

Effects of Organophosphate Pesticide Pulses on Benthic Arthropods in the Sacramento-San Joaquin Delta

Melanie McCutchan

Departments of Environmental Sciences and Economics

Abstract

Periods of elevated organophosphate (OP) pesticide levels in waterways of the Sacramento- San Joaquin Delta associated with rainfall events that occurred after application of the pesticides to crops in California's Central Valley may pose a threat to delta biota. Bioassays have shown delta water to be lethally toxic to the water flea *Ceriodaphnia dubia* during these organophosphate 'pulses'. Comparison of OP levels measured in the Delta to published toxicity data also suggests that OP pulses may be toxic to aquatic invertebrates in the Delta, particularly arthropods, many of which are food sources for commercially valuable or endangered fish species. In this study, I examined possible effects of OP pulses on benthic arthropods in the Delta using Department of Water Resources benthic monitoring data and pesticide measurements taken by the USGS from 1991-1994. I used regression analysis that incorporated major factors known to affect benthic organisms, to test for correlation between the occurrence of OP pulses and short-term declines in abundances of the four taxa studied, *Corophium stimpsoni*, *Corophium spinicorne*, *Gammarus daiberi*, and *Nippoleucon hinumensis*. I also roughly surveyed long-term trends in abundances of these four taxa from 1985-1999 to determine if possible short-term declines in abundances are set upon a background of decreasing abundance over this period. Finally, I examined toxicity of the primary OP pesticide detected in the Delta, diazinon, to a benthic arthropod that is resident to the Sacramento- San Joaquin Delta, *Hyallela azteca*. Results of the regression analysis suggest that *Corophium stimpsoni* may have been adversely affected by OP pulses in the Delta, and may be susceptible to an organophosphate dose at concentrations of 20-40 ng/l persisting for one-four days. Toxicity caused by OP pulses may be related to a possible long-term decline in *C. stimpsoni* abundances in the Delta. The regression analysis indicates no adverse effects of OP pulses on *Corophium spinicorne*, *Gammarus daiberi*, and *Nippoleucon hinumensis*. Laboratory tests of diazinon toxicity to *Hyallela azteca* suggest that this species is not susceptible to OP levels detected in the Delta, but exhibits significant mortality when exposed to OP levels comparable to those measured upstream in waterways of the lower Sacramento and San Joaquin River Basins.

Introduction

Chemical pesticides used on agricultural fields often migrate to waterways where they can adversely affect aquatic organisms (Cooper 1993, Pritchard 1993, and others). Approximately ten percent of the pesticides used in the United States are applied to crops in California's Central Valley (Wright and Phillips 1988). This intensively farmed area is drained by the Sacramento and San Joaquin rivers, whose waters converge in a 1,600 km² delta before discharging to the San Francisco Bay. Human influences have seriously altered the Delta. Along with agricultural runoff, oil pollution, urban runoff, the introduction of non-native species, and water development activities such as dredging, diking, diversion of freshwater inflow, and filling of wetlands are all probably contributing to major declines in fish and invertebrate populations in the estuary (Obrebski *et al.* 1992, Meng and Moyle 1995, Kimmerer *et al.* 1994, Pereira *et al.* 1994, Yoshiyama *et al.* 1998).

Between 1991 and 1994, workers with the United States Geological Survey detected episodes of elevated concentrations of the organophosphate (OP) pesticides, diazinon and methidathion, in the Sacramento-San Joaquin Delta (Kuivila and Foe 1995, MacCoy *et al.* 1995, Domagalski *et al.* 1997). These OP 'pulses' were associated with rainfall events occurring after application of the pesticides to crops in the Central Valley. Kuivila and Foe (1995) found water samples taken from the Sacramento and San Joaquin Rivers during peaks in organophosphate levels to be toxic to the water flea *Ceriodaphnia dubia*. Laboratory toxicity studies have demonstrated lethal and sublethal effects of organophosphate pesticides on several aquatic organisms (Ankley and Colyard 1995, Serrano *et al.* 1995, Hughes *et al.* 1997). Better understanding of organophosphate pulses' effects on estuarine organisms may be a crucial component of efforts to reverse the decline of commercially and ecologically important fish and invertebrate populations in one of the most productive estuaries in the United States.

First developed as nerve gases in the 1930s, organophosphates have replaced many of the organochlorine pesticides that were banned in the early 1970's (Walker *et al.* 1996). Agricultural use of organophosphates in the Central Valley has expanded considerably since the early 1990's (Department of Pesticide Regulation 1999). Organophosphates (OPP) are less persistent in the environment than the organochlorine pesticides, and create environmental threats that are mostly, though not exclusively, based on short-term toxicity

(Walker *et al.* 1996). For two reasons, organophosphate pesticides are able to enter delta waterways: (1) Organophosphates are relatively soluble in water, and are mobilized by precipitation (Kuivila and Foe 1995); and (2) Over half of the organophosphate pesticides diazinon and methidathion used in the Central Valley are applied to fruit and nut trees when the trees are dormant in the winter months (DPR 1995, 1998). This timing of application results in the most efficient and effective pest control, but also allows winter rains to wash the pesticides into waterways (Novartis 1997).

Laboratory studies suggest that measured OP levels detected in the Sacramento-San Joaquin Delta are not likely to be toxic to fish, though concentrations of up to ten times higher detected further upstream in the Sacramento and San Joaquin rivers and their tributaries may pose a direct threat to fish (Geiger *et al.* 1988, Foe *et al.* 1998, Hughes *et al.* 1997, USEPA AQUIRE 1999). Possible detrimental effects of organophosphate pulses on fish in the Delta are more likely to be indirect. These pulses may adversely affect estuarine fish by harming their food, the zooplankton and other invertebrates. Recorded organophosphate concentrations in the estuary have occurred at levels and durations that have been shown in laboratory studies and bioassays of contaminated river water to be toxic to both planktonic (drifting in the water column) and benthic (bottom-dwelling) invertebrates (Arthur *et al.* 1983, Kuivila and Foe 1995, Giddings *et al.* 1996, Katznelson and Munley 1997, USEPA AQUIRE 1999).

For several reasons, predicting effects on organisms in the Sacramento-San Joaquin Delta based on bioassays and laboratory toxicity tests is uncertain. Environmental factors in the Delta not present in a laboratory setting may moderate or exacerbate the toxic effects of increased organophosphate levels. Also, few studies have examined OP toxicity to resident organisms. Different species, and even populations within species can demonstrate widely varied responses to given toxicants (Reese 1972).

Given the uncertainties in determining hazards posed by a given toxicant to aquatic organisms, the U.S. Environmental Protection Agency (EPA) and other governmental agencies set relatively conservative water quality guidelines based on available toxicity data. The California Department of Fish and Game (CDFG) (1994) completed an assessment of the hazards to aquatic organisms posed by the primary OP pesticide detected in the Delta, diazinon. Based on available toxicity data, the CDFG estimated that aquatic organisms

should not be unacceptably affected if the four-day average concentration of diazinon does not exceed 40 ng /l more than once every three years on the average, and if the one-hour average concentration does not exceed 80 ng/l more than once every three years on the average. According to my estimates of pesticide levels in the Delta, average diazinon levels exceeded 40 ng/l for four days on 104 four-day occasions, and levels greater than 80ng/l occurred on 22 days during the period of January 1991 through April 1994 (Menconi and Cox 1994).

The potential of OP levels detected in the Delta to adversely affect aquatic invertebrates, as well as the fish and other organisms that depend on them for food, necessitates better understanding of OP effects on aquatic invertebrates. Between 1972 and 1988, twelve of twenty zooplankton taxa in the Sacramento-San Joaquin Estuary significantly declined in abundance (Obrebski *et al.* 1992). Laboratory tests of organophosphate toxicity to zooplankton taxa present in the estuary might shed light on whether OP pulses are contributing to this decline. Information on how OP pulses might affect benthic organisms is also crucial to understanding the impacts of these pulses on the Sacramento-San Joaquin estuarine ecosystem. Benthic organisms provide vital ecosystem functions in estuarine environments, such as cycling nutrients between sediments and the water column and providing food for birds and fish (Nichols 1988 and others).

Because zooplankton drift with the currents, it would be very difficult to relate detected OP pulses with effects on specific zooplankton populations in the field. Benthic organisms, on the other hand, are relatively sedentary, allowing for the spatial definition of effects from OP pulses. If a pulse of organophosphates was known to pass through an area, one can be fairly certain that benthic organisms sampled in that area after the pulse episode would have been exposed to the increased OP levels.

During the period 1991-1994 when the USGS monitored Sacramento and San Joaquin River water for the presence of organophosphates, the California Department of Water Resources (DWR), as it has for the past three decades, took monthly samples of benthic organisms at several sites in the Delta. In this study, I used these benthic samples and the USGS pesticide measurements to examine the relationship between occurrence of OP pulses and abundances of benthic organisms in the Delta. Because the pesticide pulses often coincided with natural processes (increased freshwater inflow and the winter season) that

might negatively affect abundances of benthic organisms, it would be misleading to examine the relationship between OP pulse events and benthic abundances without considering key factors that can affect benthic organisms. I used regression analysis that incorporated major factors that determine the distribution of benthic organisms, to investigate whether OP pulses are correlated with short-term declines in benthic abundances independent of these potentially confounding factors.

Arthropods of the benthic community are most likely to be affected by increased levels of these pesticides, as organophosphates are designed to eradicate insect pests in the arthropod phylum (Flemer *et al.* 1997, Ankley *et al.* 1995). For this reason I focused my analysis on four arthropod taxa that dominate the benthic arthropod community, *Corophium stimpsoni*, *Corophium spinicorne*, *Gammarus daiberi*, and *Nippoleucon hinumensis*. Of the organisms collected at the Department of Water Resources benthic sampling sites, D7, D4, D11, D19, and D28A (Fig. 1) over the years 1991-1994, these four taxa accounted for 90% of the number of arthropods collected in samples. These taxa also account for a significant portion of the Delta's benthic community, as arthropods comprised 27% of the total number of benthic organisms collected between 1991 and 1994 at these five sites. Following is a summary of the known life-habits and ecological roles of the four taxa studied:

- 1,2) *Corophium stimpsoni* and *Corophium spinicorne*: These *Corophium* spp. are tube dwelling, encrusting amphipods that are native to the Sacramento-San Joaquin Delta. They serve as important food sources for many fish that inhabit or migrate through the Delta such as striped bass *Morone saxatilis*, young Chinook Salmon *Onchorynshus tshawytscha*, young Sturgeon *Acipenser* spp., and catfish *Ictalurus* spp (Markmann 1986);
- 3) *Gammarus daiberi*: This amphipod is endemic to the Atlantic Coast of the United States, where its life habits and ecological functions have been comprehensively studied, though little is known about its role in the San Francisco Estuary ecosystem. First discovered in the San Francisco Estuary in 1983, *Gammarus daiberi* appears to have established sizable populations in the Delta by 1990. This amphipod is pelagic, inhabiting mid to near-bottom depths, and also occurs epibenthically. Young striped bass are known to feed on *Gammarus daiberi* (Hymanson *et al.* 1994);

4) *Nippoleucon hinumensis* (= *Hemileucon hinumensis*): This burrowing cumacean crustacean is endemic to the Western Pacific, and was first discovered in Suisun Bay in 1986 (Watling 1991, Hymanson 1994). A search of recent literature retrieved little information on the life habits or ecological role of this species.

