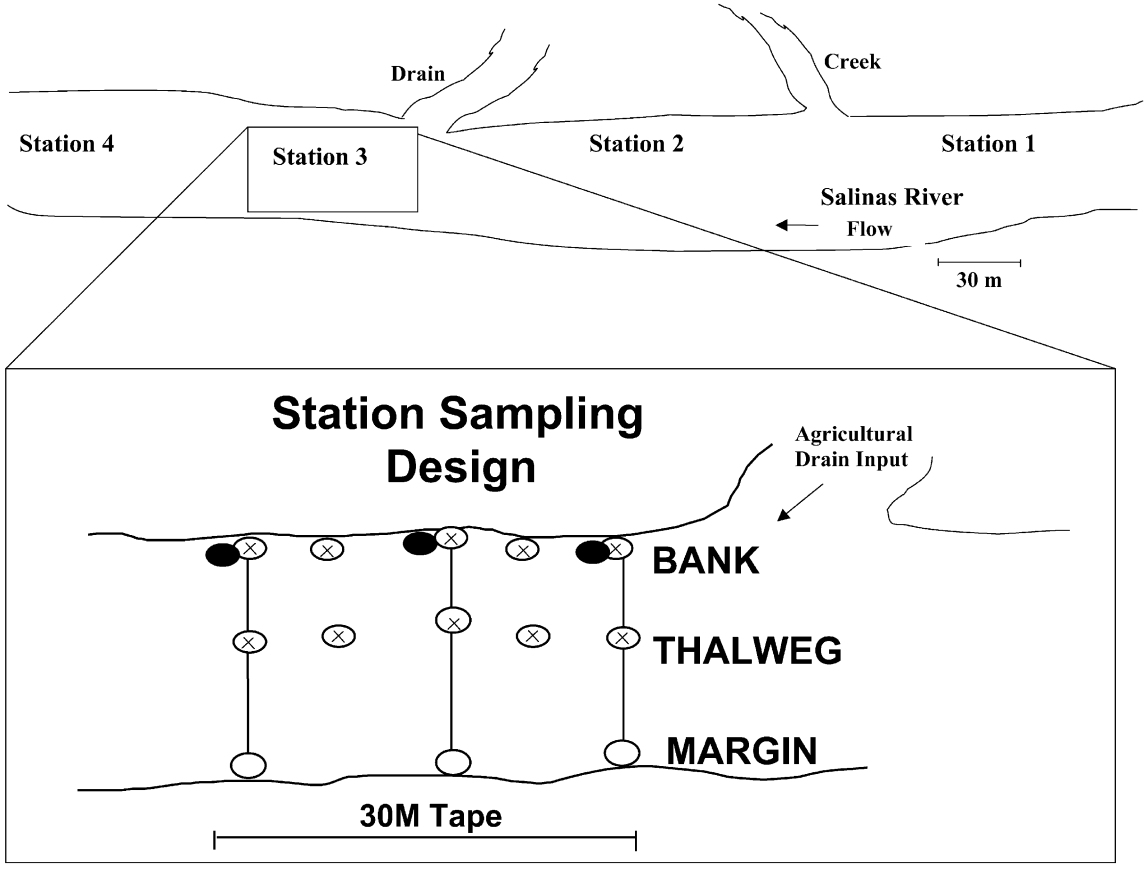

Fig. 1. Schematic diagram of Salinas River sampling design showing four stations and bank and composite samples in relation to the agricultural Creek and Drain. Note that the Creek and Drain combined to form one flow above Station 3 in May 2001. Solid circles indicate location of bank macroinvertebrate samples, open circles and circles with × indicate locations of composite macroinvertebrate samples, circles with × also indicate locations of water and sediment samples for toxicity tests.

characterized in all surveys but the May 2000. Sediment toxicity was assessed in August 2001.

Each station was delineated by a 30 m stretch of river bank marked with a transect tape. During each of the four sample periods, two separate water samples were collected for toxicity tests at 5 randomly selected points along the transect. The first was a composite of five samples of river water collected mid-river and parallel to the 30 m bank transect. The second water sample consisted of a composite of five samples of river water collected along the bank. The mid-river samples were used to assess toxicity in the whole river as influenced by the drainages. Because it was assumed there would be less dilution of the incoming creek and drain waters along the bank where they entered the river, the bank water samples were presumed to be most influenced by drainwater (Fig. 1).

2.2. C. dubia *96-h survival tests*

C. dubia 96-h toxicity tests were conducted on bank and mid-river samples using the EPA standard acute test protocol (US EPA, 1993). Each undiluted sample was tested using five replicates, each containing five *C. dubia* neonates (<24-h old). Survival was monitored daily in each replicate of each sample. Test animals were fed a YCT and *Selenastrum* mix at 48 h (3:1 alga to YCT) then solutions were renewed. Conductivity, hardness, alkalinity, pH, dissolved oxygen, and ammonia were measured in one replicate of each sample at the beginning and end of each test. Temperature was monitored continuously in the controlled temperature room by placing a probe in an additional replicate (test temperatures ranged between 24 and 26 °C). Samples for the *Ceriodaphnia* tests were refrigerated after collection and tested within 48 h.

