Aquatic Pesticide Monitoring Program

Phase 2 (2003) Bioassessment of Waterbodies Treated with Aquatic Pesticides

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INTRODUCTION

This report presents the results of the bioassessment monitoring for Phase 2 of the Aquatic Pesticide Monitoring Program (APMP). Bioassessments were conducted in 2003, in conjunction with chemical and toxicological sampling, to provide weight-of-evidence for the potential affects of aquatic pesticides in California waterbodies.

The importance of utilizing bioassessments to assess water quality (Barbour et al. 1995, Karr 1999, Resh et al. 1995, 1996, and USEPA 1996, 1998) and sediment quality is well documented nationally (Canfield et al. 1994, 1996, Long et al. 2001, US EPA 1994). As biological communities are *in-situ* integrators of water and sediment quality over time, they are sensitive and responsive to water quality changes, and have been commonly used as indicators of aquatic system health. Bioassessments can be particularly useful to evaluate the effects of point-source contaminants on biological communities, especially when evaluated alongside the chemical, physical, and toxicological conditions (Harrington and Born 2000). Although the use of bioassessments in California has primarily concentrated on high gradient, cobble-bottom lotic systems, we have monitored target aquatic pesticides from a diverse range of both lotic and lentic waterbodies located in various regions throughout the state.

The primary objective of the bioassessment portion of the study, as stated in the APMP contract, is to determine the cumulative impact of aquatic pesticides on select non-target aquatic biological communities by assessing organism diversity and biotic integrity in treated versus non-treated ecosystems. To accomplish the objective, this study employed a rapid bioassessment protocol as advocated by the EPA (2003) and the California Department of Fish and Game Aquatic Laboratory (1999). Bioassessment data was collected from multiple assemblages widely recognized as good biological indicators: benthic and epiphytic macroinvertebrates, and phytoplankton (Barbour et al. 1995, US EPA 2003). In addition, preliminary information was accrued on macroinvertebrate species assemblages for select types of lentic and lotic systems around California that have previously not been described. In-depth background information, management objectives, chemical and toxicological methods and results, and final conclusions of the APMP are described in the APMP Phase 2 (2003) Project report (Siemering 2004).

METHODS

Sampling Design

A temporally stratified study design was implemented to directly coincide with the field application of pesticides applied according to manufacturers' labels. Biological, chemical, and toxicological samples were collected before and after pesticide application from reference and treated sites. Phase 2 monitoring sampled target aquatic pesticides from a diverse range of water-body types located in various regions throughout California (**Table 1, Figure 1**). The frequency and level of sampling varied due to pesticide and site-specific issues (e.g. presence of other potential contaminants, availability of reference sites). The pesticides monitored during Phase 2 (2003) included 2,4-D, copper sulfate, diquat dibromide, fluridone, glyphosate, methoprene, and triclopyr. Although bioassessment data was gathered on these seven aquatic pesticides, this report will focus on copper sulfate, fluridone, glyphosate, and triclopyr, as they are identified as high priority herbicides because of their ubiquitous use and/or lack of information on potential effects. Information on bioassessments results for the remaining pesticides will be reported to the state water board in a separate supplemental document.

Table 1. Sampling locations

Cooperating Permit Holder /Treated Sites /Control Site / Pesticide
Marin Municipal Water District / Bon Tempe and Nicasio Reservoirs / Lake Lagunitas / copper sulfate[1]
Cal. Dept. of Food and Agriculture / Costa Ponds / untreated pond / liquid fluridone[1]
U.S. FWS and Dept. of Boating and Waterways / Lower Stone Lake / Upper Stone Lake / glyphosate[1]
Sand Bay Isle Homeowners Association / Sand Bay Isle Ponds / diquat dibromide and copper sulfate
U.S. FWS and Dept. of Boating and Waterways / treated Stone Lake slough / untreated slough / 2,4-D
Solano Irrigation District / Byrnes canal / untreated canal section / chelated copper
Potter Valley Irrigation District / treated canal / untreated canal section / chelated copper
Big Bear Municipal Water District / treated lake area / untreated lake area / granular fluridone
Contra Costa Mosquito Vector Control District / VCD pond / untreated area / methoprene
Merced Irrigation District / Atwater Canal / untreated canal section / glyphosate
Merced Irrigation District / LeGrande Canal / untreated canal section / acrolein
Ventura County Flood Control District / Doris Drain storm water canal / untreated section / glyphosate
Cal. Dept. of Food and Agriculture / Bear Creek / untreated creek section / triclopyr
Dept. of Boating and Waterways / 7 Mile slough / untreated slough area / diquat dibromide

[1] Long-term study sites

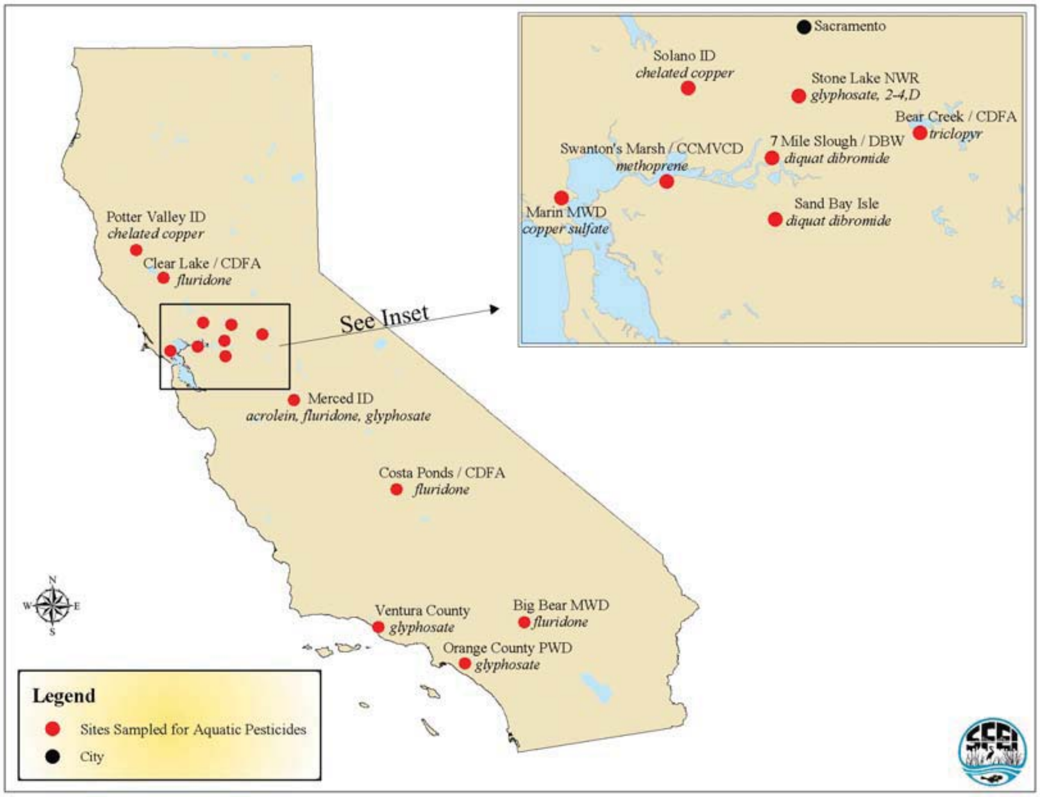


Figure 1. Map of APMP Phase 1 and 2 sampling sites

Site Descriptions

As this report will focus on four of the targeted pesticides as noted above, site descriptions will be confined to the representative systems where these four pesticides were applied in 2003. However, physical and chemical characterization of all study sites is presented in **Table 2**.

Bon Tempe Reservoir, Lake Lagunitas, and Nicasio Reservoir are all drinking water reservoirs located in Marin County, California. In the Marin Water System, Bon Tempe is considered a medium size reservoir (4000 acre feet), while Nicasio is the second largest reservoir in the Marin system (averages 20 feet deep and contains 22,400 acre feet). Lagunitas (350 acre feet) is located slightly higher in elevation than Bon Tempe and feeds into Bon Tempe. Bon Tempe and Lagunitas are filled during the wet season by runoff from a protected watershed and there are no anthropogenic inputs. Nicasio has only limited agricultural development within its watershed boundaries. Bon Tempe and Nicasio are treated every summer with copper sulfate, while Lagunitas has not been treated for at least 30 years. Nicasio has one reference site within the lake that is not treated, while Lagunitas acts as the reference site for Bon Tempe. Lagunitas is smaller and shallower than Bon Tempe, and both sites exhibit similar ranges of dissolved oxygen (particularly at the water-sediment interface), pH, and water temperature (Table 2). However, means for total organic carbon differ between Lagunitas (7.5 %) and Bon Tempe (1.4 %). Lagunitas had higher ranges of total kjeldahl nitrogen in the sediment (710 – 1700 mg/kg) than Bon Tempe (180 - 920 mg/kg). Complete chemical and toxicological data for each site can be found in APMP Phase 2 (2003) Project report (Siemering et al. 2004).

All three Marin lakes are oligotrophic, and exhibited vertical thermal stratification during the summer as water temperatures ranged from 15-30^{o}C (Table 2). Nicasio had higher nutrient values (Total nitrogen and phosphorus ranged 0.51-0.77 mg/L and less than recording limit (<RL) to 0.0313 mg/L, respectively) than Bon Tempe and Lagunitas (both sites <RL). Turbidities were low (1-2 Nephelometric Turbidity Units (NTU) for Bon Tempe and Lagunitas, 6-15 NTU in Nicasio), pH in the median range of 7-9, and redox potential (Eh) of the upper substrate revealed slight hypoxia (-160 – +210 mV). Dissolved oxygen was higher in Bon Tempe and Lagunitas (6-10 mg/L), than Nicasio (3-8 mg/L at bottom of water column).

The Potter Valley Irrigation District (ID) maintains a two-forked primary irrigation canal fed from the East Fork of the Russian River. The canal is dirt lined, shallow, at highest flow reaching less than 3 feet. Potter Valley ID is located northwest of Clear Lake in Mendocino County, and the surrounding area is primarily used for cattle grazing. The canal is treated with copper sulfate twice over the summer period. The water quality was comparatively good in terms of low organic enrichment (Total nitrogen and phosphorus ranged 0.32-1.53 mg/L and 0.022-0.0297 mg/L, respectively), average dissolved oxygen ranges (6-7 mg/L), normal range of pH (8-9), summer temperatures (18-28^{o}C), and turbidity ranged 2-16 NTU (Table 2). Water flow in this canal is regulated depending on irrigation needs so some of these parameters may experience high variability over a short time period.

Table 2. Field Measurements for APMP 2003 sample sites

Analyte	Units	7 Mile Slough	Atwater Drain	Bear Creek	Big Bear Lake	Brynes Irrigation Canal	Costa Ponds	Doris Stormwater Drain
Conductivity	umhos/cm	182; 169 – 194	398; 234 – 561	69; 66 – 74	400; 392 – 405	326; 320 – 332	218; 152 – 379	2458; 2210 – 2760
DO – Bottom	mg/L	5; 2 – 7	NR	2; 1 – 3	6; 5 – 9	NR	6; 0 – 10	NR
DO – Mid	mg/L	7; 7 – 7	7; 7 – 7	2; 1 – 2	6; 5 – 9	10; 10 – 11	8; 4 – 9	6; 6 – 7
DO – Surface	mg/L	7; 6 – 8	NR	2; 1 – 4	6; 5 – 8	11; 11 – 11	8; 5 – 10	NR
Eh	mV	NR	NR	NR	-65; -120 – -10	155; 155 – 155	-88; -110 – -78	192; 192 – 192
pH	s.u.	7; 7 – 7	7; 7 – 7	6; 6 – 7	8; 8 – 9	8; 6 – 9	7; 5 – 9	8; 8 – 8
Salinity	ppt	0; 0 – 0	0; 0 – 0	0; 0 – 0	NR	NR	NR	1; 1 – 1
Temperature	°C	21; 21 – 22	25; 23 – 27	22; 20 – 26	19; 17 – 21	18; 16 – 24	28; 24 – 31	24; 20 – 30
Turbidity	NTU	6; 5 – 6	6; 2 – 10	4; 3 – 5	4; 2 – 5	7; 5 – 8	5; 3 – 7	34; 19 – 54
Water Depth	ft	7; 6 – 8	1; 1 – 1	3; 2 – 4	10; 5 – 18	4; 4 – 4	11; 9 – 14	1; 0 – 1

Note: (Average; Range of values reported) NR=not reported.