Long Term Trends In this study, I concentrated on examining the relationship between OP pulse events and short-term (on a scale of one month) declines in abundances of the four taxa studied. Because few years of coincident pesticide and benthic sampling data is available, and because naturally caused interannual variation in abundances of delta organisms can be very high, it would be unreasonable to attempt to relate long-term trends in benthic abundances to the occurrence of OP pulses in the Delta.

Even when looking at more years of benthic sampling data than correspond to years when OPP were monitored, discerning long-term trends in abundances of the four taxa studied using the benthic sampling data is challenging for several reasons: (1) As mentioned above, interannual variation in benthic abundances caused by natural processes can be considerable. The benthos of the Delta is subject to dramatic between-year fluctuations in environmental parameters such as salinity, food availability, inter-species interactions, and other factors. Organisms' responses to these factors are not well understood, making it difficult to identify causes of long-term trends in benthic abundances. (2) Benthic sampling sites have come in and out of use over the past few decades, and comparing abundances between different sampling sites is difficult due to the importance of local conditions in determining abundances. (3) Two of the taxa I studied that are now prevalent in the benthic arthropod community are relatively recent introductions to the Sacramento-San Joaquin Delta. Despite these challenges, careful analysis of benthic abundances that takes into account major factors known to affect benthic organisms can facilitate better understanding of long-term changes in the benthos and potential causes of those changes. The Interagency Ecology Program (IEP) for the San Francisco Bay/Delta has summarized long-term trends in the benthos through the years 1972-1990 in reports by Markmann (1986) and Hymanson *et al.* (1994), and is currently working on summarizing trends through 1999.

Because the long-term trends in abundances of the four taxa studied may be of central importance to the health of the Delta ecosystem, I wanted to get a rough idea of whether or not possible short-term declines in abundance of these taxa associated with OP pulses would

be set upon a background of long-term declines in their abundances. I surveyed abundances of the four taxa studied between 1985 and 1999 at the only three benthic sampling sites in the Delta that were sampled consistently during this period. I considered how long-term trends in abundances of the four taxa might be related to what appears to be one of the primary factors affecting the benthos, freshwater inflow to the Delta (Nichols and Pamatmat 1988, Hymanson *et al.* 1994). Freshwater inflow affects benthic organisms by altering the physico-chemical parameters of the Delta environment such as salinity, sediment composition and stability, and other variables (Hymanson *et al.* 1994). I did not examine the possible individual influences of these variables, or the influences of additional variables such as food availability that might affect long-term trends in abundances of benthic organisms. I also did not test for statistical significance in these trends. My objective was to roughly outline long-term trends in abundances of the four-taxa studied to see if there is a marked decline in any of the taxa that might be cause for concern. A more thorough analysis of long-term trends in the benthos that more carefully considers the factors affecting organism distribution will be provided by the IEP report currently in production.

Toxicity Tests The final objective of this study was to examine in a more controlled setting the susceptibility of a species resident to the Sacramento-San Joaquin Delta to the OP pesticide, diazinon. I performed laboratory tests of diazinon toxicity to the benthic amphipod *Hyaella azteca* at diazinon levels comparable to those detected in the Delta and upstream in waterways of the Lower Sacramento and San Joaquin River Basins. Between 1985 and 1999, *Hyaella azteca* were collected in small numbers (<13) in benthic samples at three of the sampling sites, D28A, D19, and D4 (Fig. 1). I would have preferred to perform toxicity tests on the four arthropod species included in my correlation analysis that were more prevalent in benthic samples throughout the Delta, but I was ironically only successful in collecting *Hyaella azteca*. *H. azteca* may be more prevalent upstream from the Delta as it is commonly found in freshwater systems.

In summary, the three questions I investigated in this study were the following:

- 1) Does a rough survey of long-term trends in abundances of *Corophium stimpsoni*, *Corophium spinicorne*, *Gammarus daiberi*, and *Nippoleucon hinumensis* indicate a marked decline in any of these taxa?

- 2) Do benthic samples exhibit short-term declines in abundances of these four taxa that is correlated with organism exposure to OP pulses?
- 3) Does exposure to diazinon, at levels corresponding to those measured in the Delta and upstream in the Sacramento and San Joaquin Rivers, cause mortality of the resident amphipod *Hyallolella azteca* in laboratory toxicity tests?

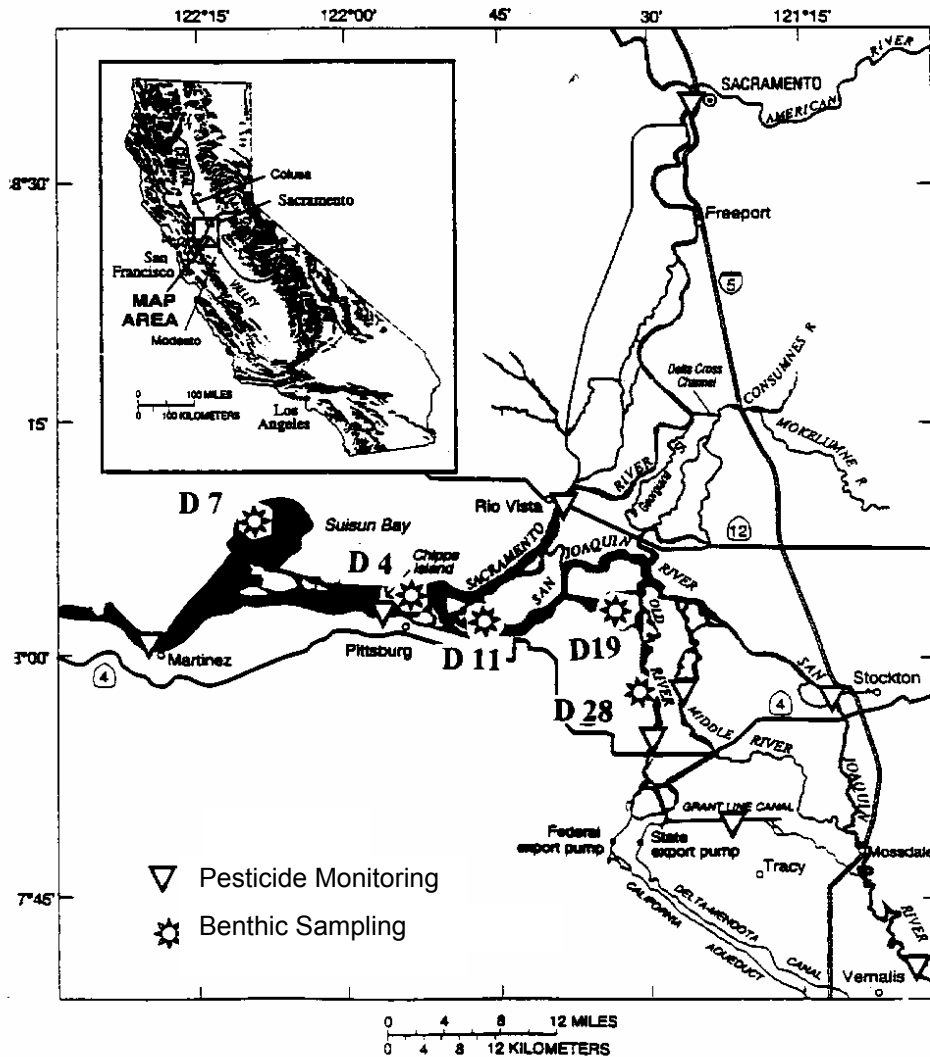


Figure 1. Map of study area. (Kuivila and Foe 1995)

Geographic setting For the period 1991-1994, pesticide measurements were taken daily at Tower Bridge in the city of Sacramento on the Sacramento River, and at Vernalis on the San Joaquin River (Fig. 1). In 1993, when the movement of the pesticide pulse was traced into the Delta, increased pesticide levels were detected as far west as Martinez (Kuivila and

Foe 1995). Increased pesticide levels would probably be diffused to very low levels east of the Carquinez Straits due to mixing with Bay waters. I limited my analysis to the Department of Water Resources benthic sites located in the interior, central and western Delta. This consisted of five sites sampled between the years 1991 and 1994: D7 in Suisun Bay; D4 on the Sacramento River; D11 near Pittsburgh; D19 at Frank's tract; and D28A near Bacon Island. At some sites, three benthic grabs (replicates) were taken at up to three spots in the center, left, and/or right sections of the channel. At sites D11, D19, and D7, one set of three replicates was taken only in the center of the channel. At site D28A, two sets of three replicates were taken, one in the left, and one in the right side of the channel. At site D4, three sets of three replicates were taken, one in each of the left, center, and right sections of the channel.

Methods

Long-term trends in abundance To get a rough picture of long-term trends in abundances of *G. daiberi*, *N. Hinumensis*, and the *Corophium spp.*, I examined their yearly abundances at the only three DWR benthic sampling sites that were sampled consistently between 1985 and 1999: site D4 near Chipps Island; site D28A at Old River; and site D7 in Suisun Bay, an area subject to highly varied salinity levels (Fig. 1). For each of the taxa, I graphed their average yearly abundances between 1985 and 1999 at each site. I used these graphs to examine trends in yearly abundances of the four taxa over these years. I looked for possible relationships between trends in abundance and freshwater inflow to the Delta using graphs of USGS river flow data measured at Freeport on the Sacramento River and at Vernalis on the San Joaquin River. I assumed that changes in salinity are inversely proportional to changes in freshwater inflow, an assumption that is well supported by previous analysis of the relationship between flow and salinity in the Delta (Hymanson *et al.* 1994).

Pesticides detected in the estuary Agricultural runoff from the Sacramento and San Joaquin Valleys could contain a variety of potentially toxic chemicals. Between 1991 and 1994, the USGS tested Sacramento and San Joaquin river waters for the presence of twenty-three pesticides. These chemicals were chosen for monitoring based on pesticide-use data for the Sacramento and San Joaquin valleys. Based on available toxicity data, I determined

whether levels of pesticides detected in the Sacramento and San Joaquin Rivers (other than the OP pesticides diazinon and methidathion) might have contributed to toxicity of delta waters between 1991 and 1994.

The organophosphate insecticides, diazinon and methidathion, and the triazine herbicides, simazine and cyanazine, were detected most often and at the highest concentrations (Menconi and Cox 1994). Laboratory toxicity tests suggest that measured levels of simazine and cyanazine are several orders of magnitude below levels that are likely to cause acute toxicity to aquatic organisms (Pauli *et al.* 1991). The arthropod species that has demonstrated the most sensitivity to any triazine herbicide in toxicity tests is *Ceriodaphnia dubia* with a 7-day LC₅₀ (the concentration that is lethal to 50% of an exposed population) of 6 parts per million (USEPA AQUIRE 1999). This level and the U.S. EPA's (1973) proposed water quality guideline for simazine of 10,000 parts per trillion, are far higher than the maximum simazine and cyanazine concentrations detected in the Delta of 1,084 and 804 parts per trillion respectively (McCoy *et al.* 1995; Pauli *et al.* 1991). The rice herbicides molinate and thiobencarb and the insecticides carbaryl and carbofuran were also detected in the Sacramento and San Joaquin Rivers. These compounds were present at levels and durations that are not likely to be acutely toxic to aquatic arthropods (Andreu-Moliner 1986, Bailey 1993, USEPA AQUIRE 1999).