2.3. H. azteca *toxicity test*

Toxicity of sediment samples from all stations was assessed in August 2001 using the 10-day survival and growth toxicity test with *H. azteca*, a genus that is resident in the Salinas River. Eight replicates, each with ten 7 to 14-day-old amphipods were tested following EPA procedures (US EPA, 2000). The amphipods were exposed to 100 ml of sediment in 300 ml beakers, each containing 175 ml of overlying water. The test temperature was 23 °C (±1 °C). The overlying water was renewed twice daily, and 1 ml of food (Yeast, Cerophyl®, and Trout Chow—YCT) was added to each test container; the containers were not aerated. After surviving animals were dried at the end of the test, growth was measured as change in mean dry weight per individual amphipod per replicate. Samples for the *Hyalella* test were collected 96-h and refrigerated prior to initiation of the test.

2.4. *Benthic macroinverebrate community characterization*

Techniques for sampling streams with sand or mud bottoms followed the California Department of Fish and Game Aquatic Bioassessment Laboratory procedures for wadeable streams (CDFG, 1999), which were adapted from the USEPA Rapid Bioassessment Protocol for use in streams and rivers (Barbour et al., 1999).

Samples were collected by placing a 30-cm wide by 24-cm high D-net (0.5-mm mesh) on the sandy river bottom or against the submerged vegetated bank substrate and then disturbing a 30×60 cm portion of substrate upstream of the net for 60 s. Two types of samples were collected: a bank sample and a composite sample, at each of three randomly selected locations per station. The bank sample was collected along the drain-side bank and was presumed to be the most influenced by the drain. The composite sample was collected at the bank, thalweg, and margin (opposite bank). All samples were fixed in the field in 95% ethanol. Samples were transferred to 70% ethanol after transport to the laboratory. All benthic macroinvertebrates were identified to species or genus following methods and quality assurance guidelines of the California Stream Bioassessment Protocol (CDFG, 1999). Physical and habitat quality assessments were conducted at each sampling station. Physical habitat characteristics included instream cover, epifaunal substrate, embeddedness, channel flow, channel alteration, sediment deposition, riffle frequency, bank vegetation, bank stability, and riparian zone cover (CDFG, 1999).

2.5. *Chemical analyses*

Because agriculture is the dominant land use in the study area and previous studies in the watershed indicated organophosphate pesticides were among the primary chemicals of concern (Hunt et al., in press), chemical analyses for this study emphasized these pesticides. Selected river samples were also analyzed for organochlorine and carbamate pesticides, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and trace metals (US EPA, 1986). Organochlorine compounds were measured by EPA Method 8080, using gas chromatograph/electron capture with method detection limits ranging from 0.3 to 5 ng/l. Organophosphate compounds were measured by EPA Method 8140/8141 using a nitrogen phosphorus specific detector (method detection limits ranging from 0.04 to 33 μg/l; chlorpyrifos detection limit = 0.05 μg/l; diazinon detection limit = 0.04 μg/l). Carbamate compounds were measured by EPA Method 632, using dual detection with UV visual mode and LCMS confirmation (method detection limits 0.054–2.5 μg/l). PCBs were analyzed as aroclors using EPA Method 8080-PCBs (detection

limits 0.04–0.11 μg/l). PAHs were analyzed using EPA Method 8310, HPLC-UV (method detection limits 0.05–1.0 μg/l). Selected water samples were also analyzed for trace metals (As, Ag, Cd, Cr, Cu, Hg, Mg, Ni, Pb, Zn) by ICP using EPA Method 200.7 (method detection limits 0.33–4.1 μg/l). Selected sediment samples were analyzed for trace metals (As, Ag, Cd, Cr, Cu, Hg, Mg, Ni, Pb, Zn) by ICP using EPA Method 6010A. Standard quality assurance procedures including measurement of standard reference materials and quantification of surrogate recoveries and matrix spikes were followed in all analyses. All chemical analyses met prescribed quality assurance guidelines.

