

**Final Report**    **January 2018**

# SMITH RIVER PLAIN

## Surface Water and Sediment Monitoring Report

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**North Coast Region,**  
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[www.waterboards.ca.gov/swamp](http://www.waterboards.ca.gov/swamp)

**Smith River Plain  
Surface Water and Sediment  
Monitoring Report  
2013 – 2015**

November 15, 2017

**Surface Water Ambient Monitoring Program (SWAMP)  
North Coast Region**

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## EXECUTIVE SUMMARY

In 2013 and 2015 the Regional Water Board implemented a monitoring program to further our understanding of water and sediment quality conditions in the tributaries to the Smith River that flow through the Smith River Plain and to evaluate if the application of agricultural pesticides are impacting the aquatic environment. The monitoring program analyzed surface water samples collected during both wet and dry seasons focusing on standard water quality measures (temperature, dissolved oxygen, conductivity, and pH), nutrients, various pesticides, dissolved copper and zinc, and toxicity.

Throughout the study period, standard water quality measures were observed to be in compliance with water quality objectives, and within acceptable limits for a healthy aquatic ecosystem. While nutrient analysis documented exceedances of the United States Environmental Protection Agency (USEPA) criteria in a number of instances, the concentrations were consistent with similar locations and settings, (i.e. alluvial flood plain and agricultural environment).

The chemical analysis of surface water samples documented the presence of several legacy (used exclusively before 2000) and current use pesticides in the tributaries of the Smith River Plain. In some cases, the concentrations of these pesticides exceeded the lowest USEPA 2014 Aquatic Life Benchmarks for fish and invertebrates. Additionally, dissolved copper (used as a fungicide) was detected in every surface water sample with, 6 of 27 samples exceeding the California Toxics Rule (CTR) Freshwater Aquatic Life Criteria for reproductive and/or acute toxicity.

Toxicity testing documenting the survival (acute toxicity) and reproductive capacity (chronic toxicity) of the test species *Ceriodaphnia dubia* in surface water samples was performed on samples collected from five locations in the Smith River Plain to evaluate if there were any observed negative impacts to the aquatic environment. In 8 of 27 samples, these tests demonstrated statistically significant reductions in reproductivity (positive for chronic toxicity), including three tests in which the “control” location (Upper Rowdy Creek) was positive for chronic toxicity. In another 2 samples, a positive acute toxic response was documented with 1 of the samples demonstrating no test species survival.

To determine the cause of the 2015 observed acute toxic response, three samples that exhibited chronic or acute toxicity were further tested utilizing a toxic identification evaluation (TIE). The TIE results identified two factors responsible for the positive toxic test results: low conductivity and the presence of agricultural chemicals.

The TIEs and associated chemical testing identified that the extremely low hardness of the tributary waters flowing through the Smith River Plain increase the likelihood of a toxic response in the test species utilized for toxicity testing. The prevalence of positive chronic toxicity results in samples collected throughout the study area (except Tilas Slough, which has higher conductivity) including the control site, suggests that the extremely low water hardness and conductivity in the tributaries are interfering with the ability of the test species to reproduce, producing false positives, or toxic responses when toxic conditions do not exist.

Additionally, one of the TIEs identified the presence of both a metal and a non-polar organic compound (pesticide) as the drivers behind the acute toxic response in which there was no test species survival. Chemical analysis of the surface water sample associated with the acute toxic response in 2015 documented that two current use pesticides, imidacloprid and permethrin, were detected in concentrations



exceeding the USEPA's Office of Pesticide Programs Aquatic Life Benchmarks and that dissolved copper concentrations exceeded the CTR Freshwater Aquatic Life Criteria for acute toxicity.

The results of this study demonstrate that chemicals and metals used as pesticides in agricultural activities are being found in low level concentrations in surface waters of the Smith River Plain, and can affect the water quality of the tributaries by contributing to toxicity. Individually the chemicals may not be in concentrations that would produce a toxic response or be directly harmful, but the extremely low hardness and conductivity may act to increase the sensitivity of aquatic life and the associated response to these low level concentrations of contaminants that may be present in the water column.

## INTRODUCTION

This report contains the results and conclusions of a sampling study performed by the North Coast Regional Water Quality Control Board (Regional Water Board) to assess the condition of surface waters (2013 and 2015) in the agricultural areas of the Smith River Plain in Del Norte County, California. Easter lily bulbs are grown in tandem with cattle grazing on the Smith River Plain, a porous 1,900 acre alluvial plain in a high rainfall region. These agricultural operations and associated chemical usage have the potential to affect the quality of both ground waters and surface waters and the beneficial uses of that water including drinking water, aquatic species, wildlife habitat and various agricultural uses. The surface water sampling included stream and sediment sampling for nutrients, metals, pesticides, and toxicity at five sites in four streams.

An Interim report was prepared for the surface water and streambed sediment<sup>1</sup> results in the fall of 2015. This report serves as a final report, incorporating new information for surface water and streambed sediments and finalizing the results previously presented in the 2015 interim report. This report borrows heavily in some sections from the 2015 interim report. However, a considerable amount of background information is contained in that report and was not duplicated here. The reader is referred to the interim reports for additional background:

([http://www.waterboards.ca.gov/water\\_issues/programs/swamp/docs/reglrpts/smith\\_riv\\_plain\\_surface\\_water\\_data\\_rep.pdf](http://www.waterboards.ca.gov/water_issues/programs/swamp/docs/reglrpts/smith_riv_plain_surface_water_data_rep.pdf))

### Surface Water and Stream Sediment Study Overview

The Regional Water Board, in its charge under the California Water Code and the Nonpoint Source Policy, will be developing a mechanism to address water quality concerns from agricultural operations in the area (e.g., waiver, permit, monitoring order). The Smith River Plain Water and Sediment Quality Study was initiated in 2013 to better understand surface water and sediment quality conditions, to help inform development of that mechanism. The surface water and sediment quality portions of the study were funded by the Regional Water Board's Surface Water Ambient Monitoring Program (SWAMP) as a special Regional Water Board study and further augmented by the Regional Water Board's discretionary funding for laboratory analysis.

This report provides a consolidated summary of the results of the 2015 surface water and sediment sampling effort and those of 2013 as presented in the 2015 report. As such, there is considerable duplication of information from the interim report, but the data and findings in this report have been updated to reflect the addition of the 2015 sampling events.

The goals of the surface water and sediment study were to gather and assess data to evaluate surface water and stream sediment conditions in the period of August 2013 to June 2015, inform the development of appropriate conditions and monitoring requirements for a regulatory mechanism (e.g., waiver, permit, monitoring order), and serve as a point of reference for future monitoring results.

The study was intended to answer the following specific questions:

- Are contaminants detected in surface waters and depositional stream sediments in agricultural areas of the Smith River Plain?
- Is sediment toxicity observed in depositional stream sediments located downstream of agricultural land use?
- Is water column toxicity observed in runoff downstream of agricultural land use?
- Is there a relationship between contaminant concentrations and agricultural activities?

Results from related surface water monitoring efforts are also presented in this report and appear in the “Related Monitoring Efforts” section (see SWAMP status and trends monitoring, page 7; SWAMP stream pollution trends monitoring, page 7; and 2010 copper and toxicity sampling, page 8).

## **MONITORING DESIGN**

### **Site Selection and Analyte Rationale**

The Regional Water Board incorporated the full field parameter, analyte list, and sampling protocols for SWAMP into the Smith River Plain Water and Sediment Quality Study and collected all samples in a manner consistent with the SWAMP SPoT Program Quality Assurance Project Plan (SWAMP 2010) and the SWAMP Quality Assurance Program Plan (SWAMP 2008a).

Sample site selection incorporated the protocols established by SWAMP (DFG-MPSL 2007 and MPSL 2009). In addition, the data collection was consistent with the Statewide SWAMP Stream Pollution Trends Monitoring (SPoT) Program (SWAMP 2008b) and the Regional Water Board’s Status and Trends Monitoring Program (Fadness 2013a).

The study was designed to obtain information on the range of constituent concentrations found in waters potentially affected by agricultural discharges and does not provide an investigation of specific sites. Thus, surface water and stream sediment site selection utilized a targeted approach to identify locations at the downstream portion of tributaries draining areas of the Smith River Plain used to grow Easter lily bulbs, based on the following criteria:

- Locations accessible to staff;
- Locations at the base of a watershed;
- Locations with adequate stream flow;
- Locations with available fine-grained depositional sediment;
- Locations amenable to seeing changes in contaminant concentration and effects over time;
- Locations most likely to characterize the accumulation of contaminants draining from agricultural lands.

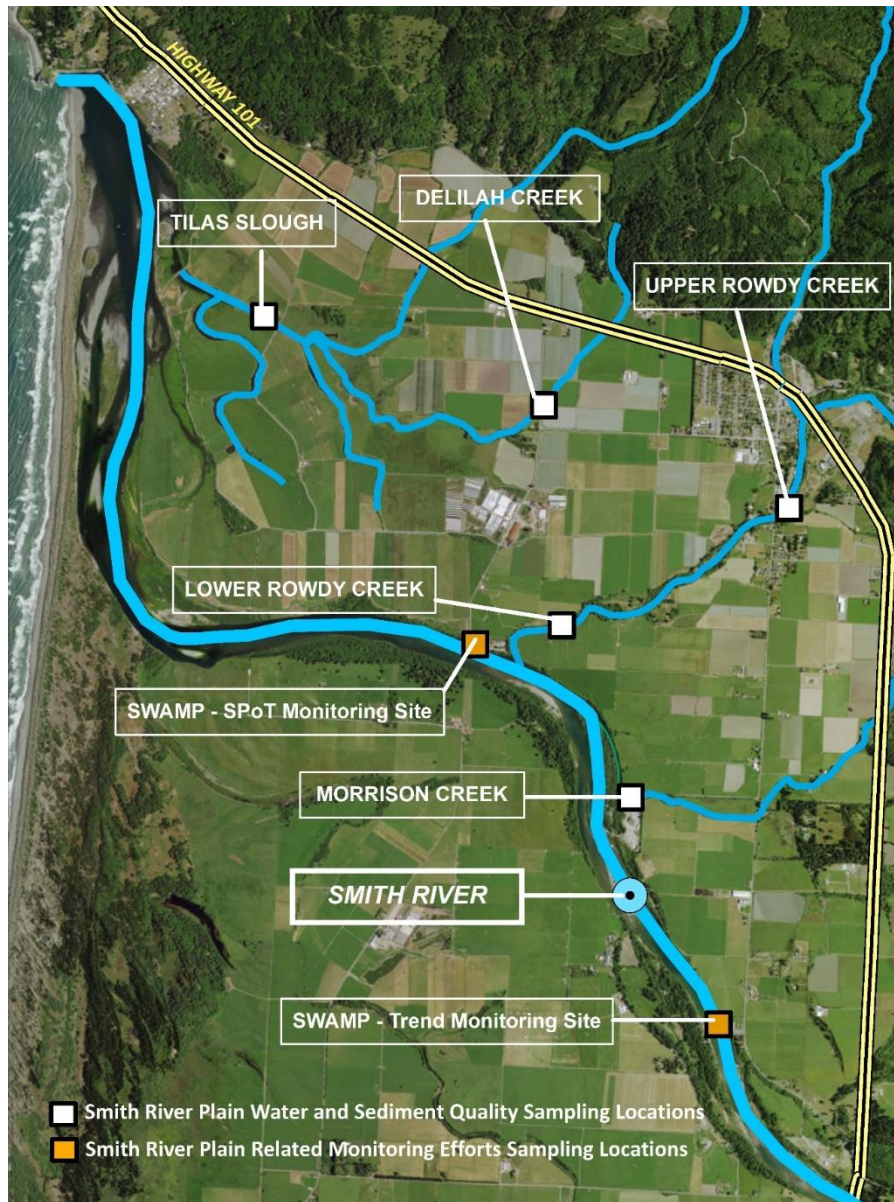


Figure 1. Surface water and sediment sampling sites, August 2013 to June 2015.