The organophosphate pesticides diazinon and methidathion were the primary compounds detected at levels and durations that are likely to cause acute toxicity to aquatic arthropods. Toxicity identification evaluations performed on samples from the Sacramento and San Joaquin rivers in January and February of 1996 and 1997 indicated that diazinon was the only contaminant responsible for toxicity to *Ceriodaphnia dubia* (Foe *et al.* 1998). Chlorpyrifos, an organophosphate insecticide that is less hydrophilic than diazinon and methidathion, was detected in the two rivers briefly in 1993 at much lower levels than diazinon and methidathion, and would probably not have contributed appreciably to toxicity of delta waters. I assumed that abundances of the four taxa studied might be affected only by increased concentrations of the organophosphate pesticides diazinon and methidathion.

Estimating benthic exposure to OP Before I could test for correlation between increased OP levels and decreased abundances of the four taxa, I had to estimate the OP exposure that organisms at a given benthic sampling site would have experienced. First, I

combined concentrations of the two OP pesticides, diazinon and methidathion, to describe the potential toxicity of a given pulse. I did this because mixtures of toxicants with similar modes of action generally produce effects that are additive (Pape-Lindstrom and Lydy 1997). For example, mixtures of diazinon and the organophosphate pesticide, chlorpyrifos, have been shown to exhibit additive toxicity when present together (Bailey et al. 1997). Methidathion may not be as toxic to aquatic invertebrates as diazinon. Upon exposure to methidathion the 96-hour LC_{50} for *Ceriodaphnia dubia* is 2000 ng/l, while the 96-hour LC_{50} for *C. dubia* exposed to diazinon is about 500 ng/l. Because the lower toxicity of methidathion as compared to diazinon may not hold true for other organisms, I did not attempt to account for methidathion's possibly less toxic effects. I added concentrations of diazinon and methidathion and used total OP level to indicate the potential toxicity of delta waters.

Second, the fact that benthic samples and pesticide concentrations were not taken/measured at the same spots, required that I estimate the time at which the pulses of diazinon and methidathion measured upstream at Freeport on the Sacramento River and at Vernalis on the San Joaquin River would reach the benthic sampling sites in the Delta. Because pesticide levels measured at Freeport and Vernalis decreased by the time a given pulse reached the benthic sampling sites, I also had to estimate the reduced pesticide levels to which organisms at a particular benthic sampling site would have been exposed.

The hydrodynamics of the numerous channels that comprise the Sacramento-San Joaquin delta are extremely complicated. Aside from variation in runoff from precipitation and snow-melt, tidal action, changes in requirements at the state and federal export pumps, and releases from upstream reservoirs can dramatically alter flows in the Delta's channels. Due to the difficulty of modeling this complex system, my estimates of the behaviour of pesticide pulses measured at Tower Bridge and Vernalis as they traveled into the Delta are rough approximations. The USGS and the DWR are currently developing hydrodynamic models that will aid the tracking of pesticides and other substances in the Delta (R.N. Oltmann, personal communication)

In February 1993, Kuivila and Foe (1995) tracked pulses of diazinon and methidathion detected at Sacramento and Vernalis as they traveled along the Sacramento and San Joaquin Rivers into the Delta. Between, 1991 and 1994, Kuivila and Foe detected other

pulses at Vernalis and Sacramento but did not follow the pulses as they entered the Delta. I used the travel times and reductions in pesticide concentrations measured by Kuivila and Foe during the February 1993 pulse to estimate the time at which, and the levels of pesticides to which organisms at the benthic sampling sites would have been exposed during the other pesticide pulses occurring between 1991 and 1994.

In February 1993, Kuivila and Foe took water samples in the ship channel along the Sacramento River at Tower Bridge in the city of Sacramento, and downstream at Rio Vista, Chipps Island and Martinez. Samples were taken at slack after ebb tide to approximate the most downstream movement of the pulse. These measurements recorded pulses of diazinon and methidathion that traveled into the Delta as far west as Martinez. On the San Joaquin River, measurements were taken at Vernalis, Stockton, Middle River, and Old River.

In the Sacramento and San Joaquin River, the maximum concentration of the pesticide pulse decreased as it traveled into the Delta, and the pulse became more dispersed. Lower pesticide levels in the Delta may have been the result of dilution caused by input from the Consumnes and Mokulumne rivers, and/or the result of dispersion caused by tidal action. Concentrations in the Old and Middle rivers might also have been affected by degradation enabled by slower flow downstream from Stockton, and by input of Sacramento River water that is diverted to the Federal and State export pumps near Tracy.

To estimate the travel times of other pesticide pulses detected between 1991 and 1994 at Sacramento and Vernalis as they entered the Delta, I determined the relationship between the travel time of the February 1993 pulse and river velocities during that pulse. Based on this relationship, I used river velocities during the other pulses to estimate the travel times of these pulses. First, I had to estimate daily velocities in the two rivers during the pesticide pulses, as velocity measurements are taken only on a monthly basis. The USGS takes monthly velocity measurements at Freeport on the Sacramento River and at Vernalis on the San Joaquin River. The USGS also takes daily discharge measurements at Freeport and Vernalis. Based on these daily measurements of river discharge, I derived estimates of average daily river velocities during the pulse events.

To accurately describe how river discharge and velocities in the Delta are related would require a complicated hydrodynamic model. I made rough approximations of this relationship by regressing monthly velocity measurements on discharge readings recorded on

the same day. Velocity and discharge measurements are influenced by the inflow of water into the two rivers, and by tidal action. Velocity and discharge were not measured at consistent times relative to the tide, so the measurements represent different flow responses to tidal action. In estimating a relationship between velocity and discharge, I omitted days on which velocity and discharge measurements were zero.

I had access to monthly velocity and discharge data taken between September 1988 and November 1999 at Freeport and Vernalis. Discharge levels in the Sacramento and San Joaquin rivers between the years 1988 and 1994 were relatively constant. Between 1995 and 1999, however, discharge was approximately three times higher than in the preceding seven years. The relationship between velocity and discharge is likely to be different for very different discharge levels. For this reason, I preferred to base my velocity estimates for the years 1991-1994 on velocity and discharge measurements taken during the years 1988-1994. I was able to use measurements taken between 1988-1994 to estimate a velocity-discharge relationship for the San Joaquin River at Vernalis. To estimate a velocity-discharge relationship for the Sacramento River at Freeport, I had to use measurements from 1988-1999 because velocity and discharge measurements at Freeport were zero for most days between 1988 and 1994.

I regressed discharge measurements on velocity measurements taken on the same day to obtain two equations, one for Freeport and one for Vernalis, that predicted river velocity based on river discharge. I used these equations to estimate daily river velocity based on discharge data taken at these two locations for the years 1991-1994 (Table 1).

Vernalis: San Joaquin River	Freeport: Sacramento River
Velocity = $-1.50 + (.98 \times \log \text{ Discharge})$	Velocity = $-13.17 + (3.45 \times \log \text{ Discharge})$
N=77 R ² = .85 P < 10 ¹⁰	N=39 R ² = .96 P < 10 ¹⁰

Table 1. Equations used to estimate river velocity based on discharge in the San Joaquin River at Vernalis and the Sacramento River at Freeport.

The river velocities measured at Freeport and Vernalis could not be directly used to estimate water travel-times into the Delta, because deeper, wider channels in the Delta as well as tidal action cause slower, and even negative, velocities in delta channels. To

approximate how river velocity at Freeport and Vernalis relates to water travel time through the Delta, I estimated a relationship between the travel time predicted by the velocities measured at Freeport and Vernalis in February 1993, and the actual travel time of the February 1993 pulse.

The actual travel times of the 1993 pulses in the two rivers, I estimated as the number of days elapsed between the day on which diazinon levels peaked at Sacramento or Vernalis and the day levels peaked at locations in the Delta near the benthic sampling sites. I assumed that the travel time of a pulse between Sacramento and Chipps Island would be representative of travel times between Sacramento and sites D4 and D11. To estimate travel times for site D7, I averaged the travel times from Sacramento to Chipps Island and Sacramento to Martinez. I used the travel time from Vernalis to Old River to approximate travel times from Vernalis to sites D19 and D28. Travel times were three days from Sacramento to Chipps Island, seven days from Sacramento to Martinez, and ten days for Vernalis to Old River. This gave estimated travel times during the February 1993 pulse of three days between Sacramento and sites D4 and D11, and five days between Sacramento and site D7. Because sites D19 and D28A are upstream of the Old River pesticide sampling site, I adjusted the estimated travel time between Vernalis and sites D19 and D28A to 8 days.

Using my estimates of river velocity based on discharge during the February 1993 pulse, I calculated a predicted travel time for that pulse to the downstream sites by multiplying the estimated velocity at Freeport and Vernalis by the river distance from Sacramento or Vernalis to the downstream sites. For sites D4 and D11, I estimated a travel time by multiplying river velocity at Freeport by the distance from Sacramento to Chipps Island (~ 96 river km). For site D7, I multiplied velocity at Freeport by the distance from Sacramento to D7 (~108 river km). For sites D19 and D28A, I multiplied velocity at Vernalis by the distance from Vernalis to the midpoint between those sites (~50 river km).

I next compared the actual travel time of the February 1993 pulse from Sacramento or Vernalis to the benthic sampling sites, to the travel time predicted by the estimated average river velocity during that pulse. I determined by what factor the actual travel time was longer than the predicted travel time. I then multiplied the travel times of the other pulses, predicted by average river velocities during these pulses, by this factor to better approximate the actual travel times of the other pulses. For example, the actual travel time of the February 1993

pulse between Sacramento and sites D4 and D11, I estimated as three days, the number of days elapsed between peaks in pesticide levels at Sacramento and Chipps Island according to Kuivila and Foe's (1995) pesticide measurements. I multiplied the average river velocity during this pulse occurring at Freeport by the distance between Sacramento and Chipps Island to get a predicted travel time of 1.2 days. I divided the actual travel time of three days by the predicted travel time of 1.2 days to get a factor of 2.6. The predicted travel times between Sacramento and sites D4 and D11 of the other pulses occurring between 1991 and 1994, I multiplied by this factor of 2.6 to estimate the actual travel time of each pulse. For sites D19 and D28A, I multiplied predicted travel times by a factor of four. For sites D7, I multiplied predicted travel times by a factor of 3.8.

I estimated the pesticide concentrations to which organisms at a particular benthic sampling site would have been exposed based on the reduction in concentrations of the February '93 pulse as it traveled into the Delta. I used the reduction in concentrations measured at Sacramento relative to concentrations occurring three days later at Chipps Island to estimate pesticide concentrations at sites D4 and D11. I averaged this reduction in concentration occurring at Chipps Island with that occurring six days later at Martinez to estimate pesticide concentrations at site D7. I used the reduction in concentrations measured at Vernalis relative to concentrations occurring ten days later at Old River to estimate pesticide concentrations at sites D19 and D28.

Dispersion of the February '93 Sacramento and San Joaquin River pulses as they moved into the Delta tended to increase the duration of elevated pesticide concentrations by a day or two. As this is a relatively minor increase and given the uncertainties in other aspects of my estimations, I did not take into account this increase in duration. I did, however, attempt to account for dispersion of higher concentrations relative to the peak over more days as the pulses traveled into the Delta. As the February '93 pulses advanced into the Delta, they 'flattened out' (Fig. 2). That is, relative to the maximum pesticide level, high pesticide concentrations were more evenly distributed over the other days comprising the pulses. Due to this dispersion, the reduction in maximum concentrations at Sacramento relative to Chipps Island and Martinez, and at Vernalis relative to Old River was greater than the reduction in the next highest concentrations at Sacramento and Vernalis relative to the downstream sites.

