ENVIRONMENTAL MONITORING OF THE CONSTRUCTED WATER QUALITY POND AT FOLSOM, CA

Final Report: CDPR Contract No. 13-C0029 -

M. Stillway, L.A. Deanovic, B.G. Hammock, S.P. Lawler and S.J. Teh

Aquatic Health Program Laboratory
School of Veterinary Medicine: Anatomy, Physiology and Cell Biology
1089 Veterinary Medicine Drive
University of California, Davis, Davis CA 95616

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Executive Summary

One of the goals of CDPR's Surface Water Program is to develop a long-term monitoring strategy that can be used to evaluate the efficacy of site-specific mitigation practices designed to reduce urban pesticide runoff. Livermore Community Park in Folsom, CA, contains a constructed water quality treatment pond (CWQTP) and is one of CDPR's local study areas with a strong chemical monitoring program. Previous studies have indicated that CWQTPs can partially mitigate urban runoff (Budd et al. 2013), but the efficacy of the CWQTP at Folsom with respect to the toxicity to aquatic organisms is unknown. This objective of this study was to determine the efficacy of the Folsom CWQTP through the use of three biological monitoring tools: 1) bioassessments, 2) laboratory toxicity testing, and 3) in-situ testing.

Data was collected between October 2013 and February 2016 for four sites: F2, F3, F5 and F100. Sites F2 and F3 are inputs to the CWQTP and drain 64 and 27 acres of residential areas, respectively. Site F5 is the output from the CWQTP and site F100 is downstream of the CWQTP in the receiving water, Alder Creek. Bioassessment samples were generally collected during the spring and fall for a total of five events; however, dewatering during the drought impacted our ability to collect data for all three biological monitoring tools of this project. The bioassessment results indicate that F2 and F3 had relatively low biological integrity before the drought compared to F5 and especially F100. F100 had the highest richness measures before the drought, while F2 had the lowest. Before the drought, F5 had consistently higher richness values than F2 and F3 suggesting that the CWQTP has a beneficial impact on the local aquatic communities. After the dewatering of F100 in summer 2014, the abundance of invertebrates at F5 and F100 dropped 10.1 and 7.3-fold respectively. Richness also declined, falling 2.6 and 3.3-fold for F5 and F100, respectively. In contrast, the drought did not appear to influence the invertebrates at F2 and F3 to the same degree. Invertebrate abundance, averaged between F2 and F3, declined by 1.5-fold over the same period, while invertebrate richness declined only 1.1-fold. The amphipods were particularly hard-hit by the drought.

The intended sampling schedule for toxicity tests and in-situ exposures was twice during each dry season and twice during the rainy season. Nine *Hyalella azteca* and *Selenastrum capricornutum* toxicity tests were performed and seven in-situ related exposures were conducted. Dilution of the samples were tested in the *H. azteca* toxicity tests to provide a comparison of the magnitude of toxicity (Toxic Units) between the inputs to and output from the CWQTP.

Site F3 generated the greatest magnitude of toxicity, with *H. azteca* exhibiting 100% mortality in every event, and with an average of 5.17 Toxic Units over the course of the project. For the remaining sites, 0.25 TUs were substituted in for non-toxic results and the average number of TUs was 3.81 at F2, 1.43 at F5 and 0.42 TUs at F100. With the exception of site F100 collected in February 2015, algae growth performance either matched or outperformed the control, and there was no other significant toxicity with this species.

The concurrent field component in this study, using habitat samplers comprised of resident invertebrates collected from nearby waterways, determined what impact storm water runoff had on the local macroinvertebrate community. In-situ exposures went through an extensive list of method

changes to address the multiple challenges experienced at the Folsom CWQTP, such as shallow water and low flow, predation of test organisms by Planaria, and dewatering at sites F5 and F100. Experiments were conducted to optimize the mesh size in the in-situ cages that would minimize Planaria interference, but still permit adequate water exchange through the cages to ensure contaminant exposure. One field study demonstrated that 160 micron mesh minimized Planaria intrusion and in a laboratory test with bifenthrin, this mesh size only decreased mortality by 22.4% averaged across all time points and concentrations for bifenthrin. Once we optimized the cage mesh size, site F100 became dry and the native amphipod populations did not recover enough to supply the 250 organisms needed to populate the in-situ cages.

CDPR shared their analytical chemistry data from the first eight events from the four sites. For many of the sampling dates in this project, grab or time-weighted composite samples were collected during runoff events. Neither of these methods readily allowed for an accurate assessment of the CWQTP efficacy for an entire storm event, only a single point in time. With these varied sample collection methods and timing approaches, it was difficult to compare toxicity test and analytical chemistry data among field events and between sites within the same event. More data is needed to thoroughly evaluate the CWQTP efficacy.

In future studies at this study area, we recommend the continuation of dilution series tests with *H. azteca*. This species was sensitive to current use pesticides and was the most reliable biological tool for evaluating whether the CWQTP was reducing the off-site movement of these contaminants. We also recommend focusing on precipitation-based events because the higher flow provides us a greater assurance that all of the samples needed to evaluate efficacy can be collected.

Background/Introduction

The California Department of Pesticide Regulation (CDPR) Surface Water Program has been monitoring urban pesticide runoff since 2008 in CA (He, 2008). Specifically in the Sacramento area of northern California, CDPR has detected 24 different pesticides (or pesticide degradates). Bifenthrin, 2,4-D, dicamba, fipronil, imidacloprid, and triclopyr are most frequently detected, and bifenthrin and fipronil are often detected at concentrations exceeding the US EPA aquatic benchmarks (Ensminger et al. 2013; Ensminger, 2014). One of the goals of CDPR's Surface Water Program is to develop a long-term monitoring strategy that can be used to evaluate the efficacy of site-specific mitigation practices designed to reduce urban pesticide use and runoff. Livermore Community Park in Folsom, CA, contains a constructed water quality treatment pond (CWQTP) and is one of CDPR's local study areas with a strong chemical monitoring program (Figure 1). Previous studies have indicated that CWQTPs can partially mitigate urban runoff (Budd et al. 2013), but the efficacy of the CWQTP at Folsom in terms of toxicity reductions is unknown.

Non-point source pollution through runoff, drainage, and spray drift accounts for a majority of all surface water pollution (Elsaesser et al., 2011; Zaring, 1996). Moreover, the application of fertilizers and pesticides to pervious areas, and the resultant overspray of these substances to adjacent impervious surfaces can further contribute to the pollutant load on the urban landscape. Much of this deposition is mobilized by surface runoff and is transported to receiving water bodies (Matamoros, 2012). Constructed wetlands are land-based water treatment systems consisting of shallow ponds or trenches

that contain floating or emergent, rooted wetland vegetation (Cole, 1998). The main advantages of constructed wetlands are their low operational costs, require minimal maintenance, and provide significant open spaces and landscape enhancement, which can also develop into a productive ecosystem (Matamoros, 2012; Lawrence et al., 2010). Constructed wetlands have the ability to mitigate pesticide pollution from various agricultural and urban non-point sources (Baker, 1992; Shultz and Liess, 2001; Shultz and Peall, 2001; Shultz et al., 2001a; in Elsaesser et al., 2011). Dense vegetation increases the effectiveness of remediating pesticide pollution (Moore et al., 2002, 2006, 2009). In addition to providing an emergent substrate for which contaminants may bind, the presence of vegetation has dramatic effects on the hydraulics of a system through increases in drag, thereby decreasing flow velocity, and resulting in increased retention times (Jadhav, 1995; in Budd, 2011). Residence time also directly influences sedimentation processes, which is a critical removal process for hydrophobic compounds (Budd et al., 2011).

This study consisted of three project tasks, used in combination to determine the efficacy of the Folsom CWQTP: 1) bioassessments, 2) laboratory toxicity testing, and 3) in-situ testing. Laboratory toxicity testing with *Hyalella azteca* and *Selenastrum capricornutum* were chosen due to *H. azteca*'s sensitivity to many current-use pesticides, and *S. capricornutum* sensitivity to some herbicides; two chemical classes frequently detected in northern California waterways. Comparing the toxicity of water collected from above and below the pond allows CDPR to determine whether the constructed water quality pond helps mitigate aquatic toxicity due to storm water runoff. Dilution tests applied in laboratory *H. azteca* toxicity tests provide comparisons of the magnitude of toxicity (Toxic Units) between upstream and downstream sites of the pond. A concurrent field component in this study, using habitat samplers comprised of resident invertebrates collected from nearby waterways, determined what impact storm water runoff had on the local macroinvertebrate community. This report summarizes the work completed by the UC Davis Aquatic Health Program Laboratory.

Figure 1. Map of CWQTP in Folsom, CA. Sites F2, F3, F5 and F100 are indicated by yellow arrows. Map was produced using Google Earth.

Materials and Methods

Task 1: Macroinvertebrate Community Survey

Five sets of benthic macroinvertebrate samples were collected and identified. Samples were collected September 3, 2013, May 6, 2014, December 5, 2014, May 18, 2015, and February 1, 2016, one sample at F2, F3, F5, and F100. The original intent was to sample in the fall and spring each year to make the samples comparable within season, but because Folsom sites were dry in fall 2014 and 2015, we delayed the sampling until there was water flowing (i.e., winter), and the animals had some time to recolonize the sites. Each sample consisted of a composite of 0.279 meter^2 benthic samples at each site collected using a 500 µm D-net. Samples were preserved in 70% ethanol, and transported to the Aquatic Health Program Laboratory (AHPL). All invertebrates were separated from the detritus, identified to the lowest feasible taxonomic resolution (usually genus), and counted.

Task 2: Laboratory Toxicity Tests

Hyalella azteca

Due to the historical toxicity to *H. azteca* observed in this area, sites were tested in dilution from the start of the project in order to quantify the number of Toxic Units (TUs) present at each site. *H. azteca* were obtained from Aquatic Research Organisms (Hampton, NH), and were acclimated to laboratory conditions 48 hours prior to test initiation. 96-hr acute water column toxicity tests consisted of five 250 mL replicate glass beakers with 100 mL of sample, 10 organisms and an one inch^2 piece of Nitex screen as artificial substrate. Reverse-osmosis water reconstituted to moderately hard standards using inorganic salts was used as the control (US EPA, 2000). Eighty percent of the test solution was renewed at the 48-hr time point, when debris and dead organisms were removed from the test chambers. *H. azteca* were fed 1 mL of YCT (yeast, organic alfalfa and trout chow) at test initiation and after water renewal at 48-hr. Mortality was scored daily. A low salinity control was included for dilutions to match the specific conductance measured in the field. Dilution series tests were evaluated using CETIS v. 1.1.2 (Tidepool Scientific Software, McKinleyville, CA, USA). LC50 and EC50s were calculated using linear interpolation methods. PMSD (percent minimum significant differences) of Dunnett's multiple comparison procedure was calculated for all tests.

