Treatment of Quail Creek with Landguard A900 Enzyme: Evaluation of pesticide and toxicity reduction and impacts on macroinvertebrates in the Salinas River

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Introduction

Chlorpyrifos, diazinon and malathion used in agriculture are among the organophosphate (OP) pesticides responsible for past surface water toxicity in central California coastal watersheds (Anderson et al., 2003; Hunt et al., 2003; Phillips et al., 2004). A number of recent studies have investigated various treatment options to reduce OP concentrations in agriculture runoff (Hunt et al., 2008). Research has shown that an integrated approach is required to reduce pesticide loading in row crop runoff sufficient to meet water quality objectives requiring no aquatic toxicity (Anderson et al., 2011). Integrated systems combine sedimentation basins to reduce particle load, water retention structures to slow runoff flow, and vegetated treatment systems to remove soluble and particle bound pesticides. While these systems reduce OP pesticides in irrigation runoff, traditional methods are only partially effective due to the higher solubility of certain OPs. Studies have shown that use of the Landguard™ enzyme reduces concentrations of specific OP pesticides to meet regulatory criteria (i.e., no toxicity; (Anderson et al., 2011)). The enzyme acts as a catalyst for the hydrolysis of the active pesticides such as diazinon and chlorpyrifos, producing metabolites with lower toxicities (Orica Watercare, 2008). The Landguard enzyme was registered as an industrial chemical by the U.S. Environmental Protection Agency in 2006, but has not been used for treating state water bodies in California. Widespread use of this technology has been impeded by concerns regarding the potential for impacts on in-stream macroinvertebrates after chronic use of the enzyme.

The California Department of Pesticide Regulation funded the University of California, Davis to evaluate the efficacy and impacts of treating agriculture tailwater with the Landguard A900 OP enzyme. The study had two primary goals: 1) Determine the effectiveness of Landguard A900 to reduce OP pesticide loading and associated toxicity in Quail Creek and the Salinas River; 2) Investigate any chronic effects of Landguard A900 use on macroinvertebrate community structure in the Salinas River. Prior to the study, a low threat permit was issued by the Central Coast Regional Water Quality Control Board for Landguard treatment of the Creek. Field trials were conducted on Quail Creek and in the Salinas River at the Quail Creek confluence by the UC Davis Marine Pollution Studies Laboratory at Granite Canyon.

Quail Creek was considered to be an appropriate site for this investigation because numerous studies have shown this water body is one of the most pesticide contaminated creeks on the central coast, and is now the focus of on-going total maximum daily load (TMDL) activities. Previous studies have shown benthic macroinvertebrate (BMI) communities in the Salinas River downstream of the Quail Creek confluence were impacted by mixtures of OP and pyrethroid pesticides. During this study, Quail Creek was treated with Landguard and reductions in toxicity and OP pesticide loading were monitored in the creek. Reductions in toxicity and OP pesticide loading were also monitored at stations in the Salinas River, downstream of the Quail Creek confluence. In addition, macroinvertebrate community structure was monitored in the Salinas River to assess changes in BMI metrics as a result of the Landguard treatment. Bioassessment results at Landguard treated stations were compared to those of the same stations prior to the Landguard treatment, as well as results from reference stations upstream of the confluence. These data allowed an evaluation of the potential impacts of chronic Landguard exposure on river BMI communities.

Methods

Study Sites

The project was designed to treat pesticides in agriculture tailwater flowing into Quail Creek upstream of its confluence with the Salinas River and to then monitor improvement of water quality in Quail Creek and the Salinas River. Quail Creek was chosen for the study location after initial screening tests in Bradley Channel (Santa Maria) and Alisal Creek (Salinas) proved that those locations were unsuitable. Quail Creek is located approximately ten km south of the City of Salinas, California. The watershed is 11,236 acres, and approximately 22% of the land use in the watershed is agriculture. Quail Creek was treated with Landguard approximately 100 meters upstream of its confluence with the Salinas River (Figures 1 and 2).

Figures 1 and 2. Map of Quail Creek project location in Monterey County, and detail of Quail Creek treatment area and Salinas River monitoring stations.

Landguard Pre-Treatment: Quail Creek Baseline Monitoring

Prior to the initiation of Landguard dosing, eight grab samples of water were collected approximately weekly in Quail Creek to investigate the presence of toxicity associated with organophosphate pesticides, in particular chlorpyrifos. For each sampling event, 2 liters of water were collected and transported on ice to the Marine Pollution Studies Laboratory for analysis. Acute toxicity tests (96 hour) with the cladoceran *Ceriodaphnia dubia* were conducted with seven of the samples. Five of the samples were also tested with the amphipod *Hyalella azteca* (U.S. EPA, 2002). These two test species were used because of their variable sensitivities to OP and pyrethroid pesticides. Amphipod exposures were

conducted in 300 mL beakers containing 100 mL of test solution and ten organisms. Daphnid tests were conducted in 20 mL glass scintillation vials, each containing 15 mL of test solution and five organisms. Both exposures consisted of five replicate containers. Test solutions were renewed daily for *C. dubia*, and at 48 hours of *H. azteca*. Daphnids were fed a combination of algae and YCT (yeast, cerophyll and trout chow mixture) daily, 2 hours prior to renewal, and amphipods were fed YCT at 48 hours, 2 hours prior to renewal. Daily survival was recorded. Dissolved oxygen, pH, and conductivity were measured with an Accumet meter and appropriate electrodes (Fisher Scientific, Pittsburgh, PA). Un-ionized ammonia was measured using a Hach 2010 spectrophotometer (Hach, Loveland, CO). Water temperature was recorded with a continuous recording thermometer (Onset Computer Corporation, Pocasset, MA). Additional daily temperatures were measured using a glass spirit thermometer.