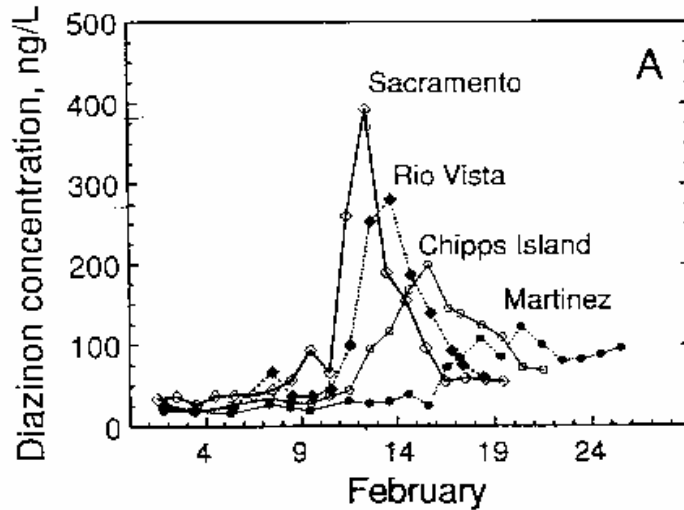


Figure 2. Diazinon concentrations from Sacramento to San Francisco Bay, February 1993 (Kuivila and Foe 1995)

To account for this, I first calculated a reduction in the maximum concentration of the February '93 pulse at Sacramento or Vernalis relative to the corresponding peak occurring a given number of days later at the corresponding benthic sampling sites. For the rest of the days of the pulse, an OP concentration on a given day at Sacramento or Vernalis was compared to concentrations at Chipps Island, Martinez, or Old River occurring the same number of days later as separated the peak concentrations. For the next four highest daily pesticide concentrations measured at Sacramento and Vernalis, I calculated the average reduction in concentration relative to concentrations corresponding to levels occurring a given number of days later at the sampling sites. I then calculated the reduction in concentrations for the remaining eight days of the thirteen-day pulse. This gave estimates of: the reduction in the maximum concentration of a given pulse once it reached the benthic sampling sites; the reduction in the ~30 percent of the days with the next highest concentrations; and the reduction in concentrations of the remaining ~60 percent of days comprising the pulse. I extrapolated this reduction in pesticide levels to the other pulses occurring between 1991 and 1994 (Table 2).

The thirteen-day pulse I used for these estimations I defined as beginning on February 8 and lasting through February 20 in both rivers. I added diazinon and methidathion levels together to determine the reductions in total OP levels as the pulses traveled through the Delta.

Site	Influence from:	Percent reduction in concentration		
		Maximum Concentration	30% next highest	Remaining 60%
D4	Sacramento	50	25	0
D11	Sacramento	50	25	0
D19	Vernalis	80	80	70
D28	Vernalis	80	80	70
D7	Sacramento	60	40	0

Table 2. Summary of adjustments made to Total OP concentrations measured at Sacramento and Vernalis to estimate level of exposure at each benthic sampling site.

Possible errors in estimations of OP exposure My estimates of the OP concentrations to which organisms at the benthic sampling sites would have been exposed and the time at which that exposure would occur are subject to several potential sources of error. The OP reductions and travel times of the pesticide pulses occurring between 1991 and 1994 would not necessarily be proportionate to those occurring during the February 1993 pulse. A possible source of error was my prediction of velocities based on flow rates using linear regression. This severely simplified what can be a complex relationship, as a river can compensate for greater flow by widening its banks or diverting its flow to other channels, rather than just by moving more rapidly. Because rivers are capable of altering their flow patterns under different flow conditions, and because flow rates and tidal action were different in the February 1993 pulse as compared to the other pulses, the relationship between river velocity and travel time in the February 1993 pulse would not necessarily hold true for the other pulses. Another possible source of error in my estimation of travel times is that I did not take into account the possible effects of pumping at the federal and state water export Pumps near Tracy.

Several factors lend uncertainty to my estimates of reductions in OP levels as the pulses moved through the Delta. Input to the Delta from the Consumnes and Mokulemne Rivers might have been lower in proportion to inflow from the Sacramento and San Joaquin Rivers during the non-February 1993 pulses as compared to the February 1993 pulse, as overall inflow to the Delta was highest in February 1993 between 1991 and 1994. This might have

led me to over-estimate the reduction in concentration of the non-February 1993 pulses caused by dilution from Consumnes and Mokulemne inflow to the Delta. Sites D19 and D28A may have been affected by OP input from Sacramento River water drawn to the federal and state export pumps, but I did not consider these effects. Especially along the slower moving San Joaquin River, OP concentrations due to degradation might have been reduced more during other pulses whose travel times were significantly longer than the that of the February 1993 pulse.

Modeling OP exposure I used regression analysis to test for correlation between elevated levels of organophosphates and decreased abundances of the four arthropod taxa studied. For a given benthic sampling event at a particular site, I considered OP levels to which organisms at that site would have been exposed during the thirty days preceding the sampling date.

To describe the OP exposure that occurred within these thirty days as an explanatory variable in my regression model, I made certain assumptions about how the four taxa I studied would be likely to respond to given doses of organophosphates. A dose describes the level and duration of exposure to a toxicant, and in this case the response I was interested in was mortality, or more exactly, decreased abundance. The dose-response relationship that an organism will exhibit upon exposure to a certain toxicant is shaped by molecular interactions between the organism and the toxicant and the consequences of those interactions at higher levels of physiological organization. Interactions at the molecular level will not necessarily increase in proportion to an increase in toxicant, and the physiological consequences of those interactions may not manifest in proportion to increased molecular interactions (Walker 1996).

Dose-response relationships are thus usually not as simple as a linear relationship in which a given increase in toxicant results in a proportionate decrease in survival. In fact, organisms generally exhibit a dose-response relationship in which the majority of organisms die at a relatively narrow range of toxin levels (Begon *et al.* 1996). This pattern of response can result when an organism's physiological ability to cope with lower doses of a toxicant is overwhelmed at a threshold range of toxicant concentrations, or by a threshold time of exposure to a certain level of toxicant (Walker 1996). Though organisms may differ in their dose-response patterns to a given toxicant, an indication of the response pattern the four

arthropod taxa examined in this study might exhibit to OPP is given by the dose-response pattern shown by the aquatic arthropod *Ceriodaphnia dubia*. In seven-day tests, diazinon concentrations of 200 ng/l have been reported to cause 90 to 100 percent mortality, while concentrations of 150 ng/l cause no mortality (Kuivila and Foe 1995).

Duration (days)	Combined diazinon and methidathion Concentration (ng/l)	Number of months in which threshold dose occurred at given sampling site between January 1991 and April 1994		
		D28A & D19	D4 & D11	D7
1	> 100	4	3	3
	> 70	5	4	4
	> 40	7	6	5
	> 20	7	9	8
4	> 40	5	4	3
	> 20	6	6	6
	> 5	16	8	7
7	> 20	5	4	3
	> 5	13	5	5

Table 3. Occurrence of threshold OP doses included in regression analysis.

The taxa studied would be likely to exhibit a dose-response pattern in which the majority of organisms die at a threshold level or duration of exposure to OPP. I constructed my regression model to test for correlation between decreased abundances of the taxa and their exposure to certain threshold concentrations persisting for different numbers of days (Table 3). I characterized the OP exposure that organisms at a sampling site would have experienced during the thirty days prior to a given sampling event as dummy variables that indicated whether or not organophosphate concentrations exceeded a threshold concentration for a given number of days during the month preceding the sampling event.

Other factors affecting abundance The OP pulses coincided with natural processes that might cause decreased abundances of the four taxa studied. Freshwater inflow associated with the pulses can dramatically affect benthic organisms. Also, many of the pesticide pulses occurred during the winter and early spring seasons when benthic abundances may be

depressed as part of natural seasonal population fluctuations. It was thus important to incorporate some of the major factors that might influence the abundance of the four benthic arthropods studied into my regression model. I included the following explanatory variables in the regression model:

- 1) WATER TEMPERATURE (SEASON): Benthic invertebrates generally exhibit seasonal population fluctuations that are largely due to periodicity in reproductive patterns. For example, in east-coast estuaries, *Gammarus daiberi* populations have been observed to substantially increase from winter levels in the Spring and Summer with large recruitment of juveniles, then decline in the Fall, and return to low overwintering populations (Bousfield 1973). At temperate and higher latitudes, water temperature has been identified as a major trigger for reproductive activity and seasonal population fluctuations of benthic invertebrates (Nichols and Pamatmat 1988). Other factors, such as algal mass related to light availability, that fluctuate with the season might also affect benthic population dynamics. I could not be certain of the reproductive periodicity and the natural seasonal abundance fluctuations in the Delta of the four taxa studied. I assumed that these natural seasonal abundance fluctuations would parallel changes in water temperature. By including water temperature as an explanatory variable, I attempted to control for the four taxa's natural seasonal abundance fluctuations. Water temperature was measured during each benthic sampling event.
- 2) LAGGED ABUNDANCE: The abundance measured in a particular month at a spot in the channel (i.e. left, right, center) at a given site is likely to be related to abundances in previous months at that spot. To account for this serial autocorrelation, I included the previous month's abundance at a particular spot (i.e. D4 center) as an explanatory variable.
- 3) SALINITY: Salinity is a primary factor affecting the distribution of benthic organisms in the estuary (Markmann 1986, Hymanson *et al.* 1994, and others). Electroconductivity (EC) measurements were taken by the D.W.R. at the benthic sampling sites during sampling events. I derived salinity from these EC readings by the equation: $\text{Salinity}_{\text{ppt}} = 100 \times \ln[(1 - \text{EC}_{\text{millisiemens}}) / 178.5]$.

- 4) **PERCENT ORGANIC CARBON:** The organic carbon content of sediment could affect food availability for the four taxa studied. It was measured as percent organic carbon, or the fraction of dry weight of organic material over dry weight of organic material plus sediments, multiplied by one hundred. Percent organic carbon was measured in a sediment grab taken at the same time as benthic samples at each site.
- 5) **GRAIN SIZE:** Grain size can limit the occurrence of certain species. For example, a tube-building organism may require certain substrate in which to build its tube (Markmann 1986). The sediment from a grab taken at the same time and place as the benthic samples was divided into three size classes, fines, sand, and gravel. Because gravel was extremely rare at the Delta sampling sites, I was able to use percent fines to indicate grain size for each sampling event.
- 6) **FLOW:** Flow rate can affect benthic organisms primarily by changing salinity, and by altering the sediment composition of a given area (Markmann 1986). I accounted for changes in salinity as well as grain size and organic carbon in the sediment by including these as variables. I nonetheless included flow rate as an explanatory variable to account for possible effects of current velocity on benthic abundances. I used flow rates measured by the USGS at Freeport on the Sacramento River to approximate changes in flow at sites D4, D11, and D7, and used flow rates measured at Vernalis on the San Joaquin River to estimate flow fluctuations at sites D19 and D28A. For each abundance corresponding to a given sampling event, the average flow rate occurring within the thirty days preceding the sampling event was included as an explanatory variable.