Selenastrum capricornutum

The green alga was obtained from the University of Texas, Star Culturing Laboratory (Austin, TX), and cultured according to standard UCD AHP protocols. The *S. capricornutum* 96-hr chronic toxicity tests consisted of four 250 mL replicate flasks with 100 mL of sample and 1 mL of 1x10^6 cells/mL *S. capricornutum*. Distilled water was used as the control. A fifth replicate flask was included for daily temperature measurements. These tests were conducted without the optional addition of EDTA outlined in EPA's test method. EDTA is a chelating agent that may alter the toxicity of metals in ambient samples. Cell growth was measured at test termination. *S. capricornutum* tests were evaluated by SWAMP (Surface Water Ambient Monitoring Program) standard statistical protocols for single-concentration toxicity tests (SWAMP Data Management Plan, Toxicity Template, 2009). The SWAMP statistical protocol involves the examination of significant differences in test organism performance by one-tailed heteroschedastic t-tests ($p<0.05$) and a categorization of the performance of organisms exposed to the ambient sample as either greater to or less than the control performance. For the purposes of this report, samples are considered toxic only when both a significant t-test result and performance below 80% of the control is observed.

Task 3: Habitat Sampler Exposures

Field work for this project began in the summer of 2013 with the deployment of leaf-litter bags for colonization by aquatic macroinvertebrates at site F100 (downstream reference site). Based on initial assessments, it was determined that 14-28 days would be a good compromise between giving stream organisms enough time to colonize the bags while ensuring that the leaves did not become too decomposed for benthic macroinvertebrate (BMI) colonization. Approximately 80 leaf-litter bags were deployed for colonization on September 1, October 1, October 15, and October 29, 2013, at site F100. This schedule allowed for the distribution of leaf-litter bags among the study sites at Folsom that had been in the water for 14-28 days prior to a potential storm. Leaf-litter bags not applied during storm deployments (i.e., greater than 28 days) were used to determine 1) efficacy in terms of appropriate substrate used for colonization, 2) tolerance of colonized organisms being moved from site F100 to

upstream sites, 3) organism tolerance of movement into organza bags [to prevent emigration of invertebrates], and 4) invertebrate tolerance for transport to UCD from Folsom.

The leaf-litter bags worked well as substrate for BMI colonization. A total of 10 macroinvertebrate taxa, including four arthropods, were identified from three leaf-litter bags deployed for 28 days. Mean abundance was 24 invertebrates/leaf-litter bag (range: 14-39).

A mock storm deployment was initiated on October 29, 2013, using leaf-litter bags colonized by resident BMIs. Six leaf-litter bags from F100 were placed into organza bags and were moved to the upstream sites of F2, F3 and F5 (2 bags each site). 48 hours later the leaf-litter bags were collected, transported to UCD and invertebrates live-sorted. All 113 invertebrates identified in the leaf-litter bags were found alive, demonstrating that resident BMIs survived the move from F100 to upstream sites, the placement into organza bags, transportation to UCD, as well as the live sorting process. Organisms were preserved in 70% ethanol for later identification.

This method was used during the November storm event that occurred between November 18 and 21, 2013. However, the invertebrate community at site F3 during this event was dominated by Planaria (flatworms), and there were very few live arthropods from this site. Based on this observation, there were concerns that the flatworms were entering the organza bags and eating the arthropods (flatworms comprise roughly 1/3 of the invertebrate population at site F3). Thus, we had the potential to miss toxic events if the flatworms scavenged invertebrates that died in response to toxicity at F3. Therefore, we changed our in-situ methodology in subsequent field events which occurred during the 2014 project year. These in-situ test chamber investigations included the use of small (1x1") biobarrels wrapped in different size-meshes, use of larger (2x2") biobarrels wrapped in mesh, and use of plastic centrifuge tubes with holes drilled through them and covered with different mesh sizes. Evaluations included the number of flatworms present in replicate test chambers upon return to UCD, the number of amphipods present in test chambers upon return to UCD (i.e., did animals escape test chambers?), as well as the appropriate number of organisms included in replicate test chambers in order to provide adequate statistical power. Based on these investigations it was determined that 50 mL centrifuge tubes with 'windows' wrapped in 160 µm mesh, with 10 amphipods per chamber, and a 96-hour exposure (to match comparability with concurrent 96-hr laboratory tests) was optimum for this portion of the project.

While ideal at keeping out predatory flatworms, the use of a reduced mesh size in in-situ replicate chambers raised concerns about the potential for the clogging of the mesh and encapsulating pre-storm F100 water, thus limiting the flow of site water and contaminants into the cages during a storm event. Early in the 2015 project year, we conducted a method test using bifenthrin and the time-to-death of amphipods to determine what effect, if any, this reduced mesh size had on the retention time of water within the replicate cages. Seventy-five cages were deployed at site F100 to allow for natural sedimentation to build up on the outside of cages, simulating a pre-storm deployment. Cages were collected and returned to UCD after 48 hr., at which time they were placed into individual replicate beakers containing synthetic control water. Ten *H. azteca* were loaded into each of the 48 beakers: either inside or outside of the cage, in order to determine differences in mortality rates between organisms inside (potentially encapsulated pre-toxicant water) and outside (theoretically no delay in exposure to toxicant). Forty-eight beakers were used in the experiment total, 24 with amphipods inside the cage and 24 with amphipods outside the cage. Sixteen beakers were used for each of the three time

points, eight of which had amphipods inside the cage, and eight with amphipods outside the cage, across the range of the aforementioned concentrations. All replicates were aerated to generate flow within the beaker to mimic stream current. Replicate beakers with amphipods were aerated for one hour prior to the addition of bifenthrin, which was spiked into each replicate beaker at nominal concentrations of 10, 20, and 40 ng/L. Actual concentrations were determined by Simone Hasenbein (see results section). Mortality was quantified for eight beakers at three time points (24, 48, and 96-hr) to determine whether the rate of mortality of organisms inside the cages was significantly reduced compared to organisms outside of the cages. Because counting mortalities inside the cages would disturb any encapsulation of the water, those beakers were terminated after mortality was quantified.

The results of this experiment were analyzed with model comparison. Proportional data (e.g. proportional survival data) have a binomial error distribution, and most biological data are over-dispersed (i.e., they exhibit more error than coin-flip data or dice rolls, which also exhibit binomial error distributions). Therefore, we fit a set of beta-binomial models to the cage-test data (binomial because the data are proportional, 'beta' refers to a parameter to account for the over dispersion). With the first set of models we asked whether there was an influence of cage in the absence of bifenthrin. The models were P~ and P~cage, where *P* is proportion mortality and *cage* is a dummy variable for cage. To assess statistical significance we first ranked the models in terms of Akaike Information Criterion corrected for small sample size (AIC_C). Generally a difference in AIC_C of ~2 is considered significant (Bolker, 2008), and models with a lower AIC_C value are better (i.e., will make better predictions). Then we asked whether the parameter estimates were reliably above or below zero for the model of interest (i.e., does the 95% confidence interval overlap zero?). In the second part of the analyses we compared the following models fit to the cage experiment data with bifenthrin (concentrations of 10, 20, 40 ng/L). These models also had beta-binomial distributions of error and included: P~, P~days, P~days+conc, P~days+conc+cage, where *P* is proportion mortality, *days* is the number of days of exposure (1,2 or 4 days), *conc* is the concentration of bifenthrin, and *cage* is a dummy variable for the presence/absence of the cage. In this case, we were most interested in whether the presence of the cage improved survival as hypothesized.

Analytical Chemistry

Water samples were collected from the bifenthrin Cage Test using amber pre-labeled and kilned glass bottles (950 mL). All samples were transported on wet ice, stored in the dark at 4°C and extracted within 24 hours of collection. Extraction was conducted using conditioned 6 mL solid phase extraction C18 cartridges (Supelclean™ 500 mg, Sigma-Aldrich) at a slow drip under vacuum. To elute pesticides, columns were rinsed twice with a 5 mL volume of a solution of hexane: ethyl acetate (1:1, v/v). Solvent elution (10 mL) from each column was collected and concentrated to 0.4 mL under a gentle stream of nitrogen. All final extracts were analyzed using gas chromatography negative chemical ionization mass spectrometry (GC-NCI-MS) an Agilent 5973 series gas chromatograph (Agilent Technologies, Palo Alto, CA), equipped with a split-splitless injector (280°C, splitless, 1.5-minute purge time). The column was a Supelco DB-5MS column (30 m x 0.25 mm with a 0.3 µm film thickness). Instrumental calibration was performed using nine sets of calibration standard solutions with each pesticide (Chem Service, West Chester, PA), the surrogate trans-permethrin D6 (EQ Laboratories, Atlanta, GA), and the internal standard dibromooctafluorobiphenyl in hexane. Quantification was based on peak area using standards of known concentration.

Quality Assurance

Quality assurance measures were included in this project to ascertain the reliability of data gathered, including whether UCD AHP toxicity testing can be duplicated, and to assess whether test species were responding typically, relative to historical test results at UCD AHP. To determine whether test species were responding typically during this study, reference toxicant tests were conducted with each event.

Reference Toxicant Tests

A Reference Toxicant (RT) test using zinc chloride as the toxicant was performed monthly for *S. capricornutum*. *H. azteca* RT tests were performed concurrently with each sampling event and used sodium chloride as the reference toxicant. Routine reference toxicant tests determine test species sensitivity to a toxicant, and whether the test species is reacting typically (within a specified range) to that toxicant. These tests generally include a laboratory control and a toxicant dilution series in laboratory control water. The LC50 or EC25 for each reference toxicant test is compared to the UCD AHP running mean to ascertain whether it falls within the acceptable range. The USEPA acceptable range is within the 95% confidence interval of the running mean. If the LC50 and/or EC25 fall out of the 95% confidence interval, test organism sensitivity is considered atypical and results of toxicity tests conducted within those months may be considered suspect. For the duration of the project period, most RT endpoints fell within the 95% confidence interval for both species. One exception is the *S. capricornutum* IC50 in April of 2015, where the RT IC50 exceeded the upper control limit based on the running mean, with a value of 55.8 mg/L. This would indicate the possibility that *S. capricornutum* cultured during that month may be less sensitive than normal. However, in the corresponding ambient test, all test acceptability criteria were met, and the Folsom sites outperformed the control in the *S. capricornutum* growth endpoint. We therefore consider these data reliable.