Salinas River Baseline Monitoring: Pre- and Post-Treatment

After the Quail Creek screening and before treatment of the creek with Landguard, monitoring was conducted in the Salinas River upstream and downstream of its confluence with Quail Creek to characterize baseline toxicity and the benthic macroinvertebrate (BMI) community in the River (Figure 1). All measurements were repeated after treatment. Pre-treatment monitoring was conducted on July 30, 2013, and post-treatment monitoring was conducted on September 12, 2013. These data serve as a baseline for comparison to toxicity monitoring and the benthic macroinvertebrate community after Landguard treatment.

Macroinvertebrate community was characterized in Salinas River at 5 reference stations above Quail Creek confluence. Methods followed a modification of the Surface Water Ambient Monitoring Program Reach Wide (RW) macroinvertebrate bioassessment protocol (Ode, 2007). The SWAMP RW bioassessment protocol was modified as a Margin-Middle-Margin protocol for this section of the Salinas River, which is dominated by sand substrate and where the majority of invertebrate habitat is provided by emergent vegetation along the margins of the channel. Five replicate transects designated stations A, B, C, D, and E were established downstream of the Quail Creek confluence. The first station (E) was at the confluence, and stations D-A were established every 20-25 meters downstream. Bioassessment sampling started at Station A, the farthest downstream station. At each station three BMI samples were collected with a D-net, one on each bank and one in the center of the channel. These three samples were composited to provide a single sample for each of the five replicate stations. In addition, the physical habitat was characterized at each station following the SWAMP protocol. Stream habitat characterization included broad descriptive ratings for epifaunal substrate/cover, sediment deposition and channel alteration. More specific categories included ratings for riparian vegetation, in-stream habitat complexity and measures of canopy cover. Salinas River discharge was measured at station A. This process was then repeated at five replicate reference stations (A-E) located upstream of the Quail Creek confluence, where Station E was the reference station farthest upstream from the confluence.

In addition to BMI sampling, water samples for toxicity testing were collected at Station E from the reference and downstream sites, and 96-hour toxicity tests were conducted with *C. dubia* and *H.*

azteca, as described above. Sediment samples were also collected at Station E from the reference and downstream sites and 10-day toxicity tests were conducted with *H. azteca* (U.S. EPA, 2000). Reference site and downstream sediments were divided among eight laboratory replicates, with ten 7-to 14-day-old amphipods in each (Chesapeake Cultures, Hayes, VA). The amphipods were exposed to 100 mL of sediment in 250 mL beakers, each containing 175 mL of overlying water. The test temperature was 23 ± 1°C. Water quality parameters, including dissolved oxygen, pH, conductivity, and ammonia, were measured at the beginning and end of each test as described above. Hardness and alkalinity were also measured at the beginning and end of each test. Overlying water was renewed twice daily, once every morning, by hand, and once every night using an automated renewal system, and 1.5 mL of YCT was added daily to each test container.

Reference site and downstream water and sediment samples from Station E were analyzed for a suite of organophosphate and pyrethroid pesticides. Organophosphate pesticides were analyzed using U.S. EPA Methods 8141. Pyrethroid pesticides were analyzed using a gas chromatograph coupled to a tandem mass spectrometer (EPA 8270M). Method reporting limits for organophosphates ranged from 13-42 ng/L in water, and 40-200 ng/g dry weight for sediment. Organophosphate method detection limits ranged from 2-10 ng/L and 10-50 ng/g dry weight for water and sediment, respectively. Method detection limits for pyrethroids ranged from 0.3-2.9 ng/L in water, and 0.106-1.6 ng/g dry weight for sediment. Pyrethroid reporting limits ranged from 2-10 ng/L and 0.5-5 ng/g dry weight for water and sediment, respectively.

Quail Creek Landguard Treatment Monitoring

Laboratory Efficacy Study

Prior to use in the field, the efficacy and dose rate of Landguard A900 was verified in the laboratory. Landguard A900 was provided by the Commonwealth Scientific and Industrial Research Organization (CSIRO - Australia, Dr. Colin Scott). Chlorpyrifos was spiked into clean laboratory culture water at the nominal concentrations of 200, 1000 and 4000 ng/L. Spiked samples were treated with an optimal enzyme dose of 100 µg/L. Chlorpyrifos concentrations were verified using enzyme-linked immunosorbent assays (ELISA) described below. Treated and untreated spiked water samples were also tested with 96-hour *C. dubia* tests as described above.

Field Monitoring

Quail Creek flow varied daily with irrigation patterns in the adjacent and upstream fields. Using sandbags and tarps to direct flow, Quail Creek was diverted through a fiberglass 60-degree V trapezoidal flume fitted with a flow-weighted dosing system and calibrated to deliver a continuous Landguard dose of 100 µg/L enzyme in the creek. The dosing system (Figure 3) was previously described (Anderson et al., 2011). Average creek flow rate was predicted based on previous studies, and was estimated to be approximately 15 L/s. Based on the estimated average creek flow and the enzyme dosing rate, a

reservoir containing 265 liters of enzyme stock solution (at 560 mg/L) could provide continuous dosing for approximately 30 hours. Because Landguard enzyme activity degrades after 48 hours, any residual enzyme solution was purged and replaced every two days.

Landguard dosing was initiated on August 13, 2013 and terminated on September 12, 2013. During the Landguard treatment period two stations were monitored in Quail Creek, one upstream and one downstream of the treatment flume to demonstrate the effectiveness of the enzyme to reduce OP pesticide loading. Water samples were collected during six separate irrigation flow events. Toxicity testing and chemical analyses were conducted during each sampling event. Toxicity tests upstream Quail Creek (= untreated) and downstream Quail Creek (= treated) included 96-hour tests with *C. dubia* and *H. azteca*, as described above.

