

**California Department of Pesticide Regulation Bioassessment Pilot Study:  
Identifying Correlations between Macroinvertebrate Communities and  
Pesticides and other Environmental Variables of Agricultural Runoff**

Juanita Bacey  
Environmental Scientist

Frank Spurlock  
Research Scientist III



**December 2007**



**CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY  
Environmental Monitoring Branch  
California Department of Pesticide Regulation  
1001 I Street Sacramento, California 95812  
Report No. EH06-01**

## ABSTRACT

This project was a collaborative effort between the California Department of Pesticide Regulation (DPR) and the Central Valley Regional Water Quality Control Board (CVRWQCB). The agencies combined limited monitoring resources to jointly collect biological, pesticide and water quality data. The primary objective of this study was to identify potential adverse impacts to the aquatic environment from pesticides and/or other elements in agricultural runoff. The approach was to compare benthic macroinvertebrate (BMI) communities at sites directly receiving agricultural runoff to physically similar sites with lesser agricultural runoff inputs.

Two waterways in San Joaquin County, California, were sampled. They are used for both irrigation supply and drainage and are not considered fish habitats. Two sites in each waterway were sampled, and all four sites monitored had similar physical habitats, having been historically modified for agricultural activities. The waterways were channelized, had banks and riparian zones with little vegetation, and had substrate consisted solely of mud and/or sand.

Samples were collected from each waterway at a site where irrigation supply entered (input) and near the end of a waterway after receiving discharges from agricultural drainage (output). All sampling occurred from February 15, 2005 through September 18, 2005. Sites were monitored semi-weekly for pesticides and nutrients. Water quality parameters consisting of temperature, pH, specific conductance (EC), dissolved oxygen (DO) and turbidity were monitored hourly, and aquatic macroinvertebrates were collected monthly.

All sites were lacking in pollutant sensitive taxa (Ephemeroptera, Plecoptera, Trichoptera). Dominant taxa found at all sites were Chironomidae, Gastropoda, Oligochaeta, and Amphipoda species. There were significant differences between Inlet1 and Outlet1 in taxa richness, abundance and community structure. At Inlet2 and Outlet2, there were no significant differences in any of the measured BMI variables.

There were significant differences in various water quality parameters between input and output sites in the same waterways. The organophosphate pesticides chlorpyrifos and dimethoate were the two most frequently detected pesticides. While occasional exceedances of the chlorpyrifos LC50 for *Ceriodaphnia dubia* occurred, dimethoate concentrations did not exceed any known aquatic toxicity benchmarks. The pyrethroids lambda cyhalothrin and bifenthrin were detected in two bed sediment samples but did not exceed any known aquatic toxicity benchmarks. Pesticide sampling was limited in duration, occurring only during the summer sampling period, and no spatial or temporal correlation between pesticide detections and BMI variables was evident.

The various pesticide, nutrient, and basic water quality variables were highly collinear, demonstrating the difficulty in inferring causality based on their correlation with BMI variables. However, it was clear that low DO concentration was related to BMI community structure. At Outlet1, extreme continuous exceedances of the DO water quality criterion was related to the presence of low DO tolerant taxa. Other water quality variables such as elevated EC or turbidity may also have had an effect, but that conclusion is far more tentative.

High nutrient levels may also be contributing to low DO concentrations by supporting increased algal and vegetation growth. That increased growth allows bacteria to thrive and deplete DO as the decaying vegetation is metabolized.

### **Acknowledgments**

We would like to offer a special thanks to Jay Rowan, Environmental Scientist with the Central Valley Regional Water Quality Control Board (CVRWQCB), without whom this study would not have been possible. Funding through CVRWQCB was provided by the Surface Water Ambient Monitoring Program (SWAMP). We would also like to thank the following environmental monitoring personnel who assisted with sample collection during the study, Milanka Ilic and Michael Mamola. Their tireless efforts allow us to report the data presented here. Thanks also to the California Department of Food and Agriculture staff, who conducted chemical analysis, and the Bidwell Institute of California State University, Chico for benthic macroinvertebrate taxonomy.

### **Disclaimer**

The mention of commercial products, their source, or use in connection with materials reported herein is not to be construed as an actual or implied endorsement of such products.

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## **1. Introduction**

This project was a cooperative effort between the California Department of Pesticide Regulation (DPR) and the Central Valley Regional Water Quality Control Board (CVRWQCB). Both agencies are working to improve monitoring methods to better protect water quality. In this study, DPR and the CVRWQCB combined monitoring resources to obtain biological, pesticide and water quality data. It also allowed DPR the opportunity to use bioassessment as a supplementary tool, in addition to chemistry, to assess potential effects of pesticides on the aquatic communities.

Bioassessment is a biological tool used to evaluate current conditions of a water body, and includes a quantitative survey of physical habitat and the biological community of a water body. By investigating the biological community rather than one species a more comprehensive survey of the health of a water body can be determined. Aquatic benthic macroinvertebrates (BMIs) are often used to represent the biological community because they are easily surveyed and provide a time-integrated measure of overall water quality conditions.

The primary objective of this study was to identify potential adverse impacts to the aquatic environment from various constituents in agricultural drainage, including pesticides. We assessed differences in BMI communities between sites that received substantial volumes of agricultural drainage and similar sites that received less drainage. A secondary goal was to characterize pesticide concentrations in surface waters in areas of high agricultural use.

## **2. Materials and Methods**

### **Site Description**

The two pairs of sites we chose are in the lower reaches of the San Joaquin river watershed in San Joaquin County, California (Figure 1). Although all sites were selected based on their proximity to agriculture and the potential for pesticide-containing runoff to enter the water bodies, the Roberts Island sites (Inlet1 and Outlet1) were selected before the development of this study. These sites were being monitored by the CVRWQCB for another ongoing study. These sites, which are a part of a water system for supply and drainage of agriculture, are not considered fish habitats.

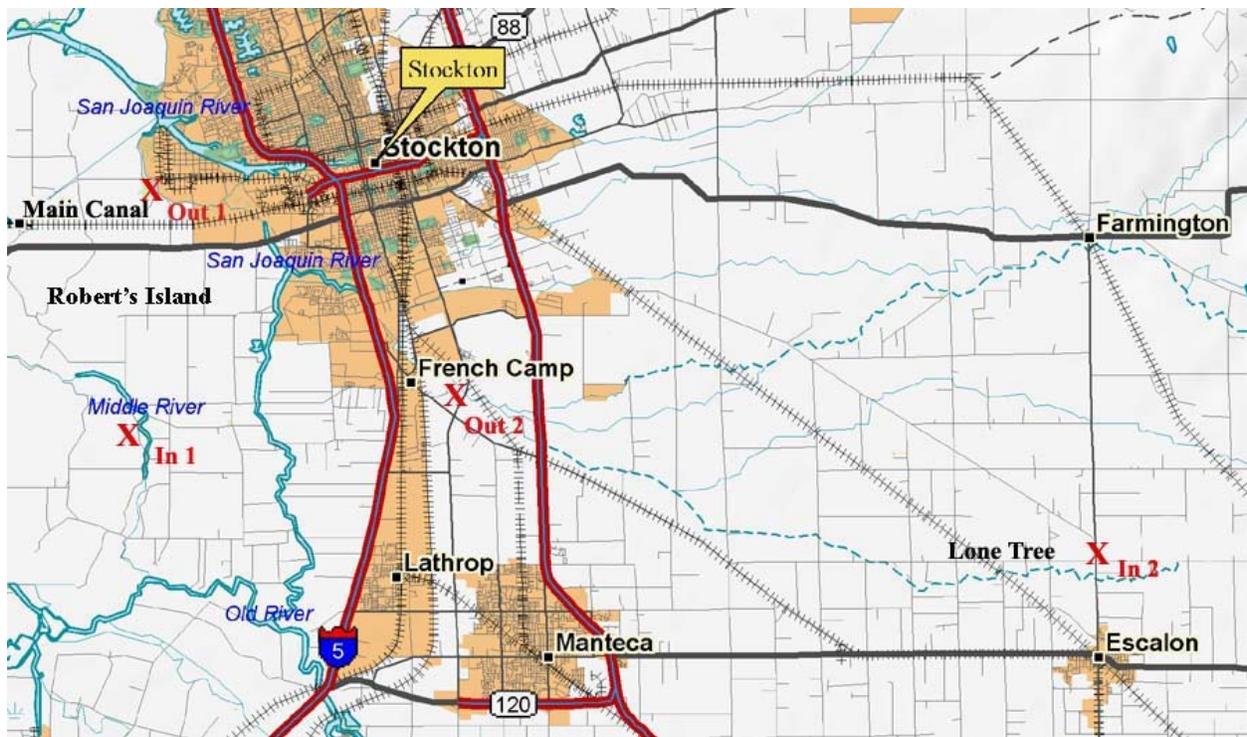
In order to achieve the objective, we compared differences between sites influenced by agricultural runoff and those with lesser influence, yet still within the same water system, and with similar physical habitat parameters. Samples were collected at the beginning of the system (input), where irrigation supply water enters, and at the end of the system (output) after the agricultural drainage water discharges into the waterway.

The Middle River and Burns Cutoff on Roberts Island in the San Joaquin Delta were selected as Inlet1 and Outlet1, respectively (Figure 1). Water from Middle River is diverted to a main canal and then into irrigation supply canals on Roberts Island. Sample collection occurred from the main canal adjacent to Middle River. While Middle River was selected as an input site because it was thought to provide cleaner, irrigation supply waters; however, initial samples and detailed site surveys indicated some agricultural drainage entered Middle River.

Sample collection of the output, Burns Cutoff, occurred from an agricultural drainage canal just before it entered Burns Cutoff. Both Middle River and Burns Cutoff flow into the San Joaquin River.

The remaining two sites are Lone Tree Creek and French Camp Slough just southeast of Stockton (Inlet2 and Outlet2, respectively). Lone Tree Creek flows into French Camp Slough, which flows into the San Joaquin River. Sample collection at Lone Tree Creek occurred below the inflow of irrigation supply water coming from Woodward Reservoir. However, pesticides were detected in initial samples collected from this site. We therefore speculated that some agricultural drainage was entering Lone Tree Creek upstream of our sampling point, beyond the entry point of the irrigation supply water.

**Figure 1. Monitoring Sites**



### **Sampling Plan**

Monitoring at all sites included BMIs, basic water quality parameters, nutrients, and selected pesticides. Physical habitat was also characterized at each sampling site. Finally, water samples for acute toxicity testing using the test species *Pimephales promelas* (Fathead minnow) and *Ceriodaphnia dubia* (water flea) were collected at the Inlet1 and Outlet1 sites.

CVRWQCB staff collected BMI and water quality samples at Inlet1 and Outlet1 from February 7, 2005, through June 24, 2005. Monthly water samples for acute toxicity testing were also collected during this time. Nutrient and pesticide analyses were not being conducted as part of the initial CVRWQCB study for these sites. On June 24, 2005, DPR staff continued the BMI and water quality sampling and added nutrient and pesticide analyses at these sites. DPR staff began sampling Inlet2 and Outlet2 on June 24, 2005, for all parameters. Sample collection at all four sites ended on September 19, 2005 (Table 1).

**Table 1. Monitoring period for all parameters measured.**

<b>Location</b>	<b>Water Quality</b>	<b>BMIs</b>	<b>Nutrients</b>	<b>Pesticides</b>
Water body 1 Inlet1 and Output 1	Feb. 7 thru Sept. 19, 2005 Hourly	Feb. 7 thru Sept. 19, 2005 Monthly	June 24 thru Sept. 19, 2005 Semiweekly	June 24 thru Sept. 19, 2005 Semiweekly
Water body 2 Inlet2 and Outlet2	Aug. 9 through Sept. 19, 2005 Hourly	June 1 thru Sept. 19, 2005 Monthly	June 24 thru Sept. 19, 2005 Semiweekly	June 24 thru Sept. 19, 2005 Semiweekly

Measured water quality parameters were temperature, DO, pH, specific conductance (EC), and turbidity; measured nutrients were alkalinity, ammonia-nitrogen, nitrate, phosphate; and measured insecticides and herbicides are listed in Table 2. Water quality parameters were measured using YSI 6600 multi-parameter probes at Inlet1 and Outlet1 and Eureka Manta® multi-parameter probes at Inlet2 and Outlet2. Probes were deployed at each site for the duration of the study. Water samples were collected and analyzed for nutrients and pesticides semi-weekly. Water samples for acute toxicity testing were collected monthly (Inlet1 and Outlet1 only). Sediment samples, collected and analyzed for pesticides, were collected twice, once at the beginning of the study and again at the end.

Hester-Dendy® (H-D) samplers were used to sample the aquatic macroinvertebrate communities. This artificial substrate sampler is best used in streams where benthic macroinvertebrate variability and abundance may be low due to heavy sedimentation and a lack of sufficient substrate for colonization. Unlike rock baskets or other benthic samplers, heavy sedimentation is not a concern for colonization of H-D samplers because they float submerged below the surface in center stream. This sampling method was adequate for the goals of this study because we were only comparing relative differences in aquatic BMI communities between input and output sites. A H-D sampler was placed at each sampling point every two weeks during the study period. Samplers were then retrieved for BMI analysis after having been submerged for four weeks.

We compared all measured data at the input and output sites, including the selected BMI taxa counts and derived metrics. Only those metrics with sufficient numbers of nonzero data points to yield meaningful statistical comparisons were used for the analyses due to the lack of statistical confidence in those metrics with zero data points. Based on weight-of-evidence and correlation, we attempted to infer whether any observed differences were due to measured water quality, nutrient or pesticide explanatory variables.

## **Sampling Methods**

### *Benthic macroinvertebrates*

Sampling was conducted per DPR SOP EQWA006, Procedure for Collecting Benthic Macroinvertebrates using a Hester-Dendy Sampler (Mamola, 2005).

### *Physical Habitat*

The physical habitat assessment consisted of completing a *Habitat Assessment Field Data Sheet* for low gradient streams (Appendix A). The U.S. EPA defined the physical habitat scoring criteria (1999): A score is determined by assessing 10 physical habitat parameters that include in-stream features (e.g. undercut banks, pools, channel flow and alteration) and riparian composition along the stream bank and beyond.

Each parameter is valued at 0 to 20 points and divided into four condition categories: poor, marginal, suboptimal, and optimal. Total scores can range from 0 to 200, with zero representing significant anthropogenic or natural impacts, and 200 representing no impacts. These scores are an observation-based score and can be subjective due to the experience or training of the individual conducting the assessment. Therefore, the score is usually determined by consensus of at least two field staff.

### *Water Samples*

Water samples were individually collected for each chemical screen. All samples were grab samples consisting of a 1-liter amber glass bottle, collected from center channel. The bottles were sealed with Teflon-lined lids. Samples were transported on wet ice and stored refrigerated at 4°C until chemical analysis.

DO, pH, EC, and water temperature and turbidity were measured *in situ* every two hours using Eureka Manta® multi-parameter sondes.

### *Sediment Samples*

Sediment samples were collected using a Teflon® coated hand scoop. The top 2 cm of sediment was placed into a 3-L Teflon® container until filled. After thoroughly mixing, sediment was transferred to 1-pint, clear glass jars for submission to the lab for pesticide analysis.