Regression analysis Abundance values were transformed from Y to $\log_{10}(Y+1)$ to minimize the effects of infrequent extreme values on mean abundances. Separately for each taxon, log abundance was regressed on the 'control' variables plus one of the variables that characterized OP exposure. The regression was run separately for each of the different threshold doses described previously. The relationship between the abundance of a given taxon and occurrence of a threshold OP dose within thirty days prior to the sampling event was interpreted based on the coefficients of the OP dose variables. If these coefficients were negative and statistically significant for $P < .05$, this was interpreted as evidence suggesting

negative correlation between abundance of a given taxon and occurrence of a threshold OP level.

Data Sources I acquired the data on benthic abundances from the Internet at the Interagency Ecology Program site. Pesticide concentrations are available in USGS open file report 95-110 (MacCoy et. al. 1995) (see Appendix A for benthic infauna and pesticide monitoring methods). The Department of Water Resources provided me with grain size, organic carbon content, and salinity measurements corresponding to benthic sampling events. I obtained flow data from the USGS Internet site that provides historical flow information in California rivers (USGS 1999). I used flow data from USGS site 11447650 at Freeport and site 11303500 at Vernalis.

Diazinon toxicity to *Hyaella azteca*

Experimental design I performed toxicity tests on the amphipod *Hyaella azteca* to determine this species' susceptibility to diazinon. Organisms were exposed to six treatments (diazinon in ng/l or parts per trillion): 25, 55, 130, 300, 700, and 1600. Each treatment consisted of four replicates. Each replicate contained ten organisms. The treatments at concentrations of 700 ng/l and below reflect diazinon concentrations that have been measured in the Delta (at, and downstream from Vernalis and Sacramento). Concentrations averaging as much as 1,000 - 7,000 ng/l persisting for several days have been measured further upstream in waterways of the Lower Sacramento and San Joaquin River Basins (Foe et al. 1998). I monitored organism survival after four and seven days of organism exposure to a control solution at 0 ng diazinon /L and the six treatments, and compared percent survival in each treatment to percent survival in the control using a Student's t-test for a small sample size ($N < 30$).

Organism collection, identification and storage I collected organisms at Bethel Island near Frank's Tract in the Delta, across from Frank's boat launching facility. I found the organisms clinging to floating algal mats that were nestled into reeds on the side of the channel opposite the boat-launching ramp. I placed clumps of algae containing the organisms into plastic and glass jars containing river water. Collection took approximately 3 hours. Organisms were transported by car to the laboratory, a trip of about 2 hours.

In the laboratory, I poured the algae and organism samples into plastic trays. I sorted organisms that appeared to be the desired amphipods without the aid of magnification into glass mason jars containing 500 ml of river water, approximately 30 organisms to a jar, in twelve jars. The jars received aeration through pipettes and their openings were covered with aluminum foil. Organisms were stored for a week in this manner.

Organisms received food every 4 days while in the lab. Food preparation consisted of the following steps: 1) 2.5 g of rabbit pellets (alfalfa) were placed in 500 ml of distilled water, and mixed at high speed in a blender for 5 minutes. 2) 2.5 g of dry yeast was added to the mixture, which was then stirred at low speed for 1 minute. 3) The mixture was then placed in a jar and allowed to settle. Organisms received approximately 20ml/l of the supernatant from this mixture while stored at ~25 organisms to a jar.

Two organisms were selected and identified under a compound microscope at 100X magnification as *Hyalloella azteca* by an expert on benthic invertebrate identification, who trained me to recognize the identifying characteristics of *Hyalloella azteca* and how this species could be distinguished from similar possibly collected species. I then selected twelve of the collected organisms, one from each jar, and identified them under a compound microscope. All twelve organisms I identified as *Hyalloella azteca*. The remaining organisms I pipetted from the jars, individually placed into Petrie dishes and identified under a dissecting microscope. All the organisms appeared to be *Hyalloella azteca*. Throughout the experiment, when organisms were removed from the pipette into water, the pipette mouth was placed below the air-water interface before ejection of the organisms.

I placed organisms in one-quart glass mason jars containing 200 ml of water and a 3 x 5 cm piece of plastic Nytex screening to provide a substrate for the *Hyalloella* to grasp. Each jar (replicate) contained ten organisms, 2 large (~5 -7 mm), 3 medium-sized (~ 3-5 mm) and 5 small organisms (~1- 3 mm). Every four days, I fed organisms in these jars 10 ml/L of the meal mixture described above. Extra organisms continued to be stored at ~30 organisms per jar and fed 20ml/L of the meal described above.

I prepared the water in which the organisms were placed by mixing 1 liter of distilled water with 1.4 g of Instant Ocean to give a salinity of approximately 1.4 ppt, the average salinity of estuarine waters during the months of December, January, and February, when organophosphate levels in the estuary were highest between 1991 and 1994. I stirred the

distilled water and instant ocean mixtures on a stirring plate for one half hour, then poured the water into jars and pipette-aerated it for 15 minutes prior to exposure of organisms to the water. Organisms were stored in these jars for two weeks prior to the toxicity testing. Every four days, I randomly selected ten jars using a random numbers table, and measured the temperature, pH and dissolved oxygen content of water in these jars, using a thermometer, pH paper and a dissolved oxygen meter, respectively. While organisms were stored and during the toxicity tests, temperatures ranged between 17 and 21 degrees Celsius, pH levels remained between approximately 6.5-7.5, and dissolved oxygen content remained between 8.8 and 11.3 mg/l.

Preparing treatment solutions I prepared solutions for the six treatments by creating stock solutions at as low a diazinon concentration as possible given the equipment available, and diluting the stock solutions to obtain the desired diazinon concentrations. I did this through the following steps:

- 1) 20 microliters (μl) of liquid diazinon (Sigma Aldrich Product No. A-8265 C) were added to 250 milliliters (ml) of distilled water to give a solution at 80 $\mu\text{l/L}$. The solution was mixed for 1.5 hours with a magnetic stirring rod on a stir plate.
- 2) 20 μl of solution from step 1 was added to 1 L of saline (1.4 parts per thousand for all treatments) water to produce Treatment 6 at 1600 nanograms (ng)/L. For all treatments, solutions were stirred for 1 hour prior to the exposure of organisms to the solutions.
- 3) Step 2 was repeated. 437.5 ml of the solution at 1600 ng/l given by step 2 was mixed with 562.5 ml of saline water to produce Treatment 5 at 700 ng/L.
- 4) 187.5 ml of remainder from Step 3 at 1600 ng/L was mixed with 812.5 ml of saline water to produce Treatment 4 at 300 ng/L.
- 5) 81.3 ml of remainder from Step 3 at 1600ng/L was mixed with 918.7 ml of saline water. This gave a solution at 130 ng/l for Treatment 3.
- 6) 34.4 ml of remainder from Step 3 at 1600 ng/L was mixed with 955.6 ml of saline water to produce Treatment 2 at 55 ng/L.
- 7) 15.6 ml of remainder from Step 3 at 1600 ng/L was mixed with 984.4 ml of saline water to produce Treatment 1 at 25 ng/L.

Toxicity tests After the six treatment solutions were mixed for one hour, I exposed the organisms to the solutions. I removed the pieces of Nytex screening from the jars containing ten organisms per jar. I then gently poured organisms and water from each of four jars into plastic trays, rinsed the jars with tap water and wiped them dry. I poured each of the control and treatment solutions into a set of four jars, 250 ml of solution per jar. All solutions had been prepared using water at a salinity of approximately 1.4 ppt, prepared by mixing 1.4 g of Instant Ocean with 1 liter distilled water on a stirring plate for one half hour. Each jar containing 250 ml of the control or treatment solutions was aerated for 15 minutes before organisms were returned to the jars. I examined organisms while they were in the plastic holding trays to ensure that all organisms to be used in the toxicity tests appeared healthy. If an organism was discolored, moved more slowly than the others, or was dead, I replaced it with a healthy organism of a similar size from the stock of organisms stored at 30 to a jar.

After four days of organism exposure to the control and treatment solutions, I monitored their mortality. I gently poured the contents of the four replicate jars in the control and each treatment into plastic trays after removing the Nytex screening from each jar with tweezers. I gently shook the screening prior to removing it from the jar to dislodge any organisms that were clinging to the screening. Because the same holding trays were used for each treatment, I examined the control and treatment jars in order of lowest to highest diazinon concentration to minimize cross-contamination.

I considered organisms to be dead if they did not move their limbs upon gentle prodding. I removed dead organisms from the trays, and recorded the number of live and dead organisms. In two of the replicates, I had to remove a live organism because of undue hardship caused by an unfortunate encounter with the pipette used for aeration. In these jars, I calculated percent survival out of nine total organisms.

Once I removed organisms from the jars, I rinsed each jar to remove any organic matter, and filled the jars with appropriate control or treatment solution. These solutions were produced following the methods outlined above that describe the first preparation of the control and treatment solutions. I aerated the solutions for 15 minutes before returning organisms to the jars. To bring the total duration of exposure to the solutions up to seven days, I exposed organisms to the reconstituted solutions for an additional three days, and

monitored survival after seven days total exposure in the same manner as I had after four days of exposure.

For each replicate (jar), I calculated percent survival after four and seven days of exposure as the number of live over the total number of dead and live organisms, multiplied by one-hundred. I tested for differences in mean percent survival between each treatment and the control, by using a Student's t-test for a small sample size ($N < 30$).

Results

Long-term trends in abundance One of the four taxa studied that exhibited a significant long-term trend of declining abundance in the Delta was *Nippoleucon hinumensis*. This species was collected at site D7 in decreasing abundances between 1990 and 1999 (Fig. 3). These declines follow a pattern of increased freshwater inflow and decreasing salinity over these years, and are probably attributable to *Nippoleucon hinumensis*' inability to tolerate less brackish water. The fact that *Nippoleucon hinumensis* has been rarely collected at upstream sites D28A and D19, and has been found in greater abundances at site D7 and at site D41 in San Pablo Bay supports the assumption that this species prefers higher salinity conditions. As illustrated by the 95% confidence intervals in Figure 3, years of higher freshwater inflow are also characterized by greater variation in flow. This variation may also affect benthic abundances by creating environmental instability.

Gammarus daiberi has been collected in relatively consistent numbers at site D4 between 1990 and 1999. At site D28A, *G. daiberi* appears to have declined after 1994. This corresponds to a dramatic increase in freshwater inflow to the Delta and may be attributable to this factor. *G. daiberi* was collected in appreciable abundances at site D7 only in 1995 and 1998.

Corophium spinicorne was collected in relatively consistent numbers at site D28A between 1985 and 1999, and was found only rarely at site D7. Abundances of this taxon at site D4 appear to have declined during and for a time after the low inflow drought years of 1987-1992, but seem to have recovered to pre-drought levels by the late 1990's.

