ELSEVIER

Agriculture, Ecosystems and Environment 75 (1999) 75–91

Agriculture Ecosystems & Environment

Patterns of aquatic toxicity in an agriculturally dominated coastal watershed in California

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Received 15 April 1998; received in revised form 12 January 1999; accepted 25 March 1999

Abstract

This study was designed to investigate the occurrence, severity, sources and causes of aquatic toxicity in a coastal river and estuary subject to non-point source pollutant inputs from adjacent agricultural and urban areas. The Pajaro River estuarine system on the central coast of California, USA, receives subsurface tile drain runoff from irrigated cropland, and seasonal surface runoff from agricultural, urban, industrial, and residential areas. Seven sites in the estuary, upstream river, tributary sloughs, and agricultural drainage ditches were selected to identify tributaries that might contribute toxic runoff to the estuary. These sites were each sampled 18 times over an 18-month period, and water samples were tested for toxicity to the mysid *Neomysis mercedis*, a resident crustacean. Results indicated toxicity in 78% of agricultural ditch samples, 25% of tributary slough samples, and 11% of river and estuary samples. Temporal patterns in the occurrence of toxicity indicated that agricultural ditches and upper river were more important than the freshwater sloughs as sources of toxic runoff to the estuary. Chemical analyses were conducted on samples collected at each site on two occasions. Organophosphate pesticides were detected in samples collected when the river flow rate was low, and persistent hydrophobic organochlorine pesticides were detected after high surface runoff. Three pesticides (toxaphene, DDT, and diazinon) were found at concentrations higher than published toxicity thresholds for resident aquatic species. Toxicity in the estuary was significantly correlated with increased river flow. Chemical causes of toxicity were investigated in two preliminary and four full Phase I Toxicity Identification Evaluations (TIEs) on six separate samples from the agricultural drainage ditches receiving tile drain discharges. The TIE results indicated that multiple compounds were responsible for toxicity in all samples evaluated, and that non-polar and perhaps polar organic compounds were present in toxic concentrations.

Keywords: Pesticides; Erosion; Runoff; *Neomysis mercedis*; Pajaro River; Estuary

1. Introduction

The Pajaro River is one of many coastal river systems in California, USA, that have been highly modified by human activity. Statewide, 89% of ripar-

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0167-8809/99/$ – see front matter
PII: S0167-8809(99)00065-1

ian woodland has been lost since European settlement (Kreissman, 1991), with 80% of coastal wetlands converted to urban or agricultural uses (Jensen et al., 1990; Barbour et al., 1991; Kreissman, 1991). As with many coastal rivers throughout the state, the lower river and adjacent coastal lagoon form a small estuary (approximately 350 ha), which is connected with the ocean predominantly during times of high river flow, when the coastal sand berm is breached. Riparian woodland and saltmarsh vegetation provide important foraging, roosting and breeding habitat for many water bird species, including the endangered brown pelican (*Pelecanus occidentalis californicus*), threatened snowy plover (*Charadrius alexandrinus*), herons (e.g., *Ardea herodias*), egrets (e.g., *Egretta thula*), and many duck and shorebird species (Swanson, 1993). The lower river and lagoon provide a migration corridor for the steelhead *Onchorhynchus mykiss* (Swanson and Lyons, 1991), an anadromous sport fish that is under consideration for protected status. Other resident fish species of economic or ecological importance include starry flounder (*Platichthys stellatus*), Pacific herring (*Clupea harengus*), topsmelt (*Atherinops affinis*), northern anchovy (*Engraulis mordax*), shiner perch (*Cymatogaster aggregata*), striped bass (*Marone saxatilis*) and tidewater goby (*Eucyclogobius newberryi*). Resident estuarine invertebrates include amphipods (genus *Corophium* and *Eogammarus*), the mud crab (*Hemigrapsus oregonensis*), and the estuarine mysid (*Neomysis mercedis*), which is an important prey item for fish (Swanson, 1993) and which was used as the toxicity indicator species in this study.

Agricultural and urban development have altered the physical and biological setting through grading, paving, flood control, and drainage activities; and the river and estuary receive runoff from urban, residential and agricultural areas. Unusual geologic features affect pollutant transport within the 3400 km^2 Pajaro River watershed. The watershed is divided along the San Andreas Fault at Chittendon Gap, and the upper watershed began draining through the lower river basin only after the two were aligned by plate movement in the recent geologic past (Gordon, 1987). Since the upper watershed is approximately 10 times larger than that which originally formed the lower river, the lower basin is subject to frequent flooding. In addition, water drainage on the coastal flood plain is impeded by subsurface clay layers. Prior to human modification, water tended to pond in wetlands and sloughs. Now, year-round agriculture is made possible by a system of levees that contain high river flows, and a system of subsurface perforated pipes (tile drains) that drain irrigated fields. An estimated 76% of the entire watershed is in rangeland (2048 km^2) or irrigated agriculture (529 km^2). In the lower river basin surrounding the study area, irrigated agriculture covers 47% (45 km^2) of the land surface, with nearly all irrigation water conveyed through sprinkler or drip systems. The remainder of the surrounding land supports a rapidly growing urban area with numerous industrial activities. During high rainfall events, local urban flooding is managed by 14 major pumping stations that deliver untreated urban storm water through levees directly into the river and tributaries.

Approximately 5.7 million kilograms of agricultural pesticides were applied in the watershed in 1994, the year in which this study was initiated, including over 3.6 million kilograms of synthetic organic pesticides (DPR, 1997). These compounds may enter waterways through pumping of subsurface tile drains following irrigation or rainfall, through surface furrow runoff following heavy rainfall (greater than approximately 5 cm in 48 h), and through erosion of contaminant-laden soil particles transported by surface water flow.

Previous studies have detected elevated levels of anthropogenic chemicals in the lower river, lagoon, and major tributaries. The California State Mussel Watch program has transplanted clams into waters of the lagoon and tributary sloughs, and has measured numerous pesticides at tissue concentrations greater than those seen in 95% of the samples collected statewide over the past 20 years (Phillips, 1988; Rasmussen, 1994). Previous analyses of water samples from the lower river, lagoon, and tributary sloughs have detected a number of pesticides and trace metals in the water column at concentrations greater than or equal to concentrations previously shown to be toxic to resident aquatic organisms or similar species (Questa Engineering Corporation, 1995). Previous investigators have speculated that observed fish and invertebrate kills in the estuary may have been due to pulses of toxic runoff (Swanson, 1993). To our knowledge, the toxicity of this runoff had not been measured prior to the current study.

This study was designed to determine whether anthropogenic contaminants entering the estuary system were likely to be associated with adverse impacts to resident aquatic organisms. The specific objectives of the study were: (1) to investigate the occurrence, severity, and temporal patterns of toxicity in the Pajaro River and its coastal lagoon; (2) to identify tributaries that may be potential sources of toxic runoff to the river and lagoon; and (3) to investigate the potential causes of observed toxicity using chemical analyses and Phase I Toxicity Identification Evaluations (TIEs).

2. Materials and methods

2.1. Study design

During the one-and-a-half year study, seven sites in the estuary and its main tributaries were each sampled 18 times, and each sample was tested for toxicity to the resident mysid crustacean *Neomysis mercedis* in 96 h exposures. Samples were collected primarily during the rainy season (November–April). In the first set of samples, concentrations of the commonly applied pesticides diazinon and chlorpyrifos were measured using enzyme-linked immunosorbent assays (ELISA). In the second and third surveys, samples from each site were screened for 74 pesticides using US Environmental Protection Agency (EPA) analytical methods (USEPA, 1982, 1994a, 1994b). Samples collected during later surveys were fractionated in TIEs to determine classes of compounds associated with observed toxicity.