## **3. Analyses**

### *Benthic Macroinvertebrate*

Bidwell Institute at the University of California, Chico, performed macroinvertebrate identification. Quality control was conducted in accordance with previously established California Department of Fish and Game (DFG) procedures. A sub-sample of 500 macroinvertebrates were identified to genera and, when possible, to species. Samples are reported in biological metrics of select taxa (Table 3). A metric is a quantitative numerical measure of a characteristic of the biota that changes in some predictable way with increased human influence (Barbour et al. 1995).

### *Pesticides*

The California Department of Food and Agriculture's (CDFA) Center for Analytical Chemistry

performed the chemical analyses. Quality control was conducted in accordance with SOP QAQC001.00 (Segawa, 1995). Reporting limits (RL) and method detection limits (MDL) are reported in Table 2. Quality control data are presented in Appendix D. Samples with no measurable concentration above the MDL are reported as non-detections. Samples with concentrations that fall between the RL and the MDL are reported as trace detections. Samples with concentrations above the RL are detections and analytical concentrations are reported.

Pyrethroid whole water samples, including any suspended sediment, were extracted with methylene chloride. Sample bottles were rinsed with extraction solvent and added to the sample extracts for analysis. The extract is passed through sodium sulfate to remove residual water.

The anhydrous extract is evaporated on a rotary evaporator and then a solvent exchange is performed with hexane. Extracts were concentrated using a Brinkmann R110 rotary evaporator (Brinkmann, Westbury, NY), and analyzed using a gas chromatograph equipped with a HP-5MS or equivalent column (Hewlett Packard, Avondale, PA) and a mass selective detector (MSD). Pyrethroid analysis results were reported on a whole sample basis (water plus suspended sediment). Reporting limits were 0.05 to 0.08 ppb.

Pyrethroid sediment samples were analyzed by two different methods at CDFA. In the older method, samples were homogenized and extracted with acetonitrile. The filtered extracts were salted out with sodium chloride. An aliquot of acetonitrile extract was evaporated to dryness in a water bath under a stream of nitrogen for solvent exchange to hexane. Extracts were then analyzed using a gas chromatograph equipped with an electron capture detector. Pyrethroids were then confirmed by a gas chromatograph equipped with mass selective detector. Reporting limits were 10.0 ppb. For sediment samples analyzed at the end of the project, they were first homogenized and then extracted with acetone and hexane solvents by shaking on an orbital shaker. The extracts were cleaned with Florisil® before being analyzed by gas chromatography with and an electron capture detector. Two columns of different polarity, the HP-5MS and DB-608, were used for confirmation. The mass selective detector was used for the analysis of resmethrin and confirmation when residues were high enough for detection. The RL for pyrethroid analytes in sediment with this method were 1.0 ppb.

Duplicate pyrethroid sediment samples were also analyzed by DFG, Water Pollution Control Laboratory (WPCL). Pressurized fluid extraction using a Dionex 200 Accelerated Solvent Extractor was used to extract pyrethroids from sediment samples (10 g) with the addition of a surrogate for quality control. Gel Permeation Chromatography was used to clean-up extracts and Florisil column chromatography was used for additional cleanup and fractionation of extracts. A gas chromatograph, equipped with electron capture detectors and dual capillary columns, is used for the analysis of pyrethroid pesticides. Pyrethroids were confirmed using GC/MS/MS. The pyrethroid reporting limits for sediment were (sediment dry weight basis): 1.0 ppb for bifenthrin, 2.0ppb for esfenvalerate and lambda-cyhalothrin, 3.0 ppb for cyfluthrin and cypermethrin and 4.0 ppb for permethrin.

CDFA lab extracted organophosphate samples with methylene chloride and the extract was passed through sodium sulfate to remove residual water. The anhydrous extract was evaporated to near dryness on a rotary evaporator and diluted to a final volume of 1.0 mL with acetone. The

extract is split. One extract was then analyzed by a Hewlett-Packard model 5890 gas chromatograph (Hewlett Packard, Avondale, PA) equipped with an Rtx OP Pesticides column (Restek, State College, PA) and a flame photometric detector (FPD) and the other with a gas chromatograph equipped with a mass selective detector to get a lower reporting limit for chlorpyrifos and diazinon. Reporting limits ranged from 0.01 to 0.05 ppb (Table 2).

For herbicide analysis at CDFA, the water samples were passed through two Oasis MCX cartridges (Waters, Millford, MA) connected in tandem. The cartridges were placed under vacuum to remove water. Then the cartridges were eluted with 5% ammonium hydroxide in methanol.

The eluant was filtered through a nylon Acrodisc 0.2 micron filter (Gelman Sciences, Ann Arbor, MI), concentrated, reconstituted in 75/25 water/methanol and analyzed by a ThermoQuest/ThermoSeparation HPLC with a Finnigan LCQ Deca mass spectrometer (Finnigan/ThermoQuest, San Jose, CA). Reporting limits are 0.05 ppb.

#### *Nutrients*

Field staff conducted the analyses using field LaMotte Smart II® colorimeters. Parameters measured were turbidity, alkalinity, nitrate, phosphate, and ammonium nitrogen. With the exception of turbidity, all samples were filtered immediately after collection using a disposable, sterile, polypropylene/polyethylene syringe and a Luer-Lok® sterile, surfactant-free, cellulose acetate membrane filter (0.45µm). Smart II colorimeters photoelectrically measure the amount of colored light absorbed by a colored sample in reference to a colorless sample (blank). Samples are reacted to produce a color by adding a reagent. Reagents were added and samples were measured in accordance with the LaMotte Smart II® test instructions (Table 4).

#### *Toxicity*

Acute toxicity testing was conducted following current U.S. EPA procedures using the test species (*Ceriodaphnia dubia* and *Pimephales promelas*). Acute toxicity was determined using a 96-hour, static-renewal bioassay in undiluted sample water.

#### *Statistical Analysis*

Differences in water quality parameters, including nutrients, were evaluated using non-parametric sign tests for median of differences of paired data, in this case using differences between input and output sites of each water body. Analyses of pesticide results were examined using the paired Prentice-Wilcoxon test (PPW), a non-parametric test for equality of paired left-censored data (Helsel, 2005). Differences in BMI counts were evaluated using paired t-tests. All differences were considered significant at  $p < 0.05$ .

To evaluate the univariate pair-wise correlations between the various BMI measurements and the water quality, nutrient and pesticide data we calculated mean water quality, nutrient and pesticide concentrations for a lag period of two weeks prior to each BMI sampling event, plotted the data (Figure 10) and tabulated the Pearson correlation matrix for the most important variables (Appendix C). In addition to the mean chlorpyrifos and dimethoate concentrations for the two-week lag period prior to BMI sampling, we also included the maximum concentrations during that period.

Macroinvertebrate taxa were also summarized into biological metrics (Table 3).

## 4. Results

### Inlet1 and Output 1

#### *Physical Habitat*

Both sites had been modified for agricultural activities. They had similar physical habitats including channelized waterways, banks and riparian zones with little vegetation, and substrate consisting solely of mud and/or sand (Figure 2).

The physical habitat assessment score obtained for Inlet1 was 55 out of a possible 200. All ten parameters (in-stream and bank and riparian zones) were in the poor and marginal categories except for one, channel flow status, which was in the optimal category. It was scored as optimal because the channel was full and water reached the base of both banks; no channel substrate was exposed. The overall score obtained for Outlet1 was 31. Similar to Inlet1, all parameters were in the poor or marginal categories except channel flow status.

**Figure 2. Inlet1 and Outlet1 on Roberts Island, San Joaquin County, California**



### *Water Quality*

Water quality parameters were measured every two hours for approximately 180 days at both sites (2/7/05 to 9/19/07), except for DO, which was measured for approximately 130 days at Inlet1 (3/26/07 to 9/19/07) due to equipment malfunction. There were significant differences between Inlet1 and Outlet1 in all water quality parameters measured based on non-parametric paired sign tests (appendix B). These differences generally reflected poorer water quality at Outlet1 as compared to Inlet1.

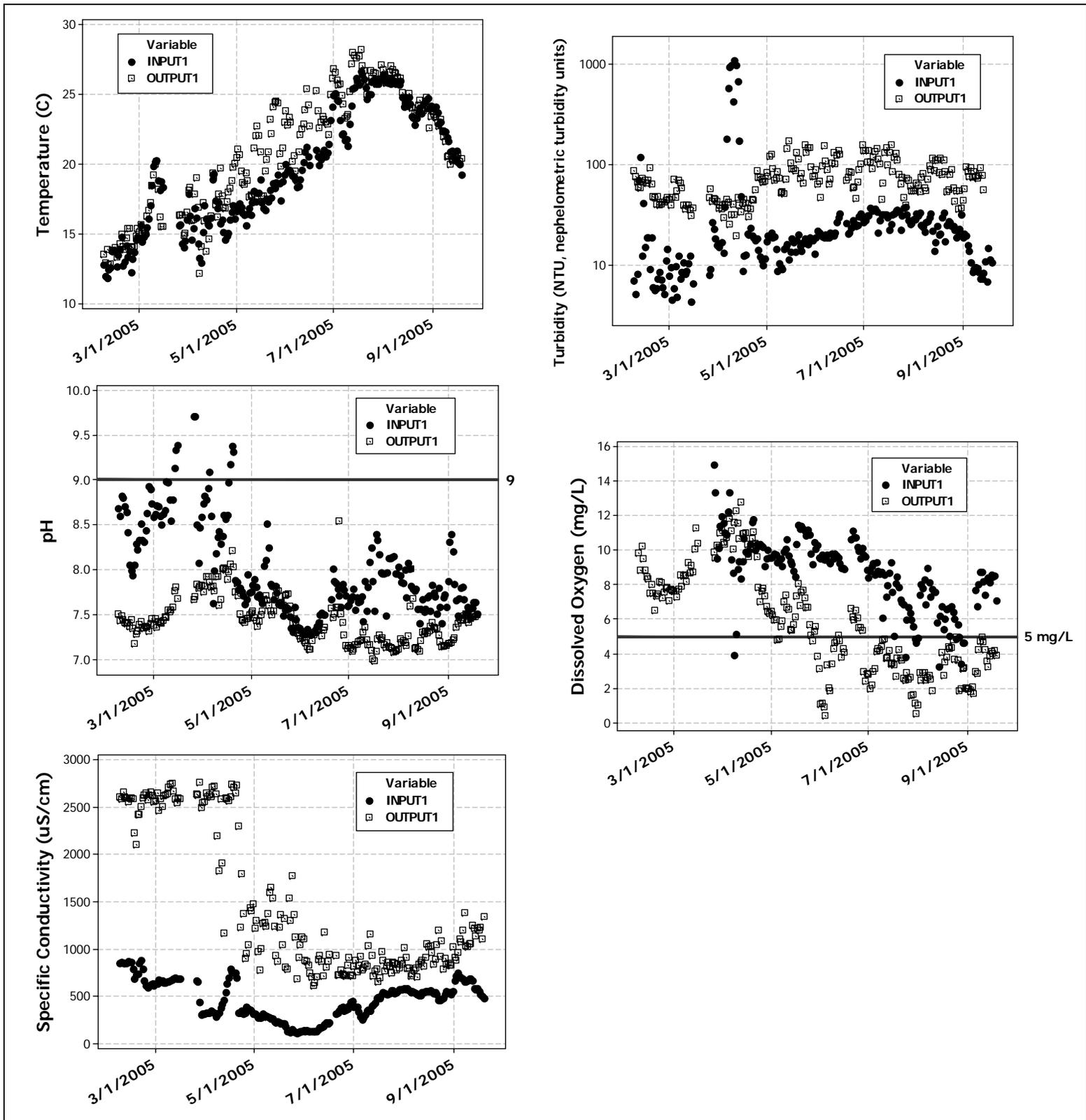
Temperature, turbidity and EC were all greater at Outlet1 than Inlet1 (Figure 3). However, the median difference in temperature was only 0.76°C. Neither site exceeded 32°C. Turbidity at Outlet1 was higher than Inlet1 (Figure 3), with medians of 71 nephelometric units (NTU) and 19 NTU, respectively, at the two sites. Similarly, EC was much higher at Outlet1 than Inlet1, with median ECs of 1080 and 501  $\mu\text{S}/\text{cm}$ , respectively, at the two sites. There are no freshwater aquatic life criteria for temperature, EC, or turbidity.

Both pH and DO levels were greater at Inlet1 compared to Outlet1. The pH criterion to sustain freshwater aquatic life is 6.5 to 9 (SWRCB, 2003). At Inlet1, daily mean pHs ranged from 7.27 to 9.72, exceeding the freshwater criterion 4 percent of the time. All of these exceedances occurred in a one month period between mid-March and mid-April (Figure 3). At Outlet1, mean daily pH ranged from 6.99 to 8.54; the criterion was not exceeded. The median pH difference between the two sites was 0.45 pH Units (Appendix B). In addition, pH at Inlet1 was somewhat more variable than Outlet1, with coefficients of variation at the two sites of approximately 3 and 7 percent, respectively (Figure 3).

DO concentrations were significantly higher at Inlet1 than at Outlet1, with median daily DO concentrations of 9.1 and 5.4 mg/L at Inlet1 and Outlet1, respectively (Appendix B). The median difference in daily DO between Inlet1 and Outlet1 was 3.88 mg/L (Appendix B). The DO ambient aquatic life water quality criterion is 5 mg/L or greater (7-day mean; U.S. EPA, 1986). Seven-day mean DO concentrations did not fall below this criterion at Inlet1 (Figure 3). In contrast, daily mean DO concentrations at Outlet1 were below the 5 mg/L criterion for 13 days in late May and early June, and again for 84 consecutive days from June 28 to the end of the study (September 19). During the latter period, the mean of the daily DO concentrations was 3.2 mg/L and was below 2 mg/L on several days.

There were obvious seasonal effects in the water quality variables at both Inlet1 and Outlet1. For example, temperatures were generally lower at both sites in spring as compared to summer, and DO was higher in spring than summer. In addition, early spring EC was much higher at Outlet1 as compared to summer (Figure 3).