2.6. ELISA tests

All samples were analyzed for chlorpyrifos and diazinon using enzyme-linked immunosorbent assays (ELISAs; Strategic Diagnostics Inc., Newark DE) following procedures recommended by Sullivan and Goh (2000). ELISA readings were compared to a 5-point standard curve, using standards provided by the manufacturer. After analysis of every group of field samples, accuracy of the ELISA method was determined by measuring an external chlorpyrifos or diazinon standard. This standard was also spiked into uncontaminated river water taken upstream of the contaminant input to account for matrix effects. 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 (CV). CVs were always less than 20%. A combined bottle blank/process blank was included during one sampling period and this indicated no contamination. Samples were tested at full strength, unless initial readings indicated that the chemical was at concentrations above the range of the test kits. In such cases, samples were diluted to known concentrations prior to definitive analysis. The lowest detectable dose was 30 ng/l for diazinon and 50 ng/l for chlorpyrifos. Twenty-seven of the 36 samples measured with ELISA kits were also measured with EPA analytical chemistry methods for comparison. The mean relative percent differences (RPD) for both chlorpyrifos and diazinon were 34.6 and 68.3%, respectively. In most cases differences were caused by detectable concentrations of chlorpyrifos or diazinon using ELISA where no chemical was detected using GC–MS (data not shown).

Dissolved oxygen (mg/l), specific conductance (μs/cm), pH, temperature (°C) and turbidity (NTU) were measured in situ using the Hydrolab Surveyor 4 and Datasonde 4x (Hydrolab, Austin, TX). 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 field-collected samples in the laboratory. Nitrate (as NO_3^-) was measured on a Hach® DR2010 spectrophotometer. Phosphate (as PO_3^-) was measured with the Hach molybdate and amino acid reagent kit. All samples were analyzed at room temperature within 48 h of collection.

2.7. Statistical analyses

Principal components analysis (PCA) was used to investigate associations between laboratory toxicity test results, macroinvertebrate community structure, physical factors, and chemical contamination. Separate analyses were conducted for the bank samples and mid-river composite samples. Macroinvertebrate community metrics selected for these analyses included the total number of ephemeroptera taxa, species richness (=total number of species or genera), abundance (=total number of organisms), the number of daphnids and amphipods (*Hyalella*) in the samples, and the percentage Chironomidae in the samples. Principal components were extracted using Systat® statistics software (v. 7.0 for Windows, 1997, Systat Inc., Evanston IL). The analysis was conducted on untransformed data with a correlation matrix and varimax rotation, and included any factors which accounted for greater than 10% of the total variance. A component loading cutoff value of 0.40 was used in selecting variables for inclusion into factors, based on suggestions by Tabachnick and Fidell (1996) that a cut-off of at least 0.32 be used and that component loading of greater than 0.45 are considered fair or better. Groupings identified through PCA were further evaluated using Spearman Rank Correlations (Systat, 7.0).

3. Results

3.1. C. dubia toxicity tests

Although there was 0% survival of *C. dubia* in all of the creek and drain samples, toxicity in the river varied seasonally and spatially (Table 1). Survival was 100% in all bank and mid-river samples collected at the reference station (Station 1) and no significant toxicity was detected at Station 2. Moderate toxicity was observed in the bank samples from Station 3 in April and May 2000. There was 0% survival in the bank samples from Stations 3 and 4 in May 2001. No mid-river samples were toxic at any station during this study. Water quality parameters were all within tolerable ranges for *C. dubia*. Dissolved oxygen was always above 8 mg/l; hardness values in these samples ranged from 78 to 1684 mg/l, and conductivity ranged from 5 to 1366 μs/cm (Table 1).

Mean total metal concentrations (As, Cd, Cr, Cu, Pb, Ni, and Zn) in the river samples for each individual metal were less than 3 μg/l. Other than the organophosphate

Table 1
Mortality of *Ceriodaphnia dubia* in Salinas River, creek, and drain samples

Date	Sample	*C. dubia* mort (%)	(S.D.)	Chlorpyr.	Diazinon	TUs
12/04/00	S1BANK	0.00	0.00	ND	0.038	0.11
	S1MID	0.00	0.00	ND	ND	0
	S2BANK	8.00	18.00	ND	0.136	0.4
	S2MID	0.00	0.00	ND	ND	0
	S3BANK	40.00	55.00	0.085	0.204	2.2
	S3MID	3.00	7.00	ND	ND	0
	S4BANK	3.00	7.00	0.068	0.098	1.59
	S4MID	9.00	12.00	0.057	0.080	1.31
	Creek	100.00	0.00	0.090	1.417	5.9
	Drain	100.00	0.00	0.278	3.188	9.37
15/05/00	S1BANK	0.00	0.00	ND	ND	0
	S1MID	0.00	0.00	ND	ND	0
	S2BANK	0.00	0.00	ND	0.168	0.49
	S2MID	0.00	0.00	ND	ND	0
	S3BANK	32.00	46.00	ND	ND	0
	S3MID	0.00	0.00	ND	ND	0
	S4BANK	0.00	0.00	ND	ND	0
	S4MID	4.00	9.00	ND	ND	0
	Creek	100.00	0.00	0.101	1.461	6.2
	Drain	100.00	0.00	0.234	0.108	4.72
05/09/00	S1BANK	0.00	0.00	ND	ND	0.11
	S1MID	0.00	0.00	ND	ND	0
	S2BANK	0.00	0.00	ND	ND	0
	S2MID	0.00	0.00	ND	ND	0
	S3BANK	0.00	0.00	ND	ND	0
	S3MID	0.00	0.00	ND	0.036	0.11
	S4BANK	3.00	7.00	ND	0.044	0.13
	S4MID	3.00	7.00	ND	0.046	0.14
	Creek	100.00	0.00	0.257	3.340	14.6
	Drain	NM	NM	NM	NM	NM
14/05/01	S1BANK	0.00	0.00	ND	ND	0
	S1MID	0.00	0.00	ND	ND	0
	S2BANK	0.00	0.00	ND	ND	0
	S2MID	0.00	0.00	ND	ND	0
	S3BANK	100.00	0.00	0.353	1.151	10.05
	S3MID	0.00	0.00	ND	ND	0
	S4BANK	100.00	0.00	0.085	0.558	3.25
	S4MID	0.00	0.00	ND	ND	0
	Creek/drain	100.00	0.00	0.609	1.66	16.4