Surface water and sediment sampling sites included Delilah Creek, Morrison Creek, Rowdy Creek (upper and lower), and Tilas Slough. The exception to the above criteria was the Upper Rowdy Creek site located at the bridge crossing on Fred Haight Drive, upstream of lily bulb farming activity representing a control site. Two sites were sampled in Tilas Slough based on access and water conditions at the time of sampling. Tilas Slough serves as a collection basin with irrigation water being reused on the fields, sometimes more than once. A tide gate at the lower end of Tilas Slough also functions to regulate the exchange of estuarine waters. Due to the unique nature of this location and the need to sample two different sites upstream of the tide gate depending on the hydrologic dynamics, the two sites separated by 1000 feet are considered to be the same reach and the data generated from both sites were combined. Additionally, a water sample was collected on March 23, 2015 from a roadside ditch that carried a combination of direct lily field runoff and road drainage into Delilah Creek. Overall, five sites were sampled on four streams (see Figure 1) and one sample collected from one roadside ditch with agricultural field connectivity.

## Timing of Sampling Events

Sampling occurred during both the wet and dry seasons. The Regional Water Board collected dry season samples on August 7-8, 2013 and June 24-25, 2015. Wet weather sample collection occurred on October 1-2 and November 5-6, 2013 and March 11-12 and again on March 23, 2015.

All wet weather sampling events were rain-triggered runoff events, initiating sampling when a storm of at least 0.5 inches of rainfall was predicted. The October 2013 sampling event followed a 5-inch rain event during the previous weekend (2 days prior). In November 2013, the sample event followed a rain event on the previous weekend (2 days prior) of approximately 0.3-0.5 inches of rain. March 11 and 12 2015 coincided with a 0.52 inch rain event on March 11. The March 23, 2015 event was preceded by three days of rain totaling 1.64 inches, with an additional 1.68 inches of rain occurring on the sampling date.

Not all stations were sampled during each sample events (Table 1). Upper Rowdy Creek and Morrison Creek were not sampled in August, 2013. A suitable sampling location on Morrison Creek had not been located until the October sampling event. The site at Upper Rowdy Creek was added to the monitoring program as a control site following the documentation of reduced survival in the water column sample collected at the Lower Rowdy Creek sampling site in August, 2013. The Lower Rowdy Creek site was not sampled during the October runoff event as it was deemed unsafe to sample due to the depth and swiftness of the flow.

**Table 1.** Sampling periodicity for the 2013 and 2015 surface water, sediment, and toxicity sampling events.

Location	Aug 7-8, 2013	Oct 1-2, 2013	Nov 5-6, 2013	Mar 11-12, 2015	Mar 23, 2015	Jun 23-24, 2015
<b>Weather</b>	dry	wet	wet	wet	wet	dry
Delilah Creek at Sarina Rd.	P, M, C, T *	P, M, C, T	P, M, C, T	P, M, C, T	P, M, C, T	P, M, C, T (sed P, M, T)
Delilah Creek Roadside Ditch					P, M, C, T	
Morrison creek		P, M, C, T	P, M, C, T (sed M, T)	P, M, C, T	P, M, C, T	(sed P, M, T)
Upper Rowdy Creek (Upstream Location)		P, M, C, T	P, M, C, T	P, M, C, T	P, M, C, T	P, M, C, T (sed P, M, T)
Lower Rowdy Creek (Downstream Location)	P, M, C, T		P, M, C, T (sed M, T)	P, M, C, T	P, M, C, T	P, M, C, T (sed P, M, T)
Tilas Slough At Westbrook Lane		P, M, C, T		P, M, C, T	P, M, C, T	P, M, C, T (sed P, M, T)
Tilas Slough at the Tide Gate	P, M, C, T		P, M, C, T			

\* P = pesticides, M = metals, C = physico-chemical field parameters, T = toxicity, sed = sediment sample

## Analytes

Chemical analysis of surface water included 328 pesticides, 2 heavy metals, nitrogen, phosphorus, and physico-chemical field parameters such as temperature, conductance, and pH. A summary list of analytes is

presented in Table 2, and a more detailed list of pesticide analytes is in Appendix B. The SWAMP program contracted all laboratory analyses to a number of state-owned laboratories. Under these contracts, the Regional Water Board is limited to a specific set of pesticide analytes for which the laboratories are capable and accredited to process. Though the list of pesticides is extensive, it was not entirely inclusive of all chemicals used in the Smith River Plain. For example, the soil fumigants 1,3-Dichloropropene (1,3-D) and metam sodium, including its breakdown product Methyl Isothiocyanate (MITC), were not on the list. To correct the data gap and analyze for these chemicals, the Regional Water Board contracted additional analysis in 2015 to Excelchem Environmental Labs, Rocklin California.

Regional Water Board staff collected standard field parameters using a YSI Datasonde during all site visits, and collected surface water grab samples for the analysis of conventional water quality constituents, water column metals concentrations, pesticides/residues, and water and sediment toxicity using the approved methods previously described.

<b>Table 2. Analytes per Sample Category.</b>	
<b>Field Measurements</b>	
Dissolved Oxygen	pH
Specific Conductivity	Temperature
<b>Conventional Water Chemistry</b>	
Boron	Chloride
Alkalinity as CaCO <sub>3</sub>	Chlorophyll-a
Hardness as CaCO <sub>3</sub>	Ortho-Phosphorous
Ammonia as N	Phosphorous as P (total)
Nitrate as N	Suspended Sediment Concentration
Nitrite as N	Total Dissolved Solids
Nitrogen, Total	Dissolved Organic Carbon
Sulfate	Total Organic Carbon
<b>Dissolved Metals (water)</b>	
Copper	Zinc
<b>Total Metals (sediment)</b>	
Arsenic	Lead
Chromium	Nickel
Copper	Zinc
<b>Organic Chemistry</b>	
Organophosphate Pesticides	Triazine Herbicides
Organochlorine Pesticides	Pyrethroids/Pyrethrins
Polychlorinated Biphenyls	Polycyclic Aromatic Hydrocarbons
Metam-sodium (MITC)	1,3-Dichloropropene (1,3-D)

Water Board staff collected streambed sediment samples on November 5, 2013 at Morrison Creek and Lower Rowdy Creek during base flow or near-base flow conditions and analyzed for metals concentrations and sediment toxicity. Stream sediment samples were collected again in 2015 at all five sampling sites and



analyzed for metals concentrations, pesticides and pesticide residues, Polychlorinated Biphenyls (PCBs), Polycyclic Aromatic Hydrocarbons (PAHs) and sediment toxicity.

## Related Monitoring Efforts

Some related monitoring efforts have occurred separate from the 2013 - 2015 sampling effort both in response to concerns and as part of an ambient monitoring program responsive to furthering the need to understand the water quality in the Smith River Plain. They are summarized below.

### *2001-2012 SWAMP Status and Trends Monitoring Program*

The Status and Trends Monitoring Program was conducted to monitor and assess ambient surface water quality in the watersheds of the North Coast Region to determine if beneficial uses are being protected. This multi-parameter monitoring project was designed to answer the following questions:

- What is the spatial variability of ambient surface water quality in the North Coast Region?
- What is the seasonal variability of ambient surface water quality in the North Coast Region?
- What is the temporal variability or trends of ambient surface water quality in the North Coast Region?
- Is there evidence that beneficial uses are not being protected in the North Coast Region?

Status and Trends sampling sites were located at the base of watersheds to capture water quality conditions influenced by the full watershed (these are known as integrator sites), at the discharge of a major tributary which drains the watershed, and at multiple locations along the main stem usually upstream or downstream of major tributary inputs.

Samples from the Smith River Watershed were collected at the following locations upstream of the Smith River Plain:

- Smith River Upstream of the South Fork Smith River (2001-2012)  
*35 site visits with an average of 5 site visits per year*
- South Fork Smith River upstream of the Smith River (2001-2012)  
*35 site visits with an average of 5 site visits per year*
- Smith River Downstream of the Dr. Fine Bridge (2001-2012)  
*35 site visits with an average of 5 site visits per year*

Regional Water Board staff measured standard field parameters using a Yellow Springs Instrument Company (YSI) 600XL Multi-Parameter Water Quality Sonde and collected grab samples for the analysis of conventional water quality constituents, water column metals concentrations, and organic chemicals, including pesticides and pesticide residues (Table 2 and Appendix A).

### *SWAMP Stream Pollution Trends Monitoring Program*

The Stream Pollution Trends (SPoT) program is a core component of SWAMP and monitors changes in water quality and land use in major California watersheds throughout the state by assessing stream sediment quality. The program is designed to evaluate the effectiveness of regulatory programs and conservation efforts at a watershed scale.

To serve their purpose as integrator sites, SPoT sites are located at the base of large watersheds containing a variety of land uses. Because depositional sediment is needed for sample collection, sites are targeted in

locations with slow water flow and appropriate micro-morphology, to allow deposition and accumulation. In the Smith River watershed, the SPoT program samples at one location in the mainstem:

- Smith River at Sarina Road (2008-2013)  
*Sampled once per year*

SPoT indicators were selected to measure contaminants previously demonstrated to be of concern in California streams. The following sediment indicators were selected: toxicity, organic contaminants (organophosphate, organochlorine, pyrethroid pesticides, and polychlorinated biphenyls (PCBs)), metal contaminants (Ag, Al, As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, and Zn), total organic carbon (TOC), and sediment grain size.

### ***2010 Smith River Plain Copper and Toxicity Sampling***

Questions and concerns expressed by members of the public prompted staff from the California Department of Fish and Wildlife and the Regional Water Board to conduct a one-time sampling event of surface water and sediment samples from tributaries draining the Smith River Plain. Four sites were sampled once each on August 18, 2010. The surface water samples were tested for copper concentrations only, while both the surface water and streambed sediment samples were tested for toxicity. The sampling was conducted during the dry season with the last rain event 68 days prior (June 10, 2010) at the following sites:

- Rowdy Creek Upstream of Smith River (used in the current study, “Lower Rowdy Creek”)
- Rowdy Creek Upstream of Highway 101
- Delilah Creek at Sarina Road (used in the current study, “Delilah Creek”)
- Delilah Creek Upstream of Highway 101



Figure 2. Surface water sampling sites, August 18, 2010.

## STUDY RESULTS

The results of the 2013-2015 study are presented below for both surface waters and streambed sediments along with results from related studies that also shed light on the conditions of the streams in the Smith River Plain.

### Analytical Results – Surface Water

#### *Physico-chemical Field Measurements*

Physico-chemical measurements obtained in the field during each sampling event are summarized by sampling site and period in Table 3. Refer to Table 2 for sampling dates by site.



**Table 3.** Summary of physico-chemical field measurements at the five surface water sampling sites, August, 2013 to June, 2015, expressed as median (range).