Figure 3. Water quality results of Inlet1 and Output 1 (daily averages of hourly results)

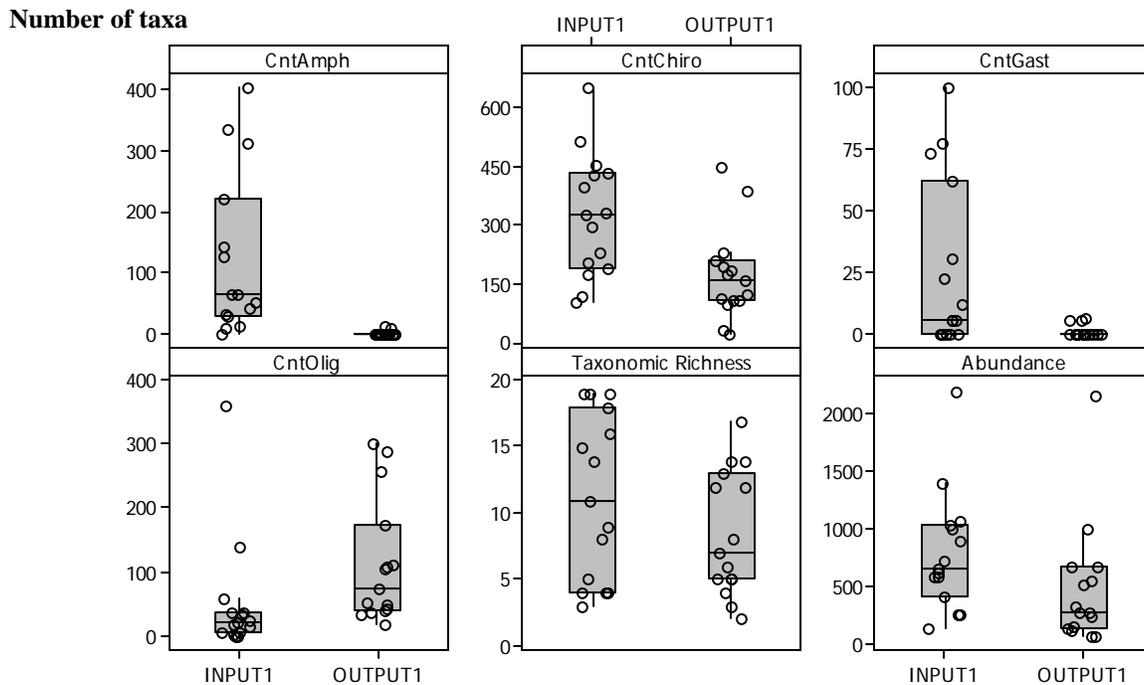


### *Benthic Macroinvertebrates*

Neither site had any of the most pollutant sensitive taxa (Ephemeroptera, Plecoptera or Trichoptera taxa (EPT)). Instead, they were dominated by taxa often found in polluted waters: Oligochaetes (aquatic worm), Chironomidae (midges), and Gastropoda (aquatic snail) (Peckarsky et al. 1990; Pennak, 1989) (Figure 4). Dominant taxa at both Inlet1 and Outlet1 were of the class Insecta, family Chironomidae: *Dicrotendipes*, *Paratanytarsus*, and *Cricotopus*. These represented 60 percent of population (mean). Also dominant were *Physa* species of the class Gastropoda, and unidentified species of the class Oligochaeta (3 and 20 percent (mean), respectively). Also, dominate at Inlet1 but not Outlet1 were *Gammarus* species of the class Crustacea, order Amphipoda (scud; 23 percent).

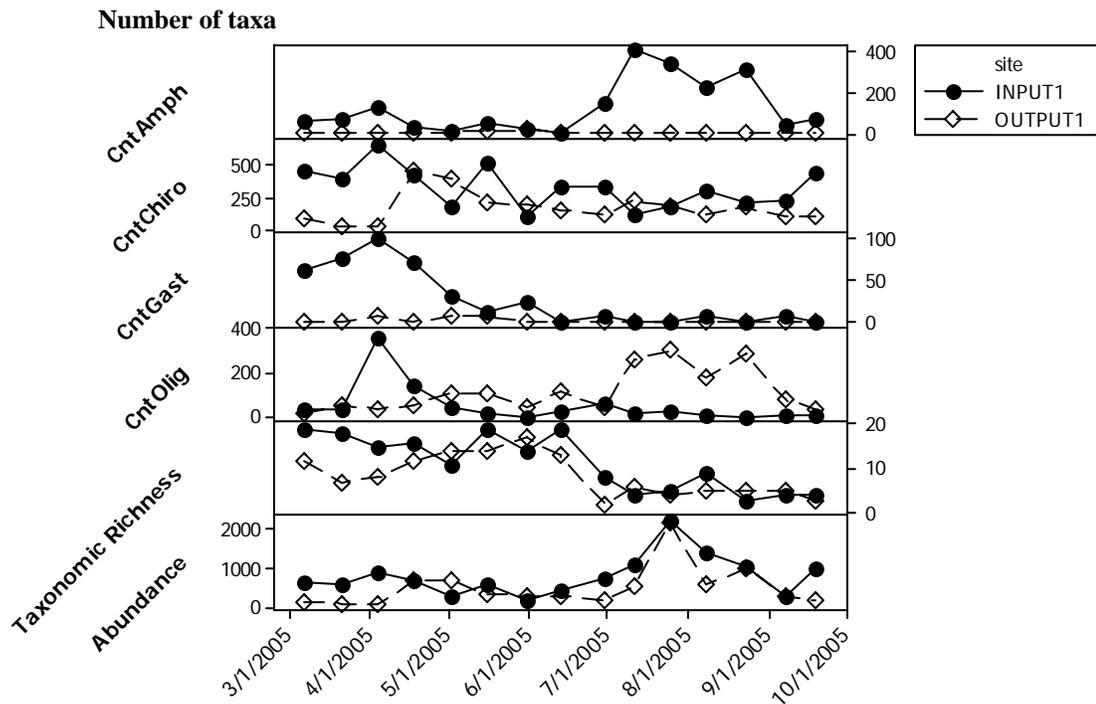
Both taxonomic richness and abundance were significantly higher at Inlet1 than Outlet1 based on paired t-tests (Appendix B, Table 6). Differences were also seen in the community structure of each site. Of the dominant taxa groups, Amphipoda, Chironomidae and Gastropoda taxa were greater at Inlet1 (Appendix B), and there were no differences in counts of the Oligochaeta taxon between sites (Figure 4, Table 6).

**Figure 4. BMI results of Inlet1 and Outlet1.**



Seasonal differences were also significant based on paired t-tests. At Inlet1 Amphipoda counts increased from a mean of 48 in the spring (3/1/2005 to 5/31/2005) to a mean of 206 in the summer (6/1/2005 to 9/19/2005). While at Outlet1 Amphipoda counts never exceeded 11 individuals, Gastropoda counts dropped from a population mean of 54 in the spring to a mean of two in the summer, and Oligochaeta taxa increased from a mean of 59 (spring) to 180 (summer). BMI time series are presented in Figure 5.

**Figure 5. BMI time series for Inlet1 and Outlet1**



### Nutrients

Nutrient monitoring began June 24, 2005, and continued through September 19, 2005. There was no significant difference in nitrate concentrations at the two sites but all other nutrients (phosphate, ammonia-nitrogen, alkalinity) were higher at Outlet1 compared to Inlet1 (Appendix B). Alkalinity concentrations ranged from 45 to 145 ppm at both sites. Ammonia-nitrogen never exceeded the acute national criterion (CMC) or the chronic national criterion (CCC) at either site (U.S. EPA, 1999). One sample (2.84 ppm) from Outlet1 did exceed the LC50 for the test species *Ceriodaphnia dubia* (1.19 ppm; Anderson and Buckley, 1998). Phosphate ranged from 0.19 to 2.93 at both sites. Nutrient time-series over the sampling period are presented in Figure 6 and Table 5, and ammonia nitrogen concentration/criterion comparisons are given in Table 7.

### Pesticide Detections

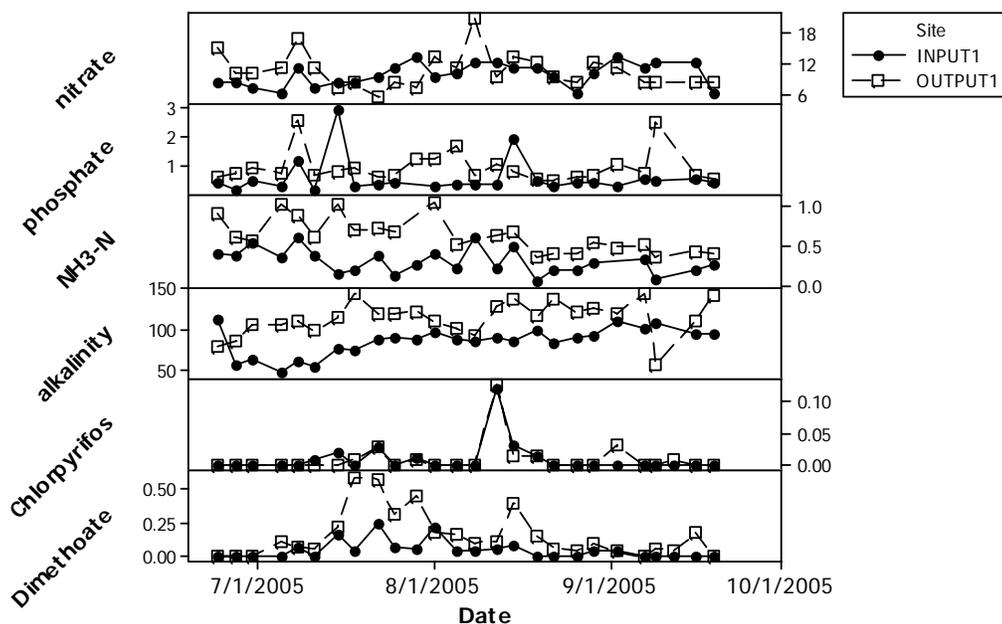
Pesticide monitoring began June 24, 2005, and continued through September 19, 2005. Water samples were collected twice weekly, and sediment samples were collected at the beginning and end of the study. At Inlet1, chlorpyrifos, dimethoate and methyl-parathion were detected in water samples, but the lone methyl parathion detection was below the reporting limit of 0.03 ppb (Table 2) and so is reported as “trace”. One of the seven chlorpyrifos detections exceeded the LC50 for the test species *C. dubia* (0.038 ppb, CDFa, 1999). That sample was collected on August 12, 2005, and had a concentration of 0.121 ppb. The remaining six chlorpyrifos detections were <0.04 ppb (Figure 6). There were 22 total dimethoate detections, of which nine were “trace” detections. The remaining 13 detections had a median of 0.061 ppb and ranged up to a maximum of 0.25 ppb.

None of the dimethoate detections exceeded the LC50s for test species plecoptera (Table 9, > 36 ppb; U.S. EPA, 2007). There were no pyrethroid detections in sediment at this site.

At Outlet1, there were detections of chlorpyrifos, dimethoate, disulfoton and DDVP in water samples. The single detections of disulfoton and DDVP each occurred on different days and were below the respective reporting limits of 0.04 and 0.05 ppb so are denoted as “trace” detections. Of the eight chlorpyrifos detections, one exceeded the known LC50 for *C. dubia*. It had a concentration of 0.127 ppb and was collected on the same day as the highest chlorpyrifos concentration was observed at Inlet1, August 12, 2005. None of the 23 dimethoate detections exceeded the known LC50 for Plecoptera test species (Table 9). There were no pyrethroid detections in sediment at this site. We used a paired Prentice-Wilcoxon nonparametric test for equality of left censored data (PPW; Helsel, 2005) to test for differences in chlorpyrifos and dimethoate concentrations between Inlet1 and Outlet1 (Appendix B). There was no significant difference in chlorpyrifos concentrations at the two sites ( $p = 0.81$ ), but dimethoate concentrations at Outlet1 were higher than at Inlet1 ( $p < 0.001$ ), with an estimated median concentration difference in paired daily samples of 0.05 ppb (Figure 6, Appendix B).

Since BMI and water quality monitoring began in February 2005 and pesticide monitoring did not begin until June 2005, we also examined reported pesticide use from March through August 2005 (DPR PUR database, 2005). Total chlorpyrifos and dimethoate use in the immediate township ranges of 39M01N05 and 39M02N05 (select sections), where the sites were located on Roberts Island, was 54 percent higher in the summer (June 1 through August 31, 2005) as compared to the spring (March 1 through May 31, 2005). The total pounds of those two active ingredients used in the reference area were 91.25 pounds during the spring and 168.35 pounds during the summer period.

**Figure 6. Nutrient and pesticide results at Inlet1 and Outlet1 (ppb).**



### *Toxicity*

There was no acute toxicity from any of the monthly water samples collected for toxicity testing from February 2005 through June 2005 at Inlet1 or Outlet1 for the test species *P. promelas* (Table 10). For the test species *C. dubia*, there was acute toxicity in the March 23, 2005, samples collected at both Inlet1 and Outlet1. Survival was zero percent at both sites (Table 10). No pesticide analyses were conducted during this time.

### **Inlet2 and Outlet2**

#### *Physical Habitat*

Both sites had been historically modified for agricultural activities. They had similar physical habitats including channelized waterways, banks and riparian zones with little vegetation, and substrate consisting solely of mud and/or sand (Figure 7). The physical habitat assessment score obtained for Inlet2 was 88. Seven parameters fell in the poor and marginal categories. Two fell in the suboptimal category (channel alteration and bank stability). This site has more vegetation along the banks compared to the other sites, however most is non-native plant species (e.g. Himalayan blackberry). One parameter fell in the optimal category (channel flow status), because the channel was full. The score obtained for Outlet2 was 79. Seven parameters were in the poor and marginal categories, while three were in the suboptimal category (channel flow status, channel alteration, vegetation protection).

**Figure 7. Inlet2 and Outlet2, near Stockton, San Joaquin County, California.**

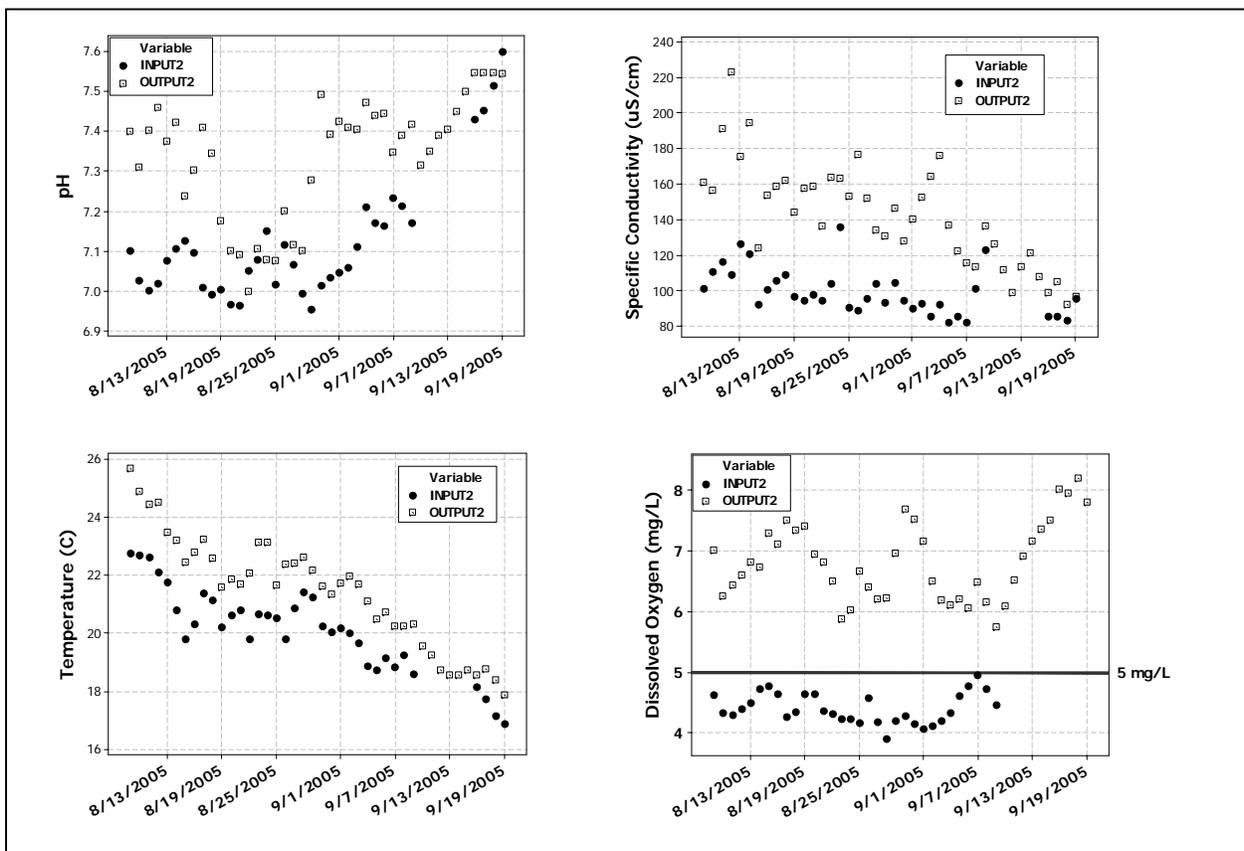


### Water Quality

Water quality parameters were measured every two hours for approximately 30 days (8/9/05 – 9/19/05). Although there were significant differences between Inlet2 and Outlet2 in all water quality parameters based on non-parametric sign tests (Appendix B), many of the differences probably had little practical importance relative to meaningful effects on BMI populations. For instance, pH at Inlet2 was consistently lower than at Outlet2, but nearly all of the pH measurements at both sites fell in a relatively narrow range of ~ 7.0 – 7.5 (Figure 8). Similarly, EC at Inlet2 was consistently lower than at Outlet2, but all EC measurements at both sites were relatively low, being less than ~ 200  $\mu\text{S}/\text{cm}$ .