There were two other outliers in the RT endpoints during the project period: Algal control growth in the May, 2015 RT test, and *H. azteca* LC50, also in May, 2015. However, there were no events conducted during this month, therefore these outliers do not impact organism sensitivity for this project. RT control charts are outlined below in Figures 2-5.

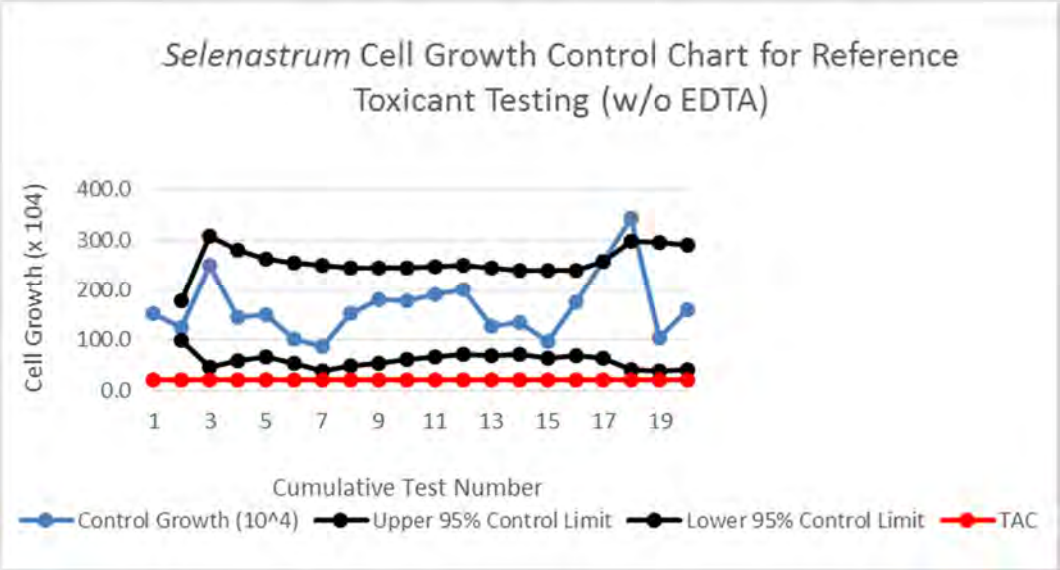

Figure 2. RT control chart for *S. capricornutum* growth. -

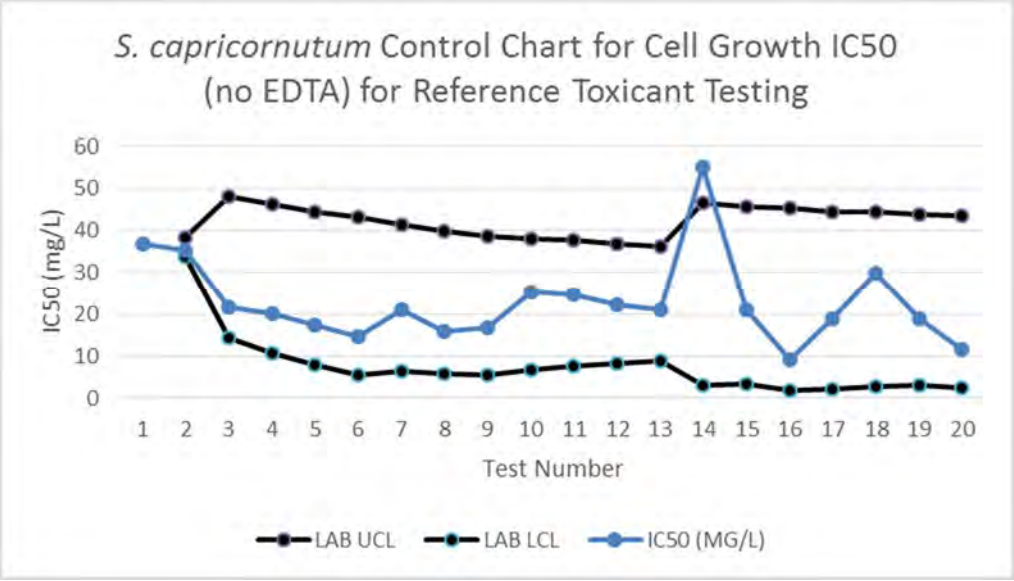

Figure 3. RT control chart for *S. capricornutum* IC50. -

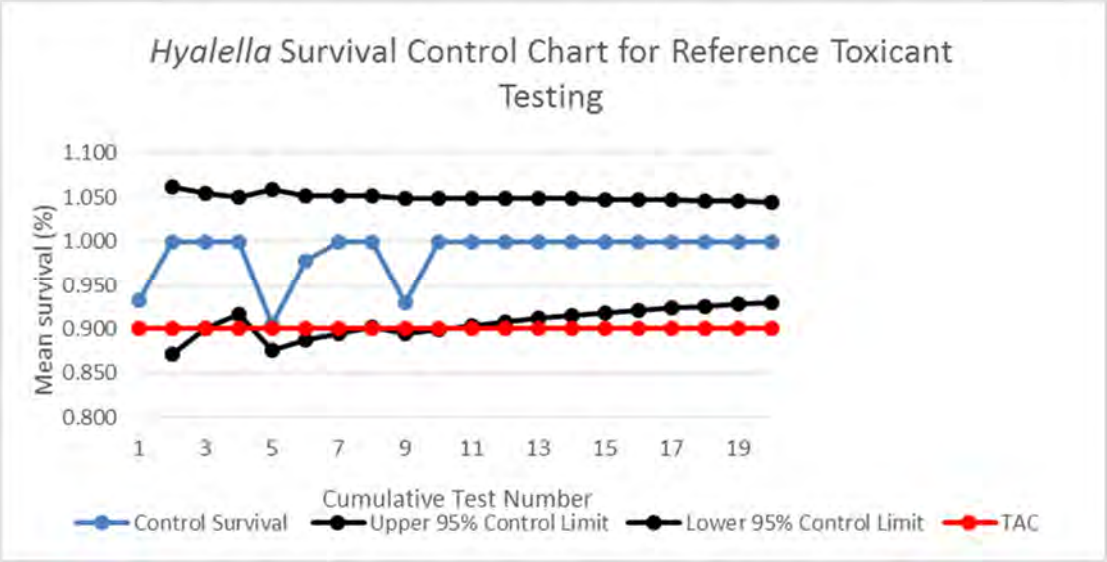

Figure 4. RT control chart for *H. azteca* survival. -

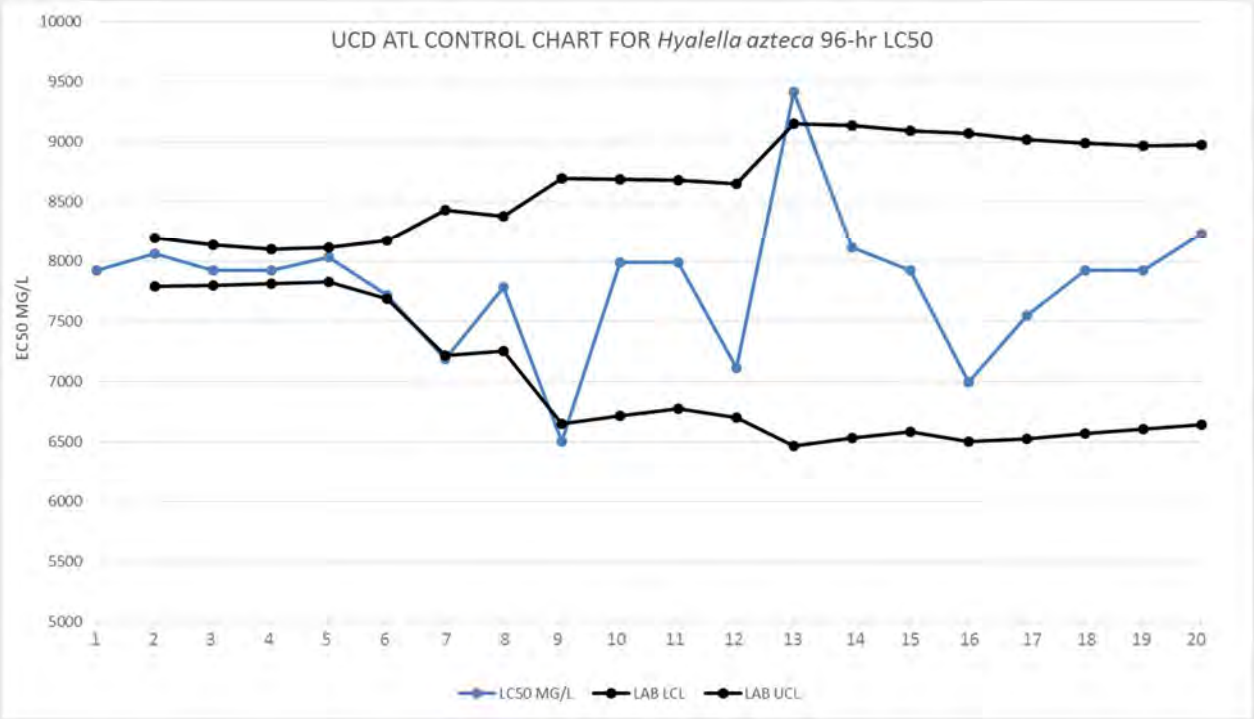

Figure 5. RT control chart for *H. azteca* LC50.

Analytical Chemistry

Quality Assurance/Quality Control for the bifenthrin cage test analysis was conducted by analyzing a method blank of deionized water (Milli-Q) to ensure that no contamination occurred during sample extraction and analysis. The surrogate trans-permethrin D6 was added to each sample, including the

blank, before extraction to monitor matrix effects and overall method performance. The instrumental limit of detection (whole water) was 0.6 ng/L bifenthrin.

Completeness

Completeness is a measure of the data obtained compared to the amount of data expected in a project. The toxicity data acquisition phase of a project is considered complete when all sites specified in a contract have been visited the number of times designated in a contract, the number of samples designated in a contract has been collected, and the number of toxicity tests designated in the contract has been successfully completed. Table A1 in Appendix A provides an outline of the following tasks.