2.2. Sample collection

Toxicity in the estuary and in five tributaries was characterized by sampling the following sites (Fig. 1). Site RRB was located in the main river upstream of the estuary, immediately downstream of the City of Watsonville. The river flow at this site varies greatly, from <0.01 to $>100\,m^3\,sec^{-1}$. Samples from site MDD were collected from the larger of two agricultural drainage ditches that drain fields on the southern side of the river (Monterey County). Site PRD was located in the lower river estuary 1.9 km from the river mouth at Monterey Bay, and 100 m downstream from the MDD drainage ditch. Site PRL was in an arm of the estuary 1.8 km from the confluence with the Pajaro River. Site BSD was located just above tide gates at the mouth of the ditch that drains Santa Cruz County agricultural and urban runoff into the estuary near site PRL. Site WAS was in the Watsonville Slough, here channeled into a ditch that drains a system of numerous year-round wetlands and agricultural fields. Site HAS was at the mouth of Harkin Slough, which receives inputs from upland agriculture and grazing land. Two of these sites, PRD and PRL, were in the estuary and were of primary concern for wildlife protection, while the other five sites were at the mouths of tributaries to the estuary. Tide gates just downstream of sites MDD, BSD, and WAS minimized backflow of brackish water from the estuary during incoming tides, and promoted unidirectional flow from the tributaries to the estuary.

Samples were collected 1 m from shore during outgoing tides using pre-cleaned 1 l amber glass bottles attached to the end of a PVC pole. Bottles were immersed just below the water surface to collect surface water but to avoid entrainment of the surface microlayer. Samples were transported and stored in the dark at $4 \pm 3^{\circ}C$. Sixteen of 18 sets of samples were tested for toxicity within 48 h of collection; two sets, collected on 12/13/94 and 1/27/95, were tested within 96 h of collection.

2.3. Toxicity testing

Toxicity was determined by exposing mysid crustaceans to each sample for 96 h and recording mortality at the end of the exposure. Of the 126 samples analyzed, 122 were tested with the resident estuarine mysid *N. mercedis*. Four samples had salinity greater than $10\,g\,kg^{-1}$; these were adjusted to $34\,g\,kg^{-1}$ with hypersaline brine so that they could be tested with the marine mysid *Holmesimysis costata*, which occurs in the kelp forests of adjacent Monterey Bay.

Juvenile mysids were obtained after laboratory release from field-collected gravid females. Ten 2–5 day-old juveniles were randomly assigned to each of five replicate glass beakers holding 400 ml of sample, except that three replicates were tested for samples collected on 12/13/94, 3/5/96, and 4/15/96. Test beakers were arranged randomly in a constant temperature room at $17^{\circ}C$, test solutions were renewed after

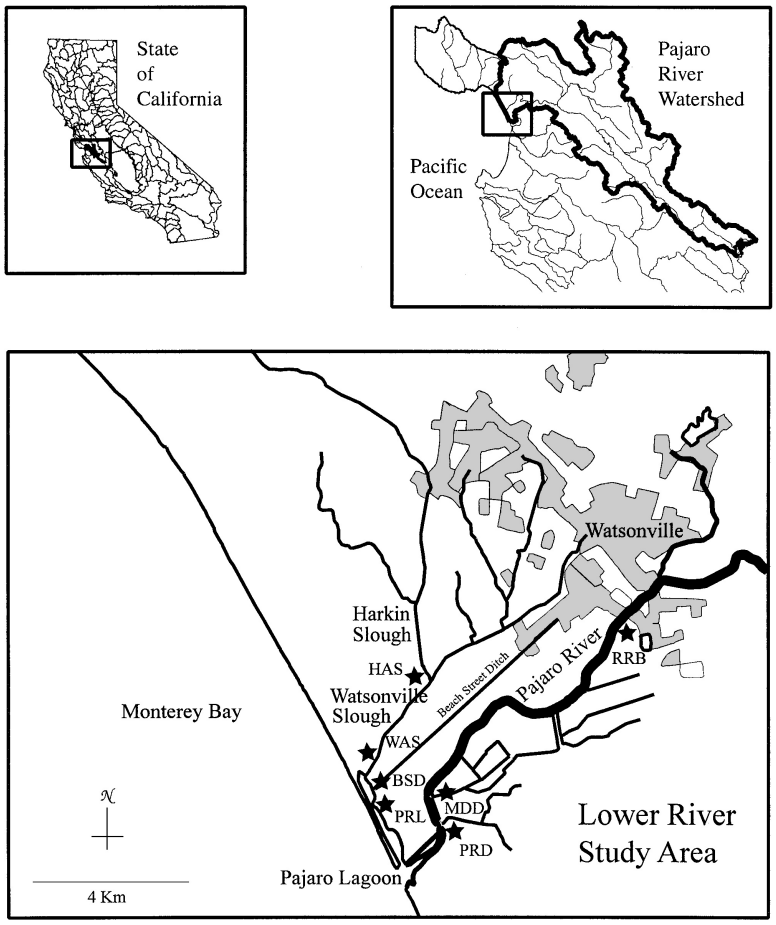

Fig. 1. Map of the study area. The State of California is shown for general location. Heavy line in second inset is the boundary of the Pajaro River watershed, with the rectangle showing the location of the study area surrounding the lower river. The Chittendon Gap, which divides the upper and lower watersheds, is at the eastern edge of the rectangle. Largest map shows the locations of study sites in the river, estuary, and main lower river tributaries. Shaded area is the approximate residential and industrial area of the City of Watsonville.

48 h, and mysids were fed 45 newly hatched *Artemia* nauplii per mysid twice daily. Four samples (estuary sites PRL and PRD in September and December, 1995) were tested with *H. costata* according to methods described by Hunt et al. (1997). These were 96 h mortality tests similar to those using *N. mercedis*.

Data quality was determined through concurrent testing of duplicate samples, high and low conductivity controls, and reference toxicant dilution series. The average variation in mysid survival among duplicate samples was 20% (average coefficient of variation; $n = 17$). High and low conductivity controls, bracketing the range measured in test samples, were prepared by adding seawater to untreated well water. *N. mercedis* control survival ranged from 75 to 100%, and was greater than 80% in 33 of 36 controls tested. Control survival for *H. costata* was always 100%. Mysids exposed at least monthly to concentrations of a reference toxicant (potassium chloride) responded similarly throughout the study, with median lethal concentration (LC50) values ranging from 124 to 157 mg l^{-1} KCl (CV = 8.8%). Water quality parameters of dissolved oxygen, pH, conductivity, hardness, total dissolved solids, and ammonia were measured in each sample at the beginning and end of each test, and at each renewal. Test temperature was recorded continuously. All test temperatures were within the range of $17 \pm 2^{\circ}$C, and measured water quality parameters did

not exceed levels expected to adversely affect the test mysids.

2.4. Chemical analysis

Enzyme-linked immunosorbent assays (ELISAs) for the pesticides diazinon and chlorpyrifos were conducted according to manufacturer's specifications (Millipore Corporation, Bedford, MA). Two replicates of each sample were analyzed, and all duplicate measurements had coefficients of variation less than 15%. However, one sample (BSD) was tested twice at full strength, found to have concentrations above the range of the test kit, and was then retested once after dilution to 33%. Measurements were compared to a three point standard curve representing the low, middle, and high concentration range of the chemical of interest. The minimum detection limits were $30\,ng\,l^{-1}$ for diazinon and $80\,ng\,l^{-1}$ for chlorpyrifos.

Organochlorine, organophosphate, and carbamate pesticides were measured using EPA methods 8080, 8140, and 632, respectively, in all samples collected on 12/13/94, and 1/27/95, and in RRB and MDD samples collected on 2/13/95. All samples were measured without prior filtration, resulting in determinations of total, rather than dissolved, pesticide concentrations. Twenty two organochlorine compounds were measured using electron capture; quantitation limits for 12/13/94 samples were $0.1\,\mu g\,l^{-1}$ for all except toxaphene ($0.2\,\mu g\,l^{-1}$); quantitation limits for 1/27/95 and 2/13/95 samples were $0.05\,\mu g\,l^{-1}$ for all except toxaphene ($1.0\,\mu g\,l^{-1}$). Twenty seven organophosphate compounds were measured using a nitrogen phosphorus specific detector with quantitation limits of $0.1–1.0\,\mu g\,l^{-1}$. Twenty five carbamates were measured using dual detection with UV visual mode and LCMS confirmation, with quantitation limits of $0.07–3.5\,\mu g\,l^{-1}$. Measurement accuracy and precision were estimated by analysis of matrix blanks and spikes of 16 different analytes. No target analytes were detected in any laboratory blanks. Analyte recovery in spiked samples ranged from 71 to 110% for organochlorines, 48 to 111% for organophosphates, and 58 to 105% for carbamates.