Temperatures ranged from 12.9°C to 29.9°C at Inlet2 and 16.8°C to 26.9°C at Outlet2, and steadily declined during the 30-day sampling period (Figure 8). The median paired temperature difference during the sample period was 1.7°C, with Inlet2 consistently colder than Outlet2. Hourly turbidity measurements were not collected at Inlet2 or Outlet2; however, semi-weekly measurements were collected. Turbidity ranged from 1 – 66 NTU at Inlet1 and 3 – 59 NTU at Outlet2. DO concentrations at Inlet2 were below 5 mg/L during the entire sampling period while those at Outlet2 never fell below this criterion (Figure 8).

**Figure 8. Inlet2 and Outlet2 Water quality results (daily average of hourly measurements).**



### *Benthic Macroinvertebrates*

Neither site had the most pollutant sensitive taxa (EPT), with the exception of Outlet2, which had one Trichoptera species (*Hydropsyche californica*). Both sites had taxa often found in polluted waters, including Oligochaetes, Chironomidae, and Gastropoda (Peckarsky et al. 1990; Pennak, 1989).

Dominant taxa at both Inlet2 and Outlet2 were of the class Insecta, family Chironomidae, subfamily Orthocladiinae (41 percent). Also dominant were *Ferrissia* species of the class Gastropoda (aquatic snail; 15 percent), and unidentified species of the class Oligochaeta (9 percent).

Based on paired t-tests there were no significant differences in taxa richness, abundance, or any of the dominant taxa (Chironomidae, Gastropoda, Oligochaeta, and Amphipoda;  $p > 0.05$ ). BMI results are presented in Table 6.

### *Nutrients*

Nutrient monitoring began June 24, 2005, and continued through September 19, 2005. There were no significant differences between Inlet2 and Outlet2 nutrient parameters except for alkalinity (Appendix B, Figure 9). The only known alkalinity water quality criteria for protection of aquatic life specifies minimum alkalinity levels of 20 ppm (as  $\text{CaCO}_3$ ). All samples from Inlet2 and Outlet2 exceeded this concentration. Ammonia nitrogen did not exceed the acute national criterion (CMC) or the chronic national criterion (CCC) at either site (U.S. EPA, 1999b; Table 7). However, one sample from Outlet2 (1.43 ppm) exceeded the LC50 for the test species *C. dubia* (1.19 ppm). Phosphate ranged from 0.05 to 2.93 ppm at both sites. All nutrient results and means are presented in Table 5. Comparisons of ammonia nitrogen concentration/criterion are presented in Table 7.

### *Pesticide Detections*

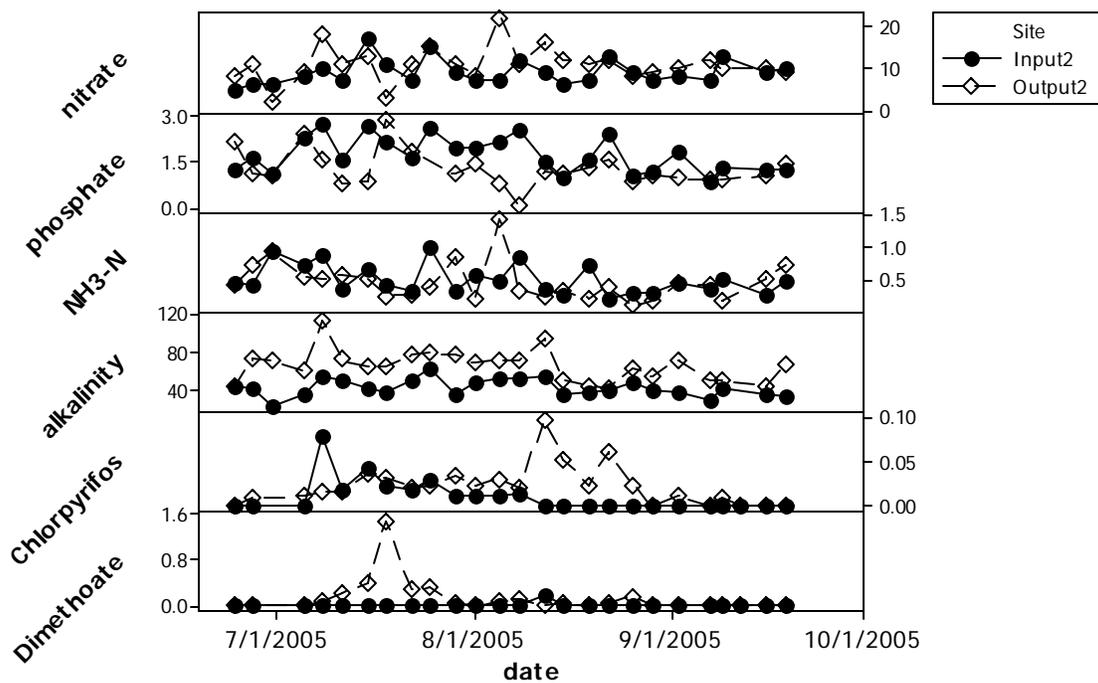
Pesticide monitoring began on June 24, 2005, and continued through September 19, 2005. Water samples were collected twice weekly, while sediment was collected twice, once at the beginning of the study and again at the end.

There were several detections of chlorpyrifos and dimethoate in water samples from Inlet2 (Table 8). By way of comparison, two of the ten chlorpyrifos detections exceeded the *C. dubia* LC50 of 0.038 ppb, 0.0819 (7/8/2005) and 0.0435 (7/15/2005), respectively. Two of the three-dimethoate detections were “trace” detections. There were no pyrethroid detections from the sediment sample collected on June 4, 2005, but there was a detection of bifenthrin (1.62 ppb, dry wt.) in the sample collected on September 19, 2005. The reported bifenthrin 10-day LC50 for the test species *Hyaella azteca* is 0.52  $\mu\text{g/g}$  organic carbon (OC), (520 ppb; Amweg et al., 2005). Organic carbon analysis was not conducted on these sediments, but typical California sediments contain 1 – 2% organic carbon. Accordingly, the estimated bifenthrin toxic units (TU) of this sample fell in the approximate range of 0.15 – 0.30 TU.

There were detections of chlorpyrifos, dimethoate, azinphos-methyl, and methyl parathion in water samples from Outlet2 (Table 8). Three of the 19 chlorpyrifos detections exceeded the chlorpyrifos *C. dubia* LC50 (0.10 ppm, 0.0539 ppm, 0.0631 ppm), while none of the 20 dimethoate detections approached the Plecoptera LC50s listed in Table 9. There was a detection of bifenthrin in a sediment sample collected on June 4, 2005 (1.02 ppb, dry wt.). There was another detection of bifenthrin and lambda cyhalothrin from a sample collected on September 19, 2005 (1.62 ppb and 2.19 ppb, respectively, dry wt.). The reported 10-day LC50s for *H. azteca* are 0.45 µg/g OC (450ppb; Amweg et al., 2005). The estimated combined *H. azteca* TU were 0.7 – 1.4 based on the 1-2% sediment OC assumption mentioned above.

Based on PPW tests there were significant differences in chlorpyrifos and dimethoate detections between Inlet2 and Outlet2 (Appendix B), with more detections of both chlorpyrifos and dimethoate at Outlet2 and at higher concentrations (Table 8, Figure 9, Appendix B).

**Figure 9. Nutrient and pesticide results at Inlet2 and Outlet2 (ppb)**



## 5. Discussion

All four sites had similar and generally poor physical habitats, having been historically modified for agricultural activities. The waterways were channelized, banks and riparian zones had little vegetation, and substrate consisted solely of mud and/or sand. We selected similar sites to minimize potential differences in BMI populations that might arise from physical habitat conditions. We did not want physical habitat to provide additional variables or stressors. Ideally, sites that differed only by the quantity of pesticides detected would have been preferred. This would have allowed us to exam the sole effects, if any, of pesticides on the BMI community.

Due to the nature of agricultural runoff where the various constituents and water quality characteristics are highly correlated, the contaminants are not limited to pesticides. Sediment and nutrients are major constituents as well.

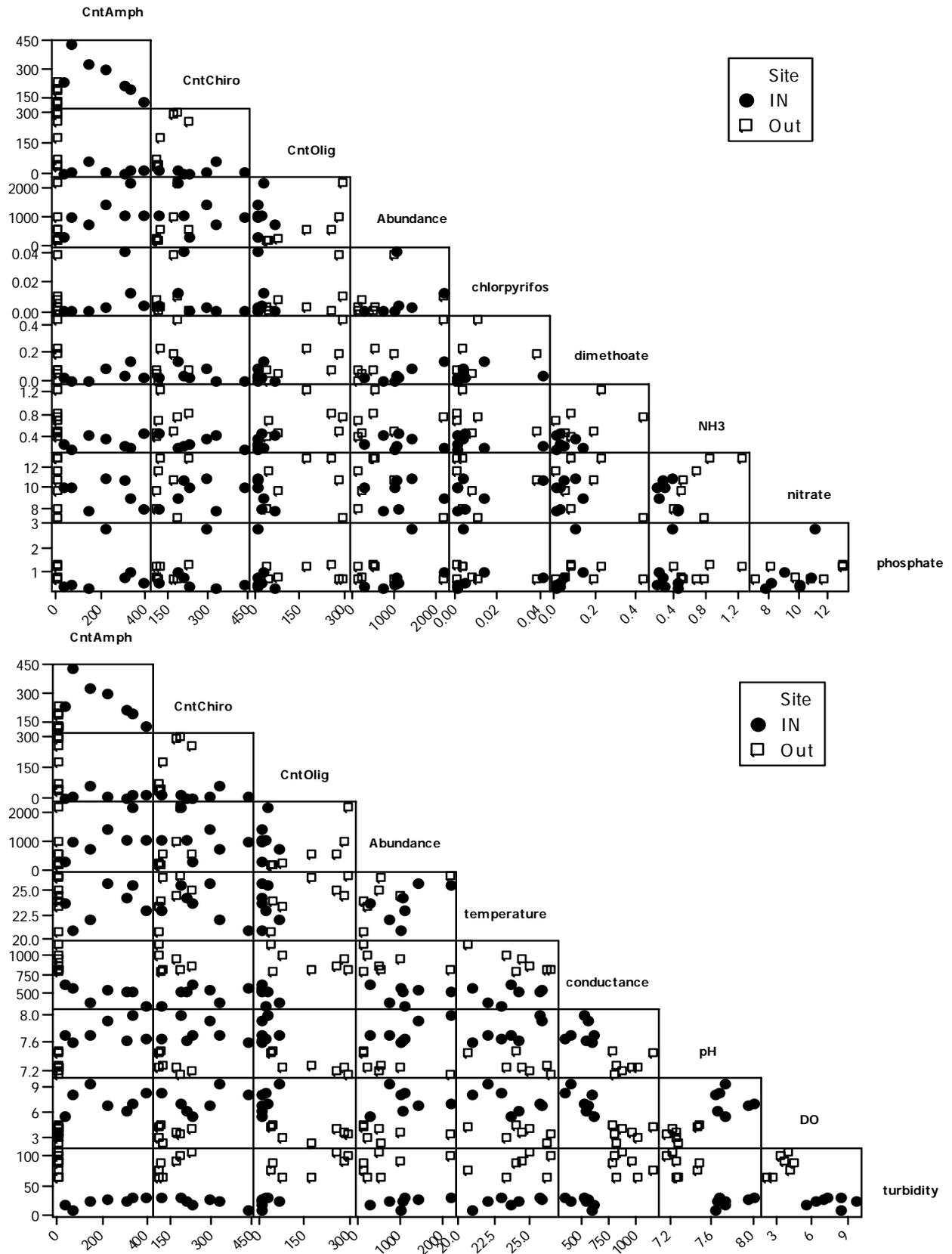
The sites selected were within the same waterbody and were in close proximity to one another (within 5 miles). They were also located in the same ecoregion and elevation. Therefore, we expected to see similarities in taxa structure, as well as seasonal changes in the populations. Based on paired t-tests we found significant differences in BMI taxa between Input and Output sites (Appendix B). However, all sites were similar in that they were lacking pollutant sensitive taxa (EPT), with the exception of one Trichoptera species found at Outlet2 (*Hydropsyche californica*). We also found significant differences in water quality.

### **Inlet1/Outlet1**

At Inlet1 and Outlet1, there was a significant difference between sites in BMI abundance, BMI taxa richness, and in counts of three of the four dominant taxa. Inlet1 had greater abundance of BMIs, greater taxa richness, and greater numbers of amphipods (scuds), chironomids (midges) and gastropods (aquatic snails). The numbers of oligochaetes (aquatic worms) at both sites were not statistically different.

The matrix plots for sites Inlet1 and Outlet1 (Figure 10) show correlation between amphipod populations and DO concentrations ( $p=0.007$ , Appendix C). It is evident that significant amphipod populations only occurred at  $DO > \sim 5$  mg/L, while at DO concentrations below this level the population remained near zero. Amphipods are scavengers and they require waters with high DO concentrations (Pennak, 1989). Generally, they rest among vegetation and debris. They are common in unpolluted waters and are tolerant to salinity. Some species can occur in very large numbers (McCafferty, 1998). The generally adequate DO concentrations (relative to the DO freshwater criterion, Table 9) at Inlet1 (Figure 3) support the presence of amphipod populations. In contrast, Outlet1 DO concentrations were continuously below the criterion for 13 and 84 consecutive days during the study. During these periods, Outlet1 DO concentrations fell below 2 mg/L several times. DO concentrations below 4 ppm are toxic to many species of invertebrates, while concentrations below 3 ppm can be stressful to most aquatic organisms (U.S. EPA, 1986). Hoback and Barnhart (1996) reported that the LC50 in hypoxia for the amphipod *Gammarus pseudolimnaeus* ranged from 1.05 to 2.0 ppm. We concluded that the extended low-DO periods at Outlet1 probably contributed to the low amphipod population at that site.