Concentrations of chlorpyrifos and diazinon were measured by ELISA (in ug/L). TUs = combined TUs for chlorpyrifos and diazinon. ND = Not detected; NM = not measured due to lack of water.

pesticides chlorpyrifos and diazinon, the only trace organic compounds detected in river water were DDE, dieldrin and two herbicides, diuron and fenuron. DDE (maximum concentration = 0.0034 μg/l) and dieldrin (maximum concentration = 0.019 μg/l) were detected in many of the water samples collected in September 2000; all concentrations were well below published effect concentrations (Phipps et al., 1995).

Acute toxicity to *C. dubia* in this study reflected concentrations of chlorpyrifos and diazinon in samples. These pesticides were detected in all of the creek and drain samples; the combined toxic units (TU: 100/LC50) of chlorpyrifos and diazinon ranged from 4.7 to 16.4 in the creek and drain. All of these samples caused 100% *C. dubia* mortality (Table 1). *C. dubia* mortality in the river samples downstream of the agriculture inputs were associated with increased TUs of these pesticides, although minimal mortality was occasionally observed even when TUs exceeded 1 (e.g. Station 4 samples from April 2000). The highest *C. dubia* mortality in the river was observed in May 2001, when combined TUs in the Stations 3 and 4 bank samples were 10 and 3.2, respectively (Table 1). It should be noted that the creek and drain combined to form one drainage during this sample period, and as a consequence, both the flow rate and combined concentrations of diazinon and chlorpyrifos were the highest measured in this study.

C. dubia mortality was highly correlated with combined TUs of chlorpyrifos and diazinon in these samples (Table 2). Turbidity and TUs of these pesticides covaried in the bank samples, and *C. dubia* mortality in the bank samples was also significantly correlated with turbidity. There was also a significant correlation between combined TUs and *C. dubia* mortality in the mid-river (composite) samples; but turbidity did not covary with TUs in the mid-river samples, and *C. dubia* mortality was not correlated with turbidity (Table 2).

3.2. H. azteca *sediment toxicity test*

Sediment toxicity in this system was assessed in August 2001 when the creek and drain combined to form one tributary to the river. Survival of amphipods *H. azteca* were greater than 84% in all bank and mid-river sediment samples from the two stations above the drainage input (Stations 1 and 2). Survival and growth of *H. azteca* was significantly inhibited in sediment samples from the combined creek/drain, as well as in bank samples collected from Stations 3 and 4. Survival in the mid-river sample from Station 4 was also significantly lower. Porewater unionized ammonia ranged between 0.003 and 0.070; hardness ranged between 59 and 252 mg/l; conductivity ranged between 698 and 780 μs/cm; and alkalinity ranged between 120 and 297 mg/l, all within the ranges tolerated by this species. Stations with the lowest survival and growth of amphipods also had the highest concentrations of porewater and bulk-phase chemical contaminants (Table 3). Chlorpyrifos was 0.113 μg/l in porewater from the combined creek/drain, exceeding the 10-day LC50 for *Hyalella* (0.086 μg/l Phipps et al., 1995). The porewater chlorpyrifos concentration was 0.046 μg/L in the station 4 bank sample (~0.5 TU). Diazinon was detected in porewater from Stations 3 and 4 bank, and from the creek/drain; all concentrations were considerably less than the 10-day LC50 for *Hyalella* (6.51 μg/l; Phipps et al., 1995). All of the toxic samples also contained relatively high

Table 2
Spearman rank correlation coefficients for selected factors significantly correlated with *Ceriodaphnia dubia* survival in laboratory toxicity tests or with selected macroinvertebrate community metrics at creek and drain study sites ($n = 16$ toxicity; or 12 macroinvertebrates)