Sample Site	Date Range	Number of Measurements	Dissolved Oxygen, mg/L	pH	Specific Conductance, uS/CM	Water Temperature, °C
Delilah Creek	Aug 2013 – June 2015	6	10.3 (9.0-10.7)	7.3 (6.2-7.8)	90 (72-290)	14.2 (10.8-14.3)
Morrison Creek	Oct 2013 – Mar 2015	4	10.5 (8.1-11.8)	7.4 (7.1-7.7)	84 (73-181)	11.0 (9.4-14.3)
Lower Rowdy Creek	Aug 2013 – June 2015	5	10.7 (10.6-12.4)	7.4 (7.2-7.5)	86 (78-96)	11.5 (11.0-14.0)
Upper Rowdy Creek	Oct 2013 – June 2015	5	11.8 (10.4-12.5)	7.4 (7.4-7.5)	85 (73-180)	13.0 (10.9-15.8)
Tilas Slough	Aug 2013 – June 2015	6	5.6* (0.65-15.0)	6.9 (6.6-7.7)	1300 (205-3730)	13.2 (10.1-17.4)

\*The dissolved oxygen minimum objective of 7.0 mg/L was not met in Tilas Slough for four of the six measurements.  
uS/cm = microSiemens per centimeter

The Regional Water Board’s Basin Plan Objectives are presented in Table 4 for reference, although all but pH were not measured at a frequency that allowed calculation of monthly means. However, most parameters were within expected ranges for the water bodies. Dissolved oxygen at Tilas Slough was below the minimum objective in four of the six sampling events and well above the specific conductance thresholds for all sampling events. These results are consistent with conditions that develop within backwater areas such as Tilas Slough that are subject to periodic inundation from overland runoff and estuarine tidal inflow (observed during some of the sampling events).

**Table 4.** Regional Water Board Basin Plan Objectives for Physico-chemical Parameters.

Parameter	Value	Threshold	
Dissolved Oxygen, mg/L	10.0	50% lower limit	50% or more of the monthly means must be more than or equal to 10.0
	7.0	minimum	
Specific Conductance, uS/cm	150	90% upper limit	90% or more of the monthly means must be less than or equal to 150
	125	50% upper limit	50% or more of the monthly means must be less than or equal to 125
pH	8.5	Maximum	
	6.5	Minimum	
Temperature		Varies	

### *Nutrients and Solids Measurements*

Both nitrogen and phosphorus were measured in surface waters, along with suspended sediment and total dissolved solids. The USEPA nutrient criteria for total nitrogen and phosphorus were exceeded in Delilah Creek, Morrison Creek, and Tilas Slough at times during the sampling period, with the highest exceedances in Delilah Creek and Tilas Slough (Table 5).

**Table 5.** Total nitrogen, phosphorus, suspended solids, and dissolved solids ranges in mg/L for the five surface water sampling sites, August, 2013 to June, 2015. Number of exceedances of the USEPA nutrient criteria in parentheses.

Sample Site	Date Range	Number of Samples	Total Nitrogen, mg/L	Total Phosphorus, mg/L	Suspended Sediment, mg/L	Total Dissolved Solids, mg/L
<b>Delilah Creek (# exceedances)</b>	Aug 2013 – June 2015	6	<b>0.990-10.5</b> (6)	0.032- <b>1.25</b> (1)	5.0-68	53-119
<b>Morrison Creek (# exceedances)</b>	Oct 2013 – Mar 2015	4	<b>0.817-1.99</b> (4)	0.023-0.10	2.0-91	42-59
<b>Lower Rowdy Creek</b>	Aug 2013 – June 2015	5	0.200-0.456	0.005-0.017	ND*-8.2	44-57
<b>Upper Rowdy Creek</b>	Oct 2013 – June 2015	5	0.147-0.448	0.008-0.023	ND*-6.4	43-59
<b>Tilas Slough (# exceedances)</b>	Aug 2013 – June 2015	6	<b>0.639-4.52</b> (6)	0.099- <b>0.464</b> (3)	ND*-21.4	109-1700

\*ND = below detection limit

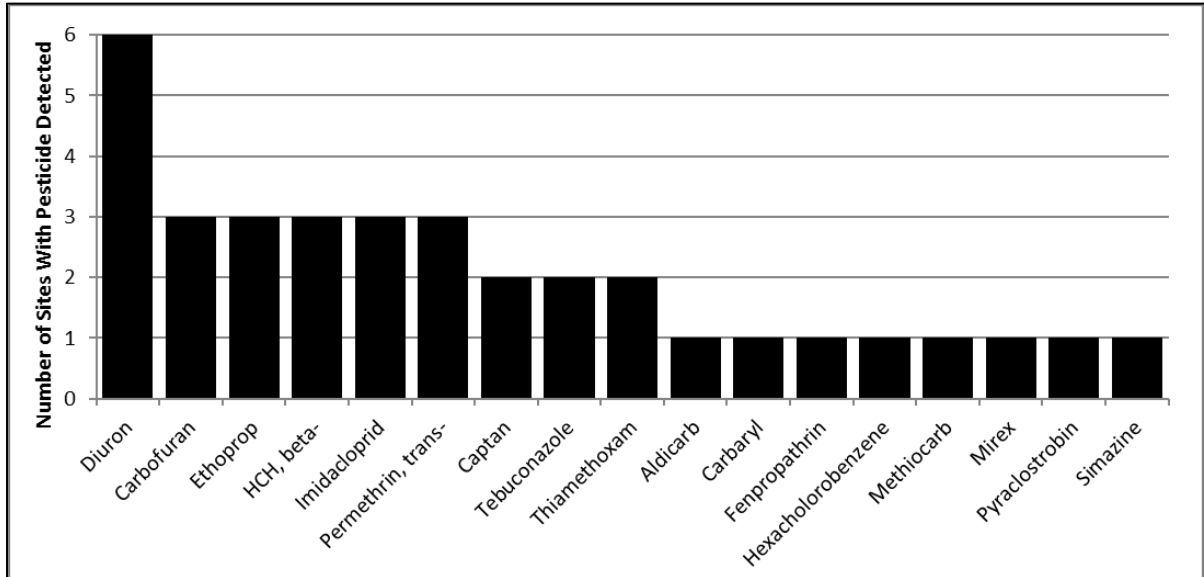
**Red text** indicates nutrient concentrations exceeding maximum USEPA nutrient criteria. Tilas Slough samples exceeded the total phosphorus criterion in three of six samples.

USEPA Ecoregion II Sub-ecoregion 1 nutrient criterion for Total Nitrogen: 0.53 mg/L

USEPA Ecoregion II Sub-ecoregion 1 nutrient criterion for Total Phosphorus: 0.325 mg/L  
mg/L = milligrams per liter

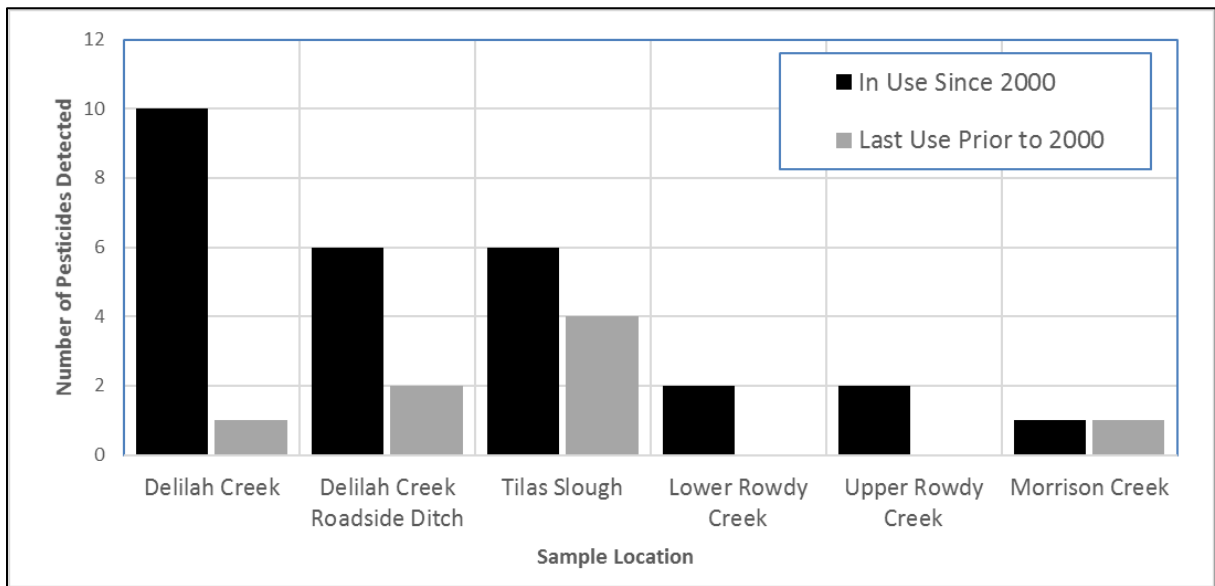
### *Pesticides*

A total of 17 detected pesticides (including isomers and degradants) were detected in surface waters in the Smith River Plain for samples collected from August, 2013 to June, 2015. The most commonly detected were diuron at all five sites and carbofuran at three of five sites, (Figure 2). Pesticide concentrations from the 2013 - 2015 surface water samples from this study met water quality thresholds at three of the five sites. The exceptions were in Delilah Creek and the Delilah Creek roadside ditch where diuron exceeded USEPA Office of Pesticide Programs (OPP) Freshwater Aquatic Life Benchmarks for Pesticide Registration on March 23, 2015.



**Figure 3.** Number of surface water sites with pesticide detections from August 2013 to June 2015.

Detected pesticides included legacy pesticides for which the last recorded use was prior to 2000 and more recently used pesticides that have been in use after 2000, (as per California Department of Pesticide Regulation (DPR) records). The highest number of detections of individual pesticides were in Delilah Creek, followed by Tilas Slough (Figure 3).



**Figure 4.** Number of individual pesticides detected at each of five surface water sampling sites in the Smith River Plain from August, 2013 to June, 2015.

Maximum pesticide concentrations from the 2013-2015 sampling period are presented in Table 6 along with available criteria and thresholds for aquatic life.

**Table 6.** Maximum concentrations of 17 detected pesticides (including isomers and degradants) and associated thresholds (criteria or standards) at five surface water and one roadside ditch sampling sites from August, 2013 to June, 2015, Smith River Plain.

Analyte, ug/L	Last Use per CaDPR*	Delilah Creek	Morrison Creek	Lower Rowdy Creek	Upper Rowdy Creek	Tilas Slough	Delilah Roadside Ditch	Threshold (ug/L)	Reference	
Aldicarb	**	ND	0.01	ND	ND	ND	ND	3	1	
Captan	2012	1.6	ND	0.277	ND	ND	ND	15	2	
Carbaryl	2013	0.087	ND	ND	ND	ND	ND	2.1	3	
Carbofuran	2009	0.008	ND	ND	0.021	0.007	ND	18	4	
Diuron	2015	57.7	0.124	0.02	0.003	3.45	39.4	26.4	5	
Ethoprop	2015	0.183	ND	ND	ND	0.158	0.019	22	5	
Fenpropathrin	**	ND	ND	ND	ND	0.0003	ND	180	6	
Hexachlorobenzene	**	ND	ND	ND	ND	0.001	ND	1	1	
Imidacloprid	2015	3.56	ND	ND	ND	2.49	2.17	1.05	5	
Lindane (HCH)	- alpha	1998	ND	ND	ND	ND	0.007	ND	0.95	7
	- beta		0.012	ND	ND	ND	0.005	0.063		
	- gamma		0.003	ND	ND	ND	0.002	0.022		
Methiocarb	2015	ND	ND	ND	ND	0.022	ND	0.1	5	
Mirex	**	ND	ND	ND	ND	ND	0.006	0.001	5	
Permethrin	-cis	2015	0.0024	ND	ND	ND	ND	0.0014	5	
	-trans	2015	0.0054	ND	ND	ND	0.0031 0.0112			
Pyraclostrobin	2010	0.0004	ND	ND	ND	ND	ND	3.1	5	
Simazine	1999	ND	ND	ND	ND	0.002	ND	4	1	
Tebuconazole	2015	7.13	ND	ND	ND	ND	13.2	12.0	5	
Thiamethoxam	2015	1.86	ND	ND	ND	ND	1.1	17.5	5	

\* "Last use per CaDPR" - 2015 is the most recently available information.