2.5. Rainfall, river flow, and total suspended solids

The rainfall gauge was located adjacent to the Pajaro River near the center of the study area. River flow and total suspended solids data were obtained from the United States Geological Survey data base (USGS, 1995). River flow in the lower river study area was estimated by adding flow measurements from the Pajaro River (at Chittendon Gap) and Corralitos Creek, the largest lower river tributary. The Chittendon Gap gauging station is approximately 15 km upstream of study site RRB, and the Corralitos Creek gauging station is approximately 10 km upstream from its confluence with the Pajaro river, 1 km upstream of site RRB. Total suspended solids were measured at the Chittendon Gap station.

2.6. Toxicity identification evaluations (TIEs)

Phase I TIEs were designed to characterize samples by isolating broad classes of compounds to determine their relationship to observed toxicity. The Phase I TIE procedures included dilution, adjustment of sample pH, EDTA chelation of cationic compounds (including many trace metals; Hockett and Mount, 1996), neutralization of oxidants (such as chlorine) with sodium thiosulfate, aeration to remove volatile compounds, inactivation of metabolically activated toxicants by addition of piperonyl butoxide (PBO), solid-phase extraction (SPE) of non-polar organic compounds on C-8 columns, and subsequent elution of extracted compounds. Volumes were adjusted so that eluates from SPE columns contained 2.5 times the concentration of non-polar organic compounds as did the original sample ($2.5\times$ addback). Each sample fraction, in which classes of compounds had been removed, inactivated, or isolated, was then tested for toxicity to the mysids. TIE procedures followed methods described by Mount and Anderson-Carnahan (1988), with minor refinements and modifications described by Bailey et al. (1996). All TIE treatments were tested with the 96 h *N. mercedis* test, as described above, except that each treatment solution (sample fraction) was divided into 12 replicate 30 ml glass scintillation vials (20 ml of solution), with one mysid placed in each. All treatments were tested at full strength; there were no dilutions.

Two preliminary TIEs were conducted on samples from sites BSD and MDD collected on 5/17/95. Both samples were tested only with solid-phase extraction, C-8 column eluate, and piperonyl butoxide (PBO)

treatments. Four complete Phase I TIEs were conducted on BSD and MDD samples collected on the following dates: BSD 3/5/96, MDD 2/13/96, MDD 3/25/96, and MDD 4/15/96. These sites were selected because they were the most consistently toxic during the TIE phase of the study. Because of funding and logistical constraints, chemical analyses were conducted at the beginning of the study, and TIEs were conducted later; no sample was the subject of both types of analysis. TIEs were always conducted within 48 h of sample collection.

2.7. Statistics

Significant sample toxicity was determined by comparison to laboratory controls using separate variance *t*-tests (alpha = 0.05), with degrees of freedom adjusted to account for variance heterogeneity (Sokal and Rohlf, 1981). Each comparison was considered to be independent from all others, an assumption that has undergone critical review in large monitoring programs (e.g. Schimmel et al., 1994). Samples were compared to either low or high conductivity controls, depending on which had the closer conductivity. All percent survival data were transformed to the arcsine square root prior to analysis.

In addition to the individual comparisons, a toxicity threshold was set at 72% of control survival. This value was determined using the 'detectable difference' method of Thursby et al. (1997), which calculates the minimum difference between sample and control that can be detected as statistically significant in 90% of the comparisons made using data from a specific test protocol, in this case, the *N. mercedis* 96 h mortality test. This is equivalent to setting the level of statistical power at 0.90 for all comparisons.

Correlations between toxicity and river flow, rainfall, sample conductivity and hardness were analyzed with non-parametric Spearman Rank correlations, with Rho values corrected for ties (alpha = 0.05). Total suspended solids data were likewise tested for correlation with river flow. All correlations were conducted using data from individual sites ($n = 18$ samples per site), and correlations with conductivity and hardness were also analyzed using data from all sites combined ($n = 126$). No inferential statistics were applied to chemistry or TIE data.

3. Results

3.1. Patterns in the occurrence of toxicity

Of the five sites in tributaries to the estuary, the agricultural drainage ditches (MDD and BSD) were the most consistently toxic; mysid survival was significantly affected in 89% of the MDD samples and 56% of the BSD samples (Figs. 2 and 3). The two sloughs produced toxic samples less often: 33% of Watsonville Slough (WAS) samples and 17% of Harkin Slough (HAS) samples produced significant mortality (Fig. 3). The upstream river site (RRB) and the two estuary sites (PRD and PRL) were toxic 11, 6 and 17% of the time, respectively.

Temporal patterns in the occurrence of toxicity were similar between the upstream river site and the downstream PRD estuary site, though the magnitude of toxicity varied (Fig. 2). In winter of 1994/95, mysid survival was lower in the downstream site (PRD), while in winter of 1995/96, the upstream site (RRB) was more toxic. The drainage ditch leading to the estuary (MDD) was always highly toxic when toxicity was observed in the estuary. At the two slough sites, the temporal toxicity patterns were nearly identical (Fig. 3), with both showing greatest toxicity in November 1994 and August 1995. This pattern, however, was distinct from that observed in the downstream estuary site (PRL) that received runoff from the sloughs. The PRL site also received runoff from the BSD agricultural drainage ditch, and on each of the three occasions that toxicity was observed at the estuary site, samples from the drainage ditch were more toxic (Fig. 3).

Samples from the estuary were toxic following periods of high rainfall and river flow (Figs. 2 and 3). However, during the periods of highest river flow (March 1995) the sampling sites were inaccessible for weeks due to extreme flooding, and no toxicity measurements could be made. Figs. 2 and 3 show no toxicity immediately prior to or following the flood, but it is unknown whether the flood waters were toxic during this event. Toxicity values from both the estuary sites were significantly correlated with rainfall and river flow (Table 1), and river flow was also significantly correlated with toxicity in the upstream river and one of the drainage ditches (Table 1; Fig. 4). All significant correlations were negative; decreasing mysid survival was correlated with increasing rainfall and river flow.

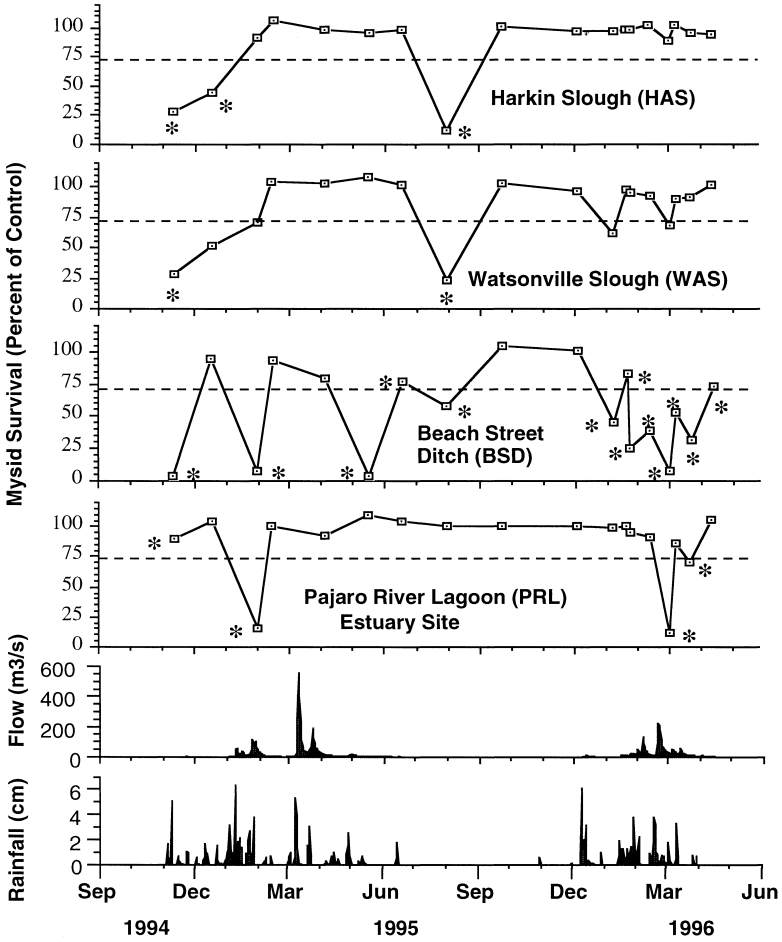

Fig. 2. Temporal trends in the occurrence of toxicity in the Pajaro River estuary and tributaries, relative to river flow and rainfall. Asterisks indicate significant differences from controls using *t*-tests. Dashed line indicates a generalized threshold for significant toxicity, based on methods described by Thursby et al. (1997); see text.