Table 2 (cont.) Field Measurements for APMP 2003 sample sites

Analyte	Units	East Fork Russian River Canals	Lake Bon Tempe	Lake Lagunitas	Lake Nicasio	MID Main Canal	Sand Bay Isle	Stone Lake Wildlife Refuge	Swanton's Marsh
Conductivity	umhos/cm	158; 155 – 161	154; 98 – 359	159; 122 – 175	172; 119 – 251	5000; 5000–500	926; 863–1009	245; 186 –367	12101; 616–2370
DO – Bottom	mg/L	NR	8; 6 – 10	8; 8 – 8	7; 3 – 8	NR	4; 0 – 7	4; 0 – 7	6; 4 – 7
DO – Mid	mg/L	6; 6 – 7	8; 6 – 10	8; 7 – 8	7; 5 – 12	8; 8 – 9	6; 2 – 8	6; 0 – 10	8; 5 – 15
DO – Surface	mg/L	NR	8; 6 – 10	8; 7 – 8	8; 4 – 13	NR	5; 2 – 7	6; 0 – 10	6; 4 – 7
Eh	mV	NR	-100; -160 – -1	-65; -65 – -65	31; -109 – 210	NR	0; 0 – 0	-49; -106 – 42	-115; -130– -103
pH	s.u.	8; 8 – 9	7; 4 – 8	9; 8 – 9	8; 7 – 9	7; 7 – 7	7; 6 – 9	6; 5 – 7	7; 7 – 8
Salinity	ppt	NR	NR	NR	NR	NR	0; 0 – 0	0; 0 – 0	11; 9 – 14
Temperature	°C	23; 18 – 28	21; 18 – 30	23; 18 – 27	19; 15 – 22	21; 20 – 23	22; 18 – 27	21; 16 – 26	21; 17 – 29
Turbidity	NTU	6; 2 – 16	2; 1 – 2	2; 1 – 2	9; 6 – 15	9; 1 – 15	5; 0 – 11	15; 7 – 30	20; 7 – 42
Water Depth	ft	3; 1 – 5	16; 9 – 28	13; 13 – 14	23; 0.3048 – 48	1; 1 – 1	4; 4 – 4	7; 3 – 22	2; 0 – 3

Note: (Average; Range of values reported) NR=not reported.

Costa Ponds are several small lakes (the largest being 5 acres) located near Porterville, in Tulare County, at the base of the Sierra Nevada foothills. The lakes were constructed as a fishing resort and are surrounded by dry scrub and limited vegetation. The water source is the Tule River. Water flows through the ponds in series beginning with the smallest pond, Pond 1 (1.0 acre). No outboard motors are allowed in the ponds and there are few uncontrolled inputs. The ponds average nine feet in depth. Pond 5 is treated with fluridone two to three times a season, while Pond 1 is not treated with fluridone, but has been treated sparingly with copper in previous years. Pond 1 copper concentrations in porewater and sediment were 12.4 ug/L and 75.3 ppb, respectively. Pond 1 was the reference site for the fluridone application in Pond 5. Both ponds exhibited low and fluctuating dissolved oxygen conditions during the summer, particularly at the bottom of the water column (0-10 mg/L). Pond 1 had lower dissolved oxygen and pH conditions than Pond 5. Nutrient content was slightly higher in Pond 1 versus Pond 5 (Total nitrogen and phosphorus were 0.91 and 0.072 mg/L, respectively, in Pond 1, while nitrogen and phosphorus ranged <RL-0.64 mg/L and <RL-0.0538 mg/L, respectively, in Pond 5). Turbidity was low (3-7 NTU), pH normal (5-9), and temperatures ranged 24-31 ^{o}C (Table 2).

Bear Creek is located between Jackson and Lodi in Calaveras County, in the low Sierra Nevada foothills. It is a natural creek surrounded primarily by lands used for cattle grazing. The creek has stagnant flow and high water depths (2-4 ft) due to the presence of several beaver dams. The creek bottom is primarily cobblestone with little sediment. The creek was experimentally treated with triclopyr this year under a limited-use research permit. The creek has not been treated with any other herbicide this year. The creek had hypoxic oxygen conditions (1-4 mg/L), low nutrient conditions (Total nitrogen and phosphorus ranged 0.3-0.54 mg/L and 0.047-0.0685 mg/L, respectively), conductivity was low (66-74 µmhos/cm), pH normal (6-7), low turbidity (3-5 NTU), and temperature ranged 22-26^{o}C (Table 2).

Stone Lake National Wildlife Refuge is located just south of Elk Grove, in Sacramento County. The lake is fed by water from the Sacramento River. There is a small dam that controls the lake level, which dampens the tidal flow in the lake and allows some areas to act as backwater sloughs with high organic matter deposition and lower oxygen levels. Additional input into northern Stone Lake comes from storm water drainages from nearby housing developments. The western lake edge is protected by levees, but direct runoff will occur from other surrounding agricultural fields. The lake is predominantly freshwater (salinities less than 3 ppt), influenced by heavy wind action, and fairly shallow (3-22 ft). Some areas of the lake are treated with glyphosate (with surfactant added) for water hyacinth control. One arm of the lake is isolated from the lake by a culvert, and did not receive glyphosate treatment in 2003. This heavily vegetated site was used as a reference for the treated area. Hypoxic conditions persisted in the lake (D.O. ranged 0-10 mg/L, with average of 4), pH was slightly acidic (5-7), turbidity ranged high (7-30 NTU), and temperatures ranged 16-26°C (Table 2). Hypoxia was more evident at the reference station (D.O. at water-sediment interface ranged 0-4 mg/L). Nutrient content ranged slightly higher in the treated areas (Total nitrogen and phosphorus ranged <RL-1.15 mg/L and <RL-0.199 mg/L, respectively) versus the reference site (nitrogen and phosphorus ranged <RL-0.81 mg/L and <RL-0.118 mg/L, respectively).

Sample Collection and Processing

Sampling for bioassessments was conducted according to aquatic system type and target biological community (benthic and epiphytic macroinvertebrate, phytoplankton), utilizing sampling protocols for lentic and lotic systems adapted from the California Department of Fish and Game Aquatic Laboratory (CDFG 1999 and 2002). Bioassessment samples were collected concurrently (both spatially and temporally) with samples for chemistry and toxicity testing (for both water and sediment), including pesticide concentrations in water, pore water, and sediment (**Table 3**). A thorough physical habitat assessment was also conducted, and Global Positioning System (GPS) coordinates were recorded for each sampling station. All samples were collected prior to pesticide application and at various post-application intervals: within 24 hours, 2 weeks post, and 4-8 weeks post. Sample collection occurred from late May through September 2003. Reference sites for each location were identified based on several *a priori* criteria: 1) same water body type, size, and chemical characteristics as treated site, 2) within same watershed as treated site, 3) no application of aquatic pesticides within the last 2-5 years, and 4) limited anthropological inputs. Reference sites were sampled at the same time period and using the same methodology as treated sites. Pre-application sampling substituted for reference sampling if no suitable reference site was found.

Table 3. Conventional parameters measured

Physical Parameters		**units**
Air Temperature		°C
Water depth		M
Sediment collection depth		Cm
Geometric profiles of water body		Cross-sections/ diagrams
Flow Rate (lotic systems)		Cfs (ft^3/s)
Inflow Volume (lotic systems)		Cubic ft
Outflow Volume (lotic systems)		Cubic ft
Flow Diversions		Describe
Current from wind action (lentic systems)		Qualitative – none, mild, moderate, strong
Anthropogenic activities/ alterations		Describe
Wildlife presence		Describe
Conventional Water Quality Parameters		**units**
	Conductivity	µmho/ cm
	Dissolved Organic Carbon	µg/L
	Dissolved Oxygen (DO)	mg/L
	Hardness (when salinity is < 5 $^0/_{00}$)	mg/L ($CaCO_3$)
	Salinity	psu (‰)
	pH	pH
	Temperature	°C
	Total Chlorophyll a	mg/m^3
	Total Phosphorous	mg/L - P
	Total Nitrogen	mg/L - N
	Total Suspended Solids	mg/L
	Alkalinity	mg/L ($CaCO_3$)
	Dissolved Calcium	mg/L
	Dissolved Magnesium	mg/L
	Dissolved Sodium	mg/L
	Turbidity	NTU
Sediment Quality Parameters		**units**
	% gravel (> 2 millimeters)	% dry weight
	% sand (2 mm > 62 µm)	% dry weight
	% fines (< 62 µm)	% dry weight
	Nitrate-Nitrogen	mg/kg
	% solids	% dry weight
	% moisture	% dry weight
	Temperature	°C
	Total Nitrogen	mg/kg
	Total Organic Carbon	mg/kg
	Pore Water Pesticide Concentration	mg/l or µg/L
	SEM-AVS (for copper treatments only)	SEM-AVS Ratio
	Eh	MV

Benthic samples were collected with a petite Ponar grab sampler (0.02 m^2) in the immediate vicinity of pesticide application. For lentic systems, a minimum of two sampling stations within the application zone and reference sites were located and sampled. Three replicate samples were randomly collected from a 5 x 5 meter area within each sampling station. Collection of ancillary water and sediment measurements, and sample matrices for toxicity testing occurred within close proximity to benthic collection and in the following collection order to minimize benthic sample disturbance: habitat assessment, water quality and chemistry, epiphytic collection, benthic collection, and sediment sampling. For lotic systems, sampling stations were selected in a linear manner in reference to the application point. Two sample reaches of equal length (minimum of 5 meters) were established sequentially downstream of the application point. As all of our lotic study sites were soft bottom systems, three replicate samples were randomly collected using a petite Ponar within each sampling reach.

The material from each benthic grab was sieved using a no. 35 (0.5 mm) sieve, and the retained material transferred into a labeled plastic jars and preserved with 95% ethanol. The preserved samples were transported, under chain of custody, to three separate laboratories for rinsing, sorting and taxonomic identification. Samples were sorted to a 300-fixed count, or the entire sampled sorted if the count was not reached. Taxonomic identification was made to the lowest practical level, which for most organisms was to genus and species.

Epiphytic macroinvertebrates were sampled at every sampling event unless there was an absence of aquatic vegetation within the water body. For lentic systems, epiphytic macroinvertebrates were sampled utilizing a transect design as outlined in the California Lentic Bioassessment Procedure (CDFG 2002). Two transects of variable length were established from near the shore out to center of the application area. Transects were chosen for habitat homogeneity between the two transects, and subjective representation of the average conditions within the area of interest. Three qualitative sweeps of standard effort (1 minute each) were taken within the submerged/emergent pelagic vegetation and within the littoral vegetation up to the shoreline along each transect. The sweeps were combined to produce one composite sample, which was placed within a plastic jar containing 95% ethanol. Three samples for each site were collected for each sampling event. For lotic systems, sampling stations were again established in a linear manner in reference to the application point and three composited samples collected within each reach. Epiphytic samples were sorted and identified in the same manner as the benthos.

Phytoplankton were sampled at three study sites: Bon Tempe and Nicasio Reservoirs, and Lake Lagunitas. Phytoplankton were collected within the application zone, in proximity to water quality collection, with site water from a Kemmerer grab bottle lowered to mid-water column. A composite sample was retrieved, placed in a polyethylene bottle, preserved with Lugol's solution and placed in a cooler, taking care to limit sample exposure to sunlight. Two samples per site, before and after treatment, were collected. The preserved samples were transported, under chain of custody, to the laboratory where they were processed following standard procedures. Samples were counted and identified in the laboratory following standard procedures, and biological metrics generated (**Table 4**).

Biological Analyses

Spatial patterns in the abundance and composition of macroinvertebrate assemblages among and within the study sites were described using classification analysis (Smith et al.1988). Classification analyses are an important tool to identify distinct biological assemblages, and determine the physical and chemical variables that define the community.

Changes over application time in the distribution, abundance, and composition of macroinvertebrate and phytoplankton communities were analyzed using a multi-metric approach. Raw abundance and species composition data were used to generate eighteen macroinvertebrate indices and three phytoplankton indices (Table 4). The biological metrics were assessed before and after application, and among treatment and reference sites. Appropriate metrics were chosen based on literature review, recommendations in the EPA’s Lake and Reservoir Bioassessment and Biocriteria Technical Guidance Document (1998), and discussions with the CDFG Aquatic Bioassessment Laboratory. The goal for metric development was twofold: 1) choose a well-balanced suite of representative metrics for soft-bottom, lentic and lotic systems in California, and 2) choose metrics that have been shown to be responsive to disturbance. Total abundance, diversity, and taxa richness are common indices used to describe biological communities (Barbour 1995, 1996, Karr et al. 1987, and Yoder 1989).