\*\* No reported use 1990-2015

ND = non-detect

Red text denotes pesticide values exceeding a criteria or threshold

ug/L = micrograms per liter

Criteria and threshold references, per Marshack (2016)

- 1: USEPA Drinking Water Standards Maximum Contaminant Levels (MCLs)
- 2: California Department of Public Health Notification and Response Levels
- 3: USEPA National Recommended Water Quality Criteria – Freshwater Aquatic Life Protection
- 4: Water Quality Control Plan for the North Coastal Basin
- 5: USEPA 2017 Aquatic Life Benchmarks (lowest value, see Table 7 below)
- 6: USEPA IRIS Reference Dose (drinking water)
- 7: California Toxics Rule (CTR)

USEPA developed Aquatic Life Benchmarks for freshwater species that are based on toxicity values reviewed by the USEPA and used in the USEPA's risk assessments developed as part of the decision-making process for pesticide registration. These are presented in Table 7. The benchmarks that were exceeded in this study are in red text. These benchmarks are not regulatory criteria or standards, rather benchmarks for use in ecological risk assessment in the pesticide registration process.

**Table 7.** USEPA Office of Pesticide Programs Aquatic Life Benchmarks (µg/L).

Pesticide	Fish		Invertebrates	
	Acute	Chronic	Acute	Chronic
Aldicarb	26	0.46	10	1
Captan	13.1	16.5	4200	560
Carbaryl	0.85	0.5	660	1500
Carbofuran	44	5.7	1.115	0.75
Diuron	200	<b>26.40</b>	80	200
Ethoprop	150	24	22	0.8
Fenpropathrin	1.1	0.091	0.265	0.064
Imidacloprid	> 41500	1200	34.5	<b>1.05</b>
Lindane (HCH)	0.85	2.9	0.5	54
Methiocarb	218	50	3.5	0.1
Permethrin	0.395	0.0515	<b>0.0106</b>	<b>0.0014</b>
Pyraclostrobin	3.1	2.35	7.85	4
Simazine	3200	960	500	40
Tebuconazole	1135	<b>12</b>	1440	120
Thiamethoxam	> 50000	20000	17.5	50000

USEPA. 2017. Aquatic Life Benchmarks. USEPA Office of Chemical Safety and Pollution Prevention (OCSPP) <https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/aquatic-life-benchmarks-pesticide-registration>

**Red text** denotes an exceedance observed in this study.

### *Pesticides – Status and Trends Monitoring Program 2001-2012*

Pesticide analysis was completed for all three Status and Trends locations in the Smith River Watershed. With the exception of the South Fork Smith River, the maximum concentrations of detected pesticides for each sampling site in the Smith River Watershed, as sampled by the SWAMP Status and Trends Program from 2001 to 2012, met thresholds and are presented in Table 8.

**Table 8.** Maximum concentrations of detected pesticides at three Smith River sampling sites from the SWAMP Status and Trends Monitoring Program, 2001-2012.

Analyte, ug/L	Last Use per CaDPR	South Fork Smith River	Lower Smith River	Upper Smith River	Threshold µg/L	Reference
<b>February 2002 Sampling Event</b>						
Endosulfan Sulfate	2001	0.0010	0.0010	0.0010	<b>0.056</b>	3
Chlordane	Legacy or No Reported Use 1990-2008	ND	0.0010	ND	<b>0.0043</b>	3
Dioxathion		ND	0.0300	ND	<i>No Thresholds</i>	
Fonofos		0.0300	ND	ND	<b>10.0</b>	5
Heptachlor Epoxide		ND	ND		<b>0.0038</b>	3
Hexachlorobenzene		0.0007	0.0007	0.0007	<b>1.0</b>	1
Methoxychlor		ND	ND		<b>0.03</b>	3
<b>April 2006 Sampling Event</b>						
Dimethoate	2005	0.0400	ND	ND	<b>1.4</b>	5
<b>February 2002 – November 2006 Sampling Events</b>						
Diazinon	2008	0.220	0.0210	0.0290	<b>0.05</b>	3

Note: Reference codes are the same as for Table 6 above; ND = non-detect.

### Metals

Surface water samples were analyzed for two metals: copper and zinc. Copper and zinc are naturally prevalent in the Smith River Watershed, but are relevant to this study since pesticide compounds that include copper and zinc are also applied to the agricultural fields of the Smith River Plain at various times throughout the year.

Copper and zinc were analyzed for the dissolved fraction because the dissolved fraction of the metal is more bioavailable and therefore more likely to negatively affect aquatic organisms. However, metals that are not dissolved can also become bioavailable under lower pH and lower dissolved oxygen conditions. The only reach where those conditions are likely to occur is at Tilas Slough.

The toxicity to aquatic organisms by copper and zinc in surface water is dependent upon the concentration of each metal and the hardness of the surface water. Metals toxicity increases as water hardness decreases, which means at a given concentration, copper or zinc will have a more pronounced negative affect on aquatic life at a lower water hardness level. Dissolved zinc was detected in samples from Delilah Creek on March 11 and 23, 2015, in Morrison Creek on March 12, 2015, in the Delilah Creek roadside ditch on March 23, 2015, and in Tilas Slough on March 12 and 23 and June 23, 2015 (Table 10).

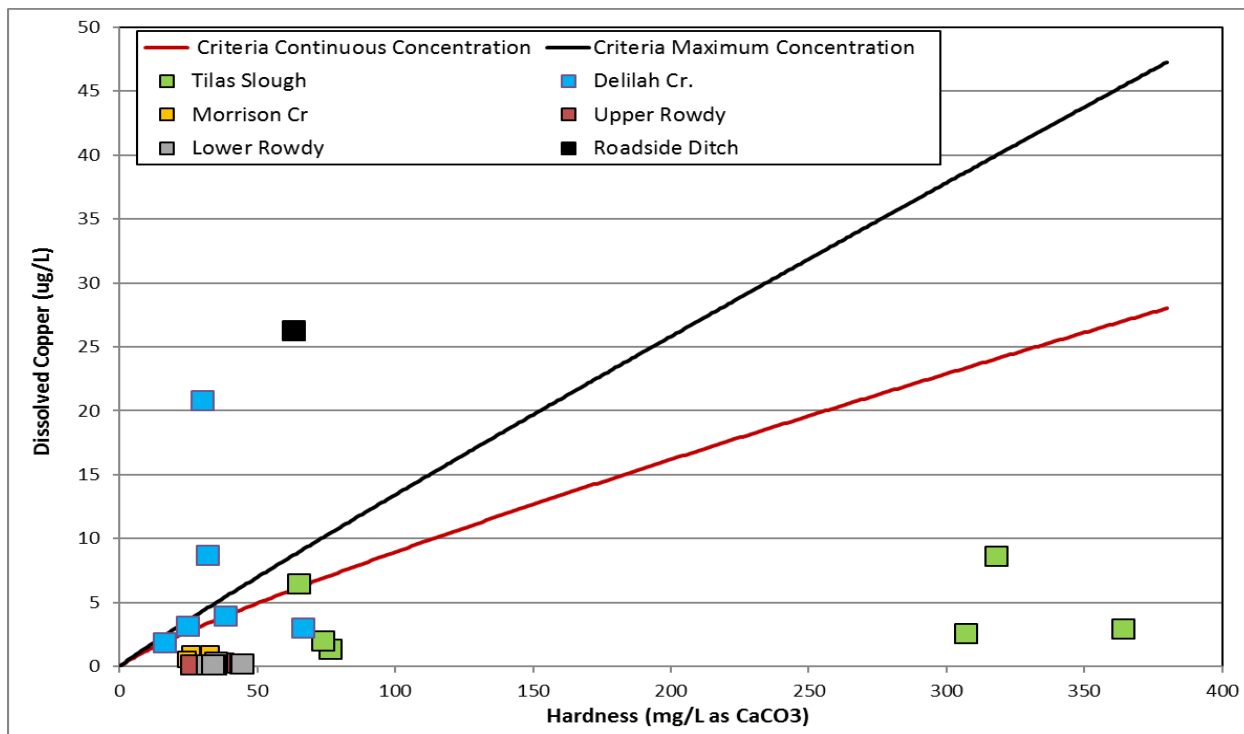
The CTR Freshwater Aquatic Life Criteria contains two standards—the criterion maximum concentration (CMC), which is the acute standard defined as the highest concentration of a pollutant to which aquatic life can be exposed for a short period of time without deleterious effects, and the criterion continuous concentration (CCC), which is the chronic standard defined as the highest concentration of a pollutant to which aquatic life can be exposed for an extended period of time without deleterious effects. For both copper and zinc, the CMC and CCC are defined as a 1-hour average and a 4-day average, respectively.

**Table 9.** Dissolved zinc and hardness values from five surface water and one roadside ditch site collected from August, 2013 to June, 2015.

Sample Site	Dissolved Zinc (ug/L) and Hardness (mg/L) by date displayed as Zinc (hardness)					
Example:	2.50 (20.0)	means	2.50 ug/L dissolved zinc and hardness of 20.0 mg/L			
Sample Dates →	Aug 7-8 2013	Oct 1-2 2013	Nov 5-6 2013	Mar 11-12 2015	Mar 23 2015	Jun 23-24 2015
Delilah Creek	ND (38.1)	ND (66.6)	ND (24.6)	1.03 (137)	0.71 (30.1)	ND (16.4)
Delilah Creek Roadside Ditch	No Sample	No Sample	No Sample	No Sample	1.22 (63)	No Sample
Morrison Creek	No Sample	ND (25.6)	ND (32.6)	0.59 (24.4)	ND (32.7)	No Sample
Lower Rowdy Creek	ND (35.0)	No Sample	ND (34.3)	ND (32.0)	ND (44.4)	ND (33.8)
Upper Rowdy Creek	No Sample	ND (37.2)	ND (34.0)	ND (26.3)	ND (44.1)	ND (32.4)
Tilas Slough	ND (76.4)	ND (65.0)	ND (73.6)	1.79 (307)	2.90 (318)	0.94 (364)

ND = non-detect

Low levels of dissolved copper were detected in every surface water sample collected as part of the Smith River Plain Water and Sediment Quality Study (Table 10). Four dissolved copper concentration/hardness pairs exceeded the criterion continuous concentration (CCC) of the CTR Freshwater Aquatic Life Criteria in Delilah Creek on August 7 and November 5, 2013, March 11, 2015, and March 23, 2015 (Table 11, Figure 5). Tilas Slough had one dissolved copper concentration/hardness pair that exceeded the CCC of the CTR Freshwater Aquatic Life Criteria on October 1, 2013 (Table 10, Figure 4). In addition, the roadside drainage ditch that flows into Delilah Creek was sampled during the rainfall event of March 23, 2015, however hardness analysis was not completed for that sample, but analyzed as part of the toxicity testing. These low level exceedances alone are not indicative of an environment that may lead to reduced reproduction or survival. However, acute toxicity was observed in the sample from Delilah Creek on March 11, 2015. See the toxicity section for additional discussion on toxicity results.