Task 1: Macroinvertebrate Community Survey

BMI community surveys were conducted five times over the duration of the project:

1. September 3, 2013
2. May 6, 2014
3. December 5, 2014
4. May 18, 2015
5. February 1, 2016

Task 2: Laboratory Toxicity Tests

A maximum of eleven events were possible during the project period. We successfully completed nine laboratory toxicity tests with both *H. azteca* and *S. capricornutum* on the following dates:

1. November 21, 2013
2. March 2, 2014
3. May 6, 2014
4. July 9, 2014
5. February 9, 2015
6. April 9, 2015
7. June 10, 2015
8. November 3, 2015
9. February 19, 2016

In some cases, not all sites had water present, thus there were instances where water samples were not collected at all sites during an event. In these instances, laboratory toxicity tests were initiated with a reduced number of treatments. These events are outlined in more detail in the results section. In the case of the December, 2015 event, sampling equipment failure precluded a complete event.

Task 3: Habitat Sampler Exposures

California's extreme drought had significant impacts on this project, most notably in terms of the field components. The lack of consistent water at the study site, especially at site F100, had a negative impact on the resident organisms, and without a BMI community present in the study area, we were unable to meet the anticipated number of field events. Out of the maximum number of possible field events (11), three field events with which parallel laboratory toxicity tests were successfully completed, on the following dates:

1. November 21, 2013

2. March 2, 2014
3. May 6, 2014

Outside of those three paralleled events, we did conduct several other field events with available resident organisms, which occurred on the following dates:

1. January 30, 2014
2. February 11, 2014
3. June 6, 2014
4. February 5, 2015 (bifenthrin cage test; counted towards field event)

Combining both paralleled and non-paralleled field events, there are seven successfully completed field tests under Task 3 for the duration of the project.

SWAMP recommends 90% completion of data. Based on the numbers of successfully completed events, divided by the number of anticipated events, we have the following percent completeness for each task:

Task 1: Five completed events / five anticipated events = 100%

Task 2: Nine completed events / maximum of eleven events = 82%

Task 3: Seven completed events / maximum of eleven events = 64%

Average completeness for the entire project combined: 82%

SWAMP-comparable Protocols

Under the protocols of the SWAMP QAPrP, QA/QC requirements include guidance regarding the maintenance of sample integrity, such as target temperature ranges for sample collection, transport, and storage, as well as holding times under which these samples should be initiated in toxicity tests. These limits are to ensure that samples collected from the field maintain their integrity, in terms of the reduction of toxicant degradation.

SWAMP protocols require samples be maintained between 0-6°C during transport and storage. For the duration of this project, sample temperatures often exceeded this limit. Temperature at sample receipt ranged from 5.6-12.5°C, and in one instance samples were received at UCD AHPL at 22°C. These sample temperature exceedances were often due to the nature of collection method, such as the collection of waters from auto-samplers from the field. These samples were then composited prior to delivery at UCD AHPL, and because of this additional processing step, samples were often unable to be chilled adequately to maintain the required temperature range. In the instance of the receiving sample temperatures of 22°C (collected June 9, 2015), these samples were composited at UCD AHPL directly after being collected from the auto-samplers at Folsom, and therefore had minimal time to chill to the proper temperature. Warmer temperatures typically tend to accelerate toxicant degradation in water samples; however since the toxicity of these samples was consistently demonstrated by high mortality in *H. azteca* toxicity tests, we consider sample integrity to have been adequately maintained in spite of the warm sample temperatures. Adding additional ice to samples in coolers directly after collection would increase the likelihood that samples will chill to proper temperatures during transport, especially during summer months.

SWAMP protocols require a 48-hour holding time from which samples are collected until they are initiated in toxicity tests. For the duration of the project, all samples collected met the applicable holding time, with the exception of samples collected on February 19, 2016. *H. azteca* are ordered from an outside vendor, and testing organisms for this sampling date exhibited high mortality during transport, and were dead upon arrival at UCD AHPL. An additional batch of organisms had to be ordered, and thus the *H. azteca* test was initiated on February 25, 2016, 96 hours past the 48-hr holding time. The *S. capricornutum* test was initiated within the 48-hr holding time, on February 19, 2016. *H. azteca* exhibited approximately 30% mortality in these treatments. It is possible that this extended holding time may have compromised the integrity of the samples in this case, and *H. azteca* toxicity may be underestimated for this event.

Results

Task 1: Macroinvertebrate Community Survey

The bioassessment results indicate that F2 and F3 had relatively low biological integrity before the drought compared to F5 and especially F100 (Figure 7). F100 had the highest richness measures before the drought, while F2 had the lowest. Before the drought, F5 had consistently higher richness values than F2 and F3 (Figure 7). After the dewatering of F100 in summer 2014, the abundance of invertebrates at F5 and F100 dropped 10.1 and 7.3-fold respectively (Figure 6). Richness also declined, falling 2.6 and 3.3-fold for F5 and F100, respectively (Figure 7). In contrast, the drought did not appear to influence the invertebrates at F2 and F3 to the same degree. Invertebrate abundance, averaged between F2 and F3, declined by 1.5-fold over the same period (Figure 6), while invertebrate richness declined only 1.1-fold (Figure 7). The amphipods were particularly hard-hit by the drought. During the first sampling event we collected 47 *Hyalella* and 299 *Crangonyx* (all sites combined), during the second we collected 20 and 101, during the third we collected 0 and 18, during the fourth we collected 0 and 22, and during the final event we collected 0 and 7. This pattern presented a problem for the planned cage tests, because the study organisms had previously been collected downstream of the pond (at F5 and F100). The taxonomic data show that *Crangonyx* declined sharply at Folsom, and *Hyalella* was extirpated from Folsom following summer 2014 (Tables 1-5).

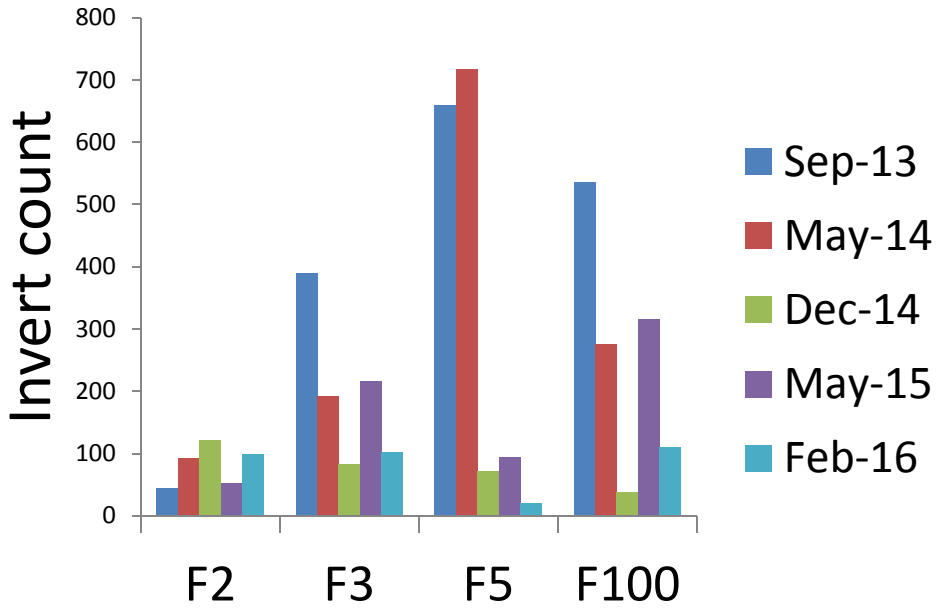

Figure 6. Total number of invertebrates by site for the five sampling events. F100 and F5 were completely dry in summer/fall 2014 and 2015.

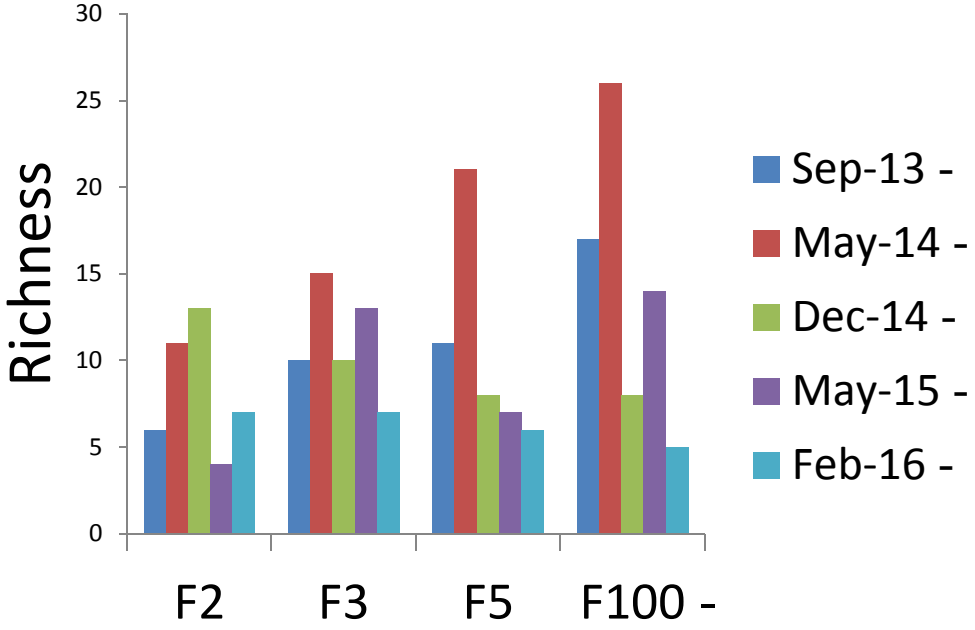

Figure 7. Taxonomic richness of invertebrates by site for the five sampling events. F100 and F5 were completely dry in summer/fall 2014 and 2015.