The percent contribution of certain groups of organisms to the total assemblage has been shown to indicate various types and extent of system stressors. In particular, high abundance of dipterans, chironomids (family within the dipteran order), and oligochaetes have been linked with organic pollution and other disturbances (contaminants such as heavy metals, industrial pollutants) (Canfield et al. 1994, 1996, Kilgour et al. 2004, Lowe and Thompson 1997). The dominance of one or few taxon can indicate stress. Dominant species greater than 35% indicate poor water quality, while less than 25% generally indicates good water quality (EPA 1996). Shifts in functional feeding groups of organisms and loss of sensitive taxa such as species within the ephemeroptera, plecoptera, and tricoptera families (EPT Index) can also signal system stress (Harrington and Born 1999, EPA 1998). Changes in the proportion of tolerant and intolerant species within a community are important when determining impacts from any contaminant. Most tolerance indices are based on sensitivity to organic enrichment (Bode et al. 1996, Hilsenhoff 1987). Taxon-specific tolerances can be found in the literature for trace metals, terrestrial pesticides, and various other industrial contaminants (Canfield et al. 1994, 1996, Chapman et al. 1987, Long et al. 2001, Mandaville 2002, Peterson et al. 1996). There are few published macroinvertebrate tolerance values specifically for aquatic pesticides, although there are toxicity threshold values for copper (Besser et al. 2003, Milani et al. 2003). Our tolerance values are based on sensitivities of western aquatic macroinvertebrates to organic pollution and some heavy metals (Ode 2003).

Table 4. Macroinvertebrate and Phytoplankton Biometrics

Metric	Unit
Macroinvertebrates	
Total Relative Abundance	Estimated no. of individuals/0.02 m^2: comprised of actual counts from 300-fixed count sub-sampling estimated to the entire sample
Richness	Total Number of Individual Taxa
Diversity	Shannon-Wiener Index
Ephemeroptera, Plecoptera, Tricoptera (EPT) Taxa	%
Intolerant Organisms	%
Tolerant Organisms	%
Tolerance Value	Tolerance Index
Dominant Taxa (Family and Species)	%
Collector	%
Filterer	%
Scraper	%
Predators	%
Shredders	%
Odonata Richness	%
Diptera Richness	%
Chironomidae Richness	%
Oligochaeata Richness	%
Phytoplankton	
Abundance	Total Number of Individuals
Diversity	Total Number of Individual Taxa
Richness	Shannon-Wiener Index

Physical and Chemical Characterization

Sediment for chemistry was collected prior to and post application at 2 weeks, and 4-6 weeks post for long-term study sites. The number and location of the sediment sampling stations was the same as those for the benthic invertebrate collection. Sediment for toxicity testing was collected at pre- and 2 weeks post application. If toxicity results from the 2-week sample events indicated persistent toxicity, then sample collection for sediment toxicity also occurred at the 4-6 post sample periods. Methods for sediment collection and analysis are found in the APMP QAP (Yee et al. 2003).

Water chemistry was monitored by field measurement (multi-parameter probe for DO, conductivity, pH, temperature, turbidity, and Eh), and laboratory analysis (Yee et al. 2003). Field water quality measurements were collected at every sampling event from the stations located for the bioassessment sampling, while water chemistry was collected pre- and immediately post-application at one representative sample station within the application zone at a frequency dependant upon the projected pesticide persistence in the water column, and site-dependent characteristics, such as flow rate, application location and rate.

Data Management and Statistical Analysis

Raw data from the three laboratories performing taxonomic identifications were standardized to the same taxonomic level to reduce error. Taxonomic protocols specific to objectives of the APMP were established for all taxonomic groups. These protocols were adapted from California's standard taxonomic effort list (CAMLnet) for macroinvertebrates (ODE 2003) and based on further discussions with the labs. Chironomid taxonomy protocols were adapted from proposed WEMAP protocols (Dan Pickard, pers. communication 2003). QA/QC for sorting was performed on ten percent of the total samples internally at all labs. External taxonomic QA/QC was conducted for five percent of all samples.

Classification analysis was performed on the raw abundance and species lists (Smith et al.1988). Analysis was run on eleven study sites, including irrigation and stormwater canals (Brynes Canal, Potter Valley Canal, and Doris Drain), small ponds and reservoirs (Bon Tempe Reservoir, Nicasio Reservoir, Lake Lagunitas, Big Bear Reservoir, Costa Ponds, and Sand Bay Pond), and Delta systems (Stone Lake and 7 Mile Slough). In summary, the data were transformed and standardized, and then a Bray-Curtis similarity matrix that uses abundances of all species was calculated to summarize the patterns of overall community composition and abundances. The result was a dendrogram showing the degree of difference (ecological distance) in the biota among the stations sampled. Numbers of species and individuals at each station were used to express species diversity rather than a formal diversity index (Lowe and Thompson 1997, Smith et al.1988).

Profile plots were generated (SAS 1995) to examine trends in the macroinvertebrate metrics over time and between reference and treatment. Means and standard deviations for each metric at each sampling station were calculated. The means were then pooled for all stations within a site and graphed. A two-way ANOVA (SAS 1995) was performed on metrics to compare differences in metrics over time and treatment against the reference conditions. Outlier analysis was conducted and standard transformations were applied to the data prior to analyses where appropriate to meet the assumptions of normality and equality of variance for the ANOVA test (Sokal and Rohlf 1995).

Spearman rank correlation analysis (SAS JMP 5.0.1 2002) was conducted between the biological metrics and select water and sediment quality parameters. The goal was to determine if any differences seen could clearly be attributed to aquatic herbicide application, and to examine seasonality or other abiotic factors.

RESULTS

Macroinvertebrate Assemblages

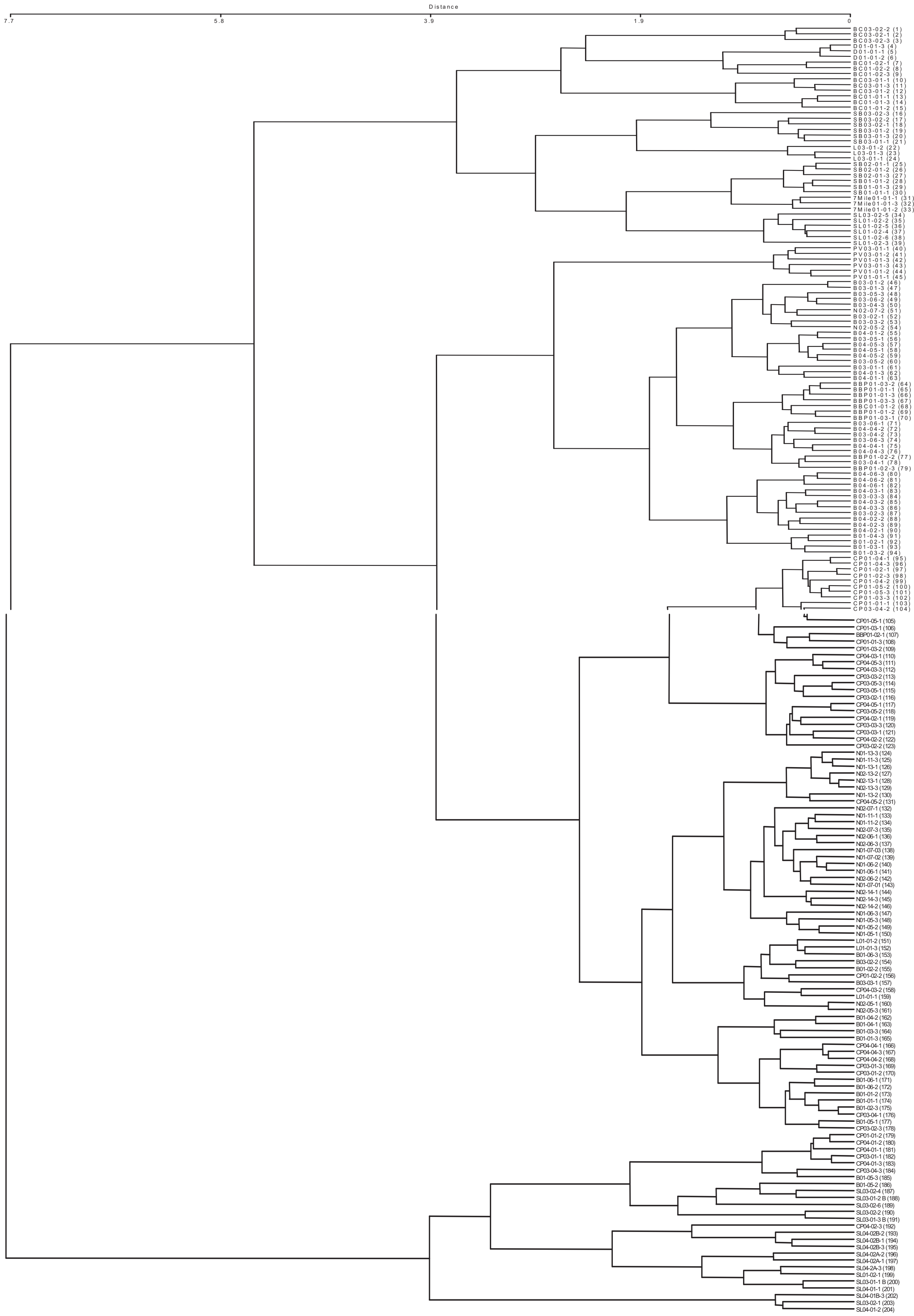
Classification analysis for macroinvertebrates revealed unique assemblages depending on site type and physical/chemical habitat characteristics. Freshwater lentic and lotic systems had distinct benthic macroinvertebrate assemblages and sub-assemblages (**Table 5**). Within the lentic assemblage, benthic macroinvertebrates aggregated into two sub-assemblages based on substrate characteristics (Table 5, **Figure 2**). The reservoirs and small ponds clustered together (Bon Tempe, Lake Lagunitas, Nicasio Reservoir, and Costa Ponds), and represent the low muck, consolidated substrate assemblage. These sites generally had lower percent fines and more sand. Species in this sub-assemblage included many worm (oligochaeta and nematoda) and dipteran larvae species (chironomidae, ceratopogonidae, and chaoboridae), finger clams (*Pisidium spp*.), and water mites (*Arrenurus spp., Forelia spp*.). Oligochaetes and dipterans (particularly chironomidae and *Chaoborus spp*.) are common in the low oxygen conditions of the profundal and sublittoral zones of lakes and reservoirs (US EPA 1998). Mites are commonly associated with ponds, and clams are generalist found in both lakes and streams. Sites with high muck and flocculent substrate, such as Stone Lake, Sand Bay Isle, and the 7 Mile delta slough, grouped together. Common and abundant species within this sub-assemblage include several species of worms and midge larvae, one snail species (*Physa spp*.), a leech (*Helobdella stagnalis*), and an amphipod (*Hyalella spp*.) (Table 5). This suite of species is considered very tolerant of low oxygen and high organic matter conditions (Ode 2003).

The lotic assemblages generally clustered together and had no clear distinction among irrigation canal sites (Table 5, Figure 2). Potter Valley Irrigation Canal was characterized by intermittent riparian vegetation and a fine-gravel substrate, while Byrnes Canal and Doris Drain had very sparse to no riparian cover and fine-sandy substrate. Main species types within the lotic systems include snails, an invasive clam (*Corbicula fluminea)*, worms, beetles, water mites, and fly larvae (midge, alderfly, and mayfly). Two of the species in particularly are indicators of canal conditions: the alderfly species (*Sialis spp*.) resides in leaf litter in intermittent backwater areas and the mayfly *Fallceon quelleri* is commonly found in disturbed riparian stream corridors. The functional feed guilds of the species (mostly predators and collectors, with a lack of shredders and grazers) indicate systems devoid of substantial allochthonous inputs.