**Figure 5.** Dissolved copper concentrations and hardness in all surface water samples collected from August 2013 to June 2015.

**Table 10.** Dissolved copper and hardness values from five surface water and one roadside ditch site collected from August, 2013 to June, 2015.

Sample Site	Dissolved Copper (ug/L) and Hardness (mg/L) by date displayed as Copper (hardness)					
	Aug 7-8 2013	Oct 1-2 2013	Nov 5-6 2013	Mar 11-12 2015	Mar 23 2015	Jun 23-24 2015
Example:	2.03 (20.0)	means	2.03 ug/L dissolved copper and hardness of 20.0 mg/L			
Delilah Creek	<b>3.96</b> (38.1)	3.03 (66.6)	<b>3.20</b> (24.6)	<b>8.71</b> (31.9)	<b>20.8</b> (30.1)	1.86 (16.4)
Delilah Creek Roadside Ditch	No Sample	No Sample	No Sample	No Sample	<b>26.3</b> (63.0)	No Sample
Morrison Creek	No Sample	0.99 (25.6)	0.94 (32.6)	0.59 (24.4)	1.02 (32.7)	No Sample
Lower Rowdy Creek	0.39 (35.0)	No Sample	0.16 (34.3)	0.18 (32.0)	0.24 (44.4)	0.17 (33.8)
Upper Rowdy Creek	No Sample	0.28 (37.2)	0.17 (34.0)	0.13 (26.3)	0.22 (44.1)	0.17 (32.4)
Tilas Slough	1.36 (76.4)	<b>6.50</b> (65.0)	2.06 (73.6)	2.62 (307)	8.64 (318)	2.98 (364)

**Red text** indicates exceedance of CTR Freshwater Aquatic Life Criteria for reproductive and/or acute toxicity.

### Metals - 2010 Smith River Plain Copper and Toxicity Sampling

Four sites were sampled for metals on August 18, 2010 (Table 11). The copper concentration /hardness pair at Delilah Creek exceeded both the CCC and the CMC of the CTR Freshwater Aquatic Life Criteria (Figure 5),

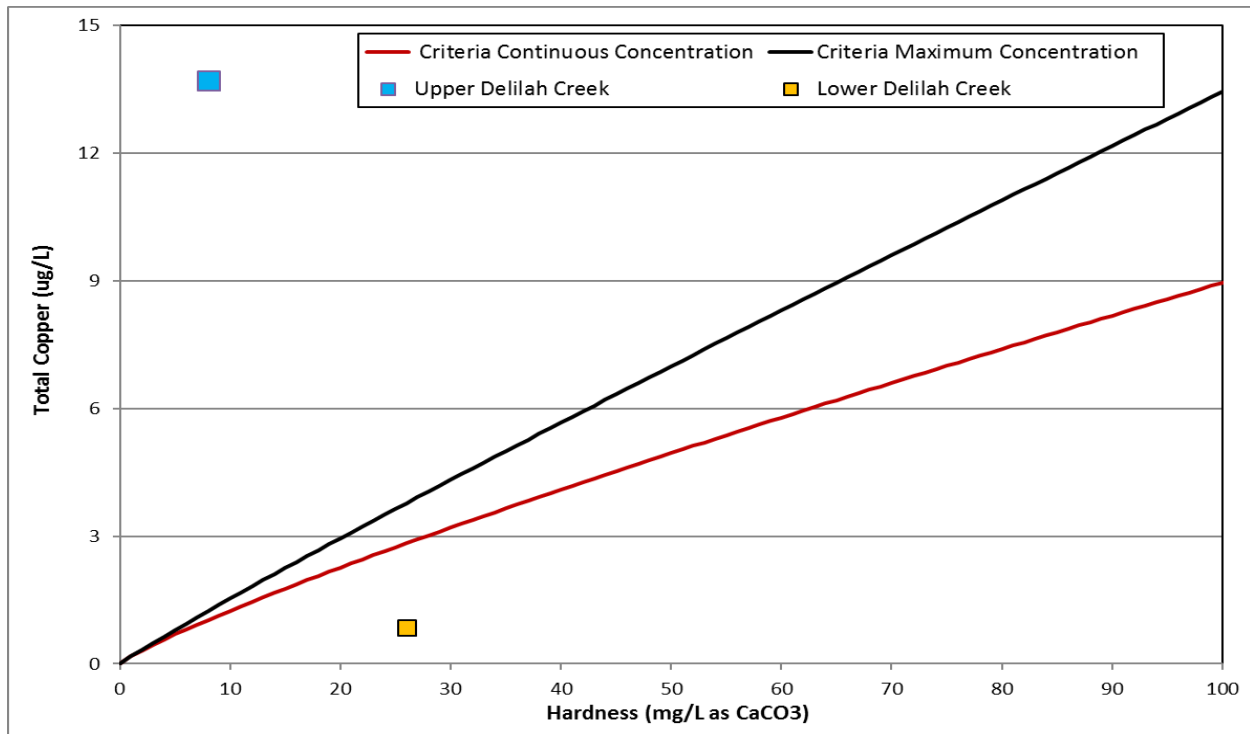


and the sample demonstrated reduced reproductivity capacity when compared to the laboratory control. See the toxicity section for additional discussion (pg. 24).

**Table 11.** Copper and hardness concentrations in Delilah and Rowdy Creek samples from August 18, 2010.

Sample Site	Total Copper, µg/L	Dissolved Copper, µg/L	Hardness, mg/L
Delilah Cr.-upper	0.85	0.36	26
Delilah Cr.-lower	13.7	3.99	8.0
Rowdy Cr.-upper	0.60	0.53	NA
Rowdy Cr.-lower	0.94	0.58	NA

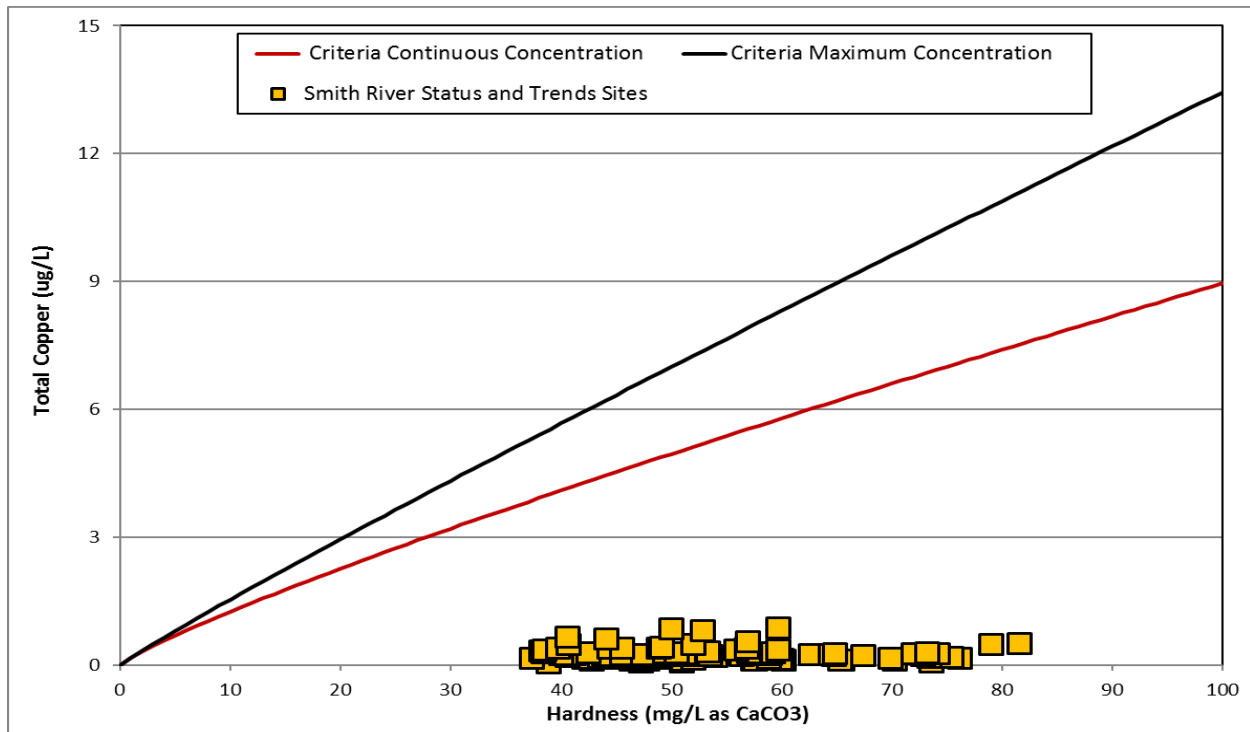
Red text indicates exceedance of the CTR Freshwater Aquatic Life Criteria.



**Figure 6.** Total copper concentrations and hardness in surface water samples from upper and lower Delilah Creek on August 18, 2010, Smith River Plain.

### *Metals - Status and Trends Monitoring Program 2001-2012*

Total metals analysis was completed for all three Status and Trends monitoring locations in the Smith River Watershed. The total copper concentration and hardness results did not exceed the CTR Freshwater Aquatic Life Criteria for reproductive or acute toxicity at any of the three sites sampled as part of the Status and Trends Monitoring Program 2001-2012 (Figure 6).



**Figure 7.** Total copper concentrations and hardness in surface water samples from the Smith River Watershed collected by the SWAMP Status and Trends Monitoring Program.

## Analytical Results – Stream Sediments

### Metals

The agricultural industry utilizes copper and zinc based compounds as fungicides during various times of the year. The Regional Water Board collected streambed sediment samples for analysis of various metals, (arsenic, chromium, copper, lead, nickel, and zinc), in Morrison Creek and Lower Rowdy Creek in 2013 and at all five surface water sites in 2015 (Table 12). In addition, the SWAMP SPoT program collected one sediment sample in the Smith River at Sarina Road each year from 2008-2013 and 2015 (Table 13).

The concentration of chromium exceeded the USEPA Probable Effects Concentration (PEC) criterion in Morrison Creek in 2013 and in 2015 in Morrison Creek, Upper Rowdy Creek, Lower Rowdy Creek, and Tilas Slough. Probable Effect Concentration (PEC) is the concentration level of an analyte that is likely to cause a biologically adverse effect if exceeded. The concentration of nickel exceeded the USEPA PEC criterion in Morrison Creek in 2013 and at all five sites in 2015.

Though the concentrations exceeded the USEPA PEC values, toxicity testing did not demonstrate reduced survival in the sediment samples from the Smith River Plain.

Chromium and nickel are not utilized in the production of lily bulbs or cattle ranching, both agricultural uses in the watershed. The presence of these metals is likely a result of the underlying geology of the Smith River watershed.

**Table 12.** Metals concentrations in stream sediments for the five surface water sampling sites in mg/Kg dry weight, August, 2013 and June, 2015.