	Toxic units		Turbidity		Habitat		Bank Cover	
	rho	PCA	rho	PCA	rho	PCA	rho	PCA
Bank samples								
C. dubia survival	−0.940***	S	−0.777***	S		NA		NA
# Ephemeroptera taxa	−0.107		−0.422		0.548*		0.707**	S
Macroinvertebrate abundance	0.039		0.287		−0.340		−0.251	
Species richness	−0.299		−0.422		0.483*		0.843***	S
% Chironomidae	0.004	NA	0.288	NA	0.140	NA	−0.103	NA
Turbidity	0.709**	S				S		
Composite samples								
C. dubia survival	−0.665**	S	−0.290			NA		
# Ephemeroptera taxa	0.189		−0.594*	S	0.602*	S		
Macroinvertebrate abundance	−0.542*		0.007		−0.081	S		
Species richness	0.174		−0.671**	S	0.680**	S		
% Chironomidae	−0.009		0.396	NA	−0.018	NA		
Turbidity	−0.110			S				

Factors identified by principal components analyses (PCA) as correlating with *C. dubia* survival or with selected macroinvertebrate community metrics are denoted by "S" (= significant component loading >0.45). See text for description of "bank" and "composite" samples. NA = not analyzed in PCA.

* $P = 0.05$.
** $P = 0.01$.
*** $P = 0.001$.

Table 3
Results of August 2001 sediment toxicity test with amphipods *Hyalella azteca*, and chemical analysis of sediment porewater (PW, ug/l) and solid-phase (SP) sediment (trace organic concentrations are in ug/kg dry wt. and trace elements are in mg/kg dry wt.)

Station Name	*H. azteca* Surv.	*H. azteca* Wt	Elisa Chlor PW	Elisa Diaz PW	Carbofur. SP	Methomyl SP	Propham SP	Propoxur SP	TDDT SP	Dieldrin SP	Endosulf SP	Ni SP	% Coarse	% Fines	% TOC
Station 1 BANK	84	0.2358	ND	ND	ND	ND	ND	ND	ND	ND	ND	20.4	90	10	ND
Station 1 MID	89	0.2215	ND	ND	ND	ND	ND	ND	ND	ND	ND	24.2	92	8	ND
Station 2 BANK	86	0.2538	ND	ND	ND	ND	ND	ND	ND	ND	ND	22.9	92	8	0.2
Station 2 MID	86	0.258	ND	ND	ND	ND	ND	ND	ND	ND	ND	26.8	91	9	0.1
Station 3 BANK	66*	0.1884*	ND	0.46	968.2	140.1	ND	802.5	ND	ND	ND	19.1	75	25	0.9
Station 3 MID	80	0.2113	ND	ND	ND	ND	ND	ND	7.6	ND	ND	25.5	80	20	ND
Station 4 BANK	49*	0.1286*	0.046	0.41	1528.7	140.1	254.8	1235.7	4.6	ND	ND	24.2	62	38	1.5
Station 4 MID	70*	0.2331	ND	ND	1656.1	178.3	789.8	2038.2	ND	ND	ND	30.6	30	70	1.1
Creek/Drain	4*	0.1333*	0.113	0.048	ND	ND	ND	ND	91.7	5.2	2.5	35.7	17	83	0.5
Control	88	0.1944													

Values with asterisk are significantly lower from the control value at $P = 0.05$. TDDT Threshold Effect Concentration (TEC) = 5.28 g/kg; TDDT Probable Effect Concentration (PEC) = 572 g/kg. Dieldrin TEC = 1.9; PEC = 61.8 g/kg. Nickel TEC = 22.7; PEC = 48.6 mg/kg, (after MacDonald et al., 2000). PW = porewater; SP = solid-phase.

concentrations of the carbamate pesticides carbofuran, methomyl, and propoxur; no sediment quality guideline values exist for these pesticides (Table 3). In addition to the herbicides barban and propham, solid-phase samples from some of the downstream stations contained detectable concentrations of DDT and dieldrin. Concentrations of DDT and dieldrin from the creek/drain sediment exceeded their respective Threshold effect concentrations (TEC) but not their Probable effect concentrations (PEC; MacDonald et al., 2000). Relatively low concentrations of trace elements were measured in sediments from these stations. Only nickel exceeded the TEC; the Ni concentrations ranged between 19.1 and 35.7 mg/kg dry wt. All other metals were below the TEC values published by MacDonald et al. (2000).