Table 1. Abundances of invertebrates by taxon for September 3, 2013. -

Taxon	F2	F3	F5	F100	Total count
Coleoptera	0	0	1	0	1
Sphaeridae	0	181	2	2	185
Caridea	0	0	1	2	3
Argia	0	1	0	103	104
Platyhelminthes	3	132	237	32	404
Dasyhelea	0	2	1	0	3
Chaoboridae/ Culicidae	0	0	1	0	1
Paratendipes	0	0	0	144	144
Rheotanytarsus	0	0	0	2	2
Allotanypus	0	0	0	1	1
Apedilum	0	13	0	0	13
Chironomus	0	3	0	0	3
Nematoda	6	2	17	123	148
Nemertea	0	0	2	2	4
Hyallela	0	0	0	47	47
Crangonyx	1	0	294	4	299
Helisoma	3	21	1	16	41
Physa	3	16	0	1	20
Ferressia	0	0	0	1	1
Fossaria	0	0	0	1	1
Collembola	0	0	0	3	3
Oligochaeta	29	19	102	51	201

Table 2. Abundances of invertebrates by taxon for May 6, 2014. -

Taxon	F2	F3	F5	F100	Total count
Crangonyx	0	1	65	35	101
Hyalella	0	0	0	20	20
Oligochaeta	73	53	59	70	255
Nematoda	0	1	1	5	7
Copepoda	0	10	1	3	14
Nemertia	1	1	3	6	11
Argia	0	0	0	21	21
Platyhelminthes	3	19	155	1	178
Physa	1	26	1	8	36
Oxyethira	0	0	0	2	2
Simuliidae	0	0	8	5	13
Ferrissia	0	0	0	9	9
Sphaeridae	0	23	1	1	25

Taxon	F2	F3	F5	F100	Total count
Baetis	0	0	0	1	1
Helisoma	4	1	4	3	12
Thienemanniella	0	0	0	22	22
Rheocricotopus	0	0	9	10	19
C/O	0	0	1	10	11
Micropsectra	4	1	125	31	161
Rheotanytarsus	0	0	0	2	2
Corynoneura	0	0	0	1	1
Parametriocnemus	0	0	11	3	14
Apedillum	0	0	0	2	2
Eukiefferiella	1	0	0	3	4
Phaenopsectra	0	0	0	1	1
Hirudina	1	0	1	1	3
Ostracoda	0	12	252	0	264
Collembola	1	34	3	0	38
Chironomous	0	0	10	0	10
Tvetenia	1	2	1	0	4
Limoniinae	0	0	2	0	2
Hydrobaenus	0	0	4	0	4
Pericoma	2	0	0	0	2
water mite	0	1	0	0	1
Fossaria	0	7	0	0	7

Table 3. Abundances of invertebrates by taxon for December 5, 2014. -

Taxon	F2	F3	F5	F100	Total count
Oligochaeta	77	34	31	15	157
Platyhelminthes	8	19	1	1	29
Physa	5	5	0	0	10
Helisoma	2	5	0	0	7
Copepoda	17	0	1	3	21
Pericoma	1	0	0	1	2
Hirudinea	1	0	0	0	1
Ostracoda	1	0	0	1	2
Collembola	3	9	26	5	43
Tipula	1	0	1	0	2
water mite	3	1	0	0	4
C/O	2	0	0	1	3
Limonia	1	0	0	0	1
Crangonyx	0	0	7	11	18

Taxon	F2	F3	F5	F100	Total count
Elmidae	0	0	3	0	3
Nematoda	0	0	1	0	1
Sphaeridae	0	4	0	0	4
Coleoptera	0	1	0	0	1
Fossaria	0	3	0	0	3
Dasyhelea	0	2	0	0	2

Table 4. Abundances of invertebrates by taxon for May 18, 2015. -

Taxon	F2	F3	F5	F100	Total count
Oligochaeta	24	6	67	167	264
Platyhelminthes	27	71	21	7	126
Physa		65		46	111
Culicidae	1	1			2
Copepoda		1			1
Ostracoda				39	39
Collembola					0
Tipula		2		2	4
water mite			1	3	4
C/O				1	1
Psychoda			1		1
Crangonyx	1	1	1	19	22
Sphaeridae		28			28
Coleoptera			2		2
Fossaria		26			26
Parametriocnemus		4	1	2	7
Helisoma		5			5
Ferrissia		5			5
Rheotanytarsus		1			1
Hydropsychidae				2	2
Micropsectra				25	25
Microtendipes				1	1
Apedillum				1	1
Corixidae				1	1

Table 5. Abundances of invertebrates by taxon for February 1, 2016. -

Taxon	F2	F3	F5	F100	Total count
Oligochaeta	29	26	10	94	159
Platyhelminthes	47	5	4		56
Physa	11	58		5	74
Helisoma	4				4
Copepoda	1		1	5	7
Ostracoda			1	4	5
Collembola		10	1		11
Crangonyx	2		3	2	7
Coleoptera		1			1
Fossaria	4	1			5
Corixidae		1			1

Task 2: Laboratory Toxicity Tests

Tables 6-8 outline *H. azteca* survival and *S. capricornutum* growth over the course of the project. *Hyalella* survival in Table 6 refers to the Folsom site tested at 100%, although samples were tested in dilution in order to determine the magnitude of toxicity (Toxic Units). In some cases, specific sites were dry upon sample collection, therefore there are no data points listed in the following tables. Individual water quality summary tables for each species are provided in Appendix B and C.

Table 6. Summary of *H. azteca* survival over the course of the project. -

Test Date	*Hyalella azteca* Survival (%)											
	Control		Low Salinity Control		F2		F3		F5		F100	
	Ave	SE	Ave	SE	Ave	SE	Ave	SE	Ave	SE	Ave	SE
11/21/13	100	0	100	0	0	0	0	0	0	0	46	11
2/27/14	98	2	100	0	2	2	0	0	52	11	92	14
5/7/14	100	0	100	0	46	9	0	0	100	0	98	2
7/9/14	100	0	-	-	0	0	0	0	-	-	-	-
2/10/15	96	4	100	0	0	0	0	0	7	4	96	2
4/9/15	98	2	84	8	10	3	0	0	0	0	87	7
6/10/15	100	0	-	-	100	0	-	-	100	0	-	-
11/3/15	100	0	-	-	6	4	0	0	-	-	-	-
2/25/16	96	2	96	2	72	8	-	-	71	8	76	2

'-'denotes no available sample for the associated date. In some instances, a site was dry and not collected; in others, sample conductivities did not warrant the use of a Low-Salinity Control.

Table 7. Summary of *H. azteca* Toxic Units and percent reduction over the course of the project. -

Sample Date	Event	Sample Type	Toxic Units							
			F2	F2 Contribution	F3	F3 Contribution	Input[1]	F5	% Reduction	F100
11/19/2013	Wet	Grab	17.5	70	14.8	30	16.7	3.1	81	1.1
2/26/2014	Wet	Auto	2.5	70	5.3	30	3.2	-	-	-
5/7/2014	Dry	Grab	1.2	70	1.6	30	1.3	<1[2]	82	<1
7/9/2014	Dry	Auto (F2) Grab (F3)	6.7	70	5.1	30	6.3	-	-	-
2/9/2015	Wet	Auto	1.7	84	3.4	16	1.9	2	15[3]	<1
4/8/2015	Wet	Auto	2.5	77	2.8	23	2.6	2.7	-3.4	<1
6/9/2015	Dry	Grab	<1	70	-	30	-	<1	-	-
11/1 2/2015	Wet	Grab	1.7	70	3.2	30	2.1	-	-	-
2/19/2016	Wet	Auto	<1	70	-	30	-	<1	-	<1

*A Toxic Unit is defined as 100% divided by the sample dilution at which 50% of the organisms die within 96 hours.

'-'denotes no available sample for the associated date. In some instances, a site was dry and not collected.

1. The combined input to the CWQTP - the weighted average of Toxic Units based on flow data when available, or the acreage of the two neighborhoods contributing to the CWQTP.

2. One quarter of a Toxic Unit was used for non-toxic samples in toxicity reduction calculations.

3. This data is not presented later in the discussion, because the samples were not representative of the storm.

Table 8. Summary of *S. capricornutum* growth and percent growth over the course of the project. -

Test Date	*Selenastrum capricornutum* cell growth ($1x10^6$)											
	Control		F2		F3		Input[1]	F5		% Improvement	F100	
	Ave	SE	Ave	SE	Ave	SE		Ave	SE		Ave	SE
11/21/13	1.465	0.13	1.472	0.42	1.840	0.84	1.582	1.589	0.95	0.4	1.935	0.16
2/27/14	1.818	0.14	2.558	0.08	2.395	0.15	2.509	2.769	0.17	10.4	2.584	0.04
5/7/14	2.242	0.02	1.970	0.09	1.821	0.02	1.925	2.149	0.12	11.6	2.261	0.06
7/9/14	1.621	0.09	1.400	0.11	1.889	0.06	1.547	-	-	-	-	-
2/10/15	1.418	0.11	1.491	0.02	1.448	0.03	1.484	1.580	0.10	6.5	0.711*	0.02
4/9/15	1.342	0.13	1.840	0.08	2.066	0.10	1.892	1.953	0.06	3.2	2.329	0.55
6/10/15	0.978	0.03	1.005	0.03	-	-	-	1.106	0.08	-	-	-
11/3/15	2.163	0.11	2.766	0.17	2.502	0.20	2.687	-	-	-	-	-
2/19/16	2.037	0.06	2.379	0.09	-	-	-	2.517	0.13	-	2.181	0.12

1. Input refers to the weighted average of algae cell density based on either acreage, or flow (when flow data was available). These weighted averages are the same for both *S. capricornutum* and *H. azteca*.

*Statistically reduced growth compared to the control (P<0.05).

'-'denotes no available sample for the associated date. In some instances, a site was dry and not collected.

H. azteca

For the duration of the project, control survival for *H. azteca* ranged from 96-100% indicating that testing organisms were healthy and test results are considered reliable. Survival for the Low Salinity Control ranged from 84-100% suggesting that the lower conductivities alone did not stress the organisms. Of the Folsom sites, site F3 generated the greatest magnitude of toxicity, with *H. azteca* exhibiting 100% mortality in every event, and with an average of 5.17 Toxic Units over the course of the project. For the remaining sites, 0.25 TUs were substituted in for non-toxic results and the average number of TUs were 3.81 at F2, 1.43 at F5 and 0.42 TUs at F100.

S. capricornutum

With the exception of site F100 collected on February 7, 2015, algae growth performance either matched or outperformed the control, and there was no other significant toxicity with this species.

Task 3: Habitat Sampler Exposures

The following section outlines the results of the in-situ resident amphipod toxicity tests. Any changes in methodology used are noted herein.

Exposure from January 29-31, 2014

Amphipods collected from site F100 were deployed to all Folsom sites and a laboratory control for a 48-hr storm exposure. Percent survivals with SE in parentheses were as follows: F2: 28% (8.76), F3: 72% (8.29), F5: 92% (2.19), and F100: 96% (1.79), and control: 95% (2.24). Mesh size was 160 µm, cage was a small (1x1”) biobarrel, and 5 amphipods/cage were used. No flatworms were found in the cages.