ANALYTE (Total)	Morrison Creek		Lower Rowdy Creek		Delilah Creek	Upper Rowdy Creek	Tilas Slough	USEPA Criteria
	2013	2015	2013	2015	2015			
Arsenic	7.46	8.14	ND	7.14	8.83	8.27	6.42	33
Chromium	115	133	ND	136	102	150	402	111
Copper	38.9	45.5	ND	30	90.2	36.2	57.7	149
Lead	9.62	9.87	ND	7.01	14.00	8.43	7.56	128
Nickel	77.3	94.8	ND	104	107	130	294	48.6
Zinc	91.1	87.7	ND	68.5	86.3	87.3	87.7	459

ND = non-detect; **Red text** denotes exceedance of USEPA PEC criterion.

The USEPA PEC criterion for chromium and zinc was exceeded in every sample collected in the Smith River at Sarina Road from the SPoT program (Table 13). Reduced survival was observed in toxicity testing at that site in 2010 only. However, the SPoT program did not identify the cause of the reduced survival.

**Table 13.** Metals concentrations in stream sediments in mg/Kg dry weight for the Smith River at Sarina Road from the SWAMP SPoT sampling program, 2008-2013 and 2015.

ANALYTE (Total)	Smith River at Sarina Road (SWAMP SPoT Program)							USEPA Criterion (PEC)
	2008	2009	2010	2011	2012	2013	2015	
Arsenic	4.79	6.74	4.71	5.00	4.53	6.00	5.10	33
Chromium	288	394	277	342	277	346	279	111
Copper	38.4	34.2	32.8	39.2	35.9	40.6	34.5	149
Lead	5.98	5.79	6.26	9.28	5.07	6.29	6.02	128
Nickel	339	336	283	299	260	340	277	48.6
Zinc	67.9	69.9	67.2	88.9	76.1	90.9	71.0	459

**Red text** denotes exceedance of USEPA PEC criterion.

### *Pesticides and Polycyclic Aromatic Hydrocarbons (PAHs)*

Analysis for the full suite of organic chemicals in stream sediments was conducted in June of 2015 at four of the sites. While a number of organic compound pesticides were detected, the only pesticide currently reported as used in the Smith River Plain was permethrin (Table 14). Presence of other chemicals is likely the result of legacy use, as chemicals may be sequestered in stream sediments for a number of years.

**Table 14.** Pesticide detections and concentrations in stream sediment samples from five surface water sampling sites collected on June 23 & 24, 2015 (mg/Kg, dry weight).

Analyte		Delilah Creek	Morrison Creek	Rowdy Creek		Tilas Slough
				Lower	Upper	
<b>Current Use Pesticides</b>						
Permethrin	-cis	<b>2.92</b>	<i>ND</i>	<i>ND</i>	<i>ND</i>	<i>ND</i>
	-trans	<b>DNQ</b>	<i>ND</i>	<i>ND</i>	<i>ND</i>	<i>ND</i>
<b>Legacy Use Pesticides</b>						
Aldrin		<b>53.3</b>	<b>DNQ</b>	<i>ND</i>	<i>ND</i>	<b>4.11</b>
DDTs	DDD(o,p')	<b>DNQ</b>	<i>ND</i>	<i>ND</i>	<i>ND</i>	<i>ND</i>
	DDD(p,p')	<b>DNQ</b>	<i>ND</i>	<i>ND</i>	<i>ND</i>	<i>ND</i>
	DDE(p,p')	<b>1.32</b>	<i>ND</i>	<i>ND</i>	<i>ND</i>	<i>ND</i>
	DDT(p,p')	<b>DNQ</b>	<i>ND</i>	<i>ND</i>	<i>ND</i>	<i>ND</i>
Lindane (HCH)	-alpha	<b>3.36</b>	<b>DNQ</b>	<i>ND</i>	<i>ND</i>	<i>ND</i>
	-beta	<b>5.16</b>	<b>DNQ</b>	<i>ND</i>	<i>ND</i>	<b>DNQ</b>
	-gamma	<b>6.24</b>	<b>0.81</b>	<i>ND</i>	<i>ND</i>	<b>DNQ</b>
Hexachlorobenzene		<b>3.09</b>	<b>DNQ</b>	<i>ND</i>	<i>ND</i>	<i>ND</i>

ND = Non-Detect. DNQ = Detected but not Quantified

There are approximately 100 different known polycyclic aromatic hydrocarbons (PAHs) in air, soil, foodstuffs, and water (Zedeck 1980). PAHs are not synthesized chemically for industrial purposes, the major source of PAHs is the incomplete combustion of organic material such as coal, oil and wood and are found in motor vehicle exhaust as well (Cherng et al. 1996 and Lewtas 1997). Specific PAHs also are used in the manufacture of dyes and pigments, plastics, pharmaceuticals, pesticides (noted as “other or inert ingredients” on pesticide labels), wood preservatives and agrochemicals. In addition, asphalt used for road construction and roofing tar may also contain PAHs.

Due to their wide use in many applications and as byproducts of combustion (including fossil fuels and forest fires), PAHs are ubiquitous in the environment. Polycyclic aromatic hydrocarbons (PAHs) were detected in all five locations in low level concentrations well below any effect levels (Table 15).

**Table 15.** PAH detections and concentrations in stream sediment samples from five sampling sites collected on June 23 & 24, 2015 (mg/Kg, dry weight).

Analyte	Delilah Creek	Morrison Creek	Rowdy Creek		Tilas Slough
			Lower	Upper	
Polycyclic Aromatic Hydrocarbons - PAHs					
Anthracene	ND	ND	ND	ND	ND
Benz(a)anthracene	ND	DNQ	ND	ND	ND
Biphenyl	DNQ	DNQ	DNQ	DNQ	DNQ
Chrysene	2.83	5.91	DNQ	DNQ	ND
Fluoranthene	4.95	8.55	DNQ	DNQ	DNQ
Benzo(b)fluoranthene	4.48	10.2	6.7	5.95	DNQ
Benzo(k)fluoranthene	ND	DNQ	ND	ND	ND
Methylfluoranthene, 2-	ND	DNQ	ND	ND	ND
Fluorene	ND	DNQ	2.6	DNQ	DNQ
Methylfluorene, 1-	ND	DNQ	DNQ	DNQ	DNQ
Naphthalene	DNQ	DNQ	DNQ	ND	DNQ
Dimethylnaphthalene, 2,6-	ND	DNQ	DNQ	DNQ	19.8
Methylnaphthalene, 1-	DNQ	DNQ	DNQ	DNQ	DNQ
Methylnaphthalene, 2-	DNQ	DNQ	DNQ	DNQ	DNQ
Perylene	DNQ	DNQ	DNQ	DNQ	29.8
Benzo(g,h,i)perylene	DNQ	8.63	DNQ	DNQ	DNQ
Phenanthrene	5.78	8.25	7.16	4.78	5.23
Methylphenanthrene, 1-	DNQ	DNQ	ND	ND	DNQ
Pyrene	3.15	8.96	2.96	DNQ	DNQ
Benzo(a)pyrene	ND	DNQ	ND	ND	ND
Benzo(e)pyrene	DNQ	DNQ	DNQ	DNQ	DNQ
Indeno(1,2,3-c,d)pyrene	ND	DNQ	ND	ND	ND

ND = Non-Detect. DNQ = Detected but not Quantified

### Toxicity Testing Results – Surface Water

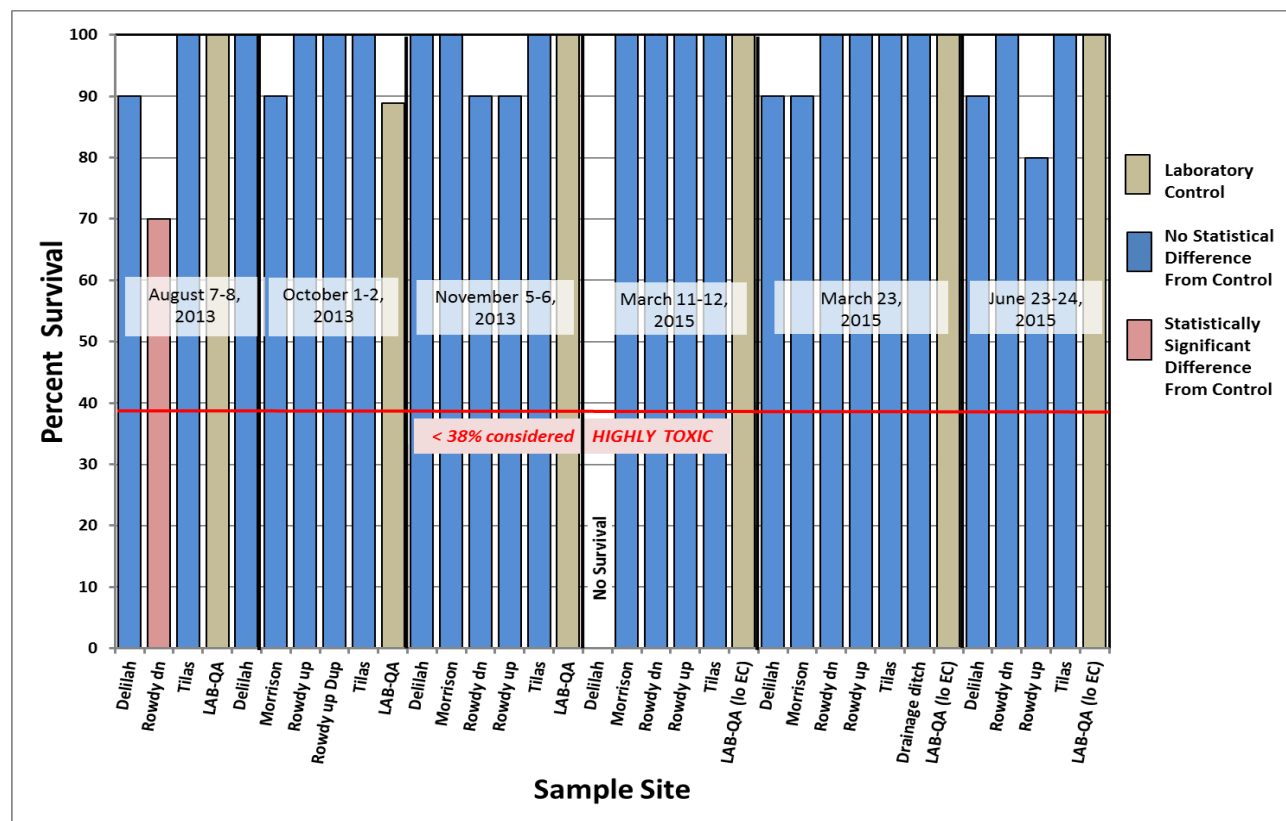
Low conductivity and low hardness water such as that in the tributaries of the Smith River Plain can negatively affect the reproduction rates of *C. dubia* in the testing environment. When the test species are reared in water with conductivity and hardness levels that are greater than the sample water their reproductive rates can be adversely affected. After observing chronic toxicity results in the absence of any documented toxicants and to examine the potential issue of false positive toxicity results (reduced reproductivity rates observed as a result of the low conductivity alone), an additional set of controls in which *C. dubia* were reared in lower conductivity water was included in the 2015 toxicity tests.

The Regional Water Board collected a total of 27 samples that were analyzed for aquatic toxicity. Of the 27 samples, two samples exhibited reduced survival (acute toxic response) and eight exhibited reduced

reproductive capacity (chronic toxic response). The surface water samples collected at the same time were analyzed for various organic compounds (i.e. pesticides) as well as dissolved copper and zinc.