3.3. Macroinvertebrate community structure

Although there was temporal and spatial variability in macroinvertebrate densities, there were sometimes measureable differences in macroinvertebrate community structure between station 1 and the downstream stations. The most obvious difference was in abundance of macroinvertebrates in the bank samples from Station 3 relative to Station 1. The average abundances of

macroinvertebrates were approximately 40% lower at Station 3 bank than at Station 1 (Table 4). In addition, lower numbers of ephemeroptera taxa were measured at Station 3 bank relative to Station 1 bank, particularly in April and September 2000. There were no large differences in species richness or the percentage of chironomidae between the upstream and downstream stations (Table 4). Similar patterns were observed in the composite sample data; lower abundances of macroinvertebrates were sometimes observed at Stations 3 and 4, particularly in April and May 2000. There were no large differences in species richness or the percentage of chironomidae in composite samples from Station 1 and the downstream stations (Table 4).

Numbers of daphnidae and *Hyalella* sp. were quantified separately to evaluate relationships between laboratory toxicity test results and taxonomically related components of the macroinvertebrate community. In September 2000, when there were relatively large densities of *Hyalella* in the bank samples from Station 1, few animals were found in any of the bank samples from the downstream stations (Table 4). In May 2001, considerably lower numbers of daphnidae were collected in the bank samples from Stations 3 and 4 relative to Station 1.

Principal components analysis (PCA) of the bank sample ecological data indicated that the majority of variance (93%) was explained by a three factors. Factor 1 (40% of the variance) contained highly significant negative loading for combined Toxic Units of diazinon and chlorpyrifos and turbidity, and *Ceriodaphnia* survival. Factor 1 also contained significant loading for the habitat score calculated from the California Department of Fish and Game Rapid Bioassessment Procedures. Factor 2 (25% of the variance) contained highly significant loading for macroinvertebrate abundance and the number of ephemeroptera taxa. Factor 3 (23% of the variance) contained highly significant loading for species richness and bank cover, a subcomponent of the habitat score.

PCA of the composite sample ecological data indicated that the majority of variance (79%) was explained by two factors. Factor 1 (44% of variance) contained highly significant loading for ephemeroptera taxa, species richness, and habitat score. This factor also contained significant negative loading for turbidity and macroinvertebrate abundance. Factor 2 (35% of variance) contained highly significant loading for *Ceriodaphnia* survival, and negative loading for combined TUs.

4. Discussion

The creek that is the subject of this study originates as an ephemeral stream in the Gabilan Range on the eastern border of the Salinas Valley. Although it carries some natural water flow during the wettest winter

Table 4
Summary of benthic macroinvertebrate community characterizations in bank and composite samples (see text for description of "bank" and "composite" samples)

	A. Bank samples				B. Composite samples			
	Station 1	Station 2	Station 3	Station 4	Station 1	Station 2	Station 3	Station 4
April 2000								
#Ephem taxa	6.3	2	3.3	5	6.3	6.3	5	4.7
Richness	16.3	10.3	15	19.7	17.3	16.7	15.3	13.7
Abundance	515	129.3	191.3	459.7	610.7	561.7	322.3	272
#Daphnids	0	0	0.3	0	0	0	0	0
# Hyalella	0.3	0	0.3	0.3	0	0	0.3	0.3
%Chironomidae	36	39	54	51	25	46	33	37
September 2000								
#Ephem taxa	5.7	4.7	2	2.7	5.3	5.7	5.7	6
Richness	20	22.3	15	16.7	20.7	24.3	23.7	22.7
Abundance	395.7	459	158.3	114.3	1092.7	662	516	644.7
#Daphnids	0.7	0.3	0	1.3	0	0.7	0.3	0
# Hyalella	25.7	1.3	0.3	1	5.7	4.3	3	3
%Chironomidae	24	26	15	13	26	14	22	24
May 2001								
#Ephem taxa	0	1	0.3	0	2.3	0.7	1	1
Richness	11.3	14.3	11.7	10.3	18.7	11	14	10.7
Abundance	3464.3	3393	1629	4314.3	4025.7	7447.7	3193	4446.3
#Daphnids	21	15	3	0	1.3	0.7	0.7	0
# Hyalella	0	0	0	0	0	0	0	0
%Chironomidae	29	36	23	16	30	31	25	13

months, headwater flows go underground above the study area. Flow in the lower portion of this creek is dominated by agricultural drainwater. Similarly, flow in the agricultural drain that enters the river near its confluence with the creek is also dominated by irrigation drainwater most of the year. The current investigation was part of a larger study designed to consider the ecotoxicologic impacts of agricultural drainwaters on certain components of the aquatic ecosystem in the Salinas River (Hunt et al., in press; Anderson et al., in press; Phillips et al., submitted for publication). As in these previous studies, the current study demonstrates that toxic concentrations of pesticides in drainwaters are entering the Salinas River.