Exposure from February 7-11, 2014

For this storm event, we switched to 96-hr exposures to make the field data more comparable to the laboratory data, and 210 µm mesh and 5 amphipods/cage were used. The switch to longer exposures did not lower the field (F100) or laboratory control survival, so we decided to move forward with 96-hr exposures. Percent survivals were as follows: F2: 63.3% (3.59), F3: 0% (0), F5: 33.3% (9.43), F100: 73.3% (6.60), and control: 96% (1.79). For this event we encountered two difficulties. First, sediment deposition buried the cages at site F3, potentially contributing to the 0% survival at this site. Second, four of the cages at site F5 were not submerged in the water when they were collected, likely killing the amphipods. No flatworms were present in the cages.

Exposure from February 26-March 2, 2014

The same methodology from the previous event was used, with the addition of using tent stakes at sites with mud substrate to reduce the likelihood of sediment burial. Percent survivals were as follows: F2: 0% (0), F3: 100% (0), F5: 100% (0), F100: 92% (4.0), and control: 96% (1.79). Flatworms were discovered in two of the cages.

Exposure from May 6-10, 2014

For this event we used 10 amphipods/cage enclosed in larger biobarrel cages (2x2”) to reduce the influence of any single amphipod on our results. Otherwise, the methodology was similar to the previous exposure (210 µm mesh, large biobarrel, 96-hr exposure). Percent survivals were as follows: F2: 6% (2.68), F3: 76% (5.22), F5: 0% (0), F100: 98% (0.81), and control: 100% (0). During this event there were many flatworms present in cages at sites F2, F3 and F5, and a small number were present in cages at site F100.

Exposure from June 2-6, 2014

Upon arrival to the Folsom sites it was discovered that F100 had no water, F2 had no flowing water, and the water level at site F3 was too shallow to deploy cages. In lieu of a regular exposure, we examined the efficacy of three different kinds of cages: 50 mL centrifuge tubes with 210 µm mesh, 50 mL centrifuge tubes with 160 µm mesh, and a large modified biobarrel with 210 µm mesh. As site F100 was dry, resident amphipods were collected from site F5. Five replicates of the three different cages were deployed in a pool of standing water at F2, and five replicates of the 50 mL centrifuge tubes with 160 µm mesh were deployed at site F5. Survival was as follows for the cages deployed at site F2: 50 mL centrifuge tubes/160 µm mesh: 46.7% (10.11), 50 mL centrifuge tubes/210 µm mesh: 3.7% (2.14), and Biobarrel/210 µm mesh: 66.7% (11.55). Survival in the 50 mL centrifuge tubes/160 µm mesh at site F5 was 100% (0). There were many flatworms observed in the cages with 210 µm, and a few very small flatworms present in the cages with 160 µm mesh. Therefore, it was determined that the 50 mL centrifuge tubes with 160 µm mesh was most suitable for use in-situ.

Exposure from February 3-5, 2015

In order to evaluate the water retention time of the new cage design, an experiment was conducted using bifenthrin, with *H. azteca* present either inside or outside of the cages, and the time to death compared between the two sets. Mortality was low and there was no influence of cage in the absence of bifenthrin (Table 9, Figure 8). The intercept model (i.e., the model without the effect of cage) strongly outperformed the model with an effect of cage, receiving an AIC_C weight proportion of 0.86. For the model with a parameter for cage, the cage confidence interval overlapped zero (95% CI: -1.61, 1.25). Thus, both the AIC_C model ranking and the confidence interval that overlaps zero indicate that the cage had no influence on survival in the absence of bifenthrin.

Table 9. Model comparison in the absence of bifenthrin. Cage is a dummy variable for the presence/absence of the cage.

Model	ΔAIC_c	df	AIC_c wt
P	0.0	2	0.86
P cage	3.6	3	0.14

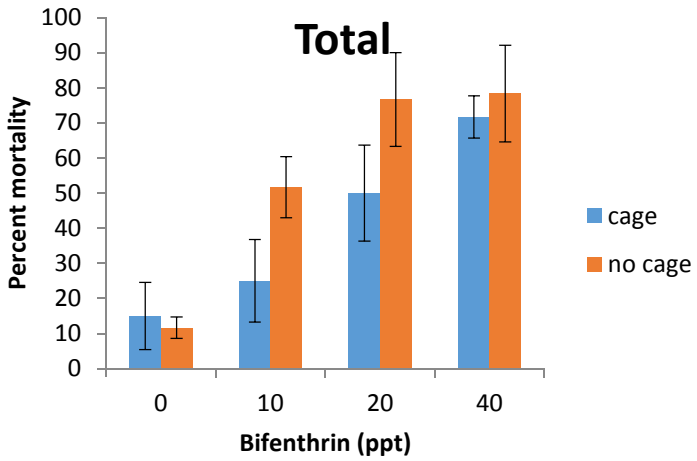

Fig. 8. The influence of cage by concentration averaged across exposure time.

Cage had a negative influence on mortality in the presence of bifenthrin (i.e., improved survival), while bifenthrin concentration and duration of the exposure both increased mortality. The top-ranked model included a parameter for each of these effects (cage, concentration, days; Table 10). The parameter estimates are as follows: days: 0.17 (95% CI: 0.00, 0.65), concentration: 0.06 (95% CI: 0.02, 0.09), and cage: -1.24 (95% CI: -2.047, -0.41). Thus, the presence of the cage decreased mortality by 22.4% averaged across all time points and treatments with bifenthrin (95% CI: 11.2, 40.0%). Table 11 outlines daily mortality rates.

Table 10. Model comparison in the presence of bifenthrin. Days is a variable for the number of days of exposure (1, 2, or 4). Conc is the concentration of bifenthrin (10, 20, or 40 ng/L). Cage is a dummy variable for the presence/absence of the cage.

Model	ΔAIC_c	df	AIC_c wt
P days+conc+cage	0.0	5	0.949
P days+conc	6.0	4	0.046
P days	11.5	3	0.003
P	12.5	2	0.002

The main purpose of the cage experiment was to determine whether the cages encapsulated clean water, sheltering *H. azteca* from toxins during storm events. A second important question was whether the cages, which were exposed to field water at site F100, caused mortality relative to the control. We found that while the cages improved survival, they did not eliminate toxicity altogether. In addition, we found that the cage did not influence survival in the absence of spiked bifenthrin. Thus, while the cages reduce toxicity of contaminated water somewhat, the experiment indicates that they are a viable method for determining toxicity in the field. The small increase in survival suggests that the test is a conservative one, meaning that mortality observed during field cage-tests will somewhat underestimate actual mortality in the field (by ~22%).

Table 11. Summary of daily mortality data for the *H. azteca* Cage Test with bifenthrin.[1]

Sample	24 Hr Survival (%)		48 Hr Survival (%)		96 Hr Survival (%)	
	Mean	SE	Mean	SE	Mean	SE
DIEPAMHR: No cage, No MeOH	90	10.0	100	0.0	90	10.0
DIEPAMHR: No MeOH, Hyalella inside cage	95	5.0	100	0.0	100	0.0
DIEPAMHR: MeOH @ 0.05%, Hyalella inside clean cage	-	-	-	-	100	0.0
DIEPAMHR: MeOH @ 0.05%, Hyalella inside cage	85	5.0	70	30.0	100	0.0
10 pptr Bifenthrin: Hyalella inside cage	75	5.0	60	40.0	90	0.0
20 pptr Bifenthrin: Hyalella inside cage	50	30.0	45	25.0	55	35.0
40 pptr Bifenthrin: Hyalella inside cage	35	15.0	25	15.0	25	5.0
DIEPAMHR: No MeOH, Hyalella outside cage	95	5.0	95	5.0	100	0.0
DIEPAMHR: MeOH @ 0.05%, Hyalella outside cage	85	5.0	90	10.0	90	0.0
10 pptr Bifenthrin: Hyalella outside cage	60	0.0	60	10.0	25	15.0
20 pptr Bifenthrin: Hyalella outside cage	65	5.0	5	5.0	0	0.0
40 pptr Bifenthrin: Hyalella outside cage	65	5.0	0	0.0	0	0.0

1. Highlighted cells indicate a significant reduction in survival compared to the laboratory control

Analytical chemistry

In the bifenthrin cage test, no pesticides were detected in the controls or the method blank. Surrogate recoveries were on average 102% with a range between 95-120%. Reported values were not corrected for surrogate recovery.

Table 12. Bifenthrin analysis results

Nominal Bifenthrin (ng/L)	Measured Bifenthrin (ng/L)	Bifenthrin Recovery (%)	Surrogate Recovery (%)
10	11.25	112.52	95.28
20	19.90	99.48	120.16
40	34.06	85.16	97.49
0	0.00	0.00	95.15
		Average	102.02
		Min	95.15
		Max	120.16

Discussion

The CWQTP at Folsom is in many ways an ideal study site; multiple collaborators working at this site provided data from flow measurements, chemical analyses, toxicity testing and bioassessments, which strongly supports the Environmental Protection Agency's integrated approach to assessing environmental chemical mixtures. These monitoring tools help us to determine whether the CWQTP is effective at reducing the offsite movement of pesticides from this residential setting. Each of our biological indicators (bioassessment, toxicity tests and in-situ exposures) had the potential to inform us about the efficacy of the pond and during this project; we had the opportunity to evaluate which methods gave us the best indication of efficacy under very challenging drought conditions.

Relevant Calculations for this Report

Data from the two inputs (Sites F2 and F3) had to be combined to represent a single input into the CWQTP. Each input value for chemical concentrations (µg/L), cell density (cells/mL) from *S. capricornutum* tests, or Toxic Units derived from *H. azteca* survival data, was calculated as a weighted average. The weight appropriation was based on flow from sites F2 and F3 (when available), or the acreage of the two residential areas supplying the CWQTP. The contribution from site F2, the larger residential area, ranged from 66 to 84%.

Data was needed from sites F2, F3 and F5 to make percent reduction calculations. Data from site F5 was used for the output values. Once the weighted average for the input value was calculated, percent reductions throughout this report were calculated with the following formula:

% Reduction = (-(Output Value/Input Value)+1) x 100

Statistical comparisons could not be made between the single input and output values, since there was no replication in each event.

Analytical Chemistry Data from DPR

Percent reduction through the CWQTP was calculated for the individual chemicals that were measured by the California Department of Food and Agriculture (CDFA) for CDPR from November 2013 to April 2015. These measurements provide the opportunity to compare our biological data to the long-term chemical monitoring data. At the time this report was written, analytical chemistry data was available for the five events that had a full data set to make reduction calculations. For chemical reduction calculations, non-detections were replaced with ½ the Reporting Limit concentrations for the particular analyte (presented in the Appendix D, Figure D1, as supplemental information). Although this method is commonly used by environmental scientists for summing data that has non-detect values within a particular dataset, we recognize that this method has inherent errors, and may overestimate the pond's reduction capabilities (Helsel, 2010).