Figure 10. Correlation matrix of key variables: Inlet1 and Outlet1.



We also found Chironomidae taxa at these agricultural sites. Cushing and Benke (2005) found that Chironomidae are abundant in the lower reaches of the San Joaquin River. They feed primarily on algae and decaying organic matter, but they can also utilize live plant material as a food source (MacRae et al. 1990; Cuda et al. 2002). One dominant midge found at the sites, *Dicrotendipes*, is an indicator of high agricultural runoff (Rae, 1989). Another dominant genus that was found, *Cricotopus*, has a symbiotic relationship with the alga *Nostoc* (McCafferty, 1998). Similar to the amphipod plots, Chironomidae counts were significantly correlated with DO (Figure 10, Appendix C). Similar to the reasoning above for amphipods, we conclude that DO is a key explanatory variable that affected the chironomid counts we measured in the H-D samplers.

Many Oligochaeta species are tolerant to low DO and are found in large numbers in organically polluted habitats (e.g. sewage, manure, decaying vegetation (McCafferty, 1998)). We did notice that aquatic weeds had a tendency to buildup at the spillway of Outlet1. The reduced water flow created by the spillway, and the buildup and decay of vegetation at this site may have contributed to the low DO concentrations. In addition, bacteria that live off decaying algae and vegetation deplete DO levels in the process. Both the low DO and decaying vegetation are most likely factors in providing habitat and favorable conditions for the oligochaetes to thrive in higher population at this site compared to Inlet1 (Figure 4, Figure 5).

During the March through May (spring) BMI sampling at Inlet1, the average gastropod count was 54. In contrast, summer samples from Inlet1 yielded an average of two gastropods per sampling, and the mean at Outlet1 was one gastropod per sampling over the entire year. Gastropoda (aquatic snails; *Physa sp.*) are tolerant to low oxygen. *Physa spp.* have lungs and are able to breath atmospheric oxygen. Therefore, they can tolerate near-anoxia, but only for short periods (Pennak, 1989). The extended periods of low dissolved oxygen in the summer at Outlet1 potentially contribute to reduced gastropod populations; however, other factors may also contribute. During spring months, there were very few gastropods found in the Outlet1 even though DO concentrations were relatively high (Figure 3).

An additional potential factor that may influence gastropod populations is salinity as reflected by EC. Kefford et al. (2003) reported that the LC50 for gastropods generally ranged from 9,000 to 14,000  $\mu\text{S cm}^{-1}$ . However, Marshall and Bailey (2004) reported that the abundance of salt sensitive species (including gastropods) is reduced at salt concentrations of 1500  $\text{mg l}^{-1}$  (~2200  $\mu\text{S cm}^{-1}$ ) following exposure to a continuous or pulse release of saline water. Horrigan et al. (2005) observed changes in macroinvertebrate community structure at salinities between 800 - 1000  $\mu\text{S cm}^{-1}$ . Observed EC levels at Outlet1 were 0.4 to 2968  $\mu\text{S cm}^{-1}$ , but there are no freshwater aquatic life criteria for this parameter. We speculate that the elevated EC levels observed at this site may be contributing to the reduced gastropod population. The seasonal differences in gastropod counts at Inlet1 also indicate additional factors probably play a role in gastropod populations.

Turbidity at Outlet1 was approximately three times greater than at Inlet1, with levels typically ranging from 0.90 – 475.3 NTU. A field study by Campbell and Doeg (1989) found that changes in turbidity have the potential to affect macroinvertebrate community structure.

One indication that major turbidity and sedimentation has occurred or is occurring is a shift from herbivorous species (e.g. many midges) to sediment-burrowing species (e.g. many aquatic worms; U.S. EPA, 1995). However, Anderson et al. (2005) reported that three test species of the taxa Amphipoda, Ephemeroptera, and Chironomidae were not directly affected by turbidity as high as 1000 NTUs. In our study, only during one week (April, 2005) did levels reach as high as 1548 NTU. We conclude that it is unlikely that turbidity had a direct impact on taxa at these sites, although long-term sedimentation obviously affects site physical habitat.

Nutrients were only monitored in summer months (Table 1). There was no significant difference in nitrate concentrations between Inlet1 and Outlet1; however, phosphate, ammonia-nitrogen and alkalinity levels were all greater at Outlet1 (Appendix B). Ammonia nitrogen did not exceed the acute national criterion (CMC), though it did exceed the known LC50 for *C. dubia* (1.19 ppm) once (2.84 ppm). Typical fresh water alkalinity can range from 20-200 ppm (Basin, 2005). Neither site exceeded this range. There is no evidence that alkalinity or ammonia nitrogen had any impact on taxa at these sites. There are no established nitrate or phosphate criteria for fresh water aquatic life. However, high levels may play a part in low DO concentrations by contributing to productivity, alga blooms, increased vegetation and ultimately higher biochemical oxygen demand.

Pesticides were monitored in the summer months only (Table 1). Dimethoate concentrations were higher at Outlet1 but much less than known aquatic toxicity thresholds. One chlorpyrifos detection each from Inlet1 and Outlet1 exceeded the known LC50 for the sensitive test species *C. dubia* (0.038 ppb). Consequently, while the data are limited, there is no evidence the pesticides we monitored had a direct cause and effect impact in BMI populations during the study period.

Finally, there is a potential pitfall of using correlation to infer causality: the combined water quality/nutrient/pesticide data are highly collinear (Figure 10). Consequently, while the Outlet1/Inlet1 amphipod counts were significantly correlated with DO, that BMI variable is also highly inversely correlated with EC and turbidity because these variables are themselves inversely correlated with DO. Several multivariate statistical techniques may be used to reduce the dimensionality of multicollinear data by creating new variables. These include principal component analysis, factor analysis and canonical variate analysis, but there are too few observations to apply these techniques here. We conclude that DO is likely an important explanatory variable related to the BMI counts not only because of their significant correlation, but also because of the well known underlying mechanism: the known adverse effect of low DO on many BMIs and on aquatic organisms in general. Any potential relationship between aquatic health and variables such as EC or turbidity is far less clear-cut, so any conclusion that these variables are related to the structure of BMI communities is much more speculative.

### **Inlet2/Outlet2**

Similar taxa were found at Inlet2 and Outlet2; there were no significant differences between sites in BMI counts, taxa richness or abundance. The one interesting observation was *H. californica* (caddisfly), which was found only at Outlet2. *H. californica* represented approximately 19 percent of the population at Outlet2 (mean). It is a filter feeding caddisfly, commonly found in riffles of low to moderately polluted flowing streams.

Although the physical habitat of all the sites was similar, Outlet2 was the largest of the waterways we surveyed. It also appeared to have variable riffles unlike the other sites, possibly explaining the presence of this species at this site.

Water quality parameters pH, temperature, and EC were significantly greater at Outlet2 than Inlet2, but these differences were relatively minor (Figure 8, Appendix B). While DO concentrations at Inlet1 were consistently less than the 5 mg/L water quality criteria (Table 5) during the entire sampling period (Figure 8), they were almost always above 4 mg/L, and never exceeded the 7-day mean criterion until the last 10 days of the study (9/10/05 – 9/19/05). At Outlet2, DO concentrations remained above 5 ppm, and similar to Inlet2, did not exceed the 7-day mean criteria. Finally, ammonia nitrogen concentrations did not exceed acute or chronic criteria at either site (Table 7).

Both chlorpyrifos and dimethoate detections at Outlet2 were more numerous and concentrations were greater than at Inlet2 (Appendix B). Inlet2 had two chlorpyrifos detections that exceeded the *C. dubia* LC50 of 0.038 ppb, while three samples exceeded that benchmark at Outlet2 (Table 8). All of these represented approximately three TUs for *C. dubia*. Both sites also had detections of pyrethroids in sediment with approximate TUs of ~ 0.5 – 1.5 for *H. azteca*.

## 6. Conclusion

Even though there were some differences in pesticide concentrations and DO concentrations between Inlet2 and Outlet2, there were no significant differences in BMI counts or community structure. We attribute this to the relatively small differences in water quality and chemistry at the two sites. In contrast, there were important seasonal and site-to-site differences in water quality and BMIs at Inlet1 and Outlet1. Although there were some detections of chlorpyrifos and dimethoate at Inlet1 and Outlet1, it is likely that other water quality characteristics are contributing to the differences in BMI populations and community composition between the two sites. It is apparent that one important variable is dissolved oxygen, which reached very low levels at Outlet1 during summer months.

In conclusion, there were samples with water column concentrations of chlorpyrifos that exceeded the LC50 of the indicator organism *C. dubia* (2 of 52 at Inlet1/Outlet1; 5 of 50 at Inlet2/Outlet2). Detections of dimethoate were more frequent, but no concentrations exceeded published aquatic toxicity thresholds. We also detected the pyrethroids lambda cyhalothrin and bifenthrin in sediment at concentrations that could potentially cause acute toxicity to *H. azteca*. However, in this limited data set we did not observe any spatial or temporal correlation between pesticide detections or concentrations, and BMI counts or community structure. However, the study did demonstrate that additional water quality characteristics beyond pesticides can have a strong influence on BMI communities in agriculturally dominated Central Valley waterways, with dissolved oxygen being one of the most important.

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## TABLES

**Table 2. Pesticides analyzed including methods and reporting limits**

<b>Organophosphate Pesticides in Water</b>				
<b><u>Method: GC/FPD</u></b>	<b><u>Method Detection Limit (ppb)</u></b>		<b><u>Reporting Limit (ppb)</u></b>	
Azinphos methyl	0.0099		0.05	
DDVP (dichlorvos)	0.0098		0.05	
Dimethoate	0.0079		0.04	
Disulfoton	0.0093		0.04	
Ethoprop	0.0098		0.05	
Fenamiphos	0.0125		0.05	
Fonofos	0.008		0.04	
Malathion	0.0117		0.04	
Methidathion	0.0111		0.05	
Methyl Parathion	0.008		0.03	
Thimet (Phorate)	0.0083		0.05	
Profenofos	0.0114		0.05	
Tribufos	0.0142		0.05	
<b><u>GC/MSD</u></b>	<b><u>ppt</u></b>		<b><u>ppt</u></b>	
Chlorpyrifos	0.7999		10	
Diazinon	1.191		10	
<b>Pyrethroid Pesticides in Water</b>				
<b><u>Method: GC/MSD</u></b>	<b><u>(ppt)</u></b>		<b><u>(ppt)</u></b>	
Fenvalerate/Esfenvalerate	22.5		50	
Permethrin	16.9		50	
Bifenthrin	2.16		5	
Lambda Cyhalothrin	7.76		20	
Cyfluthrin	55.5		80	
Cypermethrin	56.6		80	
<b>Pyrethroid Pesticides in Sediment older/newer method</b>				
<b><u>Method: GC/ECD, confirmed with GC/MSD</u></b>	<b><u>(ppb)</u></b>	<b><u>(ppb)</u></b>	<b><u>(ppb) **</u></b>	<b><u>(ppb)</u></b>
Fenvalerate/Esfenvalerate	8.0	0.143	10	1.0
Permethrin, cis/trans	6.0	0.116/0.135	10	1.0
Bifenthrin	7.0	0.108	10	1.0
Lambda Cyhalothrin/ L. Cyhalothrin epimer	9.0	0.117/0.115	10	1.0
Cyfluthrin	8.0	.183	10	1.0
Cypermethrin	8.0	.107	10	1.0
Fenopropathrin	None	.109	None	1.0
Deltamethrin	None	.0661	None	1.0
Resmethrin	None	0.87	None	1.5

Note: All MDL's and RLs listed were determined by CDFA for DPR

\* MDL for DFG, WPCL is 0.5 ppb for this pesticide; all others are greater than those listed.

\*\* For DFG, WPCL reporting limits (pyrethroids in sediment) see pesticide analyses methods on pg. 5.

**Table 3. Nutrient analyses methods**

Analyte	Detection Limit	Colorimeter range	Method
Alkalinity	10.0 ppm	0-200 ppm as CaCO <sub>3</sub>	The sample is added to a buffered indicator reagent. The color that develops will indicate the amount of alkalinity in the sample.
Ammonia-Nitrogen	0.05 ppm	0.00 – 4.00 ppm Ammonia Nitrogen	Ammonia forms a colored complex with Nessler's Reagent in proportion to the amount of ammonia present in the sample. Rochelle salt is added to prevent precipitation of calcium or magnesium in undistilled samples.
Nitrate	5.0 ppm	0.0 – 60.0 ppm	Zinc is used to reduce nitrate to nitrite. The nitrite that was originally present, plus the reduced nitrate, reacts with chromotropic acid to form a red color in proportion to the amount of nitrite in the sample.
Phosphate	0.05 ppm	0.00 – 3.00 ppm Orthophosphate	Ammonium molybdate and antimony potassium tartrate react in a filtered acid medium with dilute solution of PO <sub>4</sub> <sup>-3</sup> to form an antimony-phosphomolybdate complex. This complex is reduced to an intense blue colored complex by ascorbic acid. The color is proportional to the amount of phosphate present.
Turbidity	2 NTU	0 – 400 NTU	Absorptimetric (Colorimeter and Eureka Manta®)

**Table 4. Benthic macroinvertebrate metrics and definitions**

Taxonomic Richness	Total number of individual taxa
Abundance	Estimated number of BMIs in the sample calculated by extrapolating from the proportion of organisms counted in the subsample.
Chironomidae	Of the order Diptera (true flies) mainly consisting of midges
Amphipoda	Order of the class Crustacea, shrimp-like in form such as scuds
Gastropoda	Class of the phylum Mollusca; univalves such as snails, slugs, and abalone
Oligochaeta	Subclass of the class Clitellata; aquatic worms
<b>Modified from Harrington and Born, 1999</b>	

**Table 5. Water quality and nutrient results and criteria**

Water Quality	Criteria to support fresh water aquatic life	Inlet1 Range (ppm)		Outlet1 Range (ppm)	
Temperature	NA	8.78 - 28.08		6.09 – 31.67	
Dissolved Oxygen	5 ppm <sup>1</sup> (7-day mean minimum)	0.82 – 22.86		0.04 – 16.59	
pH	6.5 – 9.0 <sup>2</sup>	7.04 – 10.24		6.34 – 8.97	
Specific Conductance	NA	110.6 – 902.70		0.4 – 2986.5	
Turbidity	NA	3.10 – 1548.8		0.90 – 475.3	
Flow (ft/sec)	NA	0.26-1.25		0.20-0.50	
<b>Nutrients</b>			<b>Mean</b>		<b>Mean</b>
Nitrate	NA	6-13	9.6	5-21	10.4
Ammonia Nitrogen	LC <sub>50</sub> for <i>Ceriodaphnia dubia</i> = 1.19 <sup>3</sup> ppm (See Table 7 for U.S. EPA acute & chronic criterion)	0.05-0.62	0.303	0.36-2.84	0.72
Alkalinity	NA	45-112	84.56	56-145	114.24
Phosphate	NA	0.19-2.93	0.60	0.53-2.57	0.98