Tests with the cladoceran *C. dubia* showed that some river water samples downstream of the creek and drain inputs were acutely toxic, particularly in May 2001 when the drainages combined into one flow. Correlation analyses suggested that toxicity to *C. dubia* was correlated with combined TUs of chlorpyrifos and diazinon, and with turbidity in the bank samples. Correlations between *Ceriodaphnia* mortality in laboratory exposures and turbidity measured in bank samples is due to the correlation of turbidity and TUs in the field samples. Laboratory toxicity was measured on samples that were allowed to settle for 24-h before being filtered and decanted for testing, thus effectively removing the majority of particles in the samples and negating turbidity effects in the laboratory *C. dubia* tests. Toxicity usually occurred when the 96-h LC50 values for chlorpyrifos or diazinon were exceeded. It is possible that the process for removing particles also lowered concentrations particle-associated chemicals such as higher log K_{ow} organochlorine pesticides. Because this may have reduced the concentrations of these chemicals, toxicity of these samples could have been greater without the settling and filtration procedures.

Patterns of toxicity to *Ceriodaphnia* in this study were similar to those observed in a concurrent study of an adjacent watershed. In that study, Anderson et al. (in press) also found greater toxicity to *Ceriodaphnia* in bank samples collected immediately downstream of a drainwater input, and toxicity was highly correlated with combined TUs of chlorpyrifos and diazinon. Although no Toxicity Identification Evaluations (TIEs) were conducted in the current study, TIE evidence from the other drainwater study suggested that in situ toxicity in the river was due primarily to chlorpyrifos, and that turbidity was not a cause of toxicity (Phillips et al., submitted for publication). This confirmed extensive TIE evidence reported by Hunt et al. (in press), who also found that toxicity of drainwater to cladocera was caused by chlorpyrifos. In the current study, the magnitude and spatial extent of *Ceriodaphnia* toxicity in the Salinas River was less than that reported by Anderson et al. (in press) for the adjacent watershed, presumably because of differences in discharge volume. Although the volume of water in the creek and drain were not characterized in the current study, our investigations in the adjacent watershed showed that drainwater volumes entering the river were sometimes comparable to the volume of the river itself (Phillips et al., unpublished data). Our observations in the current study were that creek and drain flow volumes in this system were lower, except when the creek and drain were combined in May 2001. Therefore, although the diazinon and chlorpyrifos concentrations in the creek and drain generally exceeded their respective thresholds for toxicity to *Ceriodaphnia*, the lower volumes in these drainages resulted in less toxicity in the river than was reported in Anderson et al. (in press).

Our results using 96-h acute toxicity tests with *C. dubia* may have underestimated the magnitude and spatial extent of toxicity in this system. The 7-day LC50 for diazinon toxicity to *C. dubia* is 110 ng/l (duFresne and Mikita, 1993); the 7-d LC50 for chlorpyrifos toxicity to this species is 20 ng/l (Menconi and Paul, 1994). Concentrations of both of these chemicals exceeded these values during the course of this study. Underestimation of the spatial extent of toxicity in this study is suggested in data from the May 2001 toxicity tests when complete mortality was observed at all stations downstream of the combined drain input.

Sediments in the drain and in the Salinas River were significantly toxic to the resident amphipod species *Hyalella azteca* in laboratory exposures. All bank samples downstream of the drain input were toxic to amphipods, so the horizontal (downstream) extent of sediment toxicity cannot be defined (Table 3). Sediment toxicity occurred in samples containing the greatest porewater and bulk-phase organic chemical concentrations (Table 3). The chlorpyrifos concentration in drain porewater exceeded the 10-day LC50 for *H. azteca*, and lower concentrations of OP pesticides were measured in the other toxic samples. However, relatively high concentrations of carbamate pesticides were also measured in all of the toxic samples. Like the OPs chlorpyrifos and diazinon, the carbamates methomyl, carbofuran, and propoxur are acetylchloinesterase inhibitors, and thus would presumably act additively with the OPs. Sediment quality guideline values have not been calculated for carbamates, so we cannot determine whether the concentrations measured in these samples were sufficient to account for the observed effects. Carbamates are relatively water soluble pesticides (Ware, 1989) and K_{ow} values calculated for these compounds are lower than those for both diazinon and chlorpyrifos (Jarvinen and Ankley, 1999). Therefore, we would expect significant sediment partitioning of these compounds to the porewater phase at these stations. Concentrations of other trace organic and trace element contaminants were probably not sufficient to account for toxicity to

Hyalella. It should be noted, however, that the trace organic analyte list in the current study was limited to organophosphate, organochlorine, and carbamate pesticides, and that other compounds could have been present in these sediment samples. For example, sediment TIE evidence reported by Anderson et al. (in press) suggested that Salinas River sediment samples impacted by the adjacent agricultural drain contained compounds whose toxicity to *Hyalella* was potentiated by the addition of the metabolic inhibitor piperonyl butoxide (PBO). Because PBO inhibits a key metabolic pathway, previous studies have suggested this is an indicator of toxicity due to non-metabolocally activated chemicals such as pyrethroids pesticides (Kakko et al., 2000). This evidence, combined with pesticide use data showing extensive use of pyrethroid pesticides in the Salinas Valley, suggests that we should consider the role of this class of pesticides in future sediment toxicity studies in the Salinas River.