Table 5. The common and abundant species in each benthic assemblage and sub-assemblage

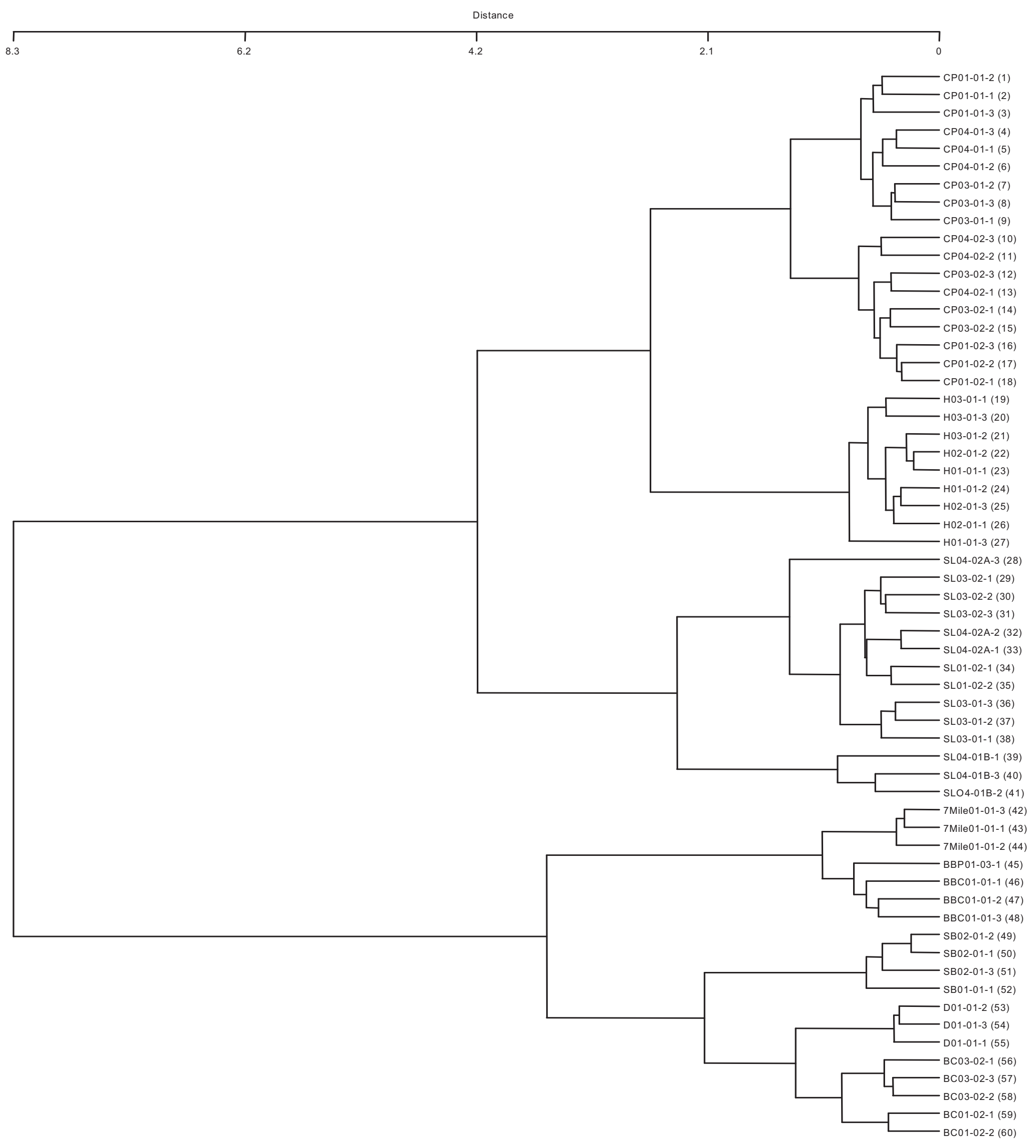
Freshwater Lentic, Soft-substrate (n=179)		**Freshwater Lotic, Soft-substrate (n=21)**	
High Muck, flocculent substrate (n=24)	**Low Muck, consolidated substrate (n=155)**	**Irrigation Canal/Stormwater, fine/sand substrate, sparse riparian cover**	
Physa spp.	Pisidium spp.	Ferrissia spp.	Limnodrilus spp. (O)
Hyalella spp.	Forelia spp.	Fallceon quilleri	Tubificid immature (O)
Helobdella stagnalis	Sphaeromias spp.	Sialis spp.	Nais bretscheri (O)
Gyraulus spp.	Chaoborus spp.	Berosus spp.	
Pisidium spp.	Polypedilum halterale group (Ch)	Corbicula fluminea	
Psectrocladius vernalis (Ch)	Phaenopsectra spp. (Ch)	Paratanytarsus spp. (Ch)	
Tanytarsus spp. (Ch)	Chironomus spp. (Ch)	Phaenopsectra spp. (Ch)	
Procladius spp. (Ch)	Procladius spp. (Ch)	Dicrotendipes spp. (Ch)	
Aulodrilus pigueti (O)	Tanytarsus spp. (Ch)	Tanytarsus spp. (Ch)	
Tubificid immature (O)	Limnodrilus spp. (O)	Chironomus spp. (Ch)	
Ilyodrilus frantzi (O)	Dero digitata (O)	Cricotopus spp. (Ch)	
Limnodrilus spp. (O)	Branchiura sowerbyi (O)	Orthocladius spp. (Ch)	
Dero digitata (O)	Nais bretscheri (O)		
Quistadrilus multisetosus (O)	Ilyodrilus templetoni (O)		
	Tubificid immature (O)		
Note: Oligochaeta (O) Chironomidae (Ch)			

Figure 2. Dendrogram of cluster analysis for benthic macroinvertebrates at 2003 study sites.



In general, epiphytic macroinvertebrate assemblages were not unique and there were many shared taxa among sites, both lentic and lotic (**Figure 3**). Common taxa included gastropods (*Physa spp*.), oligochaetes (*Dero spp., Nais spp*.), dipterans (chironomidae and ceratopogonidae), odonates (*Ischnura spp*.), ephemeropterans (*Caenis latipennis*), and trichopterans (*Oxyethira spp*.). Epiphytic abundances, however, were dominated by dipterans, oligochaetes, amphipods, and gastropods. A complete list of unique macroinvertebrate taxa compiled from all sites is shown in Appendix 1.

Figure 3. Dendrogram of cluster analysis for epiphytic macroinvertebrates at 2003 study sites.



Species Composition and Abundance

Benthic macroinvertebrate abundance (mean estimated no. individuals /0.02 m^2) for the lake/reservoir sites were low (300 to 12,500/m^2), but within ranges of other literature values (Canfield et al. 1994, VTDEC 2003) (**Table 6**). The irrigation canal abundances (21,050 to 30,350/m^2) were well within ranges of studies conducted on impacted streams (Canfield et al. 1994, Kilgour et al. 2004) (Table 6). Qualitative dip net sampling of epiphytic communities revealed densities at the higher end of literature ranges (VTDEC 2001) (Table 6). All mean abundances varied greatly across reference and treatment conditions and within each site (Table 6).

The number of taxa represented in both the benthic and epiphytic assemblages were fairly low at all sites (Table 6). Average benthic richness at reservoirs and lakes ranged 2.0 – 19.7, and ranged 16.7 – 20 at the irrigation canal site (Potter Valley). Costa Ponds exhibited the lowest richness values of all sites, most likely due to the extreme high temperatures at these ponds experienced throughout the summer in the entire water column. Mean epiphytic richness ranged 11.3 – 37 for all sites.

Table 6. Range of mean abundance and mean taxa richness pooled for all stations at each site.

Site Name	Sample Type	Site Type	Total Abundance	Number of Taxa
Bon Tempe Reservoir (n=54)	Benthos	T	28-172	5.6-10.9
Lake Lagunitas (n=9)	Benthos	R	42-250	8.7-19.7
Nicasio Reservoir (n=6)	Benthos	R	91-144	7.7-8.7
Nicasio Reservoir (n=24)	Benthos	T	42-52	7.7-9.3
Potter Valley ID (n=12)	Benthos	T	421-607	16.7-20
Costa Ponds (n=9)	Benthos	R	32-23	2-4.7
Costa Ponds (n=36)	Benthos	T	24-41	6.1-6.3
Costa Ponds (n=9)	Epiphytic	R	565-6272	16.3-20
Costa Ponds (n=9)	Epiphytic	T	184-1842	17-24
Stone Lake (n=6)	Benthos	R	6-10	3.0-3.7
Stone Lake (n=18)	Benthos	T	40-121	6.3-17
Stone Lake (n=6)	Epiphytic	R	222-667	11.3-13
Stone Lake (n=8)	Epiphytic	T	652-3784	26-37
Bear Creek (n=9)	Epiphytic	T	480-667	18.3-23

Note: Benthos abundance is no. individuals/0.02m^2 ; epiphytic abundance is no. individuals per composite sweep. Reference (R) and treated (T) conditions are noted.

Indicator Species

Dominant taxa groups represented in the macroinvertebrate assemblages for each site remained fairly constant over the course of the sampling season (**Table 7**). A complete list of the top five dominant species by sample station is found in Appendix 2. Chironomidae and Oligochaeta species occurred commonly and in the highest abundances. Assemblages did not change with regard to pesticide application, and the few new dominant species that occurred between pre and post application were most likely due to seasonal differences (after comparison to reference sites). All the dominant macroinvertebrates found at our sites are moderately-to-extremely tolerant organisms, with tolerance values at the highest levels (6-10) (Ode 2003). Taxa represented at our study sites within the dominant groups Oligochaeta (*Dero digitata, Slavina appendiculata, Ilyodrilus spp., and Limnodrilus hoffmeisteri*) and Chironomidae (*Procladius spp., Chironomus spp., and Cryptochironmus spp.*) have been found to be moderately tolerant to highly tolerant to both organic pollution and metal-contaminated sediments (Canfield et al. 1994). Other common and abundant taxa found in the study (*Aulodrilus pluriseta, Branchuira sowerbyi, Cladotanytarsus, Cryptochironomus spp., Cryptotendipes spp., Dero digitata, Limnodrilus hoffmeisteri and udekemianus, Polypedilum spp., and Procladius spp.*) have been used as potential contaminant tolerant indicators in freshwater and estuarine systems (Lowe and Thompson 1997). Tolerance determinations are species specific and sometimes pollutant specific. However, much of the current literature does not differentiate tolerances between organic pollution and industrial contaminants (including heavy metals), as these types of pollutants commonly occur together. Metals, in particular, are commonly found in close association with high organic matter due to the higher binding capacity to organics. In-situ field tolerances of invertebrates to aquatic pesticides are rare in the literature and must be extrapolated from lab-based toxicity tests. Therefore, the use of tolerance values for determining effects from aquatic pesticides is limited in this study. Moreover, many of the same species were found in systems where different pesticides were applied, indicating a tolerance of the taxa to general stress and not one specific pollutant. The key is to begin to look for indicator species for one pesticide, i.e. those that clearly change in abundance with application in treatment sites or those where there is a change in presence/absence between reference and treatment. Thus far, our study has not found any such indicator species with one year of data.

Assemblages with 12% oligochaete individuals and 6% chironomid individuals have been reported as ‘clean’, non-contaminated sites, while significantly higher proportions of these groups (80 to 90%) have been found at metal and industrial pollutant contaminated sites (Canfield et al. 1994). All of our sites, including reference, exhibited average oligochaete and chironomid abundances of 20-80% and 10-70%, respectively. However, high abundances of oligochaete and chironomid genera are commonly found in fine, high organic matter sediments and the profundal zone of lakes, so care needs to be taken when assigning contamination to assemblages. It is difficult to determine the percentage necessary to define clean versus contaminated, or whether a system's communities are baseline tolerant to a myriad of system stressors. Again, identifying indicator species with known tolerance to a specific pollutant will be necessary for assessing any degree of contamination.

Few intolerant taxa were found at any site, regardless of treatment type or reference condition, and when present, densities were very low. One intolerant species (*Oxyethira spp*., tolerance value of 3) was present in the reference site Lake Lagunitas at two and six week post application, and absent in treated Bon Tempe at those time periods. We are unable to determine at this time whether this is due to seasonality, sampling variability, or water quality changes (including herbicide application). In summary, aquatic herbicide application did not alter tolerances of the assemblages, as all the dominant macroinvertebrates were highly tolerant prior to application.