Table 1
Results of correlation analyses relating mysid survival to river flow, rainfall and other factors at seven sites in the Pajaro River estuarine system[a]

Site	River flow	Rainfall (period prior to sampling)				Conductivity	Hardness
		Week	4-Day	Day prior	Day of		
RRB	<0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
MDD	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
PRD	<0.05	n.s.	<0.025	<0.05	<0.05	n.s.	n.s.
PRL	<0.001	n.s.	n.s.	n.s.	<0.05	<0.002	n.s.
BSD	<0.025	n.s.	n.s.	n.s.	n.s.	<0.05	n.s.
WAS	n.s.	n.s.	n.s.	<0.05	n.s.	n.s.	n.s.
HAS	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

[a] Values presented are probability (p) values from Spearman Rank correlations.'n.s.' indicates correlation was not statistically significant at the $p < 0.05$ level. Significant correlations with river flow and rainfall were negative: the higher the river flow, the lower the mysid survival. Significant correlations with conductivity were positive. Site locations are shown in Fig. 1.

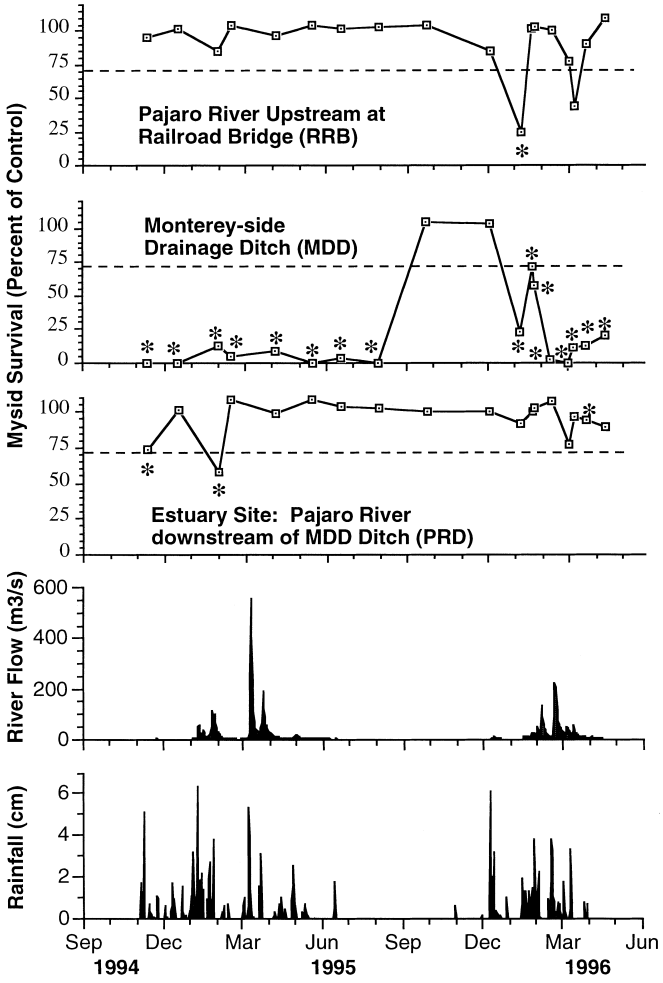

Fig. 3. Temporal trends in the occurrence of toxicity in the main Pajaro River and tributary drainage ditch, relative to river flow and rainfall. Asterisks indicate significant differences from controls using *t*-tests. Dashed line indicates a generalized threshold for significant toxicity, based on methods described by Thursby et al. (1997); see text.

Rates of mysid survival in samples from the lagoon (PRL) and its tributary drainage ditch (BSD) were significantly correlated with conductivity, with higher survival in samples with higher conductivity. There were no significant relationships between mysid survival and sample hardness (Table 1).

3.2. Relationship between toxicity and measured chemicals

All samples collected during the first sampling event (11/10/94) were screened for the pesticides chlorpyrifos and diazinon using ELISA tests. Chlorpyrifos was not detected in any of these samples (the minimum detection limit was 80 ng l^{-1}). Diazinon was detected in all samples, with values from six of the sites ranging from 0.053 to 0.474 μg l^{-1} (Table 2). The diazinon concentration at the seventh site (BSD) was measured at 1.064 μg l^{-1}, a value that fell between the 96 h no observed effect concentration (NOEC) and LC50 values for diazinon toxicity to *N. mercedis* (0.64 and 1.54 μg l^{-1}, respectively; Bailey et al., 1994). Mysid survival in this BSD sample was 4%. However, low rates of survival were also observed in samples collected on this date from MDD (0%), WAS (28%), HAS (28%) and PRD

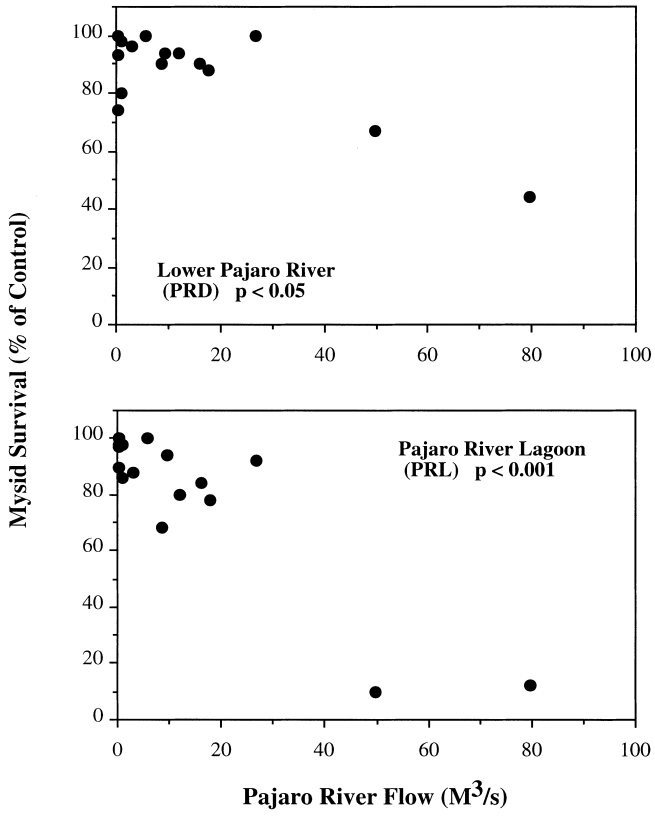

Fig. 4. Relationships between river flow and mysid survival in samples from the Pajaro River estuary. Probability values from Spearman rank correlations are shown.

Table 2
Mysid survival and measured concentrations of the pesticide diazinon ($\mu g l^{-1}$) and the trace metals copper and lead ($\mu g l^{-1}$)[a]

Site	Diazinon concentration	CV (%)	Trace metal concentrations		Mysid survival (%)
			Copper	Lead	
RRB	0.053	3.1	n.s.	n.s.	96
MDD	0.285	4.8	n.s.	n.s.	0
PRD	0.241	1.1	n.s.	n.s.	74
PRL	0.474	8.8	n.s.	n.s.	90
BSD	1.064	n.a. ($n = 1$)	19	10	4
WAS	0.472	1.2	20	9.4	28
HAS	0.122	0.0	n.s.	n.s.	28

[a] Site locations are shown in Fig. 1. Diazinon concentrations were measured using ELISA tests on samples collected 11/10/94; trace metal concentrations were measured by Questa Engineering Corporation (1995) on samples collected 11/15/94, and toxicity data were from samples also collected on 11/10/94. CV is coefficient of variation (standard deviation divided by mean, $n = 2$). Mysid survival is given as a percentage of the control survival.

(74%), indicating additional causes of toxicity in the watershed.