Chlorpyrifos was measured in each water sample using an enzyme linked immunosorbent assay, ELISA, (Modern Water, Newark, DE). ELISA procedures followed those recommended by Sullivan and Goh (Sullivan and Goh, 2000). Readings were compared to a 5-point standard curve prepared using standards provided by the manufacturer. Accuracy was determined for each batch using external standards and matrix spikes. Precision was determined by duplicate measurement of one sample per batch. Samples were tested without dilution unless necessary. Lowest detectable concentrations for this procedure were 50 ng/L for chlorpyrifos, and the reporting limit was 100 ng/L. Synoptic water samples were analyzed for OP and pyrethroid pesticides using U.S. EPA GC/MS methods described above.

In addition, the Quail Creek discharge volume was monitored continuously during the 30 day Landguard treatment period. A water level logger was placed in a protective housing approximately one meter upstream of the dosing flume to record water height. The housing consisted of one-inch PVC pipe with holes drilled every few cm, and was securely fastened to a 1 meter staff plate. The level logger continuously recorded Quail Creek height and was calibrated with daily direct measures of creek height on the staff plate, relative to a zero point on the flume. The zero point was at the bottom of the v-notch in the flume. Discharge rates were calculated based on Quail Creek height and the relationship between creek height and discharge flowing through the flume. This relationship between creek height and flow rate is based on the following formula: $941.2(H_m)^{2.58}$, where H_m is the height in the flume in meters.

Pesticide loading was calculated for each of the 6 monitored irrigation events as the instantaneous concentration measured in an upstream grab water sample during each event, multiplied by the Quail Creek discharge volume (in liters) during that irrigation event. Pesticide load reduction was calculated as the difference between the loading for the upstream sample minus the loading for the downstream sample.

Data Analysis

Toxicity data was analyzed using the test for significant toxicity (TST) to determine statistically significant toxicity (U.S. EPA, 2010). Chemical concentrations (pyrethroids, organophosphate pesticides) were compared to established LC50 values for each species to determine their potential to cause toxicity to *C. dubia* and *H. azteca*. Bioassessment metrics and habitat data were summarized and analyzed using separate-variance t-tests to determine differences in the downstream river stations before and after the enzyme treatment. Analysis of Variance was also used to determine overall statistical differences among the upstream and downstream sites in the Salinas River both before and after treatment.

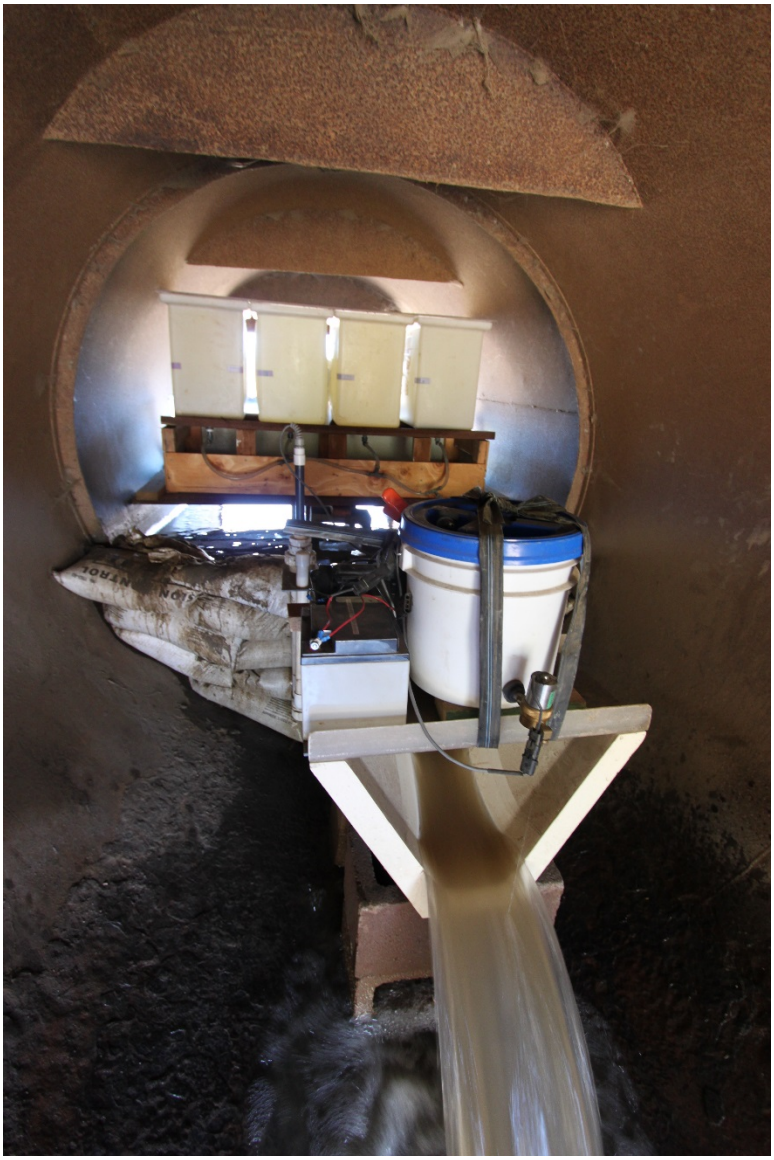

Figure 3. Landguard dosing flume and enzyme reservoir system treating Quail Creek. The dosing system was established in the 6 foot diameter culvert.

Results and Discussion

Quality Assurance

All water column toxicity tests met the test acceptability criterion of greater than 90% survival except for three of fifteen *C. dubia* tests that all had 88% control survival. Data from these tests were considered acceptable for interpretation. All toxicity test water quality parameters were within acceptable limits defined in the test protocols. All analytical blanks were non detect for organophosphates and pyrethroids. Laboratory control standards (LCS) for most chemicals ranged from 52 to 143 percent recovery for water, and 84 to 123 percent recovery for sediment. Low LCS recoveries were noted for deltamethrin/tralomethrin, esfenvalerate/fenvalerate, and dimethoate. Low LCS recoveries suggest these compounds may be biased low, but the only results that would be affected are those for dimethoate in the first Quail Creek treatment samples. Laboratory control standard duplicates for most chemicals in water ranged from 0 to 24.4 relative percent difference, and sediment RPDs ranged from 2.4% to 10%. There was high variability between three dimethoate duplicates that was related to the insufficient recovery noted above. Dibromooctafluorobiphenyl was used as a surrogate in both water and sediment pyrethroid analyses, and triphenyl phosphate was used as a surrogate for all organophosphate analyses. Percent recoveries for these surrogates in water samples ranged from 60% to 101%, and recoveries in sediment samples ranged from 71% to 128%.