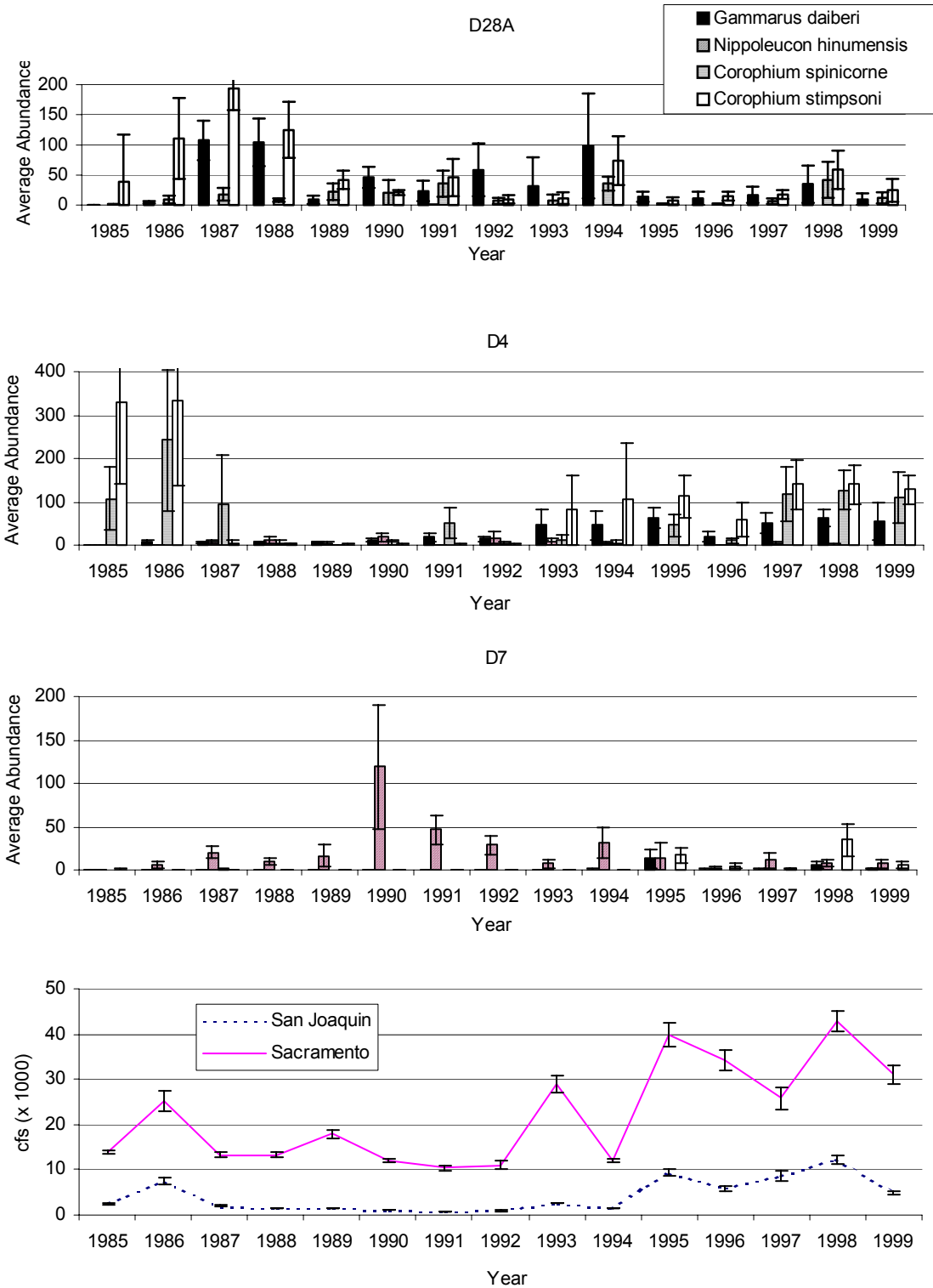


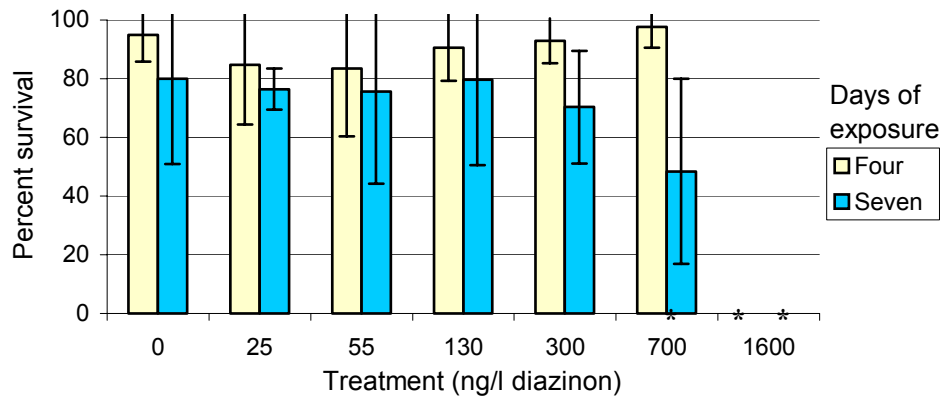
Figure 3. Average annual abundances of *Gammarus daiberi*, *Nippoleucon hinimensis*, *Corophium spinicorne* and *C. stimpsoni* at sites D7, D4 and D28A, and Sacramento And San Joaquin Rivers flow at Freeport and Vernalis between 1985 and 1999. Error bars denote 95% C.I.

At site D4, *C. stimpsoni* followed a similar pattern of decreased abundances during the low inflow years of 1987-1992, but unlike *C. spinicorne*, *C. stimpsoni* does not appear to have recovered to pre-drought numbers after 1992. This may be a result of higher freshwater inflow during the late 1990's as compared to inflow in 1985-86. At site D28A, the pattern of *C. stimpsoni* abundance fluctuations was similar to that apparent at site D4, though abundances associated with the drought years do not appear to have declined until 1989. *C. stimpsoni* was collected in appreciable abundances only between 1995-1999 at site D7, probably facilitated by higher freshwater inflow and lower salinities during those years.

Correlation analysis When monthly abundances of *Corophium spinicorne*, *Gammarus daiberi*, and *Nippoleucon hinumensis* were regressed on the 'control' variables plus one of each of the variables describing exposure to ten threshold OP doses within the thirty days preceding the benthic sampling event, none of the correlation coefficients relating abundances to the OP dose variables were significantly different from zero ($P < .05$). These results suggest that OP pulses do not cause short-term declines in abundances of these taxa. R-squared values for the regressions on different OP doses were similar for a given taxon. The regressions of abundances on the control and OP dose variables yielded R-squared values of .52 for *G. daiberi*, .68 for *C. spinicorne*, and .70 for *Nippoleucon hinumensis*.

Regression of abundances on the control and OP dose variables yielded a correlation coefficient, relating abundance to the variable that indicated the presence of a threshold dose of 20 ng/l for one day, that was negative and significantly different from zero (Correlation coefficient = -.13, $P = .026$ $R^2 = .82$). This result suggests that OP pulses may cause short-term declines in *C. stimpsoni* abundance in the Delta.

Diazinon toxicity to *Hyalella azteca* After four days of exposure to diazinon, *Hyalella azteca* survival appeared to be adversely affected only in the treatment with the highest diazinon level of 1600 ng/l, in which no organisms survived (Fig. 4). Survival after four and seven days in the treatment with the next highest diazinon levels was not significantly different from the control. The zero survival rate after four day-exposure to a diazinon level of 1600 ng/l and the lack of negative effects after four and seven-day exposure to 700 ng/l, suggests that four-day survival of *H. azteca* may be negatively affected at diazinon levels considerably lower than 1600 ng/l, but higher than 700 ng/l.



Notes: * Survival significantly different from control for $P < 0.05$

Error bars represent 95% Confidence Interval

Figure 4. Survival of *Hyallela azteca* after four and seven day exposure to diazinon concentrations that reflect levels detected in the Delta and further upstream in the Sacramento and San Joaquin Rivers. * represents survival significantly different from controls ($p < 0.05$); error bars represent 95% C.I.

Discussion

Long-term trends Over the years 1985-1999, all four species show some indication of declines in abundance at at least one of the three sites examined. These declines might be explained by increased freshwater inflow to the Delta during the late 1990s, by the residual effects of severe salinity intrusion that occurred during the drought years of the late 1980s, or by other factors I did not consider. It is important to remember that decreased abundances at these three sites would not necessarily reflect decreased abundances in the Delta. For example, an increase in salinity at a given site may result in decreased abundances at that site, but upstream areas that had previously been inhospitable to a given organism may see an increase in abundance of that species.

The regression analysis suggests that short-term declines in abundances of *C. stimpsoni* are correlated with exposure to organophosphates. *C. stimpsoni* abundances at sites D4 and D28A may not have recovered to pre-drought levels during the late 1990s due to the effects of OP pulses.

OP effects on *Corophium stimpsoni* The regression analysis suggests that abundances of *Corophium stimpsoni* are negatively correlated with exposure to an OP dose of 20 ng/l for one day. I used the equation provided by the regression, that relates *C. stimpsoni* abundances to the control variables and the variable indicating exposure to an OP dose of 20 ng/l for one

day, to calculate the percent decline in abundance that is associated with the occurrence of a one-day, 20 ng/l dose. The regression model estimates that exposure to this dose is related to a 25% reduction in abundance. This is not as large a decline in abundance as would be expected if 20 ng/l for one day represents a threshold dose that is lethal to the majority of organisms. The fact that *C. stimpsoni* abundances were not significantly correlated (for $P < .05$) with exposure to the next highest OP doses of 40 ng/l for one day, 20 ng/l for 4 days, or any higher doses, suggests that a threshold OP dose for *C. stimpsoni* would be at a concentration of between 20-40 ng/l for one-four days. Available data of OP toxicity to aquatic arthropods indicates that this degree of susceptibility to OPP would be on the very sensitive end of the spectrum. For example, the aquatic arthropod most sensitive to diazinon based on available toxicity data is *Chironomus tentans* with a 96-hour LC_{50} of 30 ng/l (Kuivila and Foe 1995, Novartis 1997).

An alternative to the explanation of lethal toxicity as a cause for the apparent negative correlation between a one-day 20 ng/l OP dose and *C. stimpsoni* abundances, is the possibility that some *C. stimpsoni* may respond to the increased OP levels by drifting downstream. *C. stimpsoni* has been collected only once from 1985-1999 west of D7 in Suisun Bay at sites D6 in the Carquinez straits and D41 in San Pablo Bay, suggesting that this species would not drift out of the system I studied. *C. stimpsoni* drifting from sites D19 and D28A would end up in the federal export pumps as little if any San Joaquin water flows toward the Bay. In months where the benthic samples were taken 3- 7 days after the OP pulse events, *C. stimpsoni* drifting downstream from sites D4 and D11 might return to the benthos and show up in samples taken at D7, thereby decreasing the correlation between declining abundance and OP pulses events at this site. I considered the hypothesis that *C. stimpsoni* respond to the pulses by entering the water column too complex to test with the model I constructed.

Bioavailability The regression analysis demonstrates no negative correlation between abundances of *Corophium spinicorne*, *Gammarus daiberi*, and *Nippoleucon hinumensis*, and their exposure to threshold doses of organophosphates. This suggests that OP levels were too low to cause significant lethal toxicity to these organisms. Another possible explanation of these results is that toxicity was not detected because OP levels in the water column were not representative of what was bioavailable to the organisms. Given the possibility that

correlation between *C. stimpsoni* abundances and OP pulses does not represent a causative relationship, OPP may not have been bioavailable to the benthos. In estuarine environments where fresh water mixes with saline waters, the fresh water may slip over the denser brackish water. If this partitioning had occurred in the Delta, the OPP associated with fresh river water might not have mixed well with more saline bottom waters. Benthic organisms might then have been exposed to less toxic bottom waters. However, in the area encompassing the benthic sampling sites, such a phenomenon is not likely to occur (Kathy Kuivila, personal communication). Particularly during periods of high freshwater inflow associated with the OP pulses, salinities are relatively homogenous in this region. Due to these low salinity differences and the shallow channel depths in this part of the Delta, benthic organisms would probably not have been exposed to water column OP levels lower than those measured by the USGS using depth-integrated samples.