In 2015, three samples that documented reduced survival or reproductive capacity were further analyzed by a Phase 1 Toxicity Identification Evaluation (TIE). A TIE does not identify the individual chemical that is responsible for the toxic response but is used to characterize the class of toxicants that are responsible for the toxic response. The results from the TIE are evaluated against the water chemistry analysis to determine the conditions or analytes responsible for the observed toxic responses.

A statistically significant difference ( $p < 0.05$ ) in the survival rate of *C. dubia* was observed in the August 2013 sample collected at the lower Rowdy Creek site, and an acute toxic response was observed in the March 11, 2015 Delilah Creek sample where there was no survival of the test species. (Figure 8).



**Figure 8.** Percent survival of *Ceriodaphnia dubia* or *Hyalella azteca* in surface water samples from five sites, 2013 & 2015.

Note: "Statistically Significantly Difference" denotes that survival was lower than the laboratory control with statistical significance ( $p < 0.05$ ).

*Hyalella azteca* was used for the Tilas Slough samples from March 23 and June 23, 2015 due to high conductivity.

A statistically significant difference ( $p < 0.05$ ) in the reproductive capacity of *C. dubia* was observed in samples collected in October 2013 and June 2015 at Delilah Creek, in October 2013 and March and June 2013 at the downstream Rowdy Creek site, in March and June 2015 at the upstream Rowdy Creek site, and in March and June 2015 at the Morrison Creek site (Figure 9).

Comparison of the controls in the March 11, 2015 Delilah Creek wet weather sample (runoff sample) for both moderate and low conductivity water revealed no significant difference between controls, indicating that low conductivity likely was not responsible for the acute toxic response during this sample event. To further evaluate the cause of the toxic response, a TIE was initiated to determine what was responsible for the documented toxic response. The results of the TIE determined that a metal was likely driving the toxic response and that a non-polar organic compound (typically a man-made pesticide/chemical) may have played a role as well. Analysis of water chemistry concurrently collected showed exceedances of the USEPA Freshwater Aquatic Life Benchmarks for Pesticide Registration OPP Aquatic Life Benchmarks for imidacloprid and permethrin and an exceedance of the CMC of the CTR Freshwater Aquatic Life Criteria for dissolved copper (See Table 16).

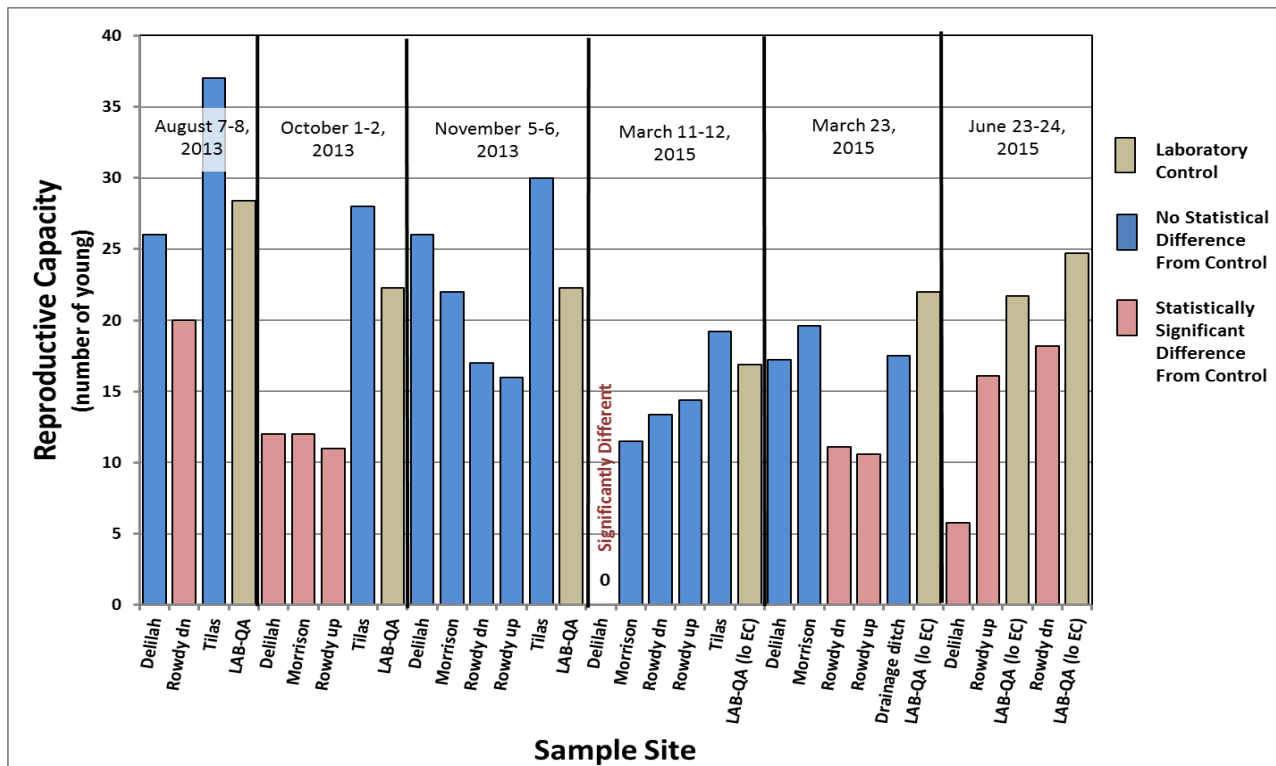
**Table 16.** Analytes documented to be within samples tested for toxicity that were exceeding various USEPA and CTR Freshwater Aquatic Life Criteria.

Station Name	Sample Date	Toxic Response	Analytes Exceeding Established Criteria
Lower Rowdy Creek	8/7/2013	Acute	None
Delilah Creek	10/1/2013	Chronic	None
Morrison Creek	10/1/2013	Chronic	None
Upper Rowdy Creek	10/1/2013	Chronic	None
Delilah Creek	3/11/2015	Acute	Copper, (dissolved)
	3/11/2015	Acute	Imidacloprid
	3/11/2015	Acute	Permethrin, (cis-)
	3/11/2015	Acute	Permethrin, (trans-)
Lower Rowdy Creek	3/23/2015	Chronic	None
Upper Rowdy Creek	3/23/2015	Chronic	None
Lower Rowdy Creek	6/23/2015	Chronic	None
Upper Rowdy Creek	6/23/2015	Chronic	None
Delilah Creek	6/24/2015	Chronic	None

The March 23, 2015 samples demonstrated a reduced reproductive capacity in both the lower and upper Rowdy Creek samples (Figure 9). As with the March 11, 2015 samples, controls were conducted at both moderate and low level conductivity. Likewise, in this case, the conductivity was not considered a contributor to the toxicity.

Reproductive capacity demonstrated a statistically significant reduction in the June 23, 2015 sample from Lower Rowdy Creek with respect to the low conductivity control, and the laboratory concluded that toxicity was most likely due to a combination of low conductivity as well as some unknown contaminant (Figure 9).

Likewise, reproductive capacity demonstrated a statistically significant reduction in the June 23-24, 2015 samples from Delilah Creek and Upper Rowdy Creek (Figure 9). A TIE was initiated by the laboratory that indicated that the observed toxicity likely was a result of low conductivity and hardness with no suggestion of any toxicants.



**Figure 9.** Reproductive capacity of *Ceriodaphnia dubia* in surface water samples from four streams, 2013 & 2015.

Note: “Statistically Significantly Different” denotes that reproductive capacity was lower than the laboratory control with statistical significance.

### 2010 Surface Water Toxicity

The surface water sample collected at Delilah Creek on August 18, 2010 exhibited reduced reproduction, but the other three sampling sites tested as part of that same sampling event did not demonstrate any surface water toxicity. No evaluation of the cause of toxicity was performed. However, the Delilah Creek sample exceeded the copper CTR Freshwater Aquatic Life Criteria for acute and reproductive toxicity.

### Toxicity Testing Results – Sediment

*Hyalella azteca* was used as the test organism in stream sediment toxicity tests. Toxicity testing of stream sediment for the 2013-2015 study was conducted for samples collected on November 5-6, 2013 at Morrison Creek and Lower Rowdy Creek, and on June 23-24, 2015 at Morrison Creek, Delilah Creek, Upper and Lower Rowdy Creek, and Tilas Slough. No toxicity was observed in any of the samples.

The stream sediment samples collected on August 18, 2010 from upper and lower Rowdy Creek, Delilah Creek at Sarina Road (same site used in 2013-2015 study), and Delilah Creek upstream of Highway 101 did not demonstrate any streambed sediment toxicity.



## FINDINGS AND CONCLUSIONS

The studies of 2013 - 2015 were conducted to assess surface water quality in the agricultural lands of the Smith River Plain. The following are notable characteristics of the study design and implementation:

### Sampling Summary

- Sampling sites included surface water sites in and around lands used for cattle grazing and lily bulb production in the Smith River Plain. The study included one control sampling site located on (upper) Rowdy Creek upstream of the agricultural area.
- Sample sites between August 2013 and June 2015, included:
  - Five stream sample sites in four drainages,
  - One roadside ditch sample with field-drainage connectivity,
  - 27 individual surface water sampling events.
- Sample matrices included surface water and sediment with the recognition that that some chemicals may become sequestered in sediment and later be released as toxicants in the water column.
- Sampling events included dry and wet periods.
- Analytes included chemicals in use by the industry.
- This list of analytes was expanded to include additional chemicals in 2015 (metam sodium, MITC, and 1,3-D).
- Toxicity testing included both surface water and sediments to help determine the potential for ecosystem impacts beyond a comparison to water quality criteria, which are derived from a number of sources.
- In some instances, a toxicity identification evaluation (TIE) was performed to assist in identifying the likely toxicant.
- Sampling did not always immediately follow the application of agricultural chemicals due to resource constraints, it did however cover the application timeframes for most chemicals associated with lily bulb production over the three-year study period.
- Related sampling efforts were used to inform the study by providing a background perspective on conditions and assisting in analyte selection.
- Natural conditions, such as weather and hydrology, and the use of toxicological test species, can lead to considerable variation in the individual measurements from this type of study. In addition, resource constraints limit the amount and timing of sample events, analytes, and toxicity tests.

### Findings

With these limitations in mind, the following findings can be drawn from the study regarding the presence of chemicals and toxicity in surface waters that may inform future sampling and studies:

1. Basic Water Quality

- a. The basic field parameters of dissolved oxygen, pH, conductivity, and water temperature were within acceptable limits for a healthy aquatic ecosystem with the exception of dissolved oxygen in Tilas Slough on about two-thirds of the sampling events.
- b. Nitrogen was elevated in every sampled collected in Delilah and Morrison Creeks and Tilas Slough. Phosphorus was elevated in one instance in Delilah Creek and three of six samplings in Tilas Slough. While nutrient analysis documented exceedances of the USEPA criteria in a number of instances, the concentrations were consistent with similar locations and settings, (i.e. alluvial flood plain and agricultural environment).