Samples collected during the next three surveys (12/13/94, 1/27/95, and 2/13/95) were screened for 74 pesticides using EPA analytical methods. Samples collected on 12/13/94 were characterized primarily by the presence of carbamates, though diazinon, 4,4′-DDE, and 4,4′-DDT were each detected in individual sam-

Table 3
Concentrations of organochlorine, organophosphate, and carbamate pesticides ($\mu g\,l^{-1}$) measured on three sampling dates[a]

Sampling date and river flow									
	13 Dec 1994 (River flow = $0.4\,m^3\,s^{-1}$)			27 Jan 1995 (River flow = $79.6\,m^3\,s^{-1}$)			13 Feb 1995 (River flow = $3.0\,m^3\,s^{-1}$)		
Site	Chemical	Conc.	Mysid survival (%)	Chemical	Conc.	Mysid survival (%)	Chemical	Conc.	Mysid survival (%)
RRB	4, 4′-DDE	0.13	102	n.d.	n.d.	85	Diuron	0.4	105
	4, 4′-DDT	0.26							
	Carbaryl	0.07							
	Diuron	1.4							
MDD	Diuron	2.8	0	4, 4′-DDT	0.26	13	n.d.	n.d.	5
	Oxamyl	2.8							
PRD	Carbaryl	0.14	102	4, 4′-DDT	0.13	58	n.s.	n.s.	109
	Diuron	0.8							
	Oxamyl	0.4							
PRL	Diuron	9.6	105	Dieldrin	0.20	15	n.s.	n.s.	100
	Oxamyl	0.6		4,4′-DDE	0.35				
				4,4′-DDT	0.77				
				Toxaphene	2.4				
BSD	Copper[b]	17	95	Dieldrin	0.26	8	n.s.	n.s.	93
				4,4′-DDE	0.57				
				4,4′-DDT	1.1				
				Toxaphene	3.9				
				Dacthal[b]	0.41				
				DDT[b]	0.13				
WAS	Diazinon	0.2	51	Dieldrin	0.06	71	n.s.	n.s.	105
	Diuron	12.0		4,4′-DDE	0.18				
	Oxamyl	0.5		4,4′-DDT	0.22				
	Copper[b]	5.4							
HAS	Diuron	6.3	44	Dieldrin	0.07	92	n.s.	n.s.	107

[a]Mysid survival and river flow data are provided for reference. Site locations are shown in Fig. 1. Concentrations are only reported when they exceeded quantitation limits.
[b]Table includes additional chemicals measured in concurrent studies from the same sites in samples collected 12/14/94 and 1/21/95 (Questa Engineering Corporation, 1995). n.d. indicates no chemicals detected, n.s. indicates the site was not sampled. Mysid survival data are given as a percent of control survival.

ples (Table 3). River flow at the time of this survey was $0.4\,m^3\,s^{-1}$. River flow increased to $79.6\,m^3\,s^{-1}$ at the time of the next survey (1/27/95), and samples collected then were dominated by persistent hydrophobic organochlorine pesticides (Table 3). Only two sites were analyzed in the last survey (2/13/95), and only the herbicide diuron was detected then.

With the exception of carbaryl, all pesticides found at estuary site PRD were found at higher concentrations in immediate tributaries (RRB and MDD), and all pesticides found at MDD were found in more dilute concentrations in the estuary (PRD; Table 3). There were no consistent trends in chemical concentrations between the estuary site PRL and either of it's immediate tributaries (WAS and BSD) in samples collected on 12/13/94. However, in samples collected during the high runoff (1/27/95), all pesticides found at the estuary site (PRL) were found at higher concentrations in the tributary drainage ditch (BSD; Table 3). The other immediate tributary (WAS) had many of the same pesticides detected, but at lower concentrations than those found in the estuary. Toxicity values for the 1/27/95 samples from these sites follow a similar pattern: lower survival in tributaries with high organochlorine concentrations, and higher (but still relatively low) survival in estuary waters with more dilute concentrations (Table 3). Ammonia did not appear to be related to toxicity, since concentrations were generally low, and the sample with the highest un-ionized ammonia reading ($0.115\,mg\,l^{-1}$ in MDD 9/25/95) had 100% survival.

3.3. Toxicity identification evaluations

Phase I TIEs were performed on samples collected later in the study than those analyzed for sample chemistry. All TIE samples were collected after moderate rainfall that produced subsurface tile drainage to the ditches but minimal surface runoff. In the preliminary TIEs, samples from the two agricultural drainage ditches (BSD and MDD) collected on 5/17/95 were found to be toxic, with survival rates of 29 and 0%, respectively. The BSD sample was slightly less toxic (43% survival) after removal of non-polar organic compounds by solid phase extraction (SPE). Non-polar constituents eluted from the SPE column and tested at 2.5 times their original concentration were as toxic as the original sample (29% survival). In the 5/17/95 MSD sample, all mysids died in the original sample and in each treatment. Thus, SPE treatment did not remove toxicity, but the column eluate was toxic. Addition of PBO did not mitigate toxicity in either sample. Control blanks for all treatments were non-toxic. No other treatments were applied to these samples.

In full Phase I TIEs conducted on additional samples collected from the drainage ditches during later surveys (February through April, 1996), toxicity was mitigated by a number of treatments. Sample dilution greatly reduced toxicity of all samples (Fig. 5). Toxicity was substantially reduced when samples were adjusted to both pH 3 and pH 11 and then restored to initial pH for testing. Aeration removed some toxicity in the BSD sample, but aeration at low and especially high pH was more effective than aeration at initial pH. SPE treatment reduced toxicity slightly in the BSD sample, and the column eluate was toxic. There was some reduction in toxicity after addition of the chelating agents EDTA and STS.

Three samples collected from the MDD drainage ditch over a 7-week period had similar toxicity profiles that were different in some respects from the BSD sample (Fig. 5). Toxicity was substantially reduced or removed in all three MDD samples by adjusting them to both low and high pH. Aeration was ineffective at removing toxicity except at low and high pH. SPE treatment did not reduce toxicity, and the column eluate was not toxic. There were minor reductions in toxicity after addition of EDTA to two of the samples, and after addition of STS to one of those two. The addition of PBO did not reduce toxicity in any samples from either site.

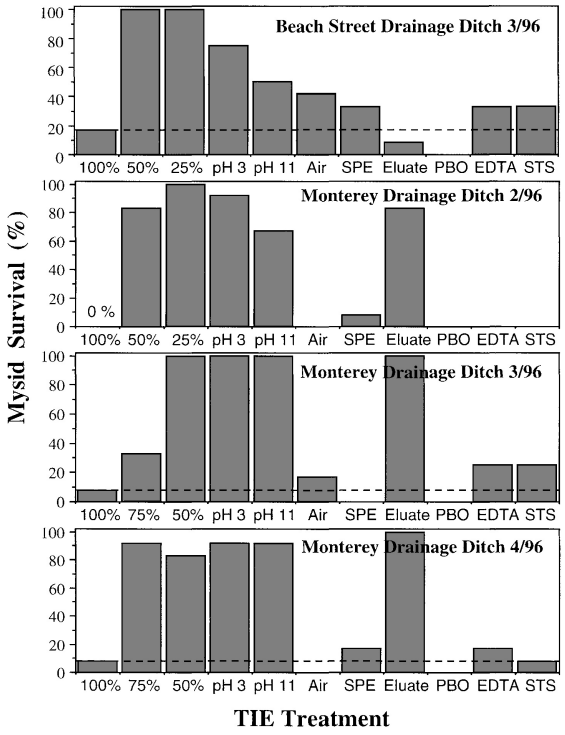

Fig. 5. Results of phase I toxicity identification evaluations (TIEs). Histogram bars represent mysid survival in the original sample (100%) and the following TIE treatments: sample dilutions (75, 50, or 25%), pH adjustments, aeration, C-8 solid-phase extraction (SPE), C-8 column eluate (2.5 × addback), and additions of piperonyl butoxide (PBO), EDTA, and sodium thiosulfate. Dashed line is the mysid survival in the untreated sample (baseline).

The possible contributions of nitrate and/or phosphate to sample toxicity were also investigated in samples from BSD and MDD collected on 3/25/96. Nitrate concentrations determined using a Chemet® Nitrate kit were 110 ppm for both samples. While relatively high for surface waters, this value is well below the *N. mercedis* NOEC value of 500 ppm (Carol DiGiorgio, University of California at Davis, personal communication). A sample from the MDD ditch collected on 4/15/96 was treated with several different anion exchange columns to remove any anionic compounds such as nitrates or phosphates. Mysid survival in the original sample was 40%. Survival after treatment with the Varian Bond Elut® SAX and DEA anion exchange columns was 50 and

30%, respectively, indicating little, if any reduction in toxicity due to removal of anionic compounds.