Table 7. Dominant macroinvertebrate taxa in descending order for reference (R) and treated (T) sites before and after herbicide application

Site Name	Sample Type	Site Type	Pre-Application (Sample Date)	2wk Post Application (Sample Date)	6wk Post Application (Sample Date)
Bon Tempe Reservoir *LM	Benthos	T	Limnodrilus spp. Phaenospectra spp. Procladius spp. Tubificidae (5-22-03)	Procladius spp. Limnodrilus spp. (7-16-03)	Limnodrilus spp. Polypedilum spp. Aulodrilus japonicus Cryptochironomous spp. (8-13-03)
Lake Lagunitas *LM	Benthos	R	Limnodrilus spp. Procladius spp. Chironomous spp. (5-22-03)	Dero app. Aulodrilus app. Dicrotendipes spp. (7-16-03)	Aulodrilus japonicus Aulodrilus pigueti (8-13-03)
Potter Valley ID *LR	Benthos	T	Limnodrilus spp. Limnodrilus hoffmeisteri Microchironomus spp. (7-22-03)	Limnodrilus spp., Limnodrilus hoffmeisteri Tubificid immature (8-6-03)	NA
Costa Ponds *LM	Benthos	R	Procladius spp. Dero digitata Limnodrilus hoffmeisteri Branchiura sowerbyi (6-15-03)	Limnodrilus spp. Procladius spp. Tubificidae Arrenurus spp. Cryptochironomous spp. (7-2-03)	Limnodrilus spp. Procladius spp. Dero trifida Chaoborus spp. (7-31-03)
	Benthos	T	Dero spp. Limnodrilus spp. Procladius spp. Bezzia spp. (6-15-03)	Dero spp. Limnodrilus spp. Procladius spp. Bezzia spp. Cryptochironomus spp. (7-2-03)	Dero spp. Limnodrilus spp. Procladius spp. Branchiura spp. (7-31-03)
Stone Lake *HM	Benthos	R	NA	Limnodrilus spp. Chironomus spp. Haemonias spp. (6-5-03)	Limnodrilus spp. Hyallela spp. Haemonias spp. (7-2-03)
	Benthos	T	Ilyodrilus spp. Slavina spp. Aulodrilus spp. (5-8-03)	Dicrotendipes spp Limnodrilus spp. Ilyodrilus spp. (6-5-03)	Aulodrilus spp. Hyallela spp. Limnodrilus spp. Polypedilum spp. (7-2-03)
Stone Lake	Epiphytic	R	NA	Hyallela spp. Ischnura spp. Physa spp. (6-5-03)	Crangonyx spp. Hyallela spp. Haemonias spp. (7-2-03)
	Epiphytic	T	Hyallela spp Limonia spp. (5-8-03)	Hyallela spp Limonia spp. Dero spp. (6-5-03)	Stylaria spp. Hyallela spp. Dero spp./ Nais spp. (7-2-03)
Bear Creek	Epiphytic	T	Dero spp. Dasyhelea spp. Hyalella spp. (7-23-03)	Dero spp. Dasyhelea spp. Hyalella spp. (8-6-03)	NA

*Assemblages denoted as LM= low muck lentic , HM= high muck lentic, LR= lotic riparian cover

Abiotic Variables

Appendix 3 shows mean values of macroinvertebrate metrics pooled for each study site, while the raw, un-pooled metric results by station within a site are given in Appendix 4. Correlations between macroinvertebrate metrics and various chemical and physical parameters (pesticide concentration, total organic carbon, and percent fines) revealed some weak, but significant relationships ($p>0.05$) (**Table 8**). Although the relationships are not strong, they do make ecological sense. As dissolved copper concentrations in the sediment increase, the percent abundance of the tolerant group, Chironomidae, increases. As depth and total organic carbon (TOC) increase in the system, the percentage of tolerant taxa increases, while species richness is reduced. Correlations run with dissolved oxygen, pH, and water temperature were not statistically significant. These results indicate that many physical variables, including both the substrate factors and pesticide sediment concentrations, are affecting the composition of the biological communities. At this time it is not clear to what extent each variable is driving changes within the biota. Further analysis and more data are necessary to elucidate the relationships.

Table 8. Spearman rank correlations between abiotic variables and biological metrics.

Metric	Abiotic Variable	R Value	P Value
Percent Chironomidae	Dissolved Copper	0.2969	0.0004
Percent Tolerant Taxa	TOC	0.28996	0.0001
Percent Tolerant Taxa	Depth	0.27634	0.0077
Diversity	Total Nitrogen in Sediment	-0.23314	0.0013
Diversity	Percent Fines	0.23884	0.0021
Species Richness	Depth	-0.32439	0.0016
Species Richness	TOC	-0.20278	0.008
Total Relative Abundance	Dissolved Fluridone	-0.38543	0.0117
Total Relative Abundance	Depth	-0.39292	0.0001

Results by Pesticide Type

Copper Sites

Bon Tempe and Lagunitas

Comparisons between Bon Tempe and Lagunitas Reservoirs showed significant differences in some metrics, over time. Pre-application metrics for total relative abundance, diversity, species richness, percent chironomidae, and percent oligochaeta were significantly different from both post application events ($p<0.001$) in both Bon Tempe and Lagunitas (**Figures 4-10**). This change is most likely a seasonal trend and not related to the copper applications, as it occurs in both the treated and reference sites. There were, however, significant differences in several of the metrics over the entire sampling season between the treated and reference site. Species richness, diversity, and percent oligochaeta were significantly higher in Lagunitas over all time periods than Bon Tempe, while percent dominant taxa was lower in Lagunitas than Bon Tempe. Although percent tolerant taxa do not differ significantly over the sampling period at any site, the metric is higher at Bon Tempe than Lagunitas. These results suggest that conditions in Bon Tempe favor a less diverse, more tolerant benthic community than in Lagunitas. It is unclear at this time whether these metric trends are due to the repeated copper applications in Bon Tempe or the natural conditions within the reservoir, or the combination of various abiotic factors. The high amounts of total organic carbon (TOC) in Lagunitas could account for the greater increase in oligochaeta populations, which are known to flourish in high organic conditions. This is also supported by the significant correlation found between TOC and percent tolerant taxa, of which all of the oligochaeta species in these reservoirs were found to be highly tolerant species.

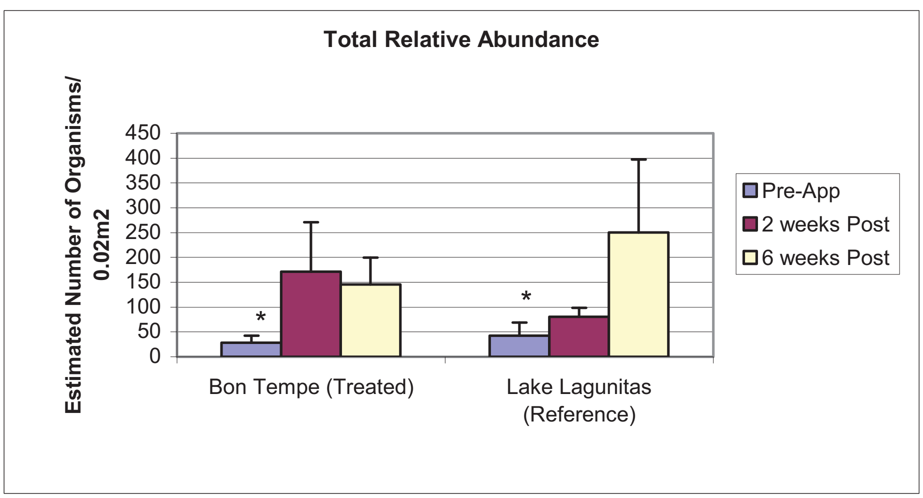


Figure 4. Mean values pooled by site for benthic macroinvertebrate total relative abundance in Marin Reservoirs. Stars denote statistical significance.

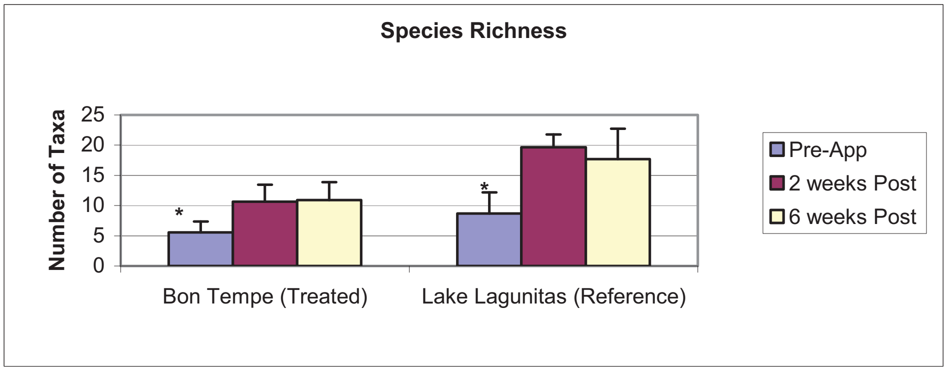


Figure 5. Mean values pooled by site for benthic macroinvertebrate species richness in Marin Reservoirs. Stars denote statistical significance.

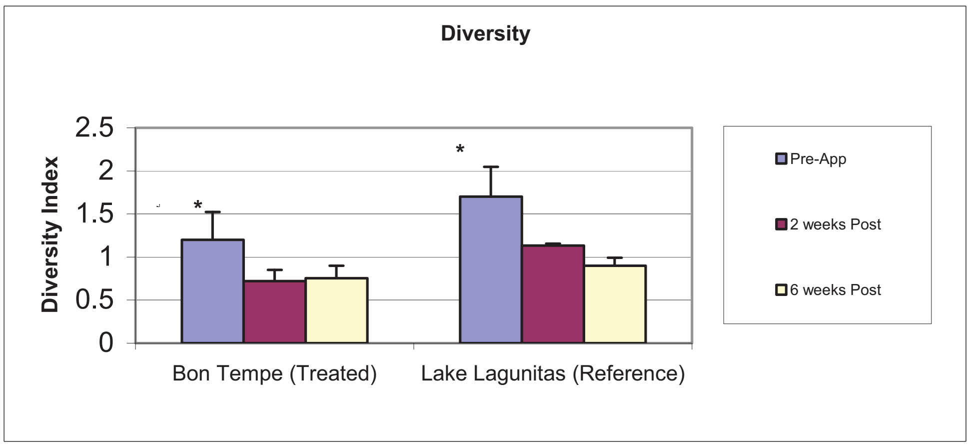


Figure 6. Mean values pooled by site for benthic macroinvertebrate diversity in Marin Reservoirs. Stars denote statistical significance.

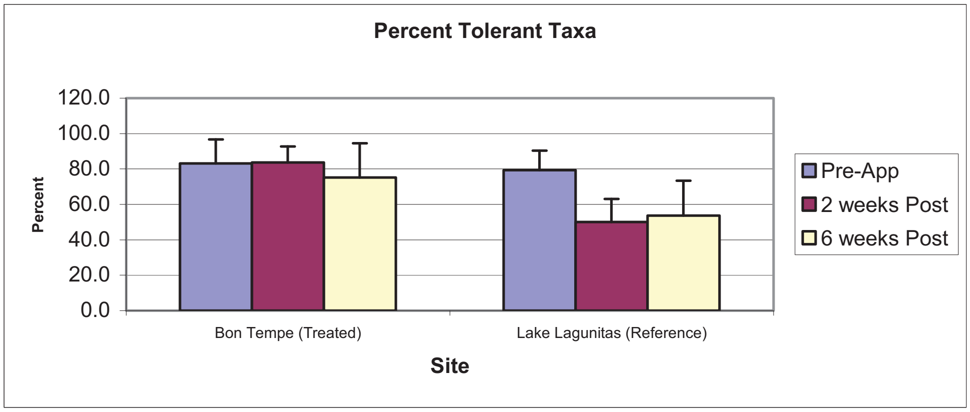


Figure 7. Mean values pooled by site for benthic macroinvertebrate percent tolerance in Marin Reservoirs. Stars denote statistical significance.

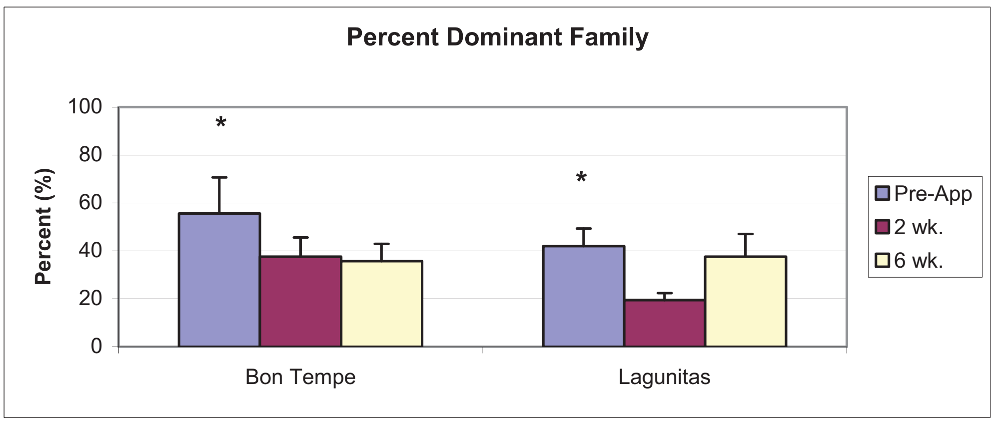


Figure 8. Mean values pooled by site for benthic macroinvertebrate percent dominance in Marin Reservoirs. Stars denote statistical significance.

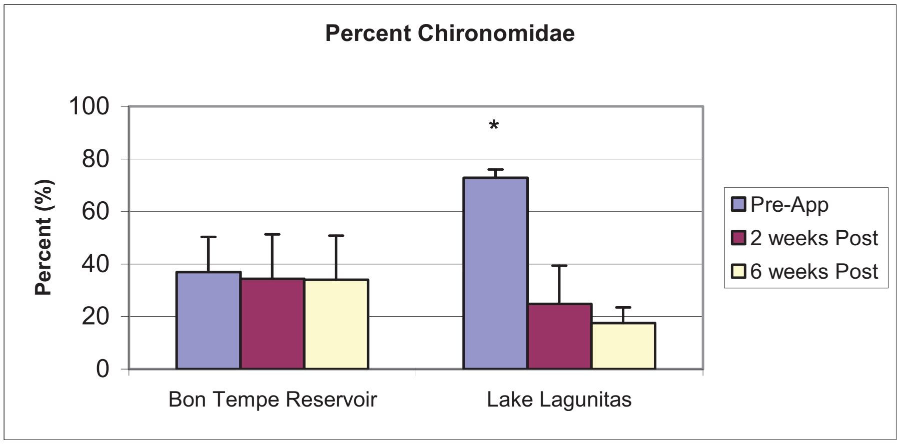


Figure 9. Mean values pooled by site for benthic macroinvertebrate percent chironomidae in Marin Reservoirs. Stars denote statistical significance.