In fact, benthic organisms may have been exposed to higher OP levels than those detected in the water column. Although OPPs are relatively hydrophilic, they may partition into sediment organic carbon at higher levels than are present in water. The partitioning coefficient (K_{oc}), a log-based constant that describes the distribution of a compound between organic carbon and water, has been estimated at between 2 and 3 for diazinon (Domagalski and Kuivila 1993). This means that diazinon may be present in organic carbon associated with sediments at levels that are 100 to 1000 times higher than those in the water column. Benthic organisms' exposure to OPP in the sediment would depend on their feeding habits, degree of association with the sediment, and other factors.

Possible sources of error Potential sources of error in the model I used to test for correlation between OP pulses and benthic abundances lend a degree of uncertainty to the results suggested by this model. I have already discussed possible inaccuracies in my estimates of the time at which, and OP levels to which organisms were exposed (See 'Methods'). Another aspect of my model that might have been inaccurate is the assumption of a linear relationship between organism abundance and the environmental variables affecting the benthos. Ecological data often do not exhibit linear relationships (Hymanson et al 1994).

Limitations of the model The model I constructed was not capable of describing and testing for certain possible effects of the OP pulses that could have important ecological

ramifications. For example, the model was limited by the manner in which it described the potential toxicity of the OP pulses. It did not take into account possible interactive effects of OPP and other toxins such as heavy metals that might have been associated with agricultural runoff, or may have already been present in the Delta. The model also did not account for the possible effects of organism exposure to multiple OP pulses. Prior exposure to elevated OP levels might stress organisms and increase the toxicity of subsequent pulses. Alternatively, previous exposure might acclimate organisms by, for example, inducing enzymatic defense mechanisms, making later pulses less toxic. I was unable to investigate relationships between organism abundance and multiple pulse exposure due to the paucity of pesticide pulse events that were available for analysis.

Yet another limitation of my model was that it was only able to test for possible acute effects of the OP pulses on the four taxa studied. A toxicant may produce adverse, but not lethal, effects on given organisms at much lower concentrations than those that produce lethal effects (Walker *et al.* 1996). For example, the diazinon concentration that produces a 25% reduction in reproduction of *Ceriodaphnia dubia* is 120 ng/l compared to an LC₅₀ of about 500 ng/l for this organism (Foe and Shepline 1993). Sub-lethal effects might adversely affect the longer-term viability of benthic populations.

The ‘noise’ of variability, in both monthly abundance measurements and major factors affecting the benthos, may have limited my model’s ability to detect abundance changes associated with OP pulses. Due to stochastic processes and the irregularity of the benthic environment, benthic organisms are generally distributed in a spatially heterogeneous manner (Nichols 1985). This heterogeneity means that variation between the numbers of organisms collected in replicates at a particular sampling site can be very high. In some sampling events, abundances between replicates varied by two to three orders of magnitude.

The simultaneous occurrence of the pesticide pulses and dramatic changes in the hydrological regime of the Delta may have also contributed to ‘noise’ that hindered the detection of changes in benthic abundances related to OP exposure. The increased freshwater inflow that accompanied the OP pulses dramatically changed physico-chemical parameters such as current velocity, salinity and substrate composition that can affect benthic organisms. These factors may have been more important in determining benthic abundances than exposure to OPP, and may have masked any changes due to the OP pulses. This

possibility raises the question: If natural factors are more important in determining benthic abundances, are potential effects from the OP pulses still relevant? OP pulses' effects might still be important if they weaken organisms' ability to adjust to or recover from periods of environmental instability.

OP effects on *Hyalella azteca* According to my tests of diazinon toxicity to *Hyalella azteca*, OP levels greater than, or equal to 1600 ng/l cause 100% mortality to *H. azteca* after four days of exposure. These results suggest that waters upstream of Vernalis on the San Joaquin River and in tributaries of the Sacramento and San Joaquin Rivers may at times be lethally toxic to *H. azteca*. Average OP levels of 1000-7000 ng/l possibly persisting for several days have been observed in these areas (Foe 1995, Foe *et al.* 1998). Results of the toxicity tests suggest that levels and durations of elevated OP concentrations detected from 1991-1994 in the Delta would not be lethal to *H. azteca*. OP levels as high as 800 ng/l were detected at Vernalis, but levels comparable to this did not persist for more than two days, and *Hyalella* mortality was not different from the control after four days of exposure to diazinon at 700 ng/l. However, OP levels of greater than 100 ng/l persisted for up to 14 days at Vernalis. *H. azteca* may suffer mortality at these concentrations when exposed for longer than the seven days employed in the toxicity tests.

Ecological implications The regression analysis, and an examination of long-term trends in *C. stimpsoni* abundances, suggests that OPP in the Delta may cause short-term declines in abundances of *C. stimpsoni*, and may have contributed to a trend of decreasing *C. stimpsoni* abundances between 1985 and 1999. What might be the ecological ramifications of reduced numbers of this taxon? As mentioned previously, *C. stimpsoni* is an important food source for several fish species that inhabit or migrate through the Delta, such as striped bass *Morone saxatilis*, young Chinook Salmon *Onchorynshus tshawytscha*, young Sturgeon *Acipenser* spp., and catfish *Ictalurus* spp. (Markmann 1986). Decreased *C. stimpsoni* abundances might lead to a decline in food availability for these fish and other predators, depending on the predators' flexibility in choosing prey species and the possibility that other prey species might increase due to reduced competition with *C. stimpsoni*.

Stomach content analysis of juvenile striped bass showed that peaks in Corophium abundance in bass stomachs coincided with decreased consumption of other prey, suggesting that bass are somewhat flexible in their prey selection (Markmann 1986). However,

Corophium species may serve as an important food source during periods when other prey is not available. These diet analyses did not discriminate between the two Corophium species, and it is probable that predators would not discriminate either. Abundances of *C. spinicorne* might compensate for decreased *C. stimpsoni* abundances due to reduced competition with the latter. However, *C. spinicorne* appears to prefer microhabitats containing coarser substrate than those in which *C. stimpsoni* thrives.

The following points suggest that OP levels detected upstream from the Delta may be more likely to cause acute toxicity to aquatic invertebrates than OP levels detected in the Delta: 1) Short-term abundances of three of the four dominant arthropod taxa that I studied in the correlation analysis did not appear to be affected by OP levels in the Delta 2) Available toxicity data suggests that OP levels in the Delta would tend to acutely affect only the most sensitive invertebrate species 3) *Hyaella azteca* appears to be susceptible to OPP at levels detected upstream from the Delta, while OP levels comparable to those measured in the Delta do not appear to cause lethal toxicity to *H. azteca*. The Central Valley Regional Water Quality Control Board has performed bioassays using *Ceriodaphnia dubia*, of water from the San Joaquin and Sacramento Rivers, and from tributaries and agricultural drains in the Lower Sacramento and San Joaquin Basins (Foe and Sheipline 1993, Foe 1995, Foe *et al.* 1998). These studies have found significant toxicity to *C. dubia* in many watercourses in these areas and have attributed most of this toxicity to the organophosphates diazinon and methidathion, though other pesticides may have also contributed to toxicity. The ecological effects of pesticide pulses on the biota of Central Valley waterways is not well understood.

Summary and conclusions The regression analysis of benthic monitoring data in this study suggests that *C. stimpsoni*, an important food source for several fish, is adversely affected by OP pulses entering the Delta, while the three other species that dominate the benthic arthropod community, *C. spinicorne*, *N. hinumensis*, and *G. daiberi*, are not. Bioassays of contaminated water and laboratory toxicity tests using these species would help shed light on the validity of these conclusions. My rough analysis of long-term trends in abundances of these four species indicates that OP pulses may be contributing to a decline in abundances of *C. stimpsoni* over the years 1985-1999. The IEP report on long-term trends in benthic abundances after 1990 might help determine the importance of various factors in determining *C. stimpsoni* abundances. Laboratory toxicity tests performed in this study

suggest that the resident amphipod *Hyallolela azteca* would not be affected by OP levels present in the Delta, but would exhibit mortality upon exposure to OP levels detected upstream in water-ways of the lower Sacramento and San Joaquin basins. Further toxicity studies on *Hyallolela azteca* would provide better understanding of this resident amphipod's dose-response pattern when exposed to OPP. Bioassays of OP contaminated river and delta waters would help determine if OP toxicity to *H. azteca* would be altered by water quality parameters specific to river and delta waters.

Most assessments of the impacts of OP pulses on the Delta and lower Sacramento and San Joaquin basins have employed two strategies: 1) Comparisons of OP levels measured in water-ways to published laboratory toxicity data on mostly non-resident species 2) Bioassays of delta and river water using national standardized test species such as *Ceriodaphnia dubia*. These strategies yield uncertain conclusions, because resident species might respond very differently to OPP than species for which toxicity data exists, and environmental conditions not present in a laboratory setting might alter the effects of OP doses.

Any successful plan for assessing the impacts of OPP on delta and river biota should employ a variety of strategies to paint a more complete picture of potential threats or lack thereof. Better understanding of organisms' ecological roles in the Sacramento and San Joaquin River Basins and Delta (as prey for endangered or commercially valuable fish, for example) would help determine which organisms should be given priority in further studies. Bioassays and laboratory toxicity tests using resident species would provide more ecologically relevant information on OP toxicity to organisms in the Delta and River Basins. Toxicity tests in which organisms are exposed to multiple pulses, or mixtures of OPP and other toxicants present in delta and river water, might provide a more accurate evaluation of potential toxicity in the field. In situ toxicity tests that use resident organisms in enclosures could facilitate better understanding of OP pulse effects under field conditions.

Because adverse ecological effects can be difficult to causally link to a given toxicant, environmental managers may take preemptive, or cautionary steps to lessen potential threats posed by a toxicant. Assessments of OP impacts on the Sacramento-San Joaquin River Basins and Delta have already led the Department of Pesticide Regulation (DPR) to institute programs designed to curb OP runoff into water-ways through voluntary measures taken by growers. These measures include adjustment of application techniques and other integrated

pest management practices (DPR 1998). In 1996 and 1997, OPP applied to orchards in the Central Valley declined considerably, as did toxicity of surface waters to *C. dubia* (Bennett 1998, DPR 1998). These changes are probably attributable not to the DPR-recommended practices, but to the consistent rainfall patterns in these years that prevented growers from taking heavy spray rigs used to apply OPPs onto saturated fields (Bennett 1998, DPR 1998). Further study of organophosphate pulses' impacts on water courses in the Lower Sacramento-San Joaquin basins and Delta will help managers determine if stricter measures should be taken to control input of organophosphates into this productive aquatic system.

Acknowledgements

Many thanks to Bruce Thompson and Sarah Lowe at the San Francisco Estuary Institute for introducing me to the idea for this project. Special thank you to Don Weston for his valuable advice and laboratory facilities. I greatly appreciated information provided to me by Christopher Foe of the Central Valley California Regional Water Quality Control Board, and Robert W. Meyer, Rick N. Oltmann, and Kathy Kuivila of the United States Geological Survey. Thanks to the Department of Water Resources for allowing me to tag along on a benthic sampling run and cruise the beautiful Sacramento-San Joaquin Delta. Finally, thank you to Tom Dudley and Kevin Kennedy for their helpful counsel and encouragement.