4. Discussion

All sites investigated in the lower Pajaro River and its immediate tributaries produced multiple samples that were acutely toxic to resident mysid crustaceans. The agricultural drainage ditches were toxic most often, followed by the freshwater sloughs, with the estuary and river sites exhibiting toxicity least often (Figs. 2 and 3). The temporal and spatial patterns of toxicity support the assumption that the main river upstream and the two agricultural drainage ditches were sources of toxic runoff to the estuary. Each available measurement showed the ditches to have higher pesticide concentrations and greater toxicity than the estuary sites into which they flowed (Table 3). The upstream river site (RRB) was not always more toxic than the downstream estuary site (PRD), but the two sites had similar temporal patterns in toxicity, indicating that toxic compounds may have been transported down the main river. In contrast, the temporal toxicity pattern in the sloughs was very different from that observed in the corresponding estuary site (PRL; Figs. 2 and 3). The slough samples were toxic in the summer, but this did not appear to affect the estuary downstream, probably because of low flow and limited transport efficiency. Toxic runoff from many of the tributaries was apparently of sufficient volume to affect the estuary sites only during times of high flow, and low summer flows appear to have had little effect downstream, regardless of the tributary toxicity observed. Seawater entering the estuary through tidal action apparently did not contribute to toxicity. No mortality was observed in any of the four high salinity samples tested with the marine mysid (*Holmesimysis*), and high conductivity in the estuary was significantly correlated with high mysid survival (Table 1).

Toxicity was statistically correlated with increased river flow at the two estuary sites, the upstream river, and one of the drainage ditches (PRD, PRL, RRB, and BSD) (Table 1; Fig. 4). High water flow in the study area was driven by local rainfall events, facilitating transport of pollutants from urban, residential, and agricultural areas throughout the watershed. High rainfall also affected the route of agricultural runoff, from soil percolation and tile drainage to direct furrow runoff during highest flows. This change was not likely to be uniform or coincident across all field types, and furrow runoff was not measured. Furrow runoff was observed during the course of the study, however, generally following rain events of at least 2–3 cm per day, depending on previous soil saturation. Transport of soil particles and soil-associated hydrophobic chemicals is far greater in surface water than in subsurface tile drainage (Carter and Bondurant, 1976), and US Geological Survey data from 1978 to 1992 (USGS, 1995) clearly show a significant relationship between flow rate and suspended sediment concentration in the Pajaro River (Fig. 6). As river flow increased from 0.01 to over 200 $m^3 s^{-1}$, the concentration of total suspended solids increased from less than 10 to more than 2000 $mg l^{-1}$, with total suspended sediment discharge reaching rates of more than 50,000 metric tons per day during flood events.

These changes in hydrology and soil transport were reflected in changes in the chemical composition of tributary and estuarine waters. The first chemical measurements made in this study were on samples collected when river flow was 0.4 $m^3 s^{-1}$, and the pesticides detected were primarily carbamates. These compounds are relatively water-soluble and more easily transported through soils to tile drains (Arienzo et al., 1994), where under normal weather conditions they might be discharged into the river and tributary ditches. River flow jumped to 79.6 $m^3 s^{-1}$ by the next sampling date, and the detected compounds were primarily organochlorine pesticides. These hydrophobic compounds tend to bind to clays during percolation (Boesten and van-der Linden, 1991), and though they have been banned for over twenty years, they are highly persistent in soils (Howard et al., 1991). Erosion of agricultural soils provides a means of introducing organochlorine pesticides into the estuary, even in the absence of recent application (either intentional or as contaminants in currently applied compounds). A similar relationship between high water flow and organochlorine pesticide concentration in runoff was observed by the Pajaro Valley Water Management Agency (PVWMA) in samples from the Beach Street ditch (site BSD). In a sample collected on 1/21/95, when Pajaro River flow was 8.3 $m^3 s^{-1}$, PVWMA measured the DDT concentration at 0.13 $\mu g l^{-1}$. After 6 days of heavy local rainfall (9.6 cm), river flow

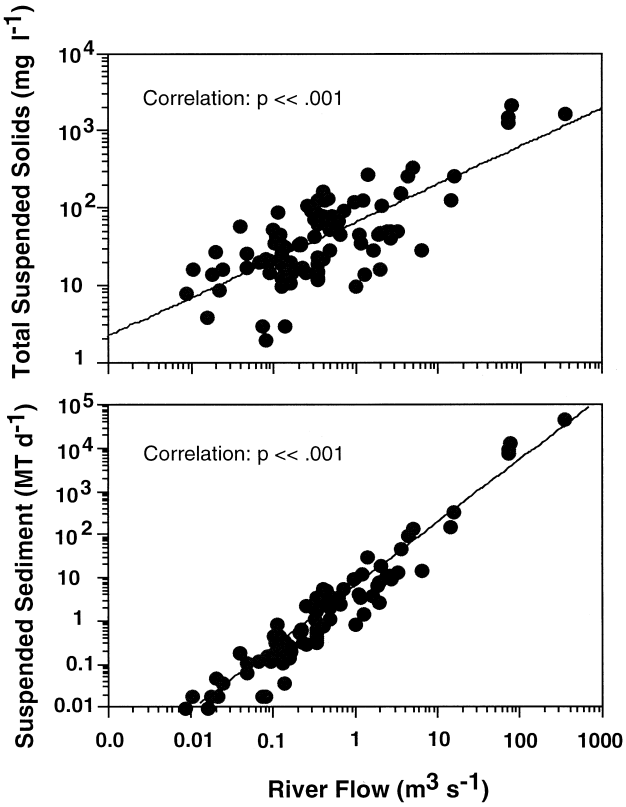

Fig. 6. Pajaro River flow and suspended sediment loads from US Geological Survey data collected at the Chittendon Gap, 1978 to 1992 (USGS, 1995). Probability values from Spearman rank correlations are shown. MT/d is metric tons per day.

rates increased by a factor of 10 to 79.6 $m^3 s^{-1}$, and DDT concentrations increased by a similar factor to 1.1 $\mu g l^{-1}$ (Table 3).

Measured chemical concentrations were often higher than concentrations associated with biological impacts in previous studies (Table 4). However, the total pesticide concentrations measured in this study may not always be directly comparable to literature values that are often presented as, or assumed to be, dissolved concentrations. The concentration of diazinon determined by ELISA analysis of the BSD sample (1.064 $\mu g l^{-1}$) was above the 96 h NOEC for *N. mercedis* (Bailey et al., 1994; Table 4). No other organophosphates or carbamates were detected at concentrations known to be toxic to crustaceans, but detection limits were higher than toxicity thresholds for some pesticides (e.g., chlorpyrifos; Table 4). All measured concentrations of DDT were above the 96 h LC50 for the resident amphipod *Corophium.* Measured concentrations of toxaphene were above the 96 h LC50s for both the mysid crustacean *Mysidopsis bahia* and early life-stages of the steelhead, a salmonid fish that supports a popular recreational fishery and uses the river as a migration corridor (Table 4). Other contaminants that may have been responsible for observed toxicity include trace metals, such as copper (Table 2), which was found above the 96 h LC50 for *H. costata* (17 $\mu g l^{-1}$; Hunt et al., 1997), and unmeasured chemicals such as hydrocarbons, PCBs, and numerous additional pesticides known to be in residential and agricultural use in the area.