Verification of Landguard Efficacy

Clean laboratory water spiked at the nominal chlorpyrifos concentrations of 200, 1000 and 4000 ng/L had measured concentrations of 152, 750 and 3300 ng/L chlorpyrifos, respectively. The measured concentrations averaged 78% of nominal. A Landguard dose of 100 µg/L reduced the lower two concentrations below the detection limit of the ELISA method. The 4000 ng/L concentration was reduced to below the reporting limit of the method (100 ng/L). Complete mortality was observed in all toxicity tests on untreated samples, whereas all treated samples had 100% survival of *C. dubia*. These results demonstrated that the Landguard A900 was actively hydrolyzing chlorpyrifos at the target dose intended for the Quail Creek field study.

Quail Creek Landguard Pre-Treatment Screening Monitoring

Samples collected on an approximately weekly basis in Quail Creek prior to initiation of Landguard treatment showed minimal toxicity to *C. dubia* (Table 1). Only one of the seven water samples tested with daphnids was toxic. Two of the five water samples tested with *H. azteca* during the pre-treatment period were toxic. No additional chemical analyses were conducted on these water samples, so it is not

clear whether toxicity to amphipods was caused by pyrethroids, but subsequent analyses on the post-treatment water samples showed pyrethroids were present at toxic concentrations in Quail Creek.

Table 1. Average percent survival of *C. dubia* and *H. azteca* in Quail Creek water samples collected before initiation of Landguard treatment (pre-treatment samples). Shaded cells indicate significant toxicity. NA indicates not analyzed.

	C. dubia % Survival		*H. azteca* % Survival	
Sample Date	Mean	SD	Mean	SD
5/14/2013	96	9	NA	NA
5/19/2013	96	9	NA	NA
7/9/2013	53	31	64	13
7/17/2013	100	0	96	5
7/23/2013	96	9	96	5
7/25/2013	96	9	96	9
7/31/2013	NA	NA	10	12
8/2/2013	84	22	NA	NA

Quail Creek Landguard Treatment Monitoring

The flow in Quail Creek during treatment was significantly less than estimated. The average flow rate during treatment was 3.3 L/s, compared to an estimated rate of 15 L/s. The creek had measurable flow approximately 77% of the time, but these flows were clearly less than measured in previous studies indicating reduced tailwater runoff in the creek. Approximately 8.5 million liters of water flowed through the treatment flume, and this water was treated with 856 g of active enzyme. Because creek discharge was less than anticipated, inactive enzyme stock solution that had been in the reservoir 48 hours needed to be discharged and renewed. A total of 1086 g of inactive enzyme was discharged to the creek.

As was observed in the pre-treatment monitoring, minimal water toxicity to *C. dubia* was detected in Quail Creek during the treatment period. Only one of the six water samples collected upstream of the Landguard treatment system between August 16 and September 10, 2013 was toxic to *C. dubia* (September 6, 2013; Table 2). The water sample collected downstream of the treatment system on this date was also toxic to *C. dubia*. Four of the six upstream samples were toxic to *H. azteca* during the treatment period and five of six downstream samples were toxic. Lower survival of *H. azteca* was associated with toxic concentrations of pyrethroid pesticides in Quail Creek.

Pesticides in Quail Creek

ELISA analysis of Quail Creek water sampled during the Landguard treatment period did not detect chlorpyrifos in any of the six samples (data not shown). Analysis of same samples using GC/MS detected chlorpyrifos in one upstream sample (63 ng/L, Table 2), and chlorpyrifos was not detected in the corresponding downstream sample, indicating it was effectively hydrolyzed by Landguard. Two other

organophosphate pesticides were detected in Quail Creek during the Landguard treatment period. Malathion and dimethoate were both detected in two of the six samples, and neither of these pesticides were reduced by Landguard. Concentrations of malathion and dimethoate were compared to published toxicity thresholds for *C. dubia* and *H. azteca*, or surrogate species (*Daphnia magna* for *C. dubia* or *Gammarus lacustis* for *H. azteca*). The 48 hour LC50 for malathion toxicity to *C. dubia* is 2,120 ng/L (Ankley et al., 1991). The 48 hour LC50 for dimethoate toxicity to *Daphnia magna* is 3,120,000 ng/L (Song et al., 1997). The 96 hour LC50 for dimethoate toxicity to *Gammarus lacustis* is 200,000 ng/L (Siemann and Yargeau, 1996). None of the organophosphate pesticides were detected at concentrations that exceeded the toxic effect thresholds for these species.

Several pyrethroids were also detected in the creek: cypermethrin, lambda-cyhalothrin and permethrin. The concentrations of these pyrethroids was not affected by the enzyme treatment system. Lambda-cyhalothrin was detected in all samples, and all detected concentrations were greater than the *H. azteca* EC50 of 2.3 ng/L (Maund et al., 1998). Cypermethrin was detected in samples from a single event at concentrations greater than the *H. azteca* LC50 of 2.3 ng/L (Weston and Jackson, 2009). Permethrin was also detected in all samples, but at concentrations less than the *H. azteca* LC50 of 21.1 ng/L (Anderson et al., 2006). The observed toxicity to *H. azteca* was likely caused by pyrethroids in the creek.