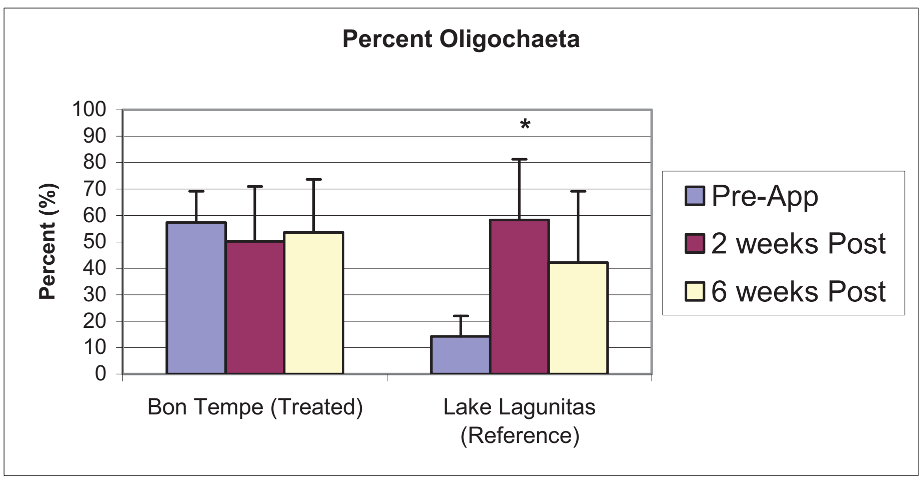
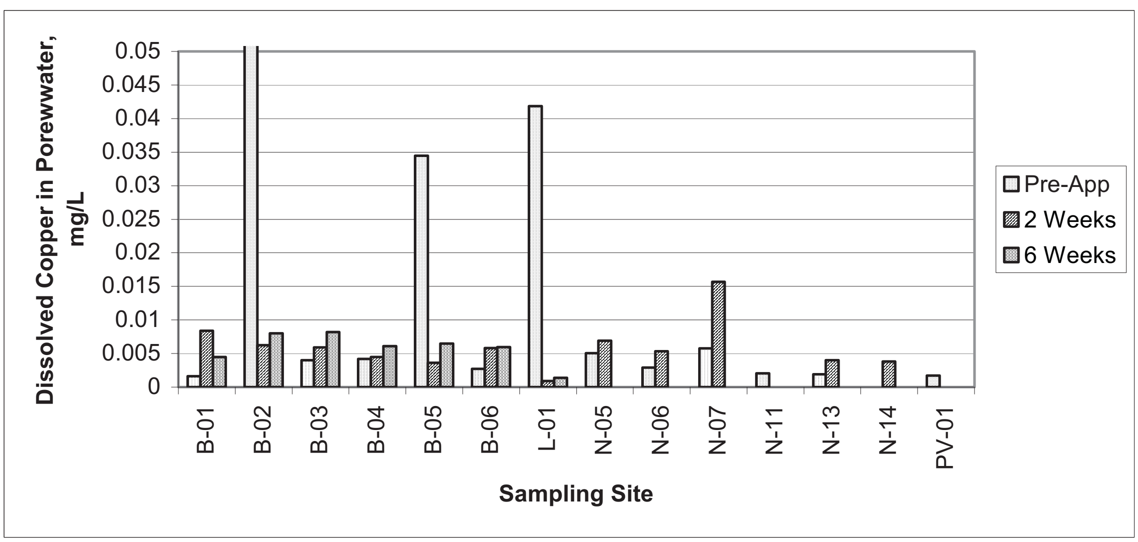


Figure 10. Mean values pooled by site for benthic macroinvertebrate percent oligochaeta in Marin Reservoirs. Stars denote statistical significance.

In general, pore water sediment copper concentrations were higher in Bon Tempe versus Lake Lagunitas (**Figure 11**). Some stations had high peaks in pre-treatment copper concentrations, and, surprisingly in the reference site, Lagunitas, as well. The Lagunitas concentrations may be attributed to naturally high bedrock copper concentrations within the Marin watershed, or from historic treatment of this system more than thirty years prior. Pre-treatment peaks were unexpected, but may be accounted for by the amount of organics in the substrate. Many factors affect the bioavailablity of copper, particularly TOC, and as TOC increases, copper availability is reduced. All the stations exhibited patchiness of TOC amounts, which may be due to natural patchiness or sampling variability, thus accounting for the variable peaks of copper concentrations seen in the sediment. High humus amounts in sediment have been shown to reduce the toxic effects of copper to macroinvertebrates (Besser et al. 2003). Perhaps biota toxicity to residual copper in Lagunitas is buffered somewhat by the higher TOC amounts, while lower humus in Bon Tempe, combined with the copper application load, increases the probability of applications impacting sensitive species. Therefore, TOC in sediment must always be evaluated alongside copper concentrations when assessing toxicity or biota response.



Note: Stations N-11 and N-13 are reference stations for Nicasio Reservoir. Pre-App application concentration for site N-14 was not available. Two-week application concentration for N-11 was not available and PV-01 was below the reporting limit of 0.003 ug/L.

Figure 11. Mean dissolved copper concentrations in porewater in mg/L for stations at Bon Tempe, Lagunitas, Nicasio, and Potter Valley.

Nicasio

Nicasio Reservoir exhibited similar benthic trends as in the Bon Tempe-Lagunitas case study. There was little difference in diversity and species richness over time between treated and reference sites (**Figures 12-14**). Relative abundance increased in both treated and reference sites, but more so in the reference sites. Reference stations in Nicasio consistently had the highest benthic abundance throughout sampling (Figure 12). There was no change in tolerance values or tolerant taxa over time. Intolerant taxa were present at pre-application in the treated site, and absent 2 weeks post, but this was most likely an outlier as no intolerant taxa were present in the reference stations. Percent oligochaeta were higher in the reference sites and percent chironomidae higher in the treated sites.

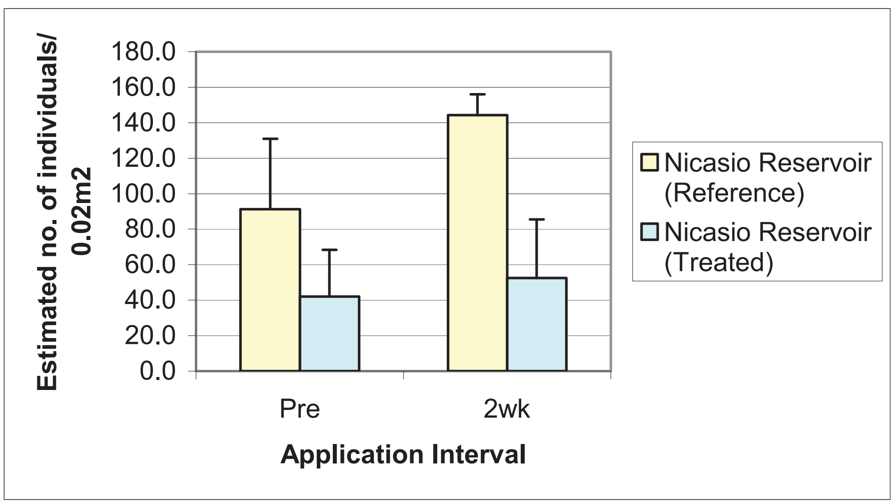


Figure 12. Mean values pooled by site for benthic macroinvertebrate total relative abundance in Nicasio.

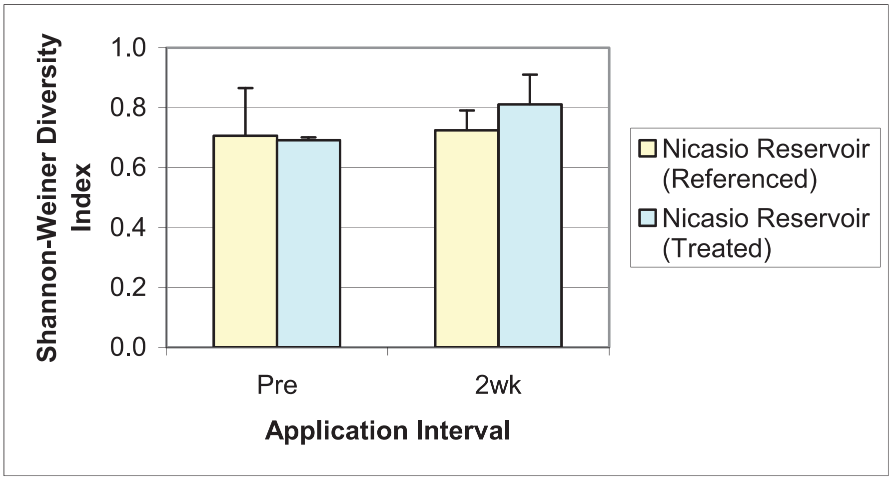


Figure 13. Mean values pooled by site for benthic macroinvertebrate diversity in Nicasio

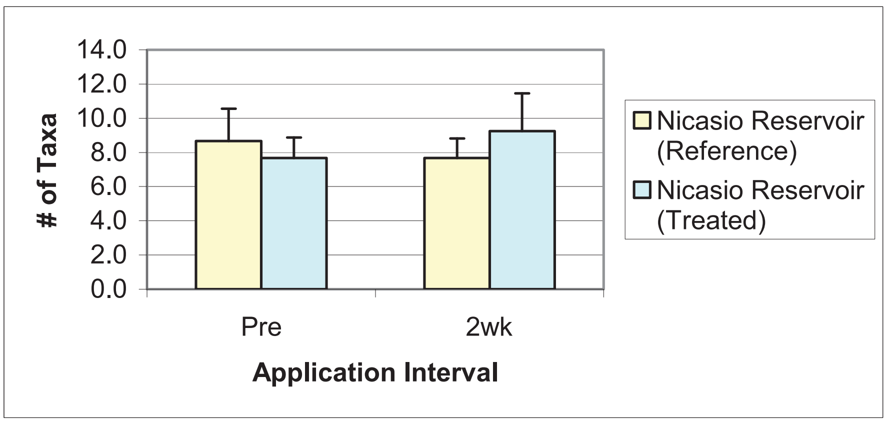


Figure 14. Mean values pooled by site for benthic macroinvertebrate species richness in Nicasio

Potter Valley

With the exception of diversity and abundance, metrics at the Potter Valley Irrigation Canal did not change over time with regard to chelated copper application. Diversity decreased at 2 weeks post application, while density increased post treatment. Oligochaeta and chironomidae dominated the assemblage, ranging 75 – 85% and 6.3 - 8.3%, respectively.

Phytoplankton

Phytoplankton metrics did not change significantly with copper sulphate applications, and varied highly between reservoir sites and sampling events (**Table 9**).

Diversity and species richness at Lake Lagunitas decreased (not significantly) from the pre-sampling event to the six weeks post-sampling event, with chlorophyta dominating in August. The treated sites (Nicassio 05 and Bon Tempe) showed an increase in diversity over the same period. Density decreased at post applications at the treated sites, while phytoplankton counts in Lagunitas are more than eight times higher at the end of the season (due to a population boom of chlorophyta (green algae)*)*. Increased summer temperatures from May to August may account for this eight-fold increase at Lagunitas, while the lack of dominance from chlorophyta in the treated sites may be due to copper applications. No noticeable nutrient pulses were observed in either Lagunitas or Bon Tempe over the sampling season that might trigger intense algal blooms.