The TIEs provided evidence that multiple compounds, including non-polar organics, were responsible for the observed toxicity. Many pesticides are non-polar organic compounds, but naturally occurring non-polar (and polar) organic toxins exist in many environments (Gribble, 1992). TIEs are generally most effective when combined with chemical analyses (e.g.,

Table 4
Pesticides detected, quantitation limits, measured concentrations, and literature values for concentrations associated with adverse biological effects to *N. mercedis* and other resident or related organisms

Chemical	Quantitation limit ($\mu g\,l^{-1}$)	Number samples detected	Analytical method	Concentration range ($\mu g\,l^{-1}$)	Effect conc. ($\mu g\,l^{-1}$)	Effect	Organism	Literature Source
Organochlorines								
Dieldrin	0.05	4	EPA8080	0.06–0.26	0.4	4-d LC50	Shrimp *Crangon*	McLeese and Metcalfe, 1980
					0.7	4-d LC50	Shrimp *Penaeus*	Parrish et al., 1974
					1.1	4-d LC50	Steelhead *Oncorhynchus*	Macek et al., 1969
4,4′-DDE	0.05	4	EPA8080	0.13–0.57	4.3	14-d EC50	Copepod *Nitocra*	Bengtsson, 1978
4,4′-DDT	0.05	6	EPA8080	0.13–1.1	0.07	4-d LC50	Amphipod *Corophium*	Reish, 1993
					0.07	4-d LC50	Amphipod *Hyalella*	Hoke et al., 1994
					0.4	4-d LC50	Fish *Menidia*	Eisler, 1970
Toxaphene	1.00	2	EPA8080	2.4–3.9	2.16	4-d LC50	Mysid *Mysidopsis bahia*	Kuhn and Chammas, 1986
					1.4	4-d LC50	Prawn *Penaeus*	Schimmel et al., 1977
					1.8	4-d LC50	Steelhead *Oncorhynchus*	Cope, 1965
Organophosphates								
Chlorpyrifos	0.10	n.d.[b]	EPA8140	n.d. (0.10)	0.045	4-d NOEC	Mysid *N. mercedis*	Bailey et al., 1994
					0.08	4-d LC50	Mysid *N. mercedis*	Bailey et al., 1994
Diazinon	0.10	1	EPA8140	0.2				
	0.03	7	ELISA[a]	0.053–1.064	0.64	4-d NOEC	Mysid *N. mercedis*	Bailey et al., 1994
					1.54	4-d LC50	Mysid *N. mercedis*	Bailey et al., 1994
Carbamates								
Carbaryl	0.07	2	EPA632	0.07–0.14	1.7	4-d LC50	Stonefly *Claassenia*	Sanders and Cope, 1968
					5.7	4-d LC50	Mysid *Mysidopsis bahia*	EPA, 1995
Diuron	0.40	7	EPA632	0.5–12.0	4300	4-d LC50	Mysid *N. mercedis*	Isaac and Phillips, 1994
Oxamyl	0.40	4	EPA632	0.4–2.8	99	4-d LC50	Mysid *N. mercedis*	Isaac and Phillips, 1994

[a]Samples collected 11/10/94 analyzed by ELISA. Samples collected from 12/13/94 to 2/13/95 were analyzed using EPA methods
[b]n.d. is 'not detected'. Chlorpyrifos was not detected, but has been shown to be toxic below the quantitation limit for this study.

Durhan et al., 1993). However, logistical constraints precluded conducting TIEs and chemical analyses on the same samples, so the chemicals identified above were not necessarily present in the TIE samples.

TIEs on both BSD drainage ditch samples implicated non-polar organic compounds that were captured on solid-phase extraction (SPE) columns, since the sample toxicity was retained in the methanol eluates from the columns. Additional toxic compounds may also have been present, since the columns did not remove all toxicity. Metals may have been present in toxic concentrations, since their removal by EDTA and STS reduced sample toxicity slightly (Fig. 5). Non-polar organic compounds may have contributed to toxicity in the MDD drainage ditch also, since the compounds retained on the SPE column were toxic in the preliminary TIE. However, there was no evidence to implicate non-polar organics in the three subsequent MDD TIE samples, since SPE column treatment did not reduce toxicity and eluates were not toxic.

In all four samples evaluated with full Phase I TIEs, sample toxicity was substantially reduced and often completely removed by both low and high pH adjustments (Fig. 5). While these results are clearly significant, their interpretation is difficult. Adjusting sample pH can shift ionic compounds to more volatile or precipitant forms, and can affect the ionization state of polar toxicants, thus making them more or less volatile (Mount and Anderson-Carnahan, 1988). The toxicity of polar organic compounds would not be affected by C-8 column SPE, and the combination of SPE and pH adjustment data might indicate that polar organic com-

pounds were at least partially responsible for observed toxicity in the MDD samples. Many compounds such as diazinon or malathion are prone to hydrolysis at either low or high pH, respectively (Bailey et al., 1996), but neither of these compounds is implicated in the MDD samples because their toxicity was not reduced by the SPE and/or PBO treatments. PBO actually increased toxicity in two of the MDD samples, providing minimal evidence that pyrethroid compounds may have been present, since their toxicity is potentiated by PBO. While additional work is clearly necessary to identify specific compounds causing toxicity in runoff to the Pajaro River, the TIE results provided evidence that non-polar and perhaps polar organics were at least partially responsible for observed toxicity, and that multiple toxic compounds were present in all samples evaluated.

Invertebrate species such as *N. mercedis* that inhabit the Pajaro River estuary are critical ecosystem components, serving as prey for numerous fish species, such as the steelhead, starry flounder, and Pacific herring that use the estuary during critical life stages. Previous studies have speculated that observed fish and invertebrate kills in the estuary were the result of toxic concentrations of anthropogenic chemicals (Swanson and Lyons, 1991; Swanson, 1993). The current study demonstrates that water in the estuary and main tributaries is frequently toxic to *N. mercedis*, and that measured pesticide concentrations were high enough to impact resident organisms, such as amphipods and early life stages of steelhead, a valued sport fish that has experienced rapid population declines.

Protection of the biological resources of the river and estuary will depend on clearly demonstrating which agricultural and other land use practices pose the greatest risk to wildlife. This study supports the assumption that toxicity is greatest during high flow events, when concentrations of particle-associated compounds are increased through surface runoff. Further, focused comparisons of the toxicity and chemical composition of surface versus subsurface runoff would help clarify causative factors, but limiting particle transport through erosion control may be among the most effective strategies to promote both sustained agricultural production and acceptable water quality in the estuary. Drainage ditch runoff was shown to be toxic even in the absence of surface flow, and TIE data indicated the presence of multiple compounds in toxic concentrations, including non-polar and possibly polar organics. Identification of toxic compounds in tile drain water would be facilitated by combined chemical analysis and toxicity confirmation studies.

5. Conclusions

Water samples in the Pajaro River estuary and its tributaries were frequently toxic to *N. mercedis*, a resident crustacean. Agricultural drainage ditches were the most frequently toxic tributaries. Toxicity was significantly correlated with increased river flow, when concentrations of particle-associated organochlorine pesticides exceeded established toxic levels. Transport of these toxic compounds into the estuary would likely be reduced through implementation of erosion control practices. Samples from drainage ditches were toxic even in the absence of surface runoff, and Phase I TIEs indicated that this toxicity was due to multiple compounds, including non-polar and possibly polar organic chemicals.

Acknowledgements

We gratefully acknowledge the contributions of colleagues who provided support, technical assistance, and/or additional information for this project. We thank Michael Perrone, Val Connor, Chris Foe, Gwen Starrett, and Val Vanway for contributions to the study design and methodology; Toby Goddard, Donna Bradford, and Bruce LeClergue for information related to land use and hydrology; Mark Stephenson and Gary Ichikawa for data on tissue chemistry; Lawrence Freeman and Pat Schifer for data on river flow and suspended sediment; Robert Holmes and Candace Miller for pesticide use data; Frank Barron for data from concurrent studies; Dave Paradies and Michelle White for assistance with maps; Carol DiGiorgio and Linda Deanovic for conducting the ELISA chemical analyses; Matt Englund, Witold Piekarski, Michelle Hester and Shirley Tudor for assistance with toxicity testing; and a number of reviewers, including Steve Bay, Val Connor, Chris Foe, Judy Pino and Bruce Thompson for thoughtful suggestions on the manuscript. Pesticide chemical analyses were conducted by APPL Laboratories, Fresno, CA. Rain-

fall data were collected by the City of Watsonville Wastewater Treatment Plant. Funding for this study was provided by the California State Water Resources Control Board through its Division of Water Quality.

References

Arienzo, M., Cristano, T., Sanchez-Martin, M., Sanchez-Camazano, M., 1994. Effect of soil characteristics on adsorption and mobility of (14C) diazinon. J. Agric. Food Chem. 42, 1803–1808.

Barbour, M., Pavlik, B., Drysdale, F., Lindstrom, S., 1991. California vegetation: diversity and change. Fremontia 19, 3–12.