Evidence from bioassessment surveys in the Salinas River suggests that certain components of the macroinvertebrate community are impacted by agricultural drainwater. Macroinvertebrate abundances were sometimes lower in the downstream bank stations relative to station 1, and the number of ephemeroptera taxa were sometimes lower at station 3 bank, the station nearest the drain input. Although the stations with the lowest macroinvertebrate abundances were also those that demonstrated the greatest toxicity and had the greatest pesticide contamination, multivariate analyses indicated that macroinverebrate abundances were not significantly correlated with toxicity or pesticide concentrations (represented as combined TUs). Species richness and the number of ephemeroptera taxa was associated with macrophyte cover in the bank samples, but was not significantly correlated with combined TUs and turbidity. Other than the total habitat score and macrophyte cover in the bank samples, no additional subcomponents of habitat quality were considered in the correlation analyses. However, it is unlikely that habitat characteristics such as riffle frequency, epifaunal substrate, or sediment deposition influenced macroinvertebrate community differences between stations because the Salinas River is heavily impacted by sedimentation throughout this study area. There were no differences between the upstream reference station and the downstream stations in terms of benthic habitat characteristics.

There is insufficient evidence to allow us to separate the relative effects of habitat, TUs, and turbidity on the observed macroinvertebrate community impacts. There are, however, several lines of evidence to suggest that contaminants associated with agriculture drainwater have the potential to impact river macroinvertebrates at this site. First, as discussed above, *Ceriodaphnia* mortality was likely not due to turbidity. Second, previous research has suggested that acute toxicity to this species is a reliable predictor of instream biological responses (see review by de Vlaming and Norberg-King, 1999). May 2001 was the only sampling survey when large numbers of daphnidae were observed in the River, and, relative to Station 1, there were significantly fewer daphnids in Stations 3 and 4 bank samples at this time. A third line of evidence involves sediment toxicity to the amphipod *H. azteca*. Use of *Hyalella* is particularly appropriate for assessing the potential for in-river biological impacts because this genus is a resident of this system. September 2000 was the only survey period when high densities of *Hyalella* were observed in the River; and relative to station 1, there were significantly fewer amphipods at all downstream bank stations at this time. Finally, our results showed that toxicity to *C. dubia* and *H. azteca* occurred in samples that contained the greatest pesticide concentrations (Tables 1 and 3) and these stations also had the greatest declines in macroinvertebrate abundances. It is possible that the lack of significant correlations between pesticides and macroinvertebrate metrics was due to the relatively small sample size. It is also likely that several stressors (i.e. pesticides, turbidity, macrophyte variability) interacted to influence macoinvertebrate communities in this system.

The agricultural creek and drain in this study were similar to other drainages in the Salinas Valley in that flow in these channels is dominated by irrigation runoff, and was consistently contaminated by toxic concentrations of organophosphate pesticides. In terms of combined TUs of chlorpyrifos and diazinon, concentrations in these drains were somewhat lower than those reported for other irrigation channels. In the study of a similar system downstream of the current study area, the mean and range of combined TUs were 16.5 and 1.0–48.5 TUs (Anderson et al., in press). The mean and range of combined TUs in the current study were 10.4 and 4.7–16.4, respectively. As discussed earlier, the differences between the magnitude of impacts reported by Anderson et al. (in press) and those observed in the current study may be due to lower volumes from the agricultural drains investigated in the current study.

5. Conclusions/future studies

This study demonstrates the utility of combining laboratory toxicity tests, chemical analyses, and bioassessments, with measures of relevant physical and abiotic factors to investigate potential ecological impacts in a semi-arid river system receiving agricultural inputs. The experimental design allowed for assessments of spatial and temporal variability in effects, and followed the general principles recently outlined for tiered, weight-of-evidence approaches to investigating effects of

stressors on aquatic ecosystems (e.g. US EPA, 2000; Burton et al., 2000). Results of this study suggest that toxic concentrations of pesticides are entering the river, and that agricultural drainwater inputs are impacting the macroinvertebrate community in the system. Drainwater may be interacting with other factors such as habitat and turbidity to affect the river ecosystem. Future work will be designed to investigate causes of sediment toxicity to *H. azteca* using TIEs and expanded chemical analyses. More extensive surveys will be conducted to investigate the spatial extent of sediment toxicity associated with selected agricultural drains. Relative impacts of pesticides and physical factors (e.g. turbidity) on resident macroinvertebrates will be assessed using laboratory dose–response experiments with selected resident species.

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