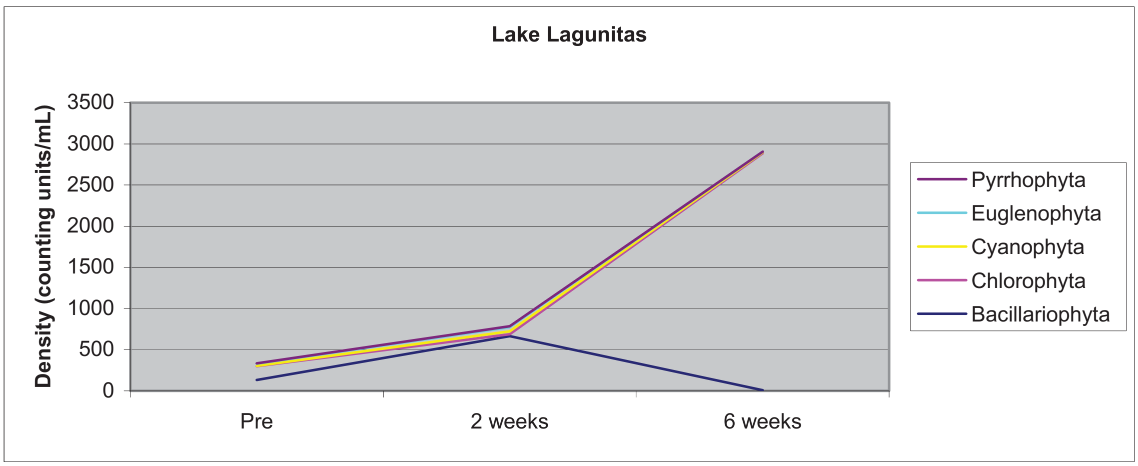
There were shifts in algal family composition with copper application, particularly for the blue-green (Cyanophyta) and green (Chlorophyta) algae (**Figure 15**). With the exception of the Bacillariophyta, the density of all algal families present in Lagunitas increased over time. In contrast, Cyanophyta and Chlorophyta densities were very low in Bon Tempe and only increased marginally two weeks after application as compared to the other algal family present (Euglenopyhta). This suggests a possible dampening effect on the green algae from the copper application. Only one algal species is present at all sites; the *Bacillariophyta fragalaria* population exhibited decreasing numbers at all treated sites and the Nicassio reference site for both post-sampling events. At Lagunitas, *B. fragalaria* density was highly variable, from 45 counting units/ml before application to 577 and 7 counting units/ml at two-weeks and six weeks post application, respectively.

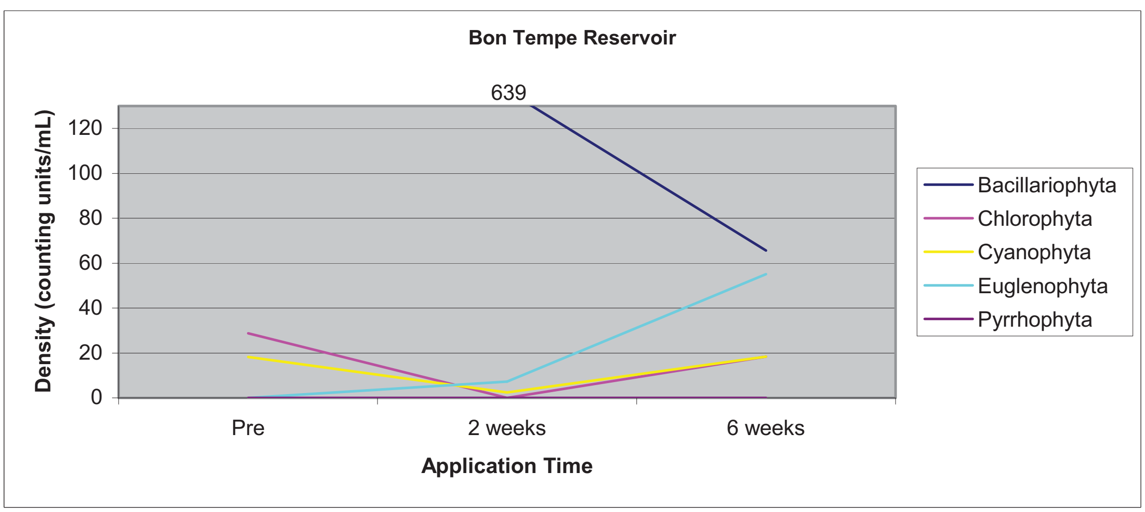
Phytoplankton assemblages have rapid turnover times and are known to be highly variable with seasonal changes in water quality (Hutchinson 1967). This makes one season of spatially and temporally limited data difficult to interpret. However, it does appear that copper applications were effective in reducing the abundance of certain algal families (Cyanophyta and Chlorophyta), while not affecting others (Bacillariophyta, Euglenophyta, and Pyrrhophyta).

Table 9. Mean phytoplankton metrics pooled by site: Shannon Diversity Index, Density and Richness for Treated (T) and Reference (R) Sites.

		Diversity Pre	**Diversity Post 2 weeks**	**Diversity Post 6 weeks**	**Density Pre**	**Density Post 2 weeks**	**Density Post 6 weeks**	**Richness Pre**	**Richness Post 2 weeks**	**Richness Post 6 weeks**
Site	Units	H' Index	H' Index	H' Index	unit counts /mL	unit counts /mL	unit counts /mL	# of taxa	# of taxa	# of taxa
Lagunitas	R	0.8799	0.4877	0.0488	335.6	785.3	2904.4	15	10	7
Nicassio 13	R	-	0.8569	-	-	3416.1	-	-	11	-
Nicassio 05	T	0.3883	0.4485	-	3060.8	1420.7	-	13	9	-
Bon Tempe	T	0.3177	0.6625	0.8354	806.2	146.4	157.5	8	7	9

Figure 15. Phytoplankton family density before and after copper sulphate application in Lagunitas and Bon Tempe.





Summary of Copper Sites

The benthic metrics indicated no acute changes in metrics with copper application. However, there may be a possible cumulative affect from repeated copper sulphate applications as seen from the reduced species richness and diversity, and increased percent dominant taxa and percent oligochaeta in Bon Tempe versus Lagunitas. Dominant and tolerant taxa were present at all sites prior to the 2003 copper treatment. Copper sulphate concentrations in the porewater showed a general increase after application at all sites and indicate long-term copper persistence in the reservoir systems at high levels, presumably from years of copper application. Some correlations between the biological metrics, pesticide concentrations, and other chemical parameters were significant. The trends in the biological metrics indicate stressed systems tolerant to high organic matter, low oxygen regimes, and metal concentrations (EPA 2003, Canfield et al. 2004). When the chemical and biological data are considered together, this lends weight to the argument that copper sulphate may be affecting in-situ biota at field concentration rates when applied repeatedly over a sustained time period in reservoir systems.

Fluridone

Profile plots for Costa Ponds indicate little change in benthic macroinvertebrate metrics (**Figures 16-20**). For the benthos, percent chironomidae, species richness, tolerance value, and diversity are all greater in the treated pond than the reference pond. With the exception of diversity, none of these metrics change over time with treatment. Diversity increased after 2 weeks in both the reference and treated sites, but the increase continued in the treated sites at the 6-week post interval. There was no change in relative abundance over time with treatment, and no difference between reference and treatment.

Epiphytic metrics varied slightly from the benthic trends (**Figures 21-23**). Percent diversity was again greater in the treated sites, but did not change over time. Percent dominant families were greater at the reference, due to high abundances of gastropods, bivalves, odonates, and oligochaeta. The treated sites had these groups as well, but higher abundances of worms. Densities were higher in the reference site than treatment, and all densities decrease over the sampling period in both ponds.

These results indicate that a contaminant tolerant assemblage was present before and after treatment (increased percent chironomidae and oligochaeta, high tolerance values, lower densities). Conditions in the reference pond are clearly different from the treated pond, with higher organic matter and lower dissolved oxygen present. This indicates the reference pond was not an appropriate baseline for the fluridone treated pond, and comparison of biological metrics between the ponds is not appropriate. Large small-scale variability was also evident in the data, making it difficult to attribute changes in metrics with the treatment.

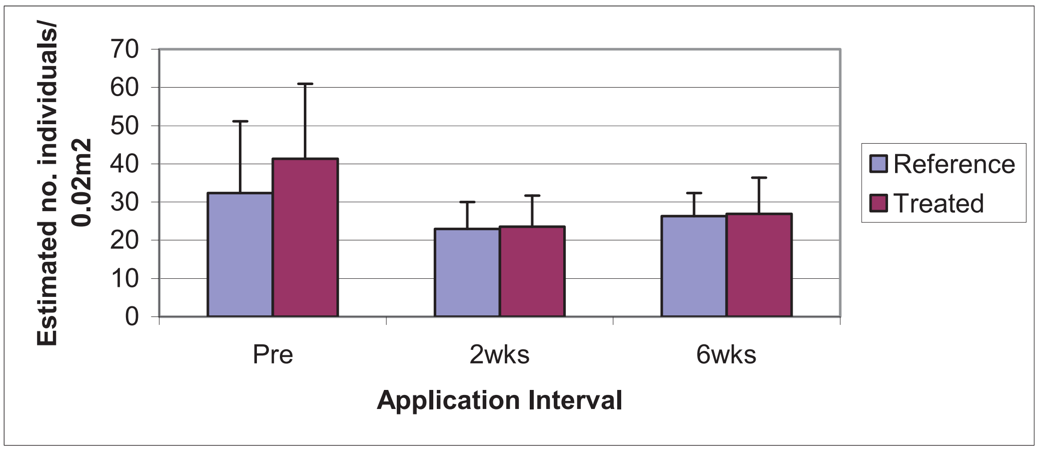


Figure 16. Mean values pooled by site for benthic macroinvertebrate total relative abundance in Costa Ponds

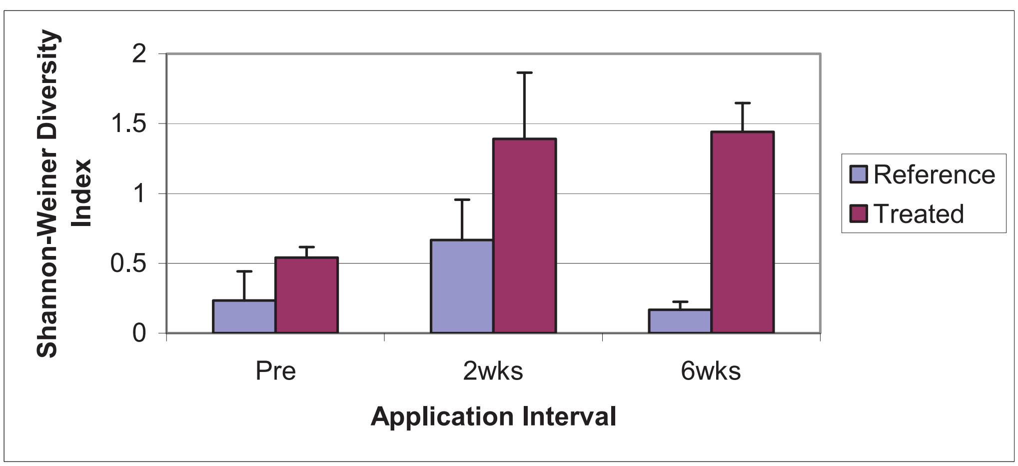


Figure 17. Mean values pooled by site for benthic macroinvertebrate diversity in Costa Ponds

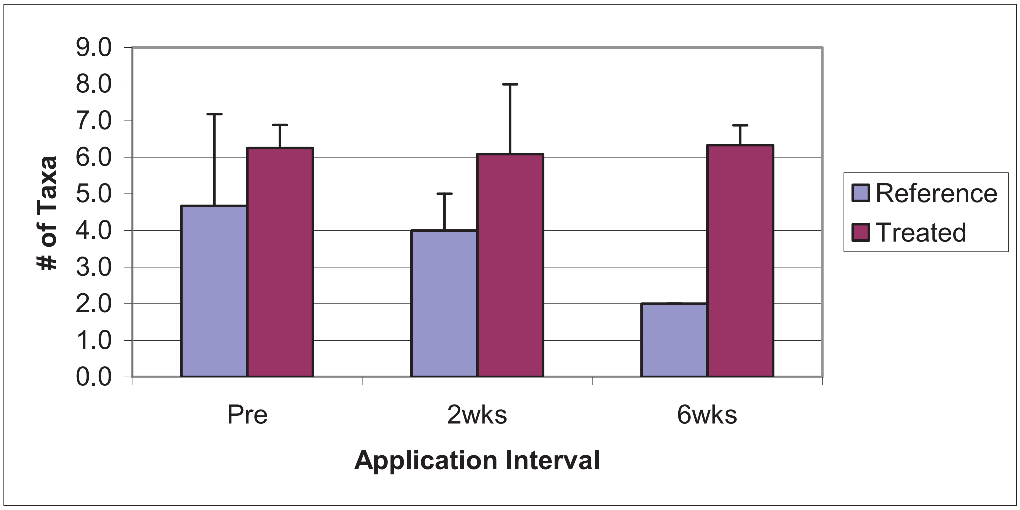


Figure 18. Mean values pooled by site for benthic macroinvertebrate species richness in Costa Ponds