The instantaneous chemical loading for each pesticide was calculated based on the total volume of water in each irrigation event sampled. The six events that were sampled during treatment ranged from approximately 50,000 liters to over 650,000 liters. The only chemical that responded to the enzyme treatment was chlorpyrifos. The upstream concentration was 63 ng/L, and no chlorpyrifos was detected downstream of the treatment. Enzyme treatment reduced the chlorpyrifos loading during that irrigation event from 0.029 grams to zero. The other chemicals were not affected by the treatment, therefore loading was calculated using the average of the upstream and downstream concentrations. Instantaneous loading of dimethoate ranged from 0.016 g to 0.638 g, and loading of malathion ranged from 0.058 g to 0.080 g. The loadings of the three detected pyrethroids were lower with the maxima for lambda-cyhalothrin, cypermethrin and permethrin at 0.017 g, 0.014 g and 0.006 g, respectively.

Moderate discharge in Quail Creek was estimated to range from approximately 3 L/s to 38 L/s (Central Coast Regional Water Quality Control Board, 2011). Average Quail Creek discharge was also previously measured during a period of higher flow in June 2000 and June 2001, and was 357 L/s and 74 L/s, respectively (Anderson et al., 2003). These figures were used in part to estimate the discharge for the current study, but because the treatment period took place later in the summer, the average moderate discharge figures were used. It was assumed that lower creek flows would prevail in late summer with decreased planting and irrigation of crops in the Quail Creek watershed. The average discharge of 3.3 L/s for the current study was on the low end of the moderate range, and a fraction of the discharge measured in 2000 and 2001. It is likely that lower measured discharge in 2013 is indicative of improved irrigation practices in the watershed.

Table 2. Average percent survival of *C. dubia* and *H. azteca* in Quail Creek samples collected upstream and downstream of the Landguard treatment system during the 30 day treatment (treatment samples), and concentrations of detected pesticides. Shaded cells indicate significant toxicity and bold values indicate concentrations less than the reporting limit. ND indicates non detect. Pesticides are as follows: CYH - lambda cyhalothrin, CYP - cypermethrin, PER - permethrin, CHL - chlorpyrifos, DIA - diazinon, DIM - dimethoate, MAL -malathion.

Sample		*C. dubia* % Survival		*H. azteca* % Survival		CYH	CYP	PER	CHL	DIA	DIM	MAL
Date	Station	Mean	SD	Mean	SD	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
8/14/2013	Upstream	80	14	86	5	2.5	ND	**2.6**	ND	ND	320	ND
	Downstream	84	17	44	23	5.7	ND	**3.4**	ND	ND	320	ND
8/20/2013	Upstream	100	0	54	11	8.2	**3.0**	11.3	63	ND	ND	179
	Downstream	100	0	46	9	8.7	**2.9**	11.2	ND	ND	ND	172
8/26/2013	Upstream	96	9	98	4	7.5	ND	**7.7**	ND	ND	ND	ND
	Downstream	100	0	100	0	2.7	ND	**8.8**	ND	ND	ND	ND
8/28/2013	Upstream	96	9	0	0	26.5	ND	**7.4**	ND	ND	ND	ND
	Downstream	96	9	0	0	24.4	ND	**6.6**	ND	ND	ND	ND
9/6/2013	Upstream	0	0	2	4	2.7	ND	15	ND	ND	1,530	137
	Downstream	0	0	0	0	2.8	ND	15	ND	ND	1,470	136
9/10/2013	Upstream	96	9	50	20	6.0	ND	11	ND	ND	427	ND
	Downstream	100	0	62	13	7.0	ND	12	ND	ND	418	ND

Table 3a and 3b. Average percent survival of *C. dubia* and *H. azteca* in water samples and percent survival and growth of *H. azteca* in Salinas River sediment samples collected upstream and downstream of the Quail Creek confluence. Salinas River samples were collected before and after the Landguard enzyme treatment period, and concentrations of detected pesticides. Shaded cells indicate significant toxicity and bold values indicate concentrations less than the reporting limit. ND indicates non detect. Pesticides are as follows: BIF - bifenthrin, CYH - lambda cyhalothrin, CYP - cypermethrin, ESF - (es)fenvalerate, FEN - fenpropathrin, PER - permethrin, CHL - chlorpyrifos, DIM - dimethoate, MAL - malathion.

Pre-Landguard Treatment of Quail Creek													
Sample Date	Water Station	*C. dubia* % Survival Mean	SD	*H. azteca* % Survival Mean	SD	BIF ng/L	CYH ng/L	CYP ng/L	ESF ng/L	PER ng/L	CHL ng/L	DIM ng/L	MAL ng/L
8/2/2013	Upstream	96	9	100	0	ND	ND	ND	ND	**6.5**	ND	ND	ND
	Downstream	92	18	100	0	6.6	**1.3**	**3.0**	ND	**16.1**	ND	ND	ND
	Sediment Station	*H. azteca* % Survival Mean	SD	*H. azteca* Growth (mg) Mean	SD	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g
	Upstream	94	11	0.221	0.054	0.446	0.652	ND	ND	**1.92**	ND	ND	ND
	Downstream	94	7	0.204	0.022	0.746	1.93	0.637	**0.247**	4.52	ND	ND	ND

Post-Landguard Treatment of Quail Creek													
Sample Date	Water Station	*C. dubia* % Survival Mean	SD	*H. azteca* % Survival Mean	SD	BIF ng/L	CYH ng/L	CYP ng/L	FEN ng/L	PER ng/L	CHL ng/L	DIM ng/L	MAL ng/L
9/13/2013	Upstream	100	0	98	4	ND	ND	ND	ND	**1.3**	ND	ND	204
	Downstream	96	9	100	0	ND	ND	ND	ND	ND	ND	ND	222
	Sediment Station	*H. azteca* % Survival Mean	SD	*H. azteca* Growth (mg) Mean	SD	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g
	Upstream	94	7	0.214	0.023	0.864	**0.512**	ND	**0.108**	**2.16**	ND	ND	ND
	Downstream	99	4	0.210	0.035	0.979	1.61	**0.762**	**0.204**	5.65	ND	ND	ND

Pre- and Post-Landguard Treatment Monitoring of the Salinas River

Water samples collected upstream and downstream of the Quail Creek confluence with the Salinas River showed no toxicity to either *C. dubia* or *H. azteca* before or after the Landguard treatment period. In addition, sediment samples collected at both locations were not toxic to *H. azteca* before or after the Landguard treatment (Tables 3a and 3b). Considering the consistent toxicity of Quail Creek water to *H. azteca*, it is likely that the dilution effect of the river greatly reduced the potential for the creek water to cause toxicity in the river samples. The Salinas River confluence is approximately 0.5 km downstream of the Landguard dosing station where Quail Creek water toxicity was detected during the treatment period.