For many of the sampling dates in this project, grab or time-weighted composite samples were collected during runoff events. Neither of these methods readily allow for an accurate assessment of the CWQTP efficacy for an entire storm event, only a single point in time. With these varied sample collection methods and timing approaches, it was difficult to compare toxicity test and analytical chemistry data among field events and between sites within the same event. Thus, discussion of pesticide reductions will be based on the concentrations of pesticides present in samples collected at each site, at the time the sample was taken.

CDFA's analytical chemistry data illustrate that the concentrations of pesticides were reduced from the input (F2 and F3) to the output (F5) when samples were collected (90.5% of insecticide detections or 19/20; Table D1 in Appendix D). Likewise, herbicide concentrations decreased in 92.9% (13/14; Figure D1 in Appendix D) of the detected compounds.

Task 1: Macroinvertebrate Community Survey

Benthic community condition is widely used in aquatic systems to assess the effects of numerous stressors, including physical disturbance, organic loading, and chemical contamination on the biota (Thompson et al., 2011). The pre-drought benthic samples suggest that the constructed water quality

pond at Folsom improves the biological integrity (i.e., richness) of downstream sites (F5 and F100), as invertebrate richness was substantially higher at F5 and F100 during the first sampling event. However, certainty is low for three reasons. First, substrate has long been known to exert a major influence on invertebrate communities (e.g., de March 1976, Rosenberg et al. 2008), and substrate was not consistent among sites. Specifically, F100 and F5 were characterized by stable substrates, including gravel, cobbles, root beds, and macrophytes, whereas F2 and F3 were characterized by unstable substrates, including sand and organic fines (note: benthic invertebrate samples were collected just below the cobbles below the culvert at F2). For example, Simuliidae was observed at both F5 and F100 but not at F2 or F3 on May 6, 2014 (Table 2), likely (at least in part) because it requires stable substrate (Eymann et al. 1988). Second, it is uncertain whether the water quality pond improves biological integrity faster than an equivalent length of stream channel. Finally, dissolved oxygen was extremely low at the upstream sites on several occasions, particularly at F3 (1.8-3 mg/L; Table A2 in Appendix A).

The benthic samples also suggest that the drought had a substantial influence on the aquatic community at Folsom, including a larger impact on the downstream communities than the communities above the pond. While richness and abundance dropped considerably at F5 and F100 following summer 2014 (when F100 dried completely), similar declines were less apparent at F2 and F3. This is likely because F100 went completely dry, and F5 almost completely during summer 2014, while to our knowledge the sampling locations at F2 and F3 never dried completely. Also, the biological community at the upstream sites is more tolerant as a group than the downstream sites, so they are potentially more resistant to drought conditions.

Task 2: Laboratory Toxicity Tests

S. capricornutum

From a regulatory perspective, all samples collected from sites F2, F3 and F5 were non-toxic to *S. capricornutum*. Dilution series tests used to calculate Toxic Units were never employed for this species, because the alga growth in these samples was generally higher than the laboratory control. Ambient samples often contain nutrients that can boost cell growth in the algae toxicity tests relative to the more nutrient limited control, and can in turn, mask the presence of toxic compounds. Runoff from residential areas may contain high concentrations of nutrients as home owners apply fertilizer(s) to their lawns. For this reason, comparisons in alga growth were made between the input and output to evaluate CWQTP efficacy. Weighted averages for the cell density in *S. capricornutum* tests were calculated for an input value in order to calculate a percent improvement in growth. These improvements ranged from 0.4 to 11.6% (n=5) and are considered negligible. The only sample that caused significant growth impairment was collected from site F100 on February 7, 2015. This source of toxicity could be from additional discharges that flow into Alder Creek between sites F5 and F100. Effect concentrations for *S. capricornutum* for the four herbicides measured by CDFA are presented in Table 13.

Table 13. Published 96-hr herbicide EC50 values for *S. capricornutum*.

Herbicide	96 h EC50 Values (µg/L)	Maximum Concentration Detected (µg/L)	Calculated TU based on most sensitive EC50 Values
2,4 D	41,772[a], 25,900[b]	3.030	.00012
Dicamba	36,375[a]	0.179	0.0000049
MCPA	18,400[c]	0.461	.000025

Herbicide	96 h EC50 Values (µg/L)	Maximum Concentration Detected (µg/L)	Calculated TU based on most sensitive EC50 Values
Triclopyr acid	50,000*[d] (acid equivalents)	0.783	.000016

a. (Fairchild et al., 1995), b. (St. Laurent et al., 1992), c. (Caux, P.Y et al., 1996), d. (Cowgill, U. and Milazzo, L. 1989)

The maximum concentrations of individual herbicides detected at any of the Folsom sites were at a minimum 8,000 times lower than the most sensitive EC50 values. Although other contaminants are likely to be present in urban runoff, the robust alga growth in these samples is congruent with the concentrations of the four herbicides that were measured. Based on the analytical chemistry data provided by CDPR, the average reduction of detected herbicides was 41.4, 41.3, 43.0 and 34.7% for Dicamba; 2,4-D; Triclopyr; and MCPA, respectively (Figure 9).

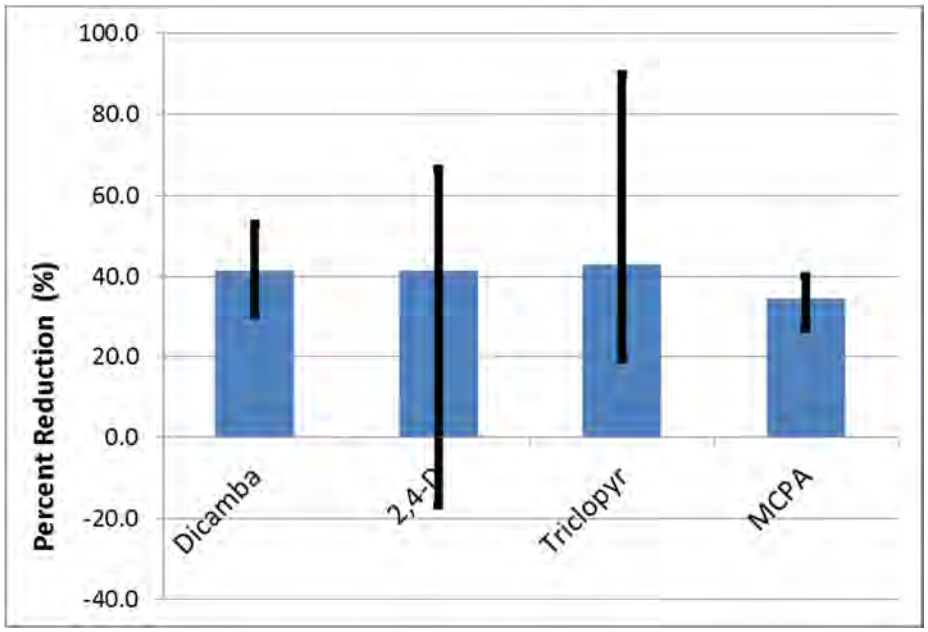

Figure 9. Average percent reductions of herbicides. Error bars delineate the maximum and minimum values.

H. azteca

Some runoff from the two residential areas (sites F2 and F3) were acutely toxic to *H. azteca* and ranged from non-toxic to acutely toxic, with a maximum of 17.5 TUs throughout the study. Percent reduction of the TU data derived from our laboratory *H. azteca* tests survival data, herein referred to as biological Toxic Units or BTUs, was calculated for the three events that had results from all three CWQTP sites and had acceptable storm representativeness (Table D2 in Appendix D). Non-toxic samples were assigned 0.25 TUs in order to calculate these reductions.

Percent reduction was also calculated for the TUs derived from insecticide concentrations and published LC50 values. Calculated TUs were assumed to be additive, thus the calculated TUs from each analyte were added to get the combined calculated TU (CCTUs) for each event. This CCTU is a reasonable

mechanism to compare the estimated toxicity from the measured analytes to the aggregate toxicity detected by our test organisms (Elsaesser et al., 2011). The number of calculated TUs for each analyte varied considerably, depending on whether we used the more sensitive or average LC50 values from Table 14. Using the more sensitive LC50 values yielded combined calculated Toxic Units approximately twice of those derived from our laboratory *H. azteca* tests. Thus, using average LC50 values underestimates toxicity by about half. Many factors contributed to discrepancies between these biological and analytical chemistry-based approaches. These factors include:

- Missing effect concentration data from some analytes, which can underestimate CCTUs,
- Other runoff-related contaminants that were present but not measured may contribute to high BTUs,
- In the presence of contaminants, low conductivity may be an additional stressor to *H. azteca* in Folsom samples and contribute to increased sensitivity, and
- Differences in sample collection type (grab vs. time-weighted vs. flow-weighted) make - comparisons among sites difficult. -

Table 14. Published 96-h insecticide LC50 and EC50 values for *H. azteca*.

Insecticide	96 h LC50 Values (µg/L)
Bifenthrin	0.0023[a], 0.0093[b], 0.010[d]
Cyfluthrin	0.0027[a]
Fipronil	0.728[e]
Imidacloprid	65.4[c],
Lambda cyhalothrin	0.0019[a],
Permethrin	0.036[a], 0.094[d]

a. (Hoffman et al., 2016), b. (Anderson et al., 2006), c. (Stoughton et al., 2008), d. (Deanovic et al., 2013), e. (EC50; Weston and Lydy, 2014),

Despite the complexity in making these comparisons, valuable information was gained from the combined calculated TUs; bifenthrin routinely contributed the highest number of calculated TUs and was therefore likely driving the toxicity to *H. azteca* in the Folsom samples.

For the percent reduction calculation for the BTUs, we opted to use the more sensitive published LC50 values, because several of the more sensitive values came from exposures conducted in our own lab using the same methods that were employed during this Folsom study. Because of the differences in sample collection procedures throughout the project, making comparisons among sampling events is problematic. Timing for one-time grab samples would ideally follow the pulse of pesticides as they move through the CWQTP. This sample timing is difficult to achieve, thus toxicity testing and analytical chemistry data has the potential to bias the data with respect to reduction calculations. For instance, premature sample collection at site F5 could have fairly low concentration of pesticides and give the impression that the CWQTP is highly effective. With this in mind, calculated BTU data from *H. azteca* toxicity tests demonstrated reductions in toxicity in the November 2013 rain (81% reduction) and the May 2014 dry event (81% reduction), and CCTUs of pesticide concentrations measured during these same events were reduced (76% and 44% reductions, respectively) in the samples collected from the output (F5) compared to the input (F2 + F3). Only one field event was collected using flow-weighted composite samplers (April 9, 2015 rain event), thus lending a representative comparison among sites. In

this case, however, BTU data calculated from acute *H. azteca* toxicity tests did not show a reduction (6% increase) in toxicity, whereas in contrast the CCTUs for this event do show a reduction of pesticide concentrations (16% reduction). Other authors have demonstrated that although pesticide concentrations are typically lower after passing through CWQTP systems, full mitigation of aquatic toxicity is not always observed (Hunt et al., 2008; Moore et al., 2007, in Budd 2011).