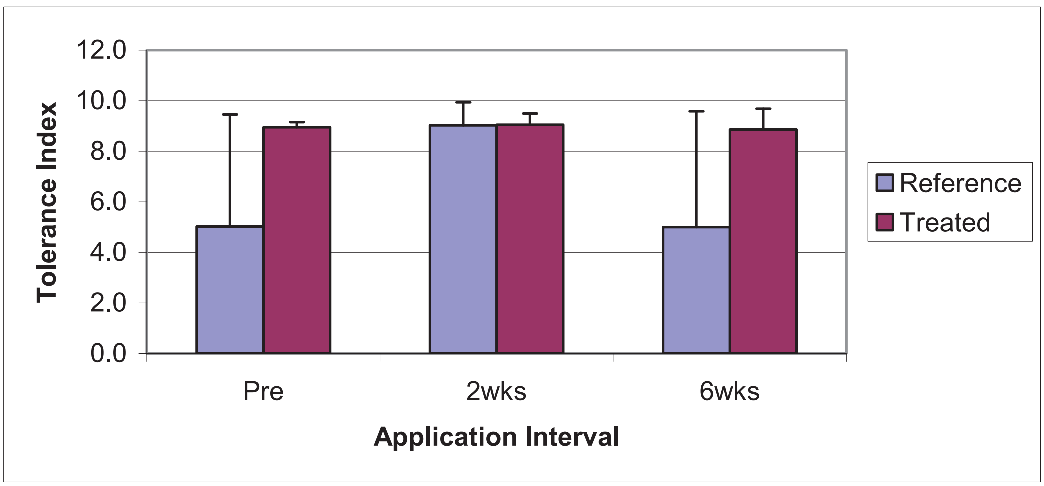


Figure 19. Mean values pooled by site for benthic macroinvertebrate tolerance values in Costa Ponds

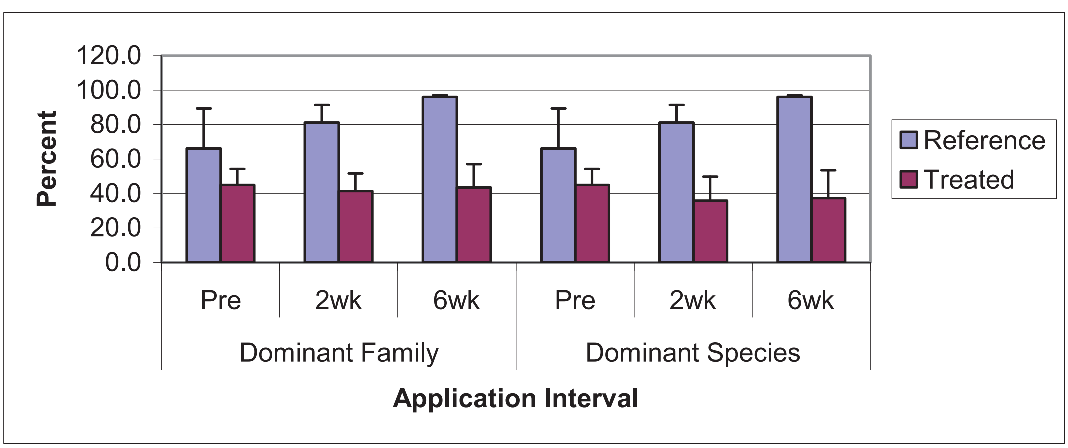


Figure 20. Mean values pooled by site for benthic macroinvertebrate percentage of dominant taxa in Costa Ponds

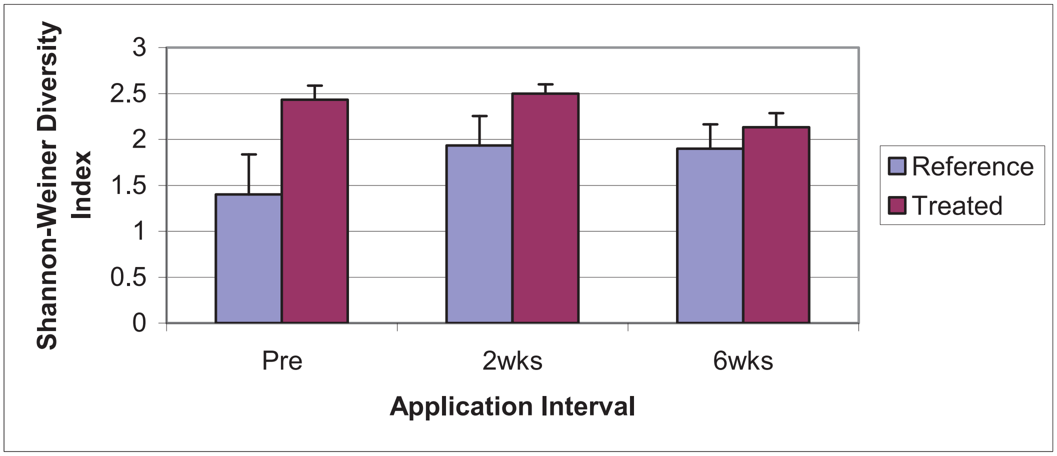


Figure 21. Mean values pooled by site for epiphytic macroinvertebrate diversity in Costa Ponds

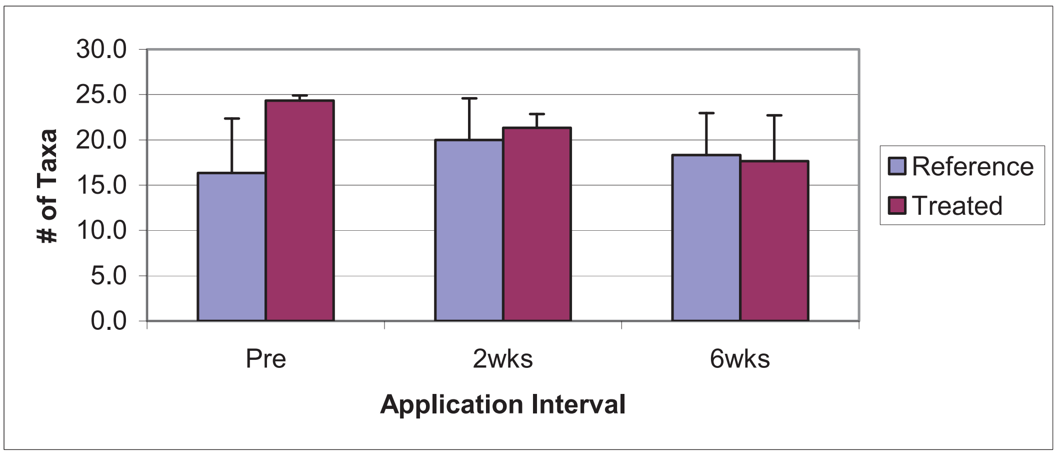


Figure 22. Mean values pooled by site for epiphytic macroinvertebrate species richness in Costa Ponds

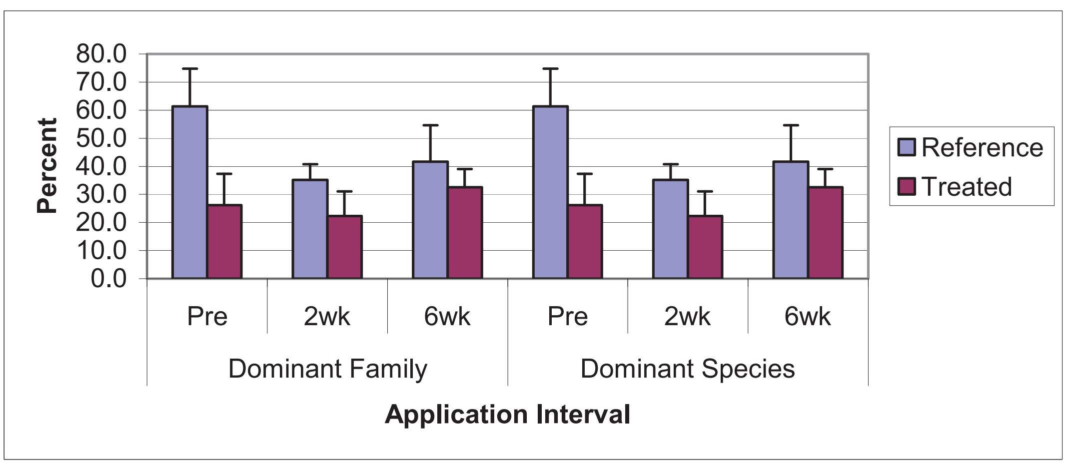


Figure 23. Mean values pooled by site for epiphytic macroinvertebrate percentage of dominant taxa in Costa Ponds

Glyphosate

Metrics for both benthic and epiphytic invertebrates were not different between reference and treated sites in Stone Lake in response to glyphosate treatment. Tolerance value, diversity, relative abundance, and percent oligochaeta did not change over time with treatment. Percent chironomidae and species richness were slightly higher in the treated sites than the reference, while dominant benthic families were slightly higher in the reference site. These results are not surprising as glyphosate is expected to quickly degrade in the water, with a half-life of 1.5 – 11.2 days in systems with high-suspended sediments. The results indicate no acute or chronic effects on in-situ biological communities from glyphosate in this study.

Triclopyr

There were no significant changes over time in epiphytic invertebrate metrics at Bear Creek following triclopyr application. Percent chironomidae, abundance, diversity, species richness, percent EPT taxa, and tolerance value showed a slight increasing trend 24 hours after triclopyr application than at pre-treatment and 2 wks post. It is surprising to see possible changes at 24 hours post, but no indications that communities remain affected 2 weeks after application. High variability in the data most likely accounts for these results and not the treatment application.

CONCLUSIONS

Benthic macroinvertebrates partition to lotic and lentic assemblages. The taxa present among assemblages at our sites are representative of organisms that thrive in habitats under environmental stress (i.e. oxygen shifts, high summer temperatures, high organic matter substrate, ephemeral water sources in canals and streams), as well as those potentially tolerating various system contaminants. Assemblages within all of the systems sampled had a dominance of highly tolerant organisms (particularly worms and midge larvae), and lower densities of organisms with moderate level tolerances (4-6). These systems are naturally stressed systems (low oxygen, high organic matter) and were already inhabited by tolerant organisms prior to pesticide application. Whether these assemblage compositions consistently reflect impacts from pesticide application or other natural conditions (or the combination of the two) is yet to be determined.

There were no acute changes in macroinvertebrate communities with any pesticide application. However, initial results indicate that in-situ biological communities (benthos and phytoplankton) may be responding to copper sulfate when applied repeatedly in a system, while there were no indications macroinvertebrate communities (both benthos and epiphytic invertebrates) were affected by fluridone, chelated copper, glyphosate, or triclopyr. There were weak but significant relationships between the biological metrics and several abiotic factors, including TOC, depth, and copper sulphate and fluridone porewater concentrations. We are unable to determine at this time which factors or combination of factors are driving the communities. Sediment and porewater concentrations of some pesticides (copper sulphate and fluridone) were not linear predictors of benthic toxicity or community effect at this time.

Out of the eighteen biological metrics generated to describe the macroinvertebrate communities and detect possible impacts from pesticides, seven were shown to be relevant, non-redundant metrics for lentic, soft-bottom systems. These are diversity, species richness, tolerance value, total relative abundance, percent chironomidae, percent oligochaeta, and dominant family. Of these, percent chironomidae, percent oligochaeta, species richness, and diversity were consistently effective at detecting changes in the biological communities in systems where aquatic pesticides were repeatedly applied. EPT taxa, percent dipteran, and percent odonata were either redundant metrics, or not relevant for lentic or soft-bottom, non-vegetated lotic systems.

The current limitations of the study include low sample size, limited study sites, a lack of least-impacted reference sites, and only one year of bioassessment data. Identifying appropriate reference conditions is necessary to differentiate impacts from abiotic factors or pesticides. As it is difficult to find non-impacted reference for California systems, particularly for those where pesticides are normally applied, determining a range of least-impacted conditions will be a challenging, but important focus in the next year. There was a high degree of sampling variability at many levels in the study, particularly within station replicates. More replicates within a station are therefore advocated. More in-depth analysis is also needed on the current dataset to determine the influence of physical and chemical parameters on the metrics. Future informational needs include determining the effects of seasonality, confirming identified assemblages, further investigating abiotic effects, and incorporating new levels of biotic indicators (i.e. looking at morphological deformities in macroinvertebrates for certain pesticides to assess sub-lethal in-situ affects).

In conclusion, macroinvertebrate assessments may be useful for assessing the acute and chronic effects of pesticides, but the current results are preliminary and require more data. Bioassessments did not detect short-term community effects from pesticide use, but revealed possible cumulative impacts from long-term repeated applications in some systems. The APMP recommends specific and focused macroinvertebrate bioassessments for pesticides with shown activity or possible effects (i.e. copper sulphate). Bioassessment sampling will be conducted again in 2004 during Phase 3 of the APMP, after which the two concurrent seasons of data will be re-evaluated. Bioassessment results should then be integrated with the chemical and toxicological data to establish credible indications of when water bodies and their biota are impaired from aquatic pesticide use.

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APPENDICES