Concentrations of chemicals in the water and sediment samples from Salinas River were generally quite low (Tables 3a and 3b). The only OP detected in the river was malathion in the post-treatment water samples. The upstream and downstream malathion concentrations were similar and indicated that the chemical was not coming from the creek input. Four pyrethroids were detected in the pre-treatment water samples, including bifenthrin, lambda-cyhalothrin, cypermethrin and permethrin. The downstream concentrations were higher than the upstream concentrations, indicating the creek was likely contributing to the load. A similar trend was noted with the sediment concentrations. In addition to the pyrethroids listed above, esfenvalerate was detected in the pre-treatment samples, and fenpropathrin was detected in the post-treatment samples. Sediment pyrethroid concentrations were always higher in the samples downstream of the Quail Creek input. It should be noted that 2013 use data for broccoli crops grown in the Quail Creek watershed showed that a number of newer classes of pesticides were used on broccoli, in addition to pyrethroid pesticides (see discussion below), and this analysis does not include other pesticides used in lettuce and other row crops in this watershed. Of these, the neonicotinoids are of particular concern because of their potential to cause water toxicity and to impact macroinvertebrate communities in receiving systems (Van Dijk et al., 2013). Neonicotinoids were not measured as part of the current study but should be included in future monitoring of central coast watersheds. Previous DPR data has shown frequent detections of the neonicotinoid imidacloprid in Quail Creek (Starner and Goh, 2012).

River discharge was measured during both pre- and post-treatment surveys. The average rate in the river did not change significantly between the surveys (1175 L/s to 1190 L/s). This average fell between the average rate measured at the Chualar USGS gauging station (2237 L/s, approximately 11 km upstream) and the average rate measured at the Spreckels USGS gauging station (680 L/s, approximately 11 km downstream), indicating gradual infiltration as the river approaches the Pacific Ocean. The average discharge rate for Quail Creek was 3.3 L/s, which was diluted by the river flow approximately 350 times. The 856 g of active enzyme and the 1086 g of inactive enzyme were heavily diluted once in the river.

Macroinvertebrate Community Response

Analysis of the Salinas River benthic macroinvertebrate (BMI) community before and after Landguard treatment evaluated whether chronic use of the enzyme affected resident biota after 30 days of treatment. Taxonomic groups in the samples collected downstream of the Quail Creek input were statistically compared to determine if there were significant differences between pre- and post-treatment samples. To investigate whether differences were caused by the enzyme or by changes in chemical inputs from Quail Creek, Salinas River BMIs were further evaluated by examining BMI data from the upstream references site collected both before and after treatment.

Physical habitat was characterized using SWAMP procedures at the upstream reference stations and downstream of the Quail Creek input before and after the Landguard treatment period to determine the potential for this factor to influence BMI community structure. This section of the Salinas River has a very low gradient, and sand is the dominant substrate. There are no cobbles, and therefore, no measure of embeddedness in the physical habitat characterization. Epifaunal substrate/cover at both sites and both times was characterized as marginal with an estimated 25% stable habitat. Sediment deposition was characterized as poor with greater than 80% of the substrate changing frequently as sand moves through the system. Channel alteration was minimal at both the reference and downstream sites before and after the Landguard treatment. Riparian vegetation was significantly heavier at both sites prior to treatment, but there were no differences between upper and lower riparian vegetation during the two sampling times. The in-stream habitat complexity was not significantly different between the upstream and downstream sites, or between the pre- and post-treatments sampling times at the downstream site (Figure 4). No filamentous algae, boulders or artificial structures were observed. The results of these habitat metrics suggest that differences observed in the BMI metrics between pre- and post-treatment sampling periods were not likely influenced by habitat.

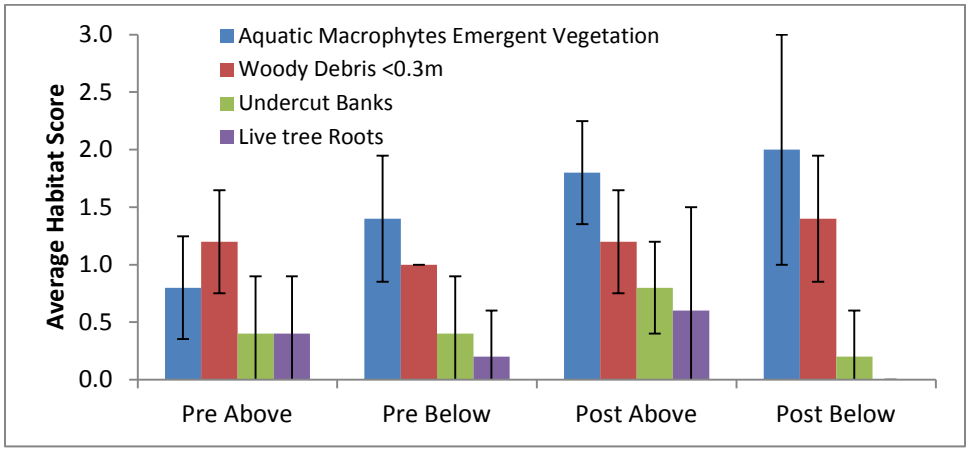

Figure 4. Average habitat scores for four in-stream categories. Error bars indicate the standard deviation of five replicate counts.

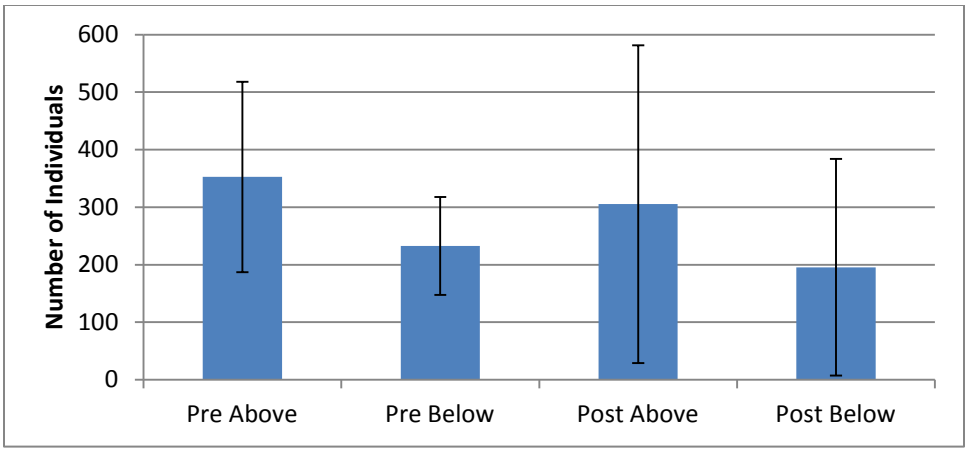

Figure 5. Total number of individuals collected as part of benthic macroinvertebrate surveys. Error bars indicate the standard deviation of five replicate counts.

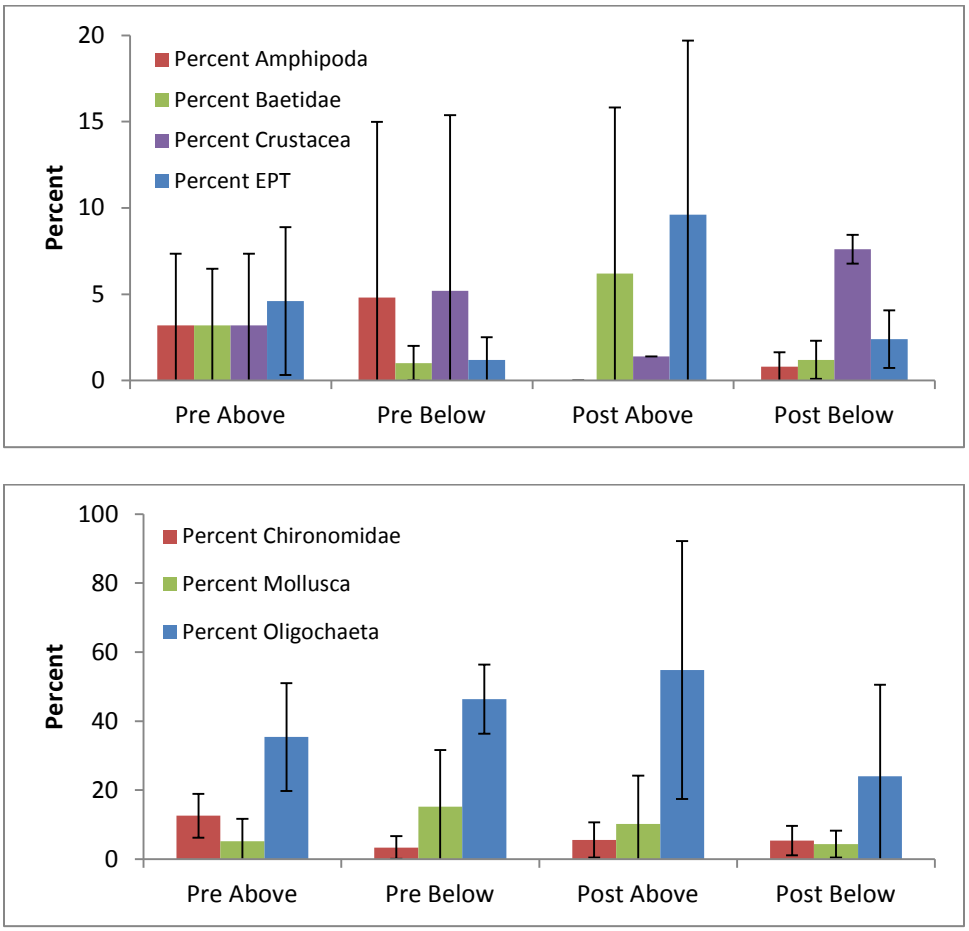

Figure 6a. Percent amphipoda, baetidae, crustacea and ephemeroptera, plecoptera and trichoptera index. 6b. Percent chironomidae, mollusca and oligochaeta. Error bars indicate the standard deviation of five replicate counts.

On average, there were fewer macroinvertebrate individuals in the samples below the Quail Creek input than those above, but these differences were not statistically significant due to high variability between replicate transects (Figure 5). Because there were no significant differences in the in-stream habitat between the upstream and downstream sites, these results indicate that the creek input likely had an influence on the resident biota. Several sensitive and tolerant taxa were grouped into Figures 6a and 6b, respectively. Only chironomids had significantly higher numbers in the upstream pre-treatment samples. There were no other significant differences between upstream and downstream samples, or between pre- and post-treatment samples. These results indicate that chronic treatment of Quail Creek with Landguard A900 had no impact on macroinvertebrate community structure in the Salinas River.