Water Quality	Criteria to support fresh water aquatic life	Inlet2 Range (ppm)		Outlet2 Range (ppm)	
Temperature	NA	12.855 – 29.89		16.76 – 26.85	
Dissolved Oxygen	5 ppm <sup>1</sup> (7-day mean minimum)	3.39 – 8.54		4.77 – 8.52	
pH	6.5 – 9.0 <sup>2</sup>	6.16 – 14.0		6.93 – 7.67	
Specific Conductance	NA	72 – 201		87 - 270	
Turbidity	NA	1.0 – 66.0		3.0 – 69.0	
Flow (ft/sec)	NA	0.69-1.69		0.60-1.80	
<b>Nutrients</b>			<b>Mean</b>		<b>Mean</b>
Nitrate	NA	5-17	9	2-22	10.9
Ammonia Nitrogen	LC <sub>50</sub> for <i>Ceriodaphnia dubia</i> = 1.19 <sup>3</sup> ppm (See Table 7 for U.S. EPA acute & chronic criterion)	0.20-1.01	0.50	0.09-1.43	0.46
Alkalinity	NA	23-56	43.36	43-114	66.12
Phosphate	NA	0.88-2.77	1.76	0.05-2.93	1.28

**NA: Not Available**

1. Ambient aquatic life water quality criteria for DO (freshwater) (U.S.EPA, 1986)
2. U.S. EPA national recommended ambient water quality criteria for fresh water aquatic life protection (SWRCB, 2003)
3. Anderson and Buckley, 1998

**Table 6. Dominant macroinvertebrate taxa found at all sites.**  
**Displayed as a percent of 1 (100 percent).**

<b>Inlet1 - Date</b>	<b>Amph <sup>1</sup></b>	<b>Chiro <sup>1</sup></b>	<b>Gast <sup>1</sup></b>	<b>Olig <sup>1</sup></b>	<b>TriTaxa <sup>2</sup></b>	<b>Taxa Richness (count)</b>	<b>Abundance (count)</b>
3/7/2005	0.08	0.73	0.1	0.06	0	19	619
3/21/2005	0.11	0.67	0.13	0.06	0	18	589
4/4/2005	0.14	0.72	0.11	0.04	0	15	905
4/18/2005	0.04	0.64	0.11	0.21	0	16	665
5/2/2005	0.04	0.69	0.12	0.15	0	11	253
5/16/2005	0.07	0.88	0.02	0.03	0	19	586
5/31/2005	0.09	0.73	0.16	0	0	14	140
6/13/2005	0	0.78	0	0.06	0.05	19	415
6/30/2005	0.26	0.61	0.01	0.11	0.25	8	723
7/11/2005	0.75	0.22	0	0.03	0	4	1079
7/25/2005	0.61	0.34	0	0.04	0	5	2209
8/8/2005	0.42	0.56	0.01	0.01	0	9	1407
8/23/2005	0.6	0.4	0	0	0	3	1036
9/7/2005	0.12	0.86	0.02	0.01	0	4	267
9/19/2005	0.13	0.86	0	0.01	0.25	4	1010
<b>Outlet1 - Date</b>	<b>Amph <sup>1</sup></b>	<b>Chiro <sup>1</sup></b>	<b>Gast <sup>1</sup></b>	<b>Olig <sup>1</sup></b>	<b>TriTaxa <sup>2</sup></b>	<b>Taxa Richness (count)</b>	<b>Abundance (count)</b>
3/7/2005	0	0.79	0	0.15	0	12	122
3/21/2005	0	0.31	0	0.69	0	7	77
4/4/2005	0	0.4	0.08	0.44	0	8	78
4/18/2005	0	0.89	0	0.1	0	12	672
5/2/2005	0	0.77	0.01	0.21	0	14	673
5/16/2005	0.02	0.64	0.01	0.33	0	14	328
5/31/2005	0.04	0.7	0	0.16	0	17	275
6/13/2005	0	0.58	0	0.41	0	13	273
6/30/2005	0	0.73	0	0.27	0	2	156
7/11/2005	0	0.44	0	0.5	0	6	520
7/25/2005	0	0.34	0	0.56	0	4	2174
8/8/2005	0	0.22	0	0.32	0	5	553
8/23/2005	0	0.35	0	0.58	0	5	1000
9/7/2005	0	0.46	0	0.32	0.2	5	239
9/19/2005	0	0.75	0	0.25	0	3	147

**Table 6. Dominant macroinvertebrate taxa found at all sites. Continued.**  
 Displayed as a percent of 1 (100 percent).

Inlet2 - Date	Amph <sup>1</sup>	Chiro <sup>1</sup>	Gast <sup>1</sup>	Olig <sup>1</sup>	Ephem Taxa <sup>2</sup>	TriTaxa <sup>2</sup>	Taxa Richness (count)	Abundance (count)
7/11/2005	0.44	0.43	0.05	0.07	0	0	9	229
7/26/2005	0.08	0.55	0.15	0.21	0.17	0.17	6	517
8/8/2005	0.05	0	0.84	0.11	0	0	3	19
8/23/2005	*	*	*	*	*	*	*	*
9/7/2005	0	0.85	0.01	0.08	0	0.2	5	357
9/19/2005	0	0.75	0	0.25	0	0	2	69
Outlet2 - Date	Amph <sup>1</sup>	Chiro <sup>1</sup>	Gast <sup>1</sup>	Olig <sup>1</sup>	EphTaxa <sup>2</sup>	TriTaxa <sup>2</sup>	Taxa Richness (count)	Abundance (count)
7/11/2005	0	0.36	0.01	0.03	0.33	0.17	6	1063
7/26/2005	0	0.17	0.02	0.02	0.11	0.22	9	483
8/8/2005	0.01	0.23	0.01	0.03	0	0.14	7	345
8/23/2005	0	0.39	0.05	0.04	0.17	0.17	6	1110
9/7/2005	0	0.27	0.04	0.01	0.2	0.2	5	354
9/19/2005	0	0.3	0.09	0.01	0	0.25	4	166

1. Percentage of Abundance

2. Percentage of Taxonomic Richness

Note: Only dominant taxa are listed in table. Percents were rounded up, therefore, some totals may be greater than 1 (100 percent).

**Table 7. Ammonia-Nitrogen Acute and Chronic criterion**

U.S. EPA guidelines (1999): if samples are obtained from a receiving water over a period during which pH and/or temperature is not constant, the pH, temperature, and the concentration of total ammonia in each sample should be determined. For each sample, the criterion should be determined at the pH and temperature of the sample, and then the concentration of total ammonia nitrogen in the sample should be divided by the criterion to determine a quotient. The criterion is attained if the mean of the quotients calculated over the averaging period (30 days) is less than one (U.S. EPA, 1999b)..

The acute criterion (CMC) was calculated using the following equation, where salmonid fish are not present:

$$CMC = (0.411/(1 + 10^{7.204 - pH})) + (58.4/(1 + 10^{pH - 7.204}))$$

The chronic criterion (CCC) was determined using the U.S. EPA derived table: Temperature and pH-dependent values of the CCC for Fish Early Life Stages (ELS) Present. At 15°C and above, the criterion for fish ELS absent is the same as the criterion for fish ELS present. Since temperatures measured were above 15°C this table was used.

Inlet1			Acute CMC	CMC		Chronic CCC	CCC
Site/Date	pH	Am N (ppm)	Calculated	Quotient	Temp	From EPA table	Quotient
06-24-2005	7.21	0.39	29.2052	0.0134	20.7	3.7800	0.1032
06-27-2005	7.69	0.37	14.6871	0.0252	21.5	2.2100	0.1674
06-30-2005	7.01	0.55	35.7759	0.0154	23.0	3.6500	0.1507
07-05-2005	7.86	0.36	10.8992	0.0330	21.4	1.9600	0.1837
07-08-2005	7.38	0.62	23.6095	0.0263	21.5	2.9200	0.2123
07-11-2005	7.43	0.38	22.0271	0.0173	22.4	3.1300	0.1214
07-15-2005	7.31	0.14	25.8846	0.0054	24.2	2.7600	0.0507
07-18-2005	8.16	0.2	6.1888	0.0323	25.7	0.8550	0.2339
07-22-2005	7.25	0.37	27.8714	0.0133	23.7	2.7600	0.1341
07-25-2005	7.66	0.11	15.4434	0.0071	25.6	1.7100	0.0643
07-29-2005	7.67	0.25	15.1885	0.0165	24.0	1.9400	0.1289
08-01-2005	6.89	0.4	39.4532	0.0101	25.5	2.9200	0.1370
08-05-2005	7.67	0.22	15.1885	0.0145	24.9	1.7100	0.1287
08-08-2005	7.33	0.6	25.2287	0.0238	25.4	2.4200	0.2479
08-12-2005	7.22	0.21	28.8715	0.0073	25.4	2.5700	0.0817
08-15-2005	7.35	0.57	24.5772	0.0232	24.1	2.5700	0.2218
08-19-2005	7.50	0.05	19.8902	0.0025	22.7	2.6900	0.0186
08-22-2005	7.85	0.19	11.0985	0.0171	22.0	1.7300	0.1098
08-29-2005	6.50	0.29	48.8281	0.0059	24.7	3.6200	0.0801
09-07-2005	7.00	0.33	36.0927	0.0091	21.8	3.6500	0.0904
09-09-2005	6.50	0.07	48.8281	0.0014	21.8	4.6800	0.0150
09-16-2005	6.50	0.18	48.8281	0.0037	20.3	4.6800	0.0385
09-19-2005	6.00	0.25	54.9880	0.0045	20.1	NA	

CMC quotient avg. over 30 days			CCC quotient avg. over 30 days		
June	0.0180		June	0.1404	
July	0.0189		July	0.1412	
August	0.0131		August	0.1282	
Sept	0.0047		Sept	0.0479	

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Table 7. Ammonia-Nitrogen Acute and Chronic criterion - Continued

Outlet1				Acute CMC	Quotient	Chronic CCC	Quotient
Site/Date	pH	Temp	Am N (ppm)	Calculated		Table	
06/24/2005	7.37	21.5	0.91	23.9307	0.0380	2.92	0.3116
06/27/2005	7.15	21.4	0.6	31.2058	0.0192	3.33	0.1802
06/30/2005	6.70	26.1	0.56	44.5652	0.0126	3.07	0.1824
07/05/2005	7.30	23.8	1.03	26.2139	0.0393	2.76	0.3732
07/08/2005	7.17	20.1	0.88	30.5399	0.0288	3.78	0.2328
07/11/2005	6.82	21.7	0.62	41.4493	0.0150	3.89	0.1594
07/15/2005	7.09	25.5	1.02	33.1893	0.0307	2.70	0.3778
07/18/2005	7.09	25.8	0.69	33.1893	0.0208	2.70	0.2556
07/22/2005	7.10	22.6	0.72	32.8606	0.0219	3.50	0.2057
07/25/2005	7.24	24.2	0.68	28.2045	0.0241	2.92	0.2329
07/29/2005	6.92	23.7	0.68	38.5618	0.0176	3.32	0.2048
08/01/2005	7.05	26.0	1.06	34.4930	0.0307	2.70	0.3926
08/05/2005	7.05	25.1	0.52	34.4930	0.0151	2.70	0.1926
08/08/2005	7.35	25.0	2.84	24.5772	0.1156	2.57	1.1051
08/12/2005	6.79	23.6	0.63	42.2659	0.0149	3.42	0.1842
08/15/2005	7.14	22.5	0.67	31.5380	0.0212	3.50	0.1914
08/19/2005	7.37	21.8	0.36	23.9307	0.0150	2.92	0.1233
08/22/2005	7.30	19.8	0.4	26.2139	0.0153	3.57	0.1120
08/29/2005	7.00	22.7	0.55	36.0927	0.0152	3.65	0.1507
09/07/2005	7.00	19.2	0.51	36.0927	0.0141	4.15	0.1229
09/09/2005	7.00	19.7	0.36	36.0927	0.0100	4.15	0.0867
09/16/2005	6.50	17.7	0.43	48.8281	0.0088	5.33	0.0807
09/19/2005	7.00	18.3	0.4	36.0927	0.0111	4.72	0.0847
<b>CMC quotient avg. over 30 days</b>					<b>CCC quotient avg. over 30 days</b>		
June				0.0233	June		0.2247
July				0.0248	July		0.2553
August				0.0304	August		0.3065
Sept				0.0110	Sept		0.0938

**Table 8. Pesticide Detections in Water**

Date	Inlet1 (ppb)			Outlet1 (ppb)			
	Chlorpyrifos	Dimethoate	Methyl Parathion	Chlorpyrifos	Dimethoate	DDVP	Disulfoton
06-24-2005	ND	ND	ND	ND	ND	ND	ND
06-27-2005	ND	ND	ND	ND	ND	ND	ND
07-05-2005	ND	ND	ND	ND	0.1110	ND	ND
07-08-2005	ND	0.0698	ND	ND	0.0735	ND	ND
07-11-2005	0.0105	Trace	ND	ND	0.0580	ND	ND
07-15-2005	0.0221	0.1740	Trace	ND	0.2260	Trace	ND
07-18-2005	ND	0.0424	ND	0.0104	0.6060	ND	ND
07-22-2005	0.0283	0.2560	ND	0.0297	0.5930	ND	ND
07-25-2005	ND	0.0729	ND	ND	0.3170	ND	ND
07-29-2005	0.0123	0.0566	ND	0.0102	0.4660	ND	ND
08-01-2005	ND	0.2230	ND	ND	0.1850	ND	ND
08-05-2005	ND	0.0476	ND	ND	0.1690	ND	ND
08-08-2005	ND	0.0421	ND	ND	0.1090	ND	ND
08-12-2005	0.121	0.0648	ND	0.1270	0.1190	ND	ND
08-15-2005	0.0321	0.0860	ND	0.0147	0.4090	ND	ND
08-19-2005	0.0155	Trace	ND	0.0143	0.1560	ND	ND
08-22-2005	ND	Trace	ND	ND	0.0662	ND	ND
08-26-2005	ND	Trace	ND	ND	0.0488	ND	ND
08-29-2005	ND	0.0415	ND	ND	0.0963	ND	ND
09-02-2005	ND	0.0430	ND	0.0319	0.044	ND	Trace
09-07-2005	ND	Trace	ND	ND	Trace	ND	ND
09-09-2005	ND	Trace	ND	ND	0.0639	ND	ND
09-13-2005	ND	Trace	ND	0.0105	0.0410	ND	ND
09-16-2005	ND	Trace	ND	ND	0.1860	ND	ND
09-19-2005	ND	Trace	ND	ND	Trace	ND	ND

ND=no detection; chlorpyrifos reporting limit (RL) = 0.01 ppb; dimethoate RL = 0.04 ppb.