References

- Andreu-Moliner, E S, M.M. Almar, I. Legarra, A. Nunez. 1986. Toxicity of some ricefield pesticides to the crayfish *Procambarus clarkii*, under laboratory and field conditions in Lake Albufera (Spain). *Journal of Environmental Science and Health Part B Pesticides Food Contaminants and Agricultural Wastes* 21: 529-538.
- Ankley, G. T., S. A. Collyard. 1995. Influence of piperonyl butoxide on the toxicity of organophosphate insecticides to three species of freshwater benthic invertebrates. *Comparative Biochemistry and Physiology C Pharmacology Toxicology & Endocrinology* 110:149-155.
- Arthur, J.W., J.A. Zischke, K.N. Allen and R.O. Hermanutz. 1983. Effects of Diazinon on macroinvertebrates and insect emergence in outdoor experimental channels. *Aquatic Toxicology* 4: 283-301.
- Bailey, H. C. 1993. Acute and chronic toxicity of the rice herbicides thiobencarb and molinate to opossum shrimp (*Neomysis mercedis*). *Marine Environmental Research* 36:197-215.

- Bailey, H. C., J.L. Miller, M.J. Miller, L.C. Wiborg, L. Deanovic, T. Shed. 1997. Joint acute toxicity of diazinon and chlorpyrifos to *Ceriodaphnia dubia*. *Environmental Toxicology and Chemistry* 16: 2304-2308.
- Begon, M., J.L. Harper, C.R. Townsend. 1996. *Ecology*. Oxford, Blackwell Science Ltd. 1068 pp.
- Bennett, K.P. 1998 . Occurrence of Aquatic Toxicity and Dormant Spray Pesticide Detections in the San Joaquin River Watershed, Winter 1996-97. State of California, Dept. of Pesticide Regulation, Environmental Monitoring and Pest Management Branch (various pagings).
- Bousfield, E. L. 1973. *Shallow-water gammaridean Amphipoda of New England*. Ithaca, New York, Comstock Pub. Associates 312 pp.
- Cooper, C. M. 1993. Biological effects of agriculturally derived surface water pollutants on aquatic systems. *Journal of Environmental Quality* 22:402-408.
- Department of Pesticide Regulation, State of California EMPM Branch. 1998. Occurrence of aquatic toxicity and dormant-spray pesticide detections in the Sacramento watershed, winter 1996-97. PM 98-01.
- Department of Pesticide Regulation, EMPM Branch. 1999. Pesticide use analysis and trends 1991-1996. PM 99-01.
- Interagency Ecological Program for the State of California. Sacramento http://www.iep.ca.gov/wqdata/disc_water, accessed June 20, 1999).
- Domagalski, J. L., N. M. Dubrovsky and C.R. Kratzer. 1997. Pesticides in the San Joaquin River, California: Inputs from dormant sprayed orchards. *Journal of Environmental Quality* 26:454-465.
- Domagalski, J.L., K. Kuivila. 1993. Distributions of pesticides and organic contaminants between water and suspended sediment, San Francisco Bay, California. *Estuaries* 16:416-416
- Flemer, D. A., B.F. Ruth, C.M. Bundrick and J.C. Moore. 1997. Laboratory effects of microcosm size and the pesticide chlorpyrifos on benthic macroinvertebrate colonization of soft estuarine sediments. *Marine Environmental Research* 43:243-263.
- Foe, C., L. Deanovic and D. Hinton. 1998. Toxicity identification evaluations of orchard spray storm runoff. Staff report. California Regional Water Quality Control Board, Central Valley Region, Sacramento, CA.
- Giddings, J.M., R.C. Biever, M.F. Annunziato and A.J. Hosmer. 1996. Effects of

diazinon on large outdoor pond microcosms. *Environmental Toxicology and Chemistry* 5: 618-643.

- Geiger, D.L., D.J. Call and L.T. Brooke. 1988. Acute toxicities of organic chemicals to fathead minnows (*Pimephales promelas*). *Center for Lake Superior Environmental Studies* 4.
- Hughes, G. M., T. Szegletes and J. Nemcsok. 1997. Study of the effects of brief exposure to an organophosphorous insecticide Methidathion on blood characteristics of carp *Cyprinus carpio*. *Acta Biologica Hungarica* 48:157-166.
- Katznelson, R. and T. Mumley. 1997. Diazinon in Surface Waters in the San Francisco Bay Area: Occurrence and Potential Impact. Prepared for the California State Water Resources Control Board. 1997.
- Kimmerer, W J., E. Gartside and J.J. Orsi. 1994. Predation by an introduced clam as the likely cause of substantial declines in zooplankton of San Francisco Bay. *Marine Ecology Progress Series* 113:81-93.
- Kratzer, C.R. and R.N. Biagtan. 1997. Determination of traveltimes in the Lower San Joaquin River Basin, California, from Dye-Tracer Studies during 1994-1995. United States Geological Survey. Water-Resources Investigations Report 97-4018.
- Kuivila, K.M. and C.G. Foe. 1995. Concentrations, transport, and biological effects of dormant spray pesticides in the San Francisco Estuary, California. *Environmental Toxicology and Chemistry* 14: 1141-1150.
- Kuivila, K.M. Aquatic Biogeochemist, United States Geological Survey, Water Resources Division, Sacramento, CA. 2000, personal communication.
- MacCoy, D., L. Crepeau, and K.M. Kuivila. 1995. Dissolved pesticide data for the San Joaquin River at Vernalis and the Sacramento River at Sacramento, California, 1991-94 Sacramento, Calif. U.S. Geological Survey open-file report 4004981614: 95-110
- Menconi, M. and C. Cox. 1994. Hazard assessment of the insecticide diazinon to aquatic organisms in the Sacramento-San Joaquin River system Pesticide Investigations Unit, Environmental Services Division administrative report 94-260 California. Dept. of Fish and Game. 60 pp
- Meng, L. and P.B. Moyle. 1995. Status of splittail in the Sacramento-San Joaquin estuary. *Transactions of the American Fisheries Society* 124:538-549.
- Merz, J. E., C. Vanicek, David. 1996. Comparative feeding habits of juvenile Chinook salmon, steelhead, and Sacramento squawfish in the lower American River, California. *California Fish and Game* 82:149-159.
- Nichols, F.H. and M.M. Pamatmat. 1988. The ecology of the soft-bottom benthos of San

- Francisco Bay: A community profile. Biological Report 85 (719) United States Fish and Wildlife Service. 73 pp.
- Nichols, F.H. and J.K. Thompson. 1985. Time scales of change in the San Francisco Bay Benthos. *Hydrobiologia* 129: 121-138.
- Novartis Crop Protection Inc. 1997. Technical Report. Greensboro, NC 27419-8300.
- Obrebski, S., J.J. Orsi and W. Kimmerer. 1992. Long-term trends in plankton distribution and abundance in the Sacramento-San Joaquin Estuary. Interagency Ecological Studies Program for the Sacramento-San Joaquin Estuary, Technical Report 32
- Pape-Lindstrom, P. A. and M.J. Lydy. 1997. Synergistic toxicity of atrazine and organophosphate insecticides contravenes the response addition mixture model. *Environmental Toxicology and Chemistry* 16:2415-2420.
- Pauli, B.D., R. A. Kent, M.P. Wong. 1991. Canadian water quality guidelines for cyanazine. *Ottawa, Ontario Inland Waters Directorate* 26:28.
- Pereira, W E., F.D. Hostettler, J.R. Cashman and R.S. Nishioka. 1994. Occurrence and distribution of organochlorine compounds in sediment and livers of striped bass (*Morone saxatilis*) from the San Francisco Bay-Delta Estuary. *Marine Pollution Bulletin* 28:434-441.
- Pritchard, John B. 1993. Aquatic toxicology: Past, present, and prospects. *Environmental Health Perspectives*. 100:249-257.
- Reese, C.D. 1972. Pesticides in the aquatic environment. United States Environmental Protection Agency, Office of Water Programs, Washington. 181 pp.
- Serrano, R. F., Hernandez, J.B. Pena, V. Dosda and J. Canales. 1995. Toxicity and bioconcentration of selected organophosphorus pesticides in *Mytilus galloprovincialis* and *Venus gallina*. *Archives of Environmental Contamination and Toxicology* 29:284-290.
- United States Environmental Protection Agency. 1995. AQUIRE (database), Duluth MN. <http://www.epa.gov/ecotox/>
- United States Geological Survey. Real-time water data in California. Sacramento. <http://s601dcascr.wr.usgs.gov/Sites/index.html> accessed July 15, 1999.
- Walker, C.H., S.B. Hobley, R.M. Sibley, D.B. Peakall, 1996. Principles of ecotoxicology. Taylor and Francis, London. 315 pp.
- Watling, L. 1991. Revision of the cumacean family Leuconidae. *Journal of Crustacean Biology* 11:569-582.

Yoshiyama, R. M., F.W. Fisher and P.B. Moyle. 1998. Historical abundance and decline of chinook salmon in the Central Valley Region of California. *North American Journal of Fisheries Management* 18:487-521.

Appendix A: Benthic Sampling and Pesticide Monitoring Methods

Measurements of abundance For the period, 1992-1994, the Department of Water Resources collected three replicates in the left, and/or center, and/or right side of the channel at a given sampling site. Field sampling and laboratory identification techniques are described by the D.W.R. as follows:

Benthic samples are collected from a boat with a Ponar Dredge. The Dredge has a sampling area of 0.053 m². The contents are brought to the surface and placed in a large plastic bucket. Water is then added to the sample to create a slurry. The contents of each grab sample bucket are then washed into a Standard No. 30 mesh screen (0.595 mm openings). Each sample is carefully washed with a fine spray to remove as much of the substrate as possible. All material remaining on the screen is washed into a plastic jar and preserved with buffered formalin containing Rose Bengal dye. Three replicate samples are collected at each sight. Each replicate is processed individually. At the laboratory, the volume of settleable substrate in each sample jar is estimated and recorded. The formalin fixative is poured off and the sample is thoroughly washed on a 30-mesh screen. The composition of the substrate is estimated and recorded noting the relative percentages of peat, sand, mica, organic detritus, and other materials.

The substrate is hand picked for organisms under a three diopter-illuminated magnifier. Organisms are placed in 70% ethyl alcohol for subsequent identification. A stereoscopic dissecting microscope (70-120x) is used to identify most organisms. When taxonomic features are too small for identification under the dissecting scope, the organism is permanently mounted on a slide and examined under a compound microscope.

If more than four hours of picking is required, and a sample contains many organisms but few species, a one-fourth sub-sample is chosen at random. The sub-sample is picked and the results are multiplied by four to represent the total sample. The remainder of the sample is inspected to make sure no other taxa were overlooked. A multiplication factor of 19 is used

to convert the number of organisms per grab sample to organisms per square meter using the following formula:

$$1.0 \text{ m}^2 / (0.53 \text{ m}^2) \text{ (sampling area of ponar)} = 19$$

(http://www.iep.ca.gov/wqdata/disc_water/benthic/doc.html July 10, 1999)

Pesticide measurements Pesticide concentrations were measured in water samples collected daily by the United States Geological Survey from the Sacramento River at Tower Bridge and from the San Joaquin river at Vernalis between 1991 and 1994 (Fig. 1). Beginning in January 1991 for San Joaquin river water samples and beginning August 1991 for Sacramento River samples, continuing through April 1994, samples were analyzed for a suite of 23 pesticides. Pesticides were chosen for analysis based on pesticide use patterns in the San Joaquin and Sacramento valleys (See McCoy *et al.* 1995 for details on sample collection, processing, and analysis).