Bailey, H.C., DiGiorgio, C., Kroll, K., Hinton, D.E., Starrett, G., 1994. Development of procedures for identifying pesticide toxicity in effluents and ambient waters: carbofuran, diazinon, chlorpyrifos. Report to the California State Water Resources Control Board, Sacramento, CA.

Bailey, H.C., DiGiorgio, C., Kroll, K., Miller, J.L., Hinton, D.E., Starrett, G., 1996. Development of procedures for identifying pesticide toxicity in ambient waters: carbofuran, diazinon, chlorpyrifos. Environ. Toxicol. Chem. 15, 837–845.

Bengtsson, B.E., 1978. Use of a harpacticoid copepod in toxicity tests. Mar. Pollut. Bull. 9, 238–241.

Boesten, J.J.T.I., van-der Linden, A.M.A., 1991. Modeling the influence of sorption and transformation on pesticide leaching and persistence. J. Environ. Qual. 20, 425–435.

Carter, D.L., Bondurant, J.A., 1976. Control of sediments, nutrients, and absorbed biocides in surface irrigation return flows. Environmental Protection Technology Series, EPA-600/2-76-237, US Environmental Protection Agency, 44 pp.

Cope, O.B., 1965. Sport fishery investigations. In: Effects of pesticides on fish and wildlife. USDI Fish and Wildl. Circ. 226, pp. 51–63.

DPR, 1997. Use report. California Department of Pesticide Regulation, Sacramento, CA.

Durhan, E.J., Norberg-King, T.J., Burkhard, L.P., 1993. Methods for aquatic toxicity identification evaluations: phase II toxicity identification procedures for samples exhibiting acute and chronic toxicity. US Environmental Protection Agency, Environmental Research Laboratory-Duluth Technical Report EPA/600/R-92/080, Duluth, MN.

Eisler, R., 1970. Acute toxicities of organochlorine and organophosphorus insecticides to estuarine fishes. Tech. Paper No. 46, Bureau Sport Fish. Wildl., USDI, Washington, DC, 12 pp.

Gordon, B.L., 1987. Monterey Bay Area: Natural History and Cultural Imprints. Boxwood Press, Pacific Grove, CA.

Gribble, G.W., 1992. Naturally occuring organohalogen compounds – a survey. J. Nat. Prod. 55, 1353–1395.

Hockett, J.R., Mount, D.R., 1996. Use of metal chelating agents to differentiate among sources of acute aquatic toxicity. Environ. Toxicol. Chem. 15, 1687–1693.

Hoke, R.A., Ankley, G.T., Cotter, A.M., Goldenstein, T., Kosian, P.A., Phipps, G.L., Vandermeiden, F.M., 1994. Evaluation of equilibrium partitioning theory for predicting acute toxicity of field-collected sediments contaminated with DDT, DDE, DDE and DDD. Environ. Toxicol. Chem. 13, 157–166.

Howard, P.H., Michalenko, E.M., Jarvis, W.F., Basu, D.K., Sage, G.W., Meylan, W.M., Beauman, J.A., Gray, D.A., 1991. Handbook of Environmental Fate and Exposure Data for Organic Chemicals. Lewis Publishers, Chelsea, MI.

Hunt, J.W., Anderson, B.S., Turpen, S.L., Englund, M.A., Piekarski, W., 1997. Precision and sensitivity of a 7-day growth and survival toxicity test using the west coast mysid crustacean *Holmesimysis costata*. Environ. Toxicol. Chem. 16, 824–834.

Isaac, G., Phillips, P. 1994. Toxicity of agricultural chemicals to water fleas and young mysid shrimp. California Department of Fish and Game, Environmental Services Division Administrative Report 94-2, Sacramento, CA.

Jensen, D.B., Torn, M., Harte, J., 1990. In our own hands: a strategy for conserving biological diversity in California. California Policy Seminar Research Report. University of California, Berkeley.

Kreissman, B., 1991. California, An Environmental Atlas and Guide. Bear Klaw Press, Davis, CA.

Kuhn, A., Chammas, M., 1986. Toxaphene acute and chronic results. US EPA, Narragansett, RI, 7 pp. (Technical Memo to D. Hansen, US EPA, Narragansett, RI)

Macek, K.J., Hutchinson, C., Cope, O.B., 1969. The effects of temperature on the susceptibility of bluegills and rainbow trout to selected pesticides. Bull. Environ. Contam. Toxicol. 4 (3), 174–183.

McLeese, D.W., Metcalfe, C.D., 1980. Toxicities of eight organochlorine compounds in sediments and seawater to *Crangon septemspinosa*. Bull. Environ. Contam. Toxicol. 25 (6), 921–928.

Mount, D.I., Anderson-Carnahan, L., 1988. Methods for aquatic toxicity identification evaluations: Phase I toxicity characterization procedures. US Environmental Protection Agency, Environmental Research Laboratory-Duluth Technical Report EPA/600/3-88/034, Duluth, MN.

EPA, 1995. Environmental Effects Database (EEDB) Environmental Fate and Effects Division, Office of Pesticide Programs, US Environmental Protection Agency, Washington, DC.

Parrish, P.R., Couch, J.A., Forester, J., Patrick, J.M., Jr., Cook, G.H., 1974. Dieldrin: effects on several estuarine organisms. In: Proc. 27th Annual Conf. Southeast Assoc. Game Fish Comm., Hot Springs, AK, pp. 427–434.

Phillips, P.T., 1988. California State Mussel Watch 10-year data summary, 1977–1987. Water Quality Monitoring Report No. 87-3, Division of Water Quality, State Water Resources Control Board, Sacramento, CA.

Questa Engineering Corporation, 1995. Water resources management plan for Watsonville Slough system, Santa Cruz County. Prepared for Association of Monterey Area Governments (AMBAG), Marina, CA.

Rasmussen, D., 1994. California State Mussel Watch 1987–1993 Draft Data Report 94-1 WQ, Division of Water Quality, State Water Resources Control Board, Sacramento, CA.

Reish, D.J., 1993. Effects of metals and organic compounds on survival and bioaccumulation in two species of marine gammaridean amphipod. J. Nat. Hist. 27 (4), 781–794.

Sanders, H.O., Cope, O.B., 1968. The relative toxicities of several pesticides to naiads of three species of stoneflies. Limnol. Oceanogr. 13 (1), 112–117.

Schimmel, S.C., Patrick Jr., J.M., Forester, J., 1977. Uptake and toxicity of toxaphene in several estuarine organisms. Arch. Environ. Contam. Toxicol. 5, 353–367.

Schimmel, S.C., Melzian, B.D., Campbell, D.E., Strobel, C.J., Benyi, S.J., Rosen, J.S., Buffum, H.W., Rubinstein, N.I., 1994. Statistical summary EMAP-Estuaries Virginian Province, 1991, EPA /620/R-94/005.

Sokal, R.R., Rohlf, F.J., 1981. Biometry: The Principles and Practice of Statistics in Biological Research, 2nd ed. Freeman, New York.

Swanson, M.L., Lyons, K., 1991. Pajaro River corridor management plan: a plan for increased flood protection and environmental enhancement. Mitchell Swanson and Associates, Sacramento, CA.

Swanson, M.L., 1993. Pajaro river lagoon management plan: technical appendices. Mitchell Swanson and Associates, Santa Cruz, CA.

Thursby, G.B., Heltshe, J., Scott, K.J., 1997. Revised approach to toxicity test acceptability criteria using a statistical performance assessment. Environ. Toxicol. Chem. 16, 1322–1329.

USEPA, 1982. The determination of carbamate and urea pesticides in industrial and municipal waste water. Environmental Monitoring and Support Laboratory, Office of Research and Development, US Environmental Protection Agency, EPA Method 632, January, 1982.

USEPA, 1994a. Test method for evaluating solid waste; physical/chemical methods. SW-846, 3rd ed. EPA Method 8080a, Revision 1, September 1994.

USEPA, 1994b. Test method for evaluating solid waste; physical/chemical methods. SW-846, 3rd ed. EPA Method 8140, Revision 1, September 1994.

USGS, 1995. United States Department of Interior, Geological Survey, ADAPS data base report for station number 11159200 Corralitos Creek at Freedom, CA, and station 11159000 Pajaro River at Chittenden, CA; USGS, Salinas, CA, May, 1996.