**Table 8. Pesticide Detections continued**

Date	Inlet2 (ppb)		Outlet2 (ppb)		Azinphos-Methyl	Methyl Parathion
	Chlorpyrifos	Dimethoate	Chlorpyrifos	Dimethoate		
06/24/2005	ND	ND	ND	0.0000	ND	ND
06/27/2005	ND	ND	0.0108	0.0000	Trace	ND
07/05/2005	ND	ND	0.0113	0.0000	ND	ND
07/08/2005	0.0819	ND	0.0167	0.0852	ND	ND
07/11/2005	0.0195	ND	0.0162	0.2190	ND	ND
07/15/2005	0.0435	ND	0.0363	0.3980	ND	Trace
07/18/2005	0.0230	ND	0.0337	1.4900	ND	Trace
07/22/2005	0.0188	ND	0.0217	0.2890	ND	ND
07/25/2005	0.0316	ND	0.0243	0.3160	ND	ND
07/29/2005	0.0116	ND	0.0348	0.0568	ND	ND
08/01/2005	0.0124	ND	0.0247	Trace	ND	ND
08/05/2005	0.0134	Trace	0.0317	0.0761	ND	ND
08/08/2005	0.0140	ND	0.0222	0.1240	ND	ND
08/12/2005	ND	0.1810	0.1000	Trace	ND	ND
08/15/2005	ND	ND	0.0539	0.0574	ND	Trace
08/19/2005	ND	ND	0.0232	Trace	ND	ND
08/22/2005	ND	Trace	0.0631	0.0629	ND	ND
08/26/2005	ND	ND	0.0238	0.1650	ND	ND
08/29/2005	ND	ND	ND	Trace	ND	ND
09-02-2005	ND	ND	0.0114	Trace	ND	ND
09-07-2005	ND	ND	ND	Trace	ND	ND
09-09-2005	ND	ND	0.0100	Trace	ND	ND
09-13-2005	ND	ND	0.0000	Trace	ND	ND
09-16-2005	ND	ND	0.0000	0.0000	ND	ND
09-19-2005	ND	ND	0.0000	0.0000	ND	ND

ND=no detection; chlorpyrifos reporting limit (RL) = 0.01 ppb; dimethoate RL = 0.04 ppb.

**Table 9. Selected toxicity data for detected insecticides**

Pesticide	<i>Ceriodaphnia dubia</i> (ppb)	<i>Daphnia magna</i> (48hr LC50, ppb)	<i>Amphipoda Gammarus sp.</i> (96hr LC50, ppb)	Plecoptera - varied species (96hr LC50, ppb)
Chlorpyrifos	0.038 <sup>a</sup>	0.1-1.7 <sup>b</sup>	0.07 – 0.9 <sup>b</sup>	7 - 65 <sup>b</sup>
Dimethoate	NA	580 - 6400 <b>b</b>	180 - 900 <sup>b</sup>	-43 - 510 <sup>b</sup>
<b>Pyrethroids - In sediment</b>	<i>Hyaella azteca</i> 10-day LC50 (µg/g)			
Bifenthrin	0.52 <sup>c</sup>	<b>1% OC</b>		
Lambda-cyhalothrin2	0.45 <sup>c</sup>	<b>1% OC</b>		

NOTES: NA = No data available.

Number ranges are for all studies listed in the indicated source and may represent 2-6 individual studies.

SOURCES:

- a. CA Dept. of Fish & Game, 1999
- b. U.S. EPA Ecotox Database, 2007
- c. Amweg et al, 2006

**Table 10. Water toxicity results of Inlet1 and Outlet1**

Site	Date	96hr <i>Pimephales promelas</i> survival (%)	Control	48hr <i>Ceriodaphnia dubia</i> survival (%)	Control
Inlet1	2/15/05	100	97.5	100	100
	3/23/05	92.5	97.5	0*	100
	4/19/05	100	100	100	100
	5/17/05	100	100	100	100
	6/21/05	97.5	100	100	100
Outlet1	2/15/05	100	97.5	100	100
	3/23/05	95	97.5	0*	100
	4/19/05	100	100	100	100
	5/17/05	100	100	100	100
	6/21/05	100	100	100	100

## Appendix A. Physical Habitat Assessment Field Data Sheet

### Habitat Assessment Field Data Sheet – Low Gradient Streams

STUDY #		DATE					TIME															
STREAM NAME/ LOCATION																						
LAT						LONG					STREAM CLASS											
FORM COMPLETED BY									AGENCY													
Parameters to be evaluated in sampling reach	Habitat parameter	Condition Categories																				
		Optimal			Suboptimal			Marginal			Poor											
	1. Epifaunal substrate/ Available Cover	Greater than 50% of substrate favorable for epifaunal colonization and fish cover; mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at stage to allow full colonization potential (i.e., logs/snags that are <u>not</u> new fall and not transient)			30-50% mix of stable habitat; well suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of new fall, but not yet prepared for colonization (may rate at high end of scale)			10-30% mix of stable habitat, habitat availability less than desirable; substrate frequently disturbed or removed.			Less than 10% stable habitat; lack of habitat is obvious; substrate unstable or lacking											
	<b>score</b>	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	0
	2. Pool Substrate characterization	Mixture of substrate materials, with gravel and firm sand prevalent; root mats and submerged vegetation common			Mixture of soft sand, mud or clay; mud may be dominant; some root mats and submerged vegetation present			All mud or clay or sand bottom; little or no root mat; no submerged vegetation			Hard-pan clay or bedrock; no root mat or vegetation											
	<b>score</b>	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	0
	3. Pool Variability	Even mix of large-shallow, large-deep, small-shallow, small-deep pools present.			Majority of pools large-deep; very few shallow.			Shallow pools much more prevalent than deep pools.			Majority of pools small-shallow or pools absent.											
	<b>score</b>	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	0
	4. Sediment Deposition	Little or no enlargement of islands or point bars and less than <20% of the bottom affected by sediment deposition.			Some new increase in bar formation; mostly from gravel; sand or fine sediment; 20-50% of the bottom affected; slight deposition in pools.			Moderate deposition of new gravel; sand or fine sediment on old or new bars; 50-80% of the bottom affected; sediment deposits at obstructions, constrictions and bends; moderate deposition of pools prevalent.			Heavy deposits of fine materials, increased bar development; more than 80% of the bottom changing frequently; pools almost absent due to substantial sediment deposition.											
	<b>score</b>	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	0
5. Channel Flow Status	Water reaches base of both lower banks, and minimal amount of channel substrate is exposed.			Water fills >75% of the available channel; or <25% of the channel substrate exposed.			Water fills 25-75% of the available channel, and/of riffle substrates are mostly exposed.			Very little water in channel and mostly present as standing pools.												
<b>score</b>	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	0	

		Condition Categories			
Habitat parameter	Optimal	Suboptimal	Marginal	Poor	
Parameters to be evaluated in sampling reach	6. Channel Alteration	Channelization or dredging absent or minimal; stream with normal pattern.	Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e., dredging (greater than past 20 yr) may be present, but recent channelization not present.	Channelization may be extensive; embankments or shoring structures present on both banks; and 40-80% of stream reach channelized and disruptive.	Banks shored with gabion or cement; over 80% of the stream reach channelized and disrupted. Instream habitat greatly altered or removed entirely
	<b>score</b>	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
	7. Channel Sinuosity	the bends in the stream increase the streams length 3 to 4 times longer than if it was in a straight line. (Note: channel braiding is considered normal in coastal plains and other low-lying areas. This parameter is not easily rated in these areas.)	The bends in the stream increase the stream length 2 to 3 times longer than if it was in a straight line.	The bends in the stream increase the stream length 1 to 2 times longer than if it was in a straight line.	Channel straight; waterway has been channelized for a long distance.
	<b>score</b>	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
	8. Bank Stability (score each bank)	Bank stable; evidence of erosion or bank failure absent or minimal; little potential for future problems. <5% of bank affected.	Moderately stable; infrequent, small areas of erosion mostly healed over. 5-30% of bank in reach has areas of erosion.	Mostly unstable; 30-60% of bank in reach has areas of erosion; high erosion potential during floods.	Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosion scars
	<b>score (LB)</b>	Left Bank 10 9	8 7 6	5 4 3	2 1 0
	<b>score (RB)</b>	Right Bank 10 9	8 7 6	5 4 3	2 1 0
	9. Vegetative Protection (score each bank) note: determine left or right side by facing downstream	More than 90% of streambank surfaces and immediate riparian zone covered by native vegetation, including trees, understory shrubs, or nonwoody macrophytes, vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally.	70-90% of streambank surfaces covered by native vegetation, but one class of plants is not well represented; disruption evident but not affecting full plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining.	50-70% of streambank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble remains.	less than 50% of the streambank surfaces covered by vegetation; disruption of streambank vegetation is very high; vegetation has been removed to 5 centimeters or less in average stubble heights.
	<b>score (LB)</b>	Left Bank 10 9	8 7 6	5 4 3	2 1 0
	<b>score (RB)</b>	Right Bank 10 9	8 7 6	5 4 3	2 1 0
	10. Riparian Vegetation Zone Width (score each bank riparian zone)	Width of riparian zone >18 meters; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns or crops) have not impacted zone	width of riparian zone 12-18 meters; human activities have impacted zone only minimally.	width of riparian zone 6-12 meters; human activities have impacted zone a great deal.	width of riparian zone <6 meters; limited or no riparian vegetation due to human activity.
	<b>score (LB)</b>	Left Bank 10 9	8 7 6	5 4 3	2 1 0
	<b>score (RB)</b>	Right Bank 10 9	8 7 6	5 4 3	2 1 0

**APPENDIX B. Statistical comparisons of various water quality, pesticide and BMI data**

**IN CASES 1, 2, 3, AND 5 BELOW MANOVA WAS INITIALLY CONDUCTED TO TEST FOR DIFFERENCES BEFORE ACTUALLY CONDUCTING THE INDIVIDUAL TESTS (EACH MANOVA WAS SIGNIFICANT).**

\*\*\*\*\*

**1. TEST for median of (INLET1-OUTLET1) =0 vs NE 0 FOR PAIRED WATER QUALITY DATA**

**Sign Test** for Median: temp\_diff1, cond\_diff1, pH\_diff1, DO\_diff1, turb\_diff1

Sign test of median = 0.00000 versus not = 0.00000

	N	N*	Below	Equal	Above	P	Median
temp_diff1	213	0	175	0	38	0.0000	-0.7675
cond_diff1	213	0	213	0	0	0.0000	-702.1
pH_diff1	213	0	15	0	198	0.0000	0.4475
DO_diff1	164	49	15	0	149	0.0000	3.875
turb_diff1	208	5	196	0	12	0.0000	-51.52

**RESULT: ALL p<0.000 SO ALL DIFFERENCES ARE SIGNIFICANT.**

**ALSO NOTE ESTIMATES FOR MEDIAN DIFFERENCES ARE PROVIDED:**

**OUTLET1 HAS HIGHER TEMPERATURE, CONDUCTIVITY AND TURBIDITY THAN INLET1, BUT HAS LOWER Ph AND DO.**

\*\*\*\*\*

**2. TEST for median of (INLET2-OUTLET2) =0 vs NE 0 FOR PAIRED WATER QUALITY DATA**

**Sign Test** for Median: temp\_diff2, cond\_diff2, DO\_diff2, pH\_diff2

Sign test of median = 0.00000 versus not = 0.00000

	N	N*	Below	Equal	Above	P	Median
temp_diff2	36	6	36	0	0	0.0000	-1.651
cond_diff2	36	6	36	0	0	0.0000	-50.04
DO_diff2	32	10	32	0	0	0.0000	-2.255
pH_diff2	36	6	33	0	3	0.0000	-0.2271

**RESULT: ALL p<0.000 SO ALL DIFFERENCES ARE SIGNIFICANT.**

\*\*\*\*\*

**3. TEST for median of (INPUT-OUTPUT) =0 vs NE 0.**

**NUTRIENT DATA FOR WATER BODIES 1 AND 2**

**Sign Test**

**NUTRIENTS**

Sign Test for Median: Nitratelin-o, phosphatelin, NH3\_lin-out, alklin-out, Nitrate2in-o, phosphate2in, NH3\_2in-out, alk2in-out

Sign test of median = 0.00000 versus not = 0.00000

	N	N*	Below	Equal	Above	P	Median
Nitratelin-out	25	1	14	2	9	0.4049	-1.000

phosphatelin-out	24	2	22	0	2	<b>0.0000</b>	-0.2750
NH3_lin-out	22	4	22	0	0	<b>0.0000</b>	-0.2750
alklin-out	25	1	23	0	2	<b>0.0000</b>	-35.00
Nitrate2in-out	25	1	16	1	8	0.1516	-2.000
phosphate2in-out	24	2	7	0	17	0.0639	0.2800
NH3_2in-out	25	1	10	0	15	0.4244	0.07000
alk2in-out	25	1	25	0	0	<b>0.0000</b>	-20.00

**BOLD p-values are significant**

#### 4. Paired Prentice-Wilcoxon test (PPW TEST) for chlorpyrifos and dimethoate water column concentrations at paired sites and times.

(NonPar test for equality of paired left-censored data)

REFERENCE: Helsel, D.R., 2005, Nondetects And Data Analysis, Wiley and Sons, 252 p.

A. Chlorpyrifos in water body 1

Ho: distribution of chlorlin = chlorlout

vs Ha: not =

Test Statistic: -0.237  
p value: 0.813

Conclude chlorpyrifos concentrations at INLET1 are not different than chlorpyrifos concentrations at OUTLET1

\*\*\*\*\*

B. Dimethoate in Water Body 1

Ho: distribution of dimethoatelin = dimethoatelout

vs Ha: not =

Test Statistic: -3.809  
p value: 0.000 .....Median difference is approx. -0.0545 (IN1-OUT1)

\*\*\*\*\*

C. Chlorpyrifos in water body 2

Ho: distribution of chlorpyrifos2in = chlorpyrifos2out

vs Ha: not =

Test Statistic: -2.930  
p value: 0.003 .....Median difference is approx. -0.01 (IN2-OUT2)

\*\*\*\*\*

D. Dimethoate in Water Body 2.

Ho: distribution of dimethoate2in = dimethoate2out

vs Ha: not =

Test Statistic: -3.944  
 p value: 0.000 .....Median difference indeterminate

**5. TEST OF BMI DATA INPUT/OUTPUT AT WATER BODY 1**  
**1-sample t-test on differences (inlet1-outlet1)**  
**(equivalent to paired t-test)**

**One-Sample T: in-outAmph, in-outChiron, in-outGast, in-outOlig, in-outTR, in-outAbund**

Test of mu = 0 vs not = 0

Variable	N	Mean	StDev	SE Mean	95% CI	T	P
in-outAmph	15	121.667	133.525	34.476	( 47.723, 195.610)	3.53	0.003
in-outChiron	15	150.200	223.147	57.616	( 26.625, 273.775)	2.61	0.021
in-outGast	15	25.0000	33.5900	8.6729	( 6.3985, 43.6015)	2.88	0.012
in-outOlig	15	-62.8000	155.3361	40.1076	(-148.8223, 23.2223)	-1.57	0.140
in-outTR	15	2.73333	4.35015	1.12320	( 0.32430, 5.14237)	2.43	0.029
in-outAbund	15	307.733	393.278	101.544	( 89.943, 525.523)	3.03	0.009

All variables are significantly different between Input and Output except for count of Oligochaetes

**Appendix C.**

**BMI – water quality, nutrient and pesticide correlation matrices.**

DATA = SUMMER DATA ONLY (6/30-9/19). Water quality and pesticide data are average data for the 2 weeks before the BMI sampling (e.g. the 7/25 BMI sampling data are being compared to average values of water quality/pesticide concentrations measured during 7/12-7/25, etc.) For the pesticide chlorpyrifos and dimethoate, the correlations include the averaged data as described above, and also the maximum values measured in the two-week period before BMI data collection.