Pesticide Usage Patterns for Broccoli in 2013

Previous studies in Quail Creek in the early 2000s showed that it was consistently contaminated by toxic concentrations of organophosphate pesticides, including diazinon and chlorpyrifos (Hunt et al., 2003; Anderson et al., 2003). The current study showed that diazinon was not detected and chlorpyrifos was rarely detected. Pyrethroid pesticides were detected in all of the Quail Creek water samples during the Landguard treatment period. Lambda cyhalothrin and permethrin were detected in all six samples, and cypermethrin was detected in a single sample. As expected, the pyrethroid concentrations were not reduced by Landguard because the enzyme is only active with specific organophosphate pesticides. In most cases, concentrations of lambda cyhalothrin were sufficient to account for toxicity observed in the corresponding *H. azteca* toxicity tests. Although not specifically the subject of the current study, the observation of greater detections of pyrethroid pesticides in Quail Creek reflects a larger pattern of changing pesticide use in Salinas Valley row crop agriculture.

Analysis of broccoli agriculture in the Quail Creek watershed by the Monterey County Agriculture Commissioner's office showed that broccoli was cultivated on 12 farms in 2013 (data kindly provided by Karen Stahlman, Office of MCAC). Broccoli was emphasized in this analysis because past data has shown that chlorpyrifos has been one of the primary pesticides used to treat cabbage root maggots in this crop. For the purposes of the Landguard study, it was assumed that continued use of chlorpyrifos would allow an evaluation of Landguard efficacy. The usage data shows that chlorpyrifos was only rarely used to treat broccoli in this watershed in 2013. These data do not include other crops grown in this watershed because chlorpyrifos use was thought to be restricted to broccoli. Diazinon and other organophosphates were not highly used due to new regulations (except malathion).

A total of 1,115 acres of broccoli were cultivated in 2013, representing about half the acreage in these 12 farms (2,464 total farm acres). Analysis of the pesticide use reports associated with broccoli production in the watershed showed that 2,135 acres were treated with various pesticides (note that some blocks were treated more than once during 2103 so the total acreage treated exceeded the total acreage in broccoli production). Analysis of reported pesticide use showed that chlorpyrifos was used only once on a small plot in one of the farms (1.75 acres). Use data showed that pyrethroid and neonicotinoid pesticides frequently were used to treat broccoli in 2013, and that a number of newer

classes of pesticides were also frequently used. Five pyrethroids were included in the use reports including cypermethrin (Mustang, Holster), lambda-cyhalothrin (Warrior II), permethrin (Pounce, Perm-up EC), bifenthrin (Brigade, Bifenture, Vetica), and esfenvalerate (Asana XL). Four neonicotinoids were frequently reported including imidacloprid (Prey, Posada, Montana), thiamethoxam (Actara, Platinum), clothianidin (Belay), and actamiprid (Assail). Several newer pesticides dominated the use lists in terms of frequency of use and volumes of active ingredient. These included spinosads (as bait, Seduce) and spinosyn (Radiant), profentofos (Vader), spirotetramat (tetramic acid = Movento), and oxydemeton-methyl (Metasystox). Other frequently used newer pesticides included chlorantranilipole (Coragen) and indoxacarb (Avaunt). Fungicides and herbicides were not emphasized in this analysis but the most commonly used were dacthal, pyraclostrobin and copper hydroxide.

Conclusions

This study was designed to evaluate the effectiveness of the Landguard A900 enzyme to treat organophosphate pesticides in Quail Creek, and to determine whether chronic use of Landguard adversely impacted macroinvertebrates in the Salinas River. Because concentrations of organophosphates were low in Quail Creek, it was not possible to fully evaluate Landguard efficacy. The enzyme effectively treated chlorpyrifos on the one occasion it was detected, however it did not reduce malathion on the occasions when it was detected indicating it is not effective for this organophosphate. Use data showed the organophosphate pesticides were not used on broccoli crops in this watershed in 2013. Our previous studies with Landguard have demonstrated it is very effective at removing chlorpyrifos and diazinon from agriculture runoff in farm scale ditch systems (Anderson et al., 2011; Phillips et al., 2012). A recent CSIRO laboratory study with Landguard confirmed its effectiveness at treating methyl parathion in a system that immobilized the enzyme on nonwoven polyester textiles (Gao et al., 2014).

One important aspect of this study was confirmation that chronic treatment of Quail Creek with Landguard had no discernible effect on Salinas River aquatic invertebrates. Evaluation of benthic macroinvertebrate community structure in the Salinas River showed no statistically significant impact of Landguard after 30 days of treatment of Quail Creek. Based on this, use of this technology should be considered a safe and effective option for treatment of some organophosphate pesticides. There is currently no plan by CSIRO to provide Landguard for use in California, and future plans for this and other enzyme bioremediation products remain unclear. Landguard is not currently being produced anywhere in the world as a remediant, but CSIRO is actively engaged in commercialization discussions in Europe and Asia. CSIRO has retained the bioremediation patent estate with the intention of making enzyme bioremediants available for environmental management in the future (Personal communication, Dr. Colin Scott, CSIRO). In the meantime, a separate project funded by DPR is currently evaluating the use of granulated activated carbon as an alternative to Landguard for removing toxicity associated with chlorpyrifos and other pesticides in irrigation runoff. This project will conclude in 2015.

Analysis of BMI data did indicate lower BMI metrics downstream of the Quail Creek input relative to upstream reference stations and the data suggested these were not due to differences in physical habitat. Lower BMI metrics In the Salinas River downstream of Quail Creek could be associated with contaminants entering the Salinas River from the Creek. Monitoring of Quail Creek showed that it was consistently contaminated with toxic concentrations of pyrethroid pesticides, but pyrethroid concentrations were below acute toxicity thresholds in the Salinas River. Lower pyrethroid concentrations in the Salinas River may have been due to relatively low Quail Creek flows during the course of this study. Broccoli crop use data showed that in addition to pyrethroids, a number of other classes were also used in the Quail Creek watershed in 2013, including several neonicotinoid pesticides. These were not monitored as part of the current study but have been detected in this and other agricultural watersheds in monitoring conducted by DPR's Surface Water Monitoring Program (Starner and Goh, 2012).

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