## 2. Pesticides

- a. A total of 17 detected pesticides (including isomers and degradants) were detected in surface water samples, the most common being diuron, a broad spectrum herbicide and carbofuran, a broad spectrum insecticide/nematicide. These two chemicals were detected in five and three sites, respectively. Of the 17 detected pesticides, eleven different pesticides were detected in Delilah Creek, ten in Tilas Slough, eight in the Delilah Creek roadside ditch, and 2 each in Morrison Creek and Upper and Lower Rowdy Creek water samples.
- b. The fumigant pesticides, metam sodium and its breakdown product MITC, and 1,3-D were added to the analyses for surface water and sediment in March and June of 2015, but were not detected.
- c. The lowest USEPA Freshwater Aquatic Life Benchmark for diuron was exceeded in the surface waters of Delilah Creek and the roadside drainage ditch to Delilah Creek on March 23, 2015. The latest California Department of Pesticide Regulation (DPR) reporting of diuron use in the area was in 2015, and of carbofuran in 2009. (Note: the most recently available DPR reporting is for 2015).
- d. The Delilah Creek roadside ditch was sampled during an active runoff event on March 23, 2015. As reported above, diuron exceeded the lowest USEPA Freshwater Aquatic Life Benchmark. Also of note is that Mirex, banned in 1976, was detected at 0.006 ug/L exceeding the USEPA National Recommended Water Quality Criteria for Freshwater Aquatic Life Protection value of 0.001 µg/L.
- e. Stream sediments were analyzed at four samples sites for the full suite of organic chemicals in June 2015, (see Appendix A). While a number of pesticides were detected, permethrin was the only detected pesticide currently reported as being used in the Smith River Plain. Presence of the other chemicals is likely the result of legacy use, as chemicals may be sequestered in stream sediments for a number of years.

### 3. Metals

- a. Copper and zinc based chemicals are used as pesticides in the Smith River Plain and have the potential to cause toxicity in surface water. Zinc was detected in the surface water samples from Delilah Creek, Tilas Slough, and Delilah Creek roadside ditch and copper was detected at all of the sites. Metal toxicity to aquatic organisms varies with hardness, with toxicity increasing as water hardness decreases. Dissolved zinc did not exceed the CTR Freshwater Aquatic Life Criteria for reproductive or acute toxicity on any of the sampling dates. However, dissolved copper exceeded one or both of the criteria in three samples collected from Delilah Creek and in one sample collected from Tilas Slough. Samples collected from Delilah Creek exhibited acute toxicity for samples on March 11, 2015. The toxicity laboratory performed a TIE investigation which strongly suggested that the observed toxicity was due to the presence of a metal toxicant.
- b. Dissolved copper was measured at a concentration of 26.3 ug/L on March 23, 2015 from the Delilah Creek roadside ditch. Total hardness was 63 mg/L as CaCO<sub>3</sub>. Although copper exceeded the CTR Freshwater Aquatic Life Criteria for both reproductive and acute toxicity, neither reduced survival nor reproduction was observed in toxicity testing. Exceedance of the criteria does not in itself mean that toxicity is evident, but rather the criteria is designed to be protective of aquatic life and used as a tool to assist in understanding toxic events that may occur.
- c. All of the metals in the analyte list were detected in stream sediments over the study period. Both chromium and nickel were detected in sediments at all of the sample sites in this monitoring effort and that of the SPoT Program, exceeding the USEPA Probable Effects Concentration (PEC) criteria. Although chromium and nickel exceeded the USEPA criteria, reduced survival was not observed in toxicity testing. Exceedance of the criteria does not in itself mean that toxicity is evident, but rather the criteria is designed to be protective of aquatic life and used as a tool to assist in understanding toxic events that may occur.

### 4. Water or Sediment Toxicity

- a. Low conductivity and water hardness can influence the results of toxicity tests. Typically the test species are reared at moderate levels of conductivity and hardness, and the test control populations are conducted at these same moderate levels. To assist in our understanding of the potential issue for false positive toxicity results (toxicity observed as a result of the low conductivity/hardness alone), the toxicity tests conducted in 2015 included additional sets of controls with the test species reared and tested in lower conductivity water.
- b. Reduced survival of *C. dubia* in surface water was observed in toxicity testing in lower Rowdy Creek on August 2013 and Delilah Creek on March 11, 2015. The zero survival and

associated reproduction observed for the March 11 sample from Delilah Creek prompted the initiation of a TIE from which strongly suggested that metals were the primary driver, and to a lesser extent, a non-polar organic compound may have been a contributor.

- c. Reduced reproduction of *C. dubia* was observed in 9 samples across four sites.
  - i. Delilah Creek on October 1, 2013, and June 24, 2015
  - ii. lower Rowdy Creek in August 7, 2013, March 23, 2015, and June 23, 2015
  - iii. upper Rowdy Creek on October 1, 2013 and March 12 and June 25, 2015
  - iv. Morrison Creek on October 1, 2013

The cause of the reduced reproduction is currently unknown with the exception of the June 23, 2015 samples collected from Delilah Creek and Upper Rowdy Creek. The initiation of TIEs for these samples suggested that low hardness may have had a role in the reproductive toxicity observations.

- d. TIE evaluations of the reduced reproduction observed in upper Rowdy Creek and Delilah Creek on June 23, 2015 pointed to low conductivity and low hardness as a contributor of toxicity with no suggestion of other toxicants.
- e. No water toxicity was observed in the sample collected from the Delilah Creek roadside ditch sampled on March 23, 2015.
- f. No sediment toxicity was observed in testing for Morrison and Rowdy creeks in November 2013 and Morrison, Rowdy, and Delilah creeks and Tilas Slough in June 2015. No sediment toxicity was observed in samples from the four sample sites in Delilah and Rowdy creeks in 2010.

## Discussion

Through the years, numerous pesticides have been applied to the agricultural fields of the Smith River Plain for the cultivation of Lily bulbs. Several legacy use pesticides have been detected in the tributaries which flow through the Smith River Plain. These pesticide detections were all below any water quality criteria with the exception of the pesticide Mirex (banned for use in 1976) which exceeded the USEPA National Recommended Water Quality Criteria for Freshwater Aquatic Life Protection. Of the current use pesticides detected, diuron, imidacloprid, permethrin and tebuconazole were all detected in concentrations that exceeded the lowest USEPA 2014 Aquatic Life Benchmarks for fish and invertebrates (USEPA 2017). In addition, dissolved copper was detected in every water sample, and 6 samples exceeded the CTR Freshwater Aquatic Life Criteria for reproductive and/or acute toxicity.

As part of this study, toxicity testing documenting the survival (acute toxicity) and reproduction (chronic toxicity) of test species with sample water was performed to evaluate if the application of agricultural pesticides has any impacts on the aquatic environment. Reduced reproduction of the *C. dubia* test species

(chronic toxicity testing) was documented at every site at various times throughout the sampling period, with the exception of the Tilas Slough site.

Two samples that had demonstrated reproductive toxicity (Delilah Creek June 24, 2015 and Upper Rowdy Creek June 25, 2015) and one sample that demonstrated acute toxicity (Delilah Creek March 11, 2015) were further tested utilizing a TIE to determine what may have been responsible for the observed chronic and acute toxic responses. The TIE results for the two samples with reduced reproductivity suggest that low hardness/conductivity is a stressor contributing to reduced reproductivity. No other contaminants were identified as stressors in the TIEs. The TIE result for the acute toxicity response from Delilah Creek did document the presence of a metal and, to a lesser degree, a non-polar organic compound (pesticide) as the drivers behind the toxic response. Associated metals sampling demonstrated that the copper/hardness pairing of the sample exceeded the CTR Freshwater Aquatic Life Criteria for acute toxicity.

The prevalence of reduced reproductivity results in samples collected throughout the study area including the control site, (except Tilas Slough, with higher conductivity) suggests that the extremely low water hardness and conductivity in tributaries that flow through the Smith River Plain are interfering with the ability of the test species to reproduce, producing false positives, or toxic responses when toxic conditions do not exist.

Low hardness and conductivity may act to increase the sensitivity of *C. dubia* to low level concentrations of contaminants that may be present in the water column. With this in mind, in some cases the combination of low hardness/conductivity and a contaminant(s), appeared to be a major contributor to some of the observed impairment in this data set.

The samples collected in 2010 and those collected in this current effort have documented several exceedances of the CTR Freshwater Aquatic Life Criteria for reproductive and/or acute toxicity for copper. The follow up TIE conducted on the acute toxic response sample collected at Delilah Creek on March 11, 2015 clearly identified a metal to be the main driver of toxicity and identified the presence of a non-polar organic compound as a secondary driver.

### *Study Questions and Answers*

- Are contaminants detected in surface waters and depositional stream sediments in agricultural areas of the Smith River Plain?
  - *Yes, 17 different pesticides were detected in surface waters of the Smith River Plain. Diuron was detected at the highest rate and at times exceeded the USEPA chronic aquatic life benchmark for fish. A number of pesticides were detected in sediments, most of which are a result of legacy use. Copper was detected in surface waters, which at times exceeded the CTR Freshwater Aquatic Life Criteria.*
- Is sediment toxicity observed in depositional stream sediments located downstream of agricultural land use?
  - *Sediment toxicity was not observed in stream sediment tests from samples obtained in 2010, 2013, and 2015.*
- Is water column toxicity observed in runoff downstream of agricultural land use?

- *Yes, either survival or reproductive toxicity were observed at various times at every site with the exception of Tilas Slough. Though much of the documented toxicity responses were directly related to the effect of low water hardness on the test species, two acute toxic results were documented as part of this effort in which agricultural chemicals were identified as toxicity drivers in one sample.*
- Is there a relationship between contaminant presence and agricultural activities?
  - *The chemicals used in agricultural operations on the Smith River Plain were detected in surface water and sediments, in some cases exceeding established criteria. The acute toxic response documented in the sample collected from Delilah Creek on March 11, 2015 coincides with chemical analysis documenting the exceedance of USEPA criteria and the California Toxics Rule for the protection of aquatic life.*

## Conclusions

Acute (survival) and chronic (reproduction) toxicity testing was performed at five locations in the Smith River Plain. In numerous instances, these tests demonstrated statistically significant reductions in reproductivity (positive for chronic toxicity), including three tests in which the “control” location (Upper Rowdy Creek) was positive for chronic toxicity. Additional TIE testing documented that in some cases the low conductivity associated with the sample water had a negative effect on the reproduction rates of the test species, producing some false positive results (chronic toxicity results when chronic toxicity did not exist).

Chemicals and metals used as pesticides in the agricultural activities on the Plain are being found in low level concentrations in the surface water and sediments of these tributaries. Individually the chemicals may not be in concentrations that would produce a toxic response or be directly harmful, but they may act synergistically to produce an acute or chronic toxicity response in the low hardness environment.

The study results did demonstrate that agricultural activities on the Smith River Plain are affecting the water quality of the tributaries that flow through the Plain and into the Smith River estuary. In one separate instance TIE testing was able to determine that a metal and a non-polar compound were likely responsible for an acute toxicity response from surface water collected in Delilah Creek on March 11, 2015. Chemical analysis of the surface water concurrently collected with the toxicity water samples demonstrated exceedances of USEPA criteria for the agricultural pesticides permethrin and imidacloprid and the CTR Freshwater Aquatic Life Criteria for metal copper. In addition, the results obtained from the 2010 sampling effort in Delilah Creek also suggest that Copper in the water column is the likely toxicant.

The extremely low hardness of the tributary waters flowing through the Smith River Plain play a role in increasing the likelihood of a toxic response in the test species utilized for toxicity testing. The stress placed upon the test species by the lower hardness may make the species more susceptible to the effects that copper or low concentrations of various pesticides may have in the testing process.

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**APPENDIX A - TOXICITY FINAL REPORT (2013-2015)**

**Toxicity Final Report**