**1. Count Amphipod and various water quality/pesticide data**

Correlations: CntAmph, meanchlor, maxchlor, meandimeth, maxdimeth, meanam-N, meantemp, meancond, meanDO, meanturbid

	CntAmph	meanchlor	maxchlor	meandimeth	maxdimeth	meanam-N	meantemp	meancond	meanDO
meanchlor	0.232 0.426								
maxchlor	0.158 0.589	0.993 0.000							
meandimeth	-0.258 0.374	0.206 0.481	0.211 0.470						
maxdimeth	-0.229 0.430	0.283 0.327	0.296 0.304	0.964 0.000					
meanam-N	-0.481 0.082	-0.204 0.484	-0.173 0.554	0.511 0.062	0.521 0.056				
meantemp	0.042 0.886	0.213 0.464	0.195 0.505	0.662 0.010	0.663 0.010	0.502 0.067			
meancond	-0.777 0.001	0.061 0.836	0.126 0.668	0.308 0.285	0.356 0.212	0.379 0.181	-0.011 0.969		
meanDO	0.687 0.007	-0.139 0.635	-0.190 0.516	-0.527 0.053	-0.558 0.038	-0.649 0.012	-0.428 0.127	-0.844 0.000	
meanturbid	-0.620 0.018	0.055 0.852	0.100 0.734	0.518 0.058	0.486 0.078	0.653 0.011	0.322 0.261	0.742 0.002	-0.760 0.002

Cell Contents: Pearson correlation  
P-Value

**2. Count Chironomid and various water quality/pesticide data**

Correlations: CntChiro, meanchlor, maxchlor, meandimeth, maxdimeth, meanam-N, meantemp, meancond, meanDO, meanturbid

	CntChiro	meanchlor	maxchlor	meandimeth	maxdimeth	meanam-N	meantemp	meancond	meanDO
meanchlor	-0.152 0.605								
maxchlor	-0.165 0.574	0.993 0.000							
meandimeth	-0.238 0.412	0.206 0.481	0.211 0.470						
maxdimeth	-0.300 0.298	0.283 0.327	0.296 0.304	0.964 0.000					
meanam-N	-0.428 0.127	-0.204 0.484	-0.173 0.554	0.511 0.062	0.521 0.056				
meantemp	-0.256 0.378	0.213 0.464	0.195 0.505	0.662 0.010	0.663 0.010	0.502 0.067			
meancond	-0.468 0.091	0.061 0.836	0.126 0.668	0.308 0.285	0.356 0.212	0.379 0.181	-0.011 0.969		
meanDO	0.627 0.016	-0.139 0.635	-0.190 0.516	-0.527 0.053	-0.558 0.038	-0.649 0.012	-0.428 0.127	-0.844 0.000	
meanturbid	-0.532 0.050	0.055 0.852	0.100 0.734	0.518 0.058	0.486 0.078	0.653 0.011	0.322 0.261	0.742 0.002	-0.760 0.002

Cell Contents: Pearson correlation  
P-Value

### 3. Count Oligochete and various water quality/pesticide data

Correlations: CntOlig, meanchlor, maxchlor, meandimeth, maxdimeth, meanam-N, meantemp, meancond, meanDO, meanturbid

	CntOlig	meanchlor	maxchlor	meandimeth	maxdimeth	meanam-N	meantemp	meancond	meanDO	meanturbid
meanchlor	0.212 0.466									
maxchlor	0.255 0.379	0.993 0.000								
meandimeth	0.740 0.002	0.206 0.481	0.211 0.470							
maxdimeth	0.707 0.005	0.283 0.327	0.296 0.304	0.964 0.000						
meanam-N	0.655 0.011	-0.204 0.484	-0.173 0.554	0.511 0.062	0.521 0.056					
meantemp	0.486 0.078	0.213 0.464	0.195 0.505	0.662 0.010	0.663 0.010	0.502 0.067				
meancond	0.487 0.078	0.061 0.836	0.126 0.668	0.308 0.285	0.356 0.212	0.379 0.181	-0.011 0.969			
meanDO	-0.609 0.021	-0.139 0.635	-0.190 0.516	-0.527 0.053	-0.558 0.038	-0.649 0.012	-0.428 0.127	-0.844 0.000		
meanturbid	0.801 0.001	0.055 0.852	0.100 0.734	0.518 0.058	0.486 0.078	0.653 0.011	0.322 0.261	0.742 0.002	-0.760 0.002	

Cell Contents: Pearson correlation

P-Value

**4. Abundance and various water quality/pesticide data**

Correlations: Abundance, meanchlor, maxchlor, meandimeth, maxdimeth, meanam-N, meantemp, meancond, meanDO, meanturbid

	Abundance	meanchlor	maxchlor	meandimeth	maxdimeth	meanam-N	meantemp	meancond	meanDO	meanturbid
meanchlor	0.275 0.342									
maxchlor	0.218 0.454	0.993 0.000								
meandimeth	0.573 0.032	0.206 0.481	0.211 0.470							
maxdimeth	0.519 0.057	0.283 0.327	0.296 0.304	0.964 0.000						
meanam-N	-0.178 0.544	-0.204 0.484	-0.173 0.554	0.511 0.062	0.521 0.056					
meantemp	0.498 0.070	0.213 0.464	0.195 0.505	0.662 0.010	0.663 0.010	0.502 0.067				
meancond	-0.402 0.154	0.061 0.836	0.126 0.668	0.308 0.285	0.356 0.212	0.379 0.181	-0.011 0.969			
meanDO	0.265 0.361	-0.139 0.635	-0.190 0.516	-0.527 0.053	-0.558 0.038	-0.649 0.012	-0.428 0.127	-0.844 0.000		
meanturbid	-0.127 0.665	0.055 0.852	0.100 0.734	0.518 0.058	0.486 0.078	0.653 0.011	0.322 0.261	0.742 0.002	-0.760 0.002	

Cell Contents: Pearson correlation

P-Value

**5. Taxonomic Richness and various water quality/pesticide data**

Correlations: TRichness, meanchlor, maxchlor, meandimeth, maxdimeth, meanam-N, meantemp, meancond, meanDO, meanturbid

	TRichness	meanchlor	maxchlor	meandimeth	maxdimeth	meanam-N	meantemp	meancond	meanDO	meanturbid
meanchlor	-0.190 0.516									
maxchlor	-0.168 0.567	0.993 0.000								
meandimeth	0.009 0.975	0.206 0.481	0.211 0.470							
maxdimeth	0.061 0.837	0.283 0.327	0.296 0.304	0.964 0.000						
meanam-N	0.020 0.947	-0.204 0.484	-0.173 0.554	0.511 0.062	0.521 0.056					
meantemp	0.231 0.427	0.213 0.464	0.195 0.505	0.662 0.010	0.663 0.010	0.502 0.067				
meancond	-0.280 0.333	0.061 0.836	0.126 0.668	0.308 0.285	0.356 0.212	0.379 0.181	-0.011 0.969			
meanDO	0.300 0.298	-0.139 0.635	-0.190 0.516	-0.527 0.053	-0.558 0.038	-0.649 0.012	-0.428 0.127	-0.844 0.000		
meanturbid	-0.202 0.489	0.055 0.852	0.100 0.734	0.518 0.058	0.486 0.078	0.653 0.011	0.322 0.261	0.742 0.002	-0.760 0.002	

Cell Contents: Pearson correlation  
P-Value

## Appendix D. Pesticide analyses laboratory quality control data

### Continuing Quality Control- Organophosphate Screen

Extraction Date	Sample Numbers	Percent Recovery												
		Ethoprop	Disulfoton	Malathion	Methidathion	Fenamiphos	Azinphos-Methyl	Dichlorvos	Phorate	Fonophos	Di-methoate	Methyl Parathion	Tribufos (DEF)	Profenofos
6/30/05	001	90.6	85.8	97.7	97.6	99.4	96.9	75.0	80.9	80.6	85.6	85.4	84.9	87.0
6/30/05	25,28,14,29,7,10,4	86.9	84	92.2	96.7	95.3	81.9	73.1	72.8	73.5	75.5	76.5	83.4	80.6
7/12/05	50,52,55,58,67,64,61,46	93.2	79.7	94.9	106	88.2	80.1	82.2	80.8	84.9	83.1	89.1	86.2	87.9
7/22/05	70,73,76,79,18,21,24,43,82,85,88,91	93.7	89.4	97.1	89.9	86.7	80.5	93.7	91.1	91.8	87.6	94.2	93.3	91.9
7/26/05	94,97,100,104,116,113,110,107	96.8	97.0	103	103	103	110	84.6	89.6	94.1	104	98.4	96.4	103
8/2/05	125,122,128,119,140,137,134,131	79.0	79.8	82.9	77.3	75.6	78.9	89.2	91.0	90.4	87.8	91.3	94.1	94.8
8/16/05	167, 170, 173, 176, 179,182, 185, 188	86.0	79.5	86.3	84.5	82.4	105	89.1	89.3	87.6	86.6	90.8	93.1	93.6
8/23/05	197,191,200,194,205,208,211,214	89.8	87.3	94.2	90.0	90.5	70.7	87.7	81.0	84.3	80.5	92.5	94.5	95.6
8/30/05	217, 220, 223, 229, 232, 235, 238	89.0	82.3	88.5	87.4	85.0	117	91.4	86.5	87.9	87.8	90.5	92.2	87.8
8/9/05	143,146,149,152,155,158,161,1164	84.4	77.1	96.8	93.8	87.0	108	89.4	89.2	92.3	92.2	96.4	95.4	97.4
9/8/05	241,244,247,250,253,256,259,262	98.0	84.3	97.8	95.2	89.8	94.7	85.1	82.3	86.6	85.0	90.5	85.5	92.3
9/14/05	300,303,306,308,313,316,319,322	88.3	74.0	89.1	90.6	86.3	103	85.3	88.3	88.7	88.9	92.9	92.7	91.2
9/20/05	325,328,331,334,337,338,343,346,(349),(350),(351)	74.6	72.6	75.3	73.2	71.5	72.1	87.5	87.4	92.2	98.2	103	101	103

Average Recovery		88.5	82.5	92.0	91.2	87.7	92.2	85.6	85.4	87.3	87.9	91.7	91.7	92.8
Standard Deviation		6.6	6.6	7.4	9.3	8.6	15.6	6.0	5.4	5.6	7.3	6.4	5.2	6.3
CV		7.47	7.98	8.09	10.17	9.81	16.92	6.98	6.36	6.43	8.25	6.93	5.68	6.79
Upper Control Limit		123	119	126	128	125	137	106	110	113	117	119	126	125
Upper Warning Limit		113	109	116	117	115	122	98.2	102	105	108	111	116	115
Lower Warning Limit		70.7	68.1	75.7	74.6	77.3	64.0	67.0	73.5	75.5	73.2	76.6	74.9	74.2
Lower Control Limit		60.2	58.0	65.7	63.9	67.9	49.4	59.2	66.3	68.1	64.5	68.0	64.7	64.1

\*Highlighted cells are percent recoveries exceeding control limits (none for this study)

**Continuing Quality Control- Diazinon and Chlorpyrifos on MSD**

Extraction Date	Sample Numbers	Percent Recovery	
		Diazinon	Chlorpyrifos
6/30/05	001	91.6	97.6
6/30/05	25,28,14,29,7,10,4	108	113
7/12/05	50,52,55,58,67,64,61,46	77.2	93.2
7/22/05	70,73,76,79,18,21,24,43,82,85,88,91	106	108
7/26/05	94,97,100,104,116,113,110,107	86.0	92.4
8/2/05	125,122,128,119,140,137,134,131	87.2	86.4
8/9/05	143,146,149,152,155,158,161,1164	95.2	102
8/16/05	167, 170, 173, 176, 179,182, 185, 188	96.0	103
8/23/05	197,191,200,194,205,208,211,214	86.4	96.0
8/30/05	217, 220, 223, 229, 232, 235, 238	90.0	90.8
9/8/05	241,244,247,250,253,256,259,262	90.0	92.8
9/14/05	300,303,306,308,313,316,319,322	91.2	98.0
9/20/05	325,328,331,334,337,338,343,346,(349),(350),(351)	94.0	93.6
Average Recovery		92.2	97.4
Standard Deviation		8.2	7.4
CV		8.85	7.57
Upper Control Limit		117	119
Upper Warning Limit		109	111
Lower Warning Limit		77.2	77.2
Lower Control Limit		69.2	68.8

\*Highlighted cells are percent recoveries exceeding control limits

() = Blind spikes

**Continuing Quality Control- Sediment Analysis Former Method**

Extraction Date	Sample Numbers	Percent recovery				
		bifenthrin	lambda cyhalothrin	permethrin (cis&trans)	cyfluthrin 1-4	cypermethrin 1-4
7/1/2005	502,503,509,500	114	106	111	98.0	106
Upper Control Limit		142	158	133	152	173
Upper Warning Limit		131	145	123	139	154
Lower Warning Limit		88.9	90.9	81.2	88.4	76.2
Lower Control Limit		78.4	77.4	70.8	75.6	56.8

### Continuing Quality Control- Sediment Analysis Newer Method

Extraction Date	Sample Numbers	Percent Recovery				
		bifenthrin	fenopro pathrin	lambda cyhalothrin epimer	lambda cyhalo thrin	permethrin cis
9/27/2005	551,550,553,552	79.4	69.6	62.4	74.0	72.4
Upper Control Limit		98.6	97.3	99.8	99.2	98.9
Upper Warning Limit		91.8	89.1	92.8	92.4	92.0
Lower Warning Limit		64.5	56.3	64.8	64.8	64.3
Lower Control Limit		57.6	48.1	57.9	58	57.4

\*Highlighted cells are percent recoveries exceeding control limits

All analytes spiked at 5 ppb.

### Continuing Quality Control- Pyrethroid Analysis of Water

Extraction Date	Sample Numbers	Percent Recovery					
		bifenthrin	lambda cyhalothrin	permethrin (cis&trans)	cyfluthrin 1-4	cypermethrin 1-4	fenvalerate/ esfenvalerate
6/29/05	002	62.8	62.5	77.1	75.5	93.8	68.6
6/29/2005	026, 034, 015, 030, 008, 011, 005	65.5	68.3	76.0	76.0	85.0	69.9
Average Recovery		64.2	65.4	76.6	76	89	69.3
Standard Deviation		1.9	4.1	0.8	0.4	6.2	0.9
CV		3.0	6.3	1.02	0.5	7.0	1.3
Upper Control Limit		128.9	149.0	141.7	147.2	162.8	137.2
Upper Warning Limit		116.6	136.0	130.2	134.2	146.3	124.8
Lower Warning Limit		67.5	81.5	84.4	82.1	80.2	75.0
Lower Control Limit		55.2	67.9	73.0	69.1	63.7	62.6

Sample numbers in () are blind spikes.

Highlighted recovery was below LCL.

**Blind Spike Data**

Extraction Date	Sample Number	Screen	Pesticide	Spike Level	Recovery	Percent Recovery	Exceed CL <sup>b</sup>
9/20/05	350	OP	Diazinon	200	181	90.5	No
			Chlorpyrifos	150	136	90.7	No
9/20/05	351	OP	Ethoprop	0.20	0.164	82.0	No
			Fenamiphos	0.15	0.129	86.0	No
9/20/05	349	OP	Methidathion	0.25	0.175	70.0	LWL
			Dimethoate	0.30	0.225	75.0	No

<sup>b</sup> CL=Control Limit; Upper CL (UCL), Lower CL (LCL).

Chlorpyrifos and Diazinon results are ppt, Others are ppb