Based on the analytical chemistry data collected in this study, the average reduction of detected insecticides ranged from 33.2 to 64.2%. Imidacloprid, which has the lowest octanol water partitioning coefficient (log K_{ow}, 0.57) for the analytes tested, was the only exception, with the average concentration increasing by 15.1% through the system. In a review by O'Geen et al. (2010), a positive relationship was noted between log K_{ow} values and observed pesticide removal rates, concluding that a greater than 50% reduction in pesticide concentrations were obtained in most CWQCP systems for chemicals with log K_{ow} values greater than 4.2 (Budd, 2011), which may account for this trend. Overall, these results suggest that the CWQTP is reducing the concentrations of the highly hydrophobic pyrethroids bifenthrin, permethrin, cyfluthrin and lambda-cyhalothrin, as well as fipronil (Figure 10).

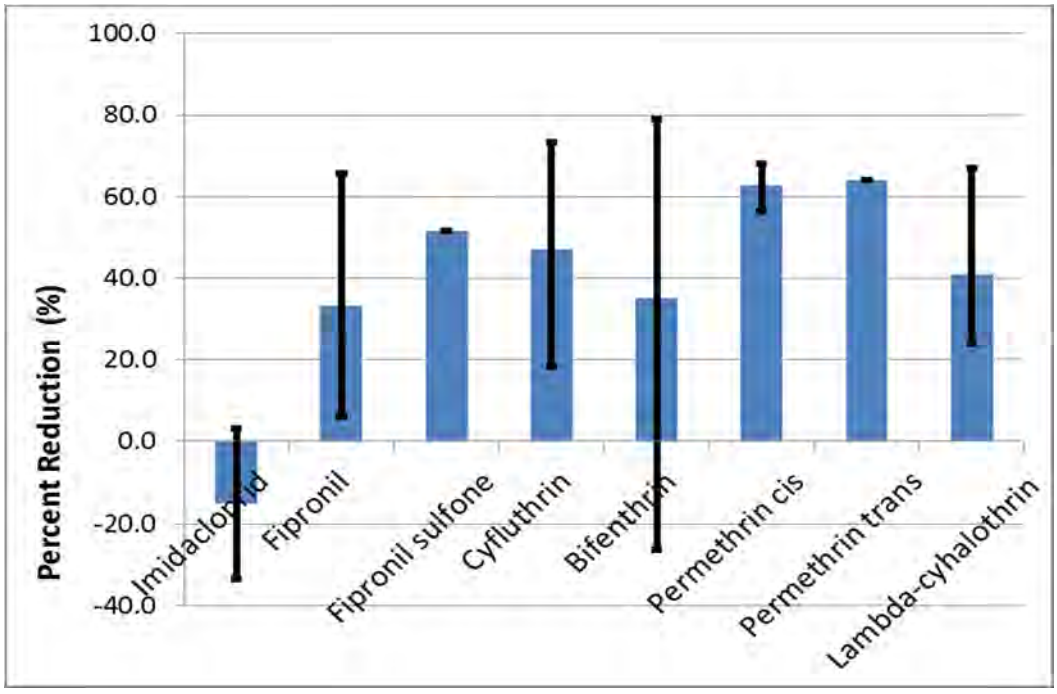

Figure 10. Average percent reductions of insecticides through the CWQTP. Error bars delineate the maximum and minimum values.

It has been well demonstrated that dense vegetation increases the effectiveness of remediating pesticide pollution (Moore et al., 2002, 2006, 2009). Budd et al. (2009) evaluated two constructed wetlands in the Central Valley and found calculated removal rates of 95-100% for pyrethroids. Specifically, they found that concentrations in the outlet flow were found to be significantly lower than those in the inlet flow for both of the constructed wetlands evaluated for permethrin, cypermethrin, bifenthrin and lambda cyhalothrin (Wilcoxon Signed Rank test, $P<0.025$). Elsaesser et al. (2011) saw a reduction of peak pesticide concentrations in water from a simulated runoff event in a constructed

wetland ranged from 46-100%. The range of peak concentrations in that study were 0.1-7 µg/L, similar to the concentrations detected at Folsom, and included the following pesticides: dimethoate, dicamba, trifloxystrobin and tebuconazole. This particular wetland study was conducted on a surface flow system with low discharge and high plant densities, with hydrologic retention times of 132-280 minutes, and the authors note that with short passage times of less than 3 hours, only a minor retention of masses can be expected. Budd (2011) in his meta-analysis of constructed wetland data, observed a positive relationship between retention time and reductions in pesticide aqueous concentrations, with greater than 50% reduction in all instances with system retention times greater than 100 hours. Overall, studies show that the rate of inflow, water residence time in the wetland, and availability of organic matter to bind contaminants, are the most important factors affecting the water purification capacity of wetlands (Budd et al., 2009).

Task 3: Habitat Sampler Exposures

For the majority of field events that occurred during this project, in-situ cages were populated with resident amphipods collected from site F100 (*Hyalella and Crangonyx*). These genera were taxonomically identified after the exposures were terminated in the laboratory, but it was near impossible to get this level of taxonomic resolution in the field prior to the exposure. Generally speaking, the vast majority of organisms used in field studies were from one genus or the other for any given event. A review of available literature did not yield any information regarding the sensitivity of *Crangonyx* to bifenthrin, the most frequently detected insecticide in this study. Should in-situ exposures continue to be employed in the future at this site, analyzing the survival data for each genera in the cages may provide some evidence regarding which is more sensitive to pyrethroids and other urban runoff contaminants.

In-situ exposures went through an extensive list of method changes to address the multiple challenges experienced at the Folsom CWQTP during this study, such as shallow water and low flow, predation of test organisms by Planaria, and dewatering at sites F5 and F100. Our first attempt at in-situ exposures, which involved the use of colonized leaf litter bags, required far too many person-hours to count living and dead organisms, and to make taxonomic identifications. Furthermore, the presence of the predatory Planaria in the vast majority of samples confounded the results and prompted the need for alternative cage designs. All subsequent exposures utilized resident amphipods (*Hyalella* and *Crangonyx* sp.) that were captured at our reference site (site F100), loaded in to cages and planted at all four study locations (F2, F3, F5 and F100). Planaria also interfered with the amphipod exposures until we eventually placed the animals in screw top cages and reduced the mesh size to 160 micron.

The presence of indigenous organisms inside in-situ chambers has been noted in other studies as being problematic. Local fauna may interact (as competitors or predators) with test organisms, biasing toxicity interpretation. Additionally, these indigenous organisms may also confound taxonomic identifications in cases where local fauna are taxonomically similar to test organisms (Chappie and Burton, 1997; Castro et al., 2003). The presence of indigenous fauna has been shown to affect growth and survival of *Chironomus* sp. in some in-situ sediment tests (Reynoldson et al., 1994; Sibley et al., 1999; Crane et al., 2000; Castro et al., 2003). In cases where indigenous organisms were present inside in-situ chambers, a reduction in mesh size was generally sufficient to minimize their presence and interaction with test organisms (Chappie and Burton, 1997; Pereira et al., 1999). Castro et al. (2003) and Sibley et al. (1999) both utilized similar mesh sizes (~200 µm) which was sufficient to keep out larger adult indigenous

organisms, however Chironomid larvae and other smaller invertebrates could still enter test chambers. Reductions from ~150 µm mesh to 50-74 µm (Pereira et al., 1999; Chappie and Burton, 1997, respectively) prevented the smaller indigenous fauna from entering test chambers, but significantly reduced flow. The reduction from 200 µm to 160 µm mesh in the test chambers used at Folsom in the current study reduced Planaria interference and also ensured adequate flow through test chambers (as demonstrated by the bifenthrin cage test). However, once site F100 became dry, native amphipod populations did not recover enough to supply the 250 organisms needed to populate the in-situ cages and we were unable to conduct further testing under this task.

Recommendations for Future Studies

During the driest months (typically summer) of the study, Sites F2, F3 and F5 often became large standing puddles of water and Site F100 dried up completely. This dewatering period limited our ability to conduct in-situ exposures, find benthic macroinvertebrates and collect the needed samples from both CWQTP inputs and the output. In the interest of water conservation, Folsom residents may continue to minimize their water use for lawn irrigation and car washing in future summers, even following wet years. Thus, focusing on precipitation-based events in the future at this site might be more cost-effective and informative.

CDPR has expressed an interest in using biological monitoring tools that are better suited for regulatory decisions. For this reason, the following recommendations emphasize future studies that support this goal.

1. - We would like to have the opportunity to continue laboratory toxicity testing with *H. azteca* at Sites F2, F3 and F5. *H. azteca* have proved to be sensitive to current use pesticides in urban runoff and also helpful in terms of evaluating CWQTPs.
2. - Since all samples related to the CWQTP were non-toxic to *S. capricornutum* and EC50 concentrations for the herbicides in CDFA's analytical scan were several orders of magnitude above the concentrations found in Livermore Park, we would not continue to test with algae. Instead, we would recommend testing with another sensitive invertebrate such as *Chironomous dilutus*.
3. - Determine any missing 96-h LC50 values for invertebrate test species that are routinely used by CDPR for all insecticides within CDFA's analytical scan, which would help determine species' sensitivity and help with TU comparisons.
4. - Now that flow weighted composite samplers have been installed at sites F2, F3 and F5, the pesticide concentrations, loading and toxicity can be more accurately represented a full storm event. Thus, more reliable mitigation evaluations of the CWQTP can be made. We would like to work closely with Loren Oki and DPR in obtaining flow data in order to calculate pesticide loads and pesticide mass reductions, rather than concentration reductions, in determining the effectiveness of the CWQTP.

Two additional suggestions are:

1. - Continue developing in-situ methods at a mitigation site that has year round flow, because they utilize resident organisms exposed to changes in pesticide concentrations for the duration of the storm, and data can be coupled to CLAM or passive sampling analytical chemistry data.

2. - Consider sampling at other sites that only have one input to a CWQTP to make data interpretation easier, improve data completeness and reduce the overall cost of these monitoring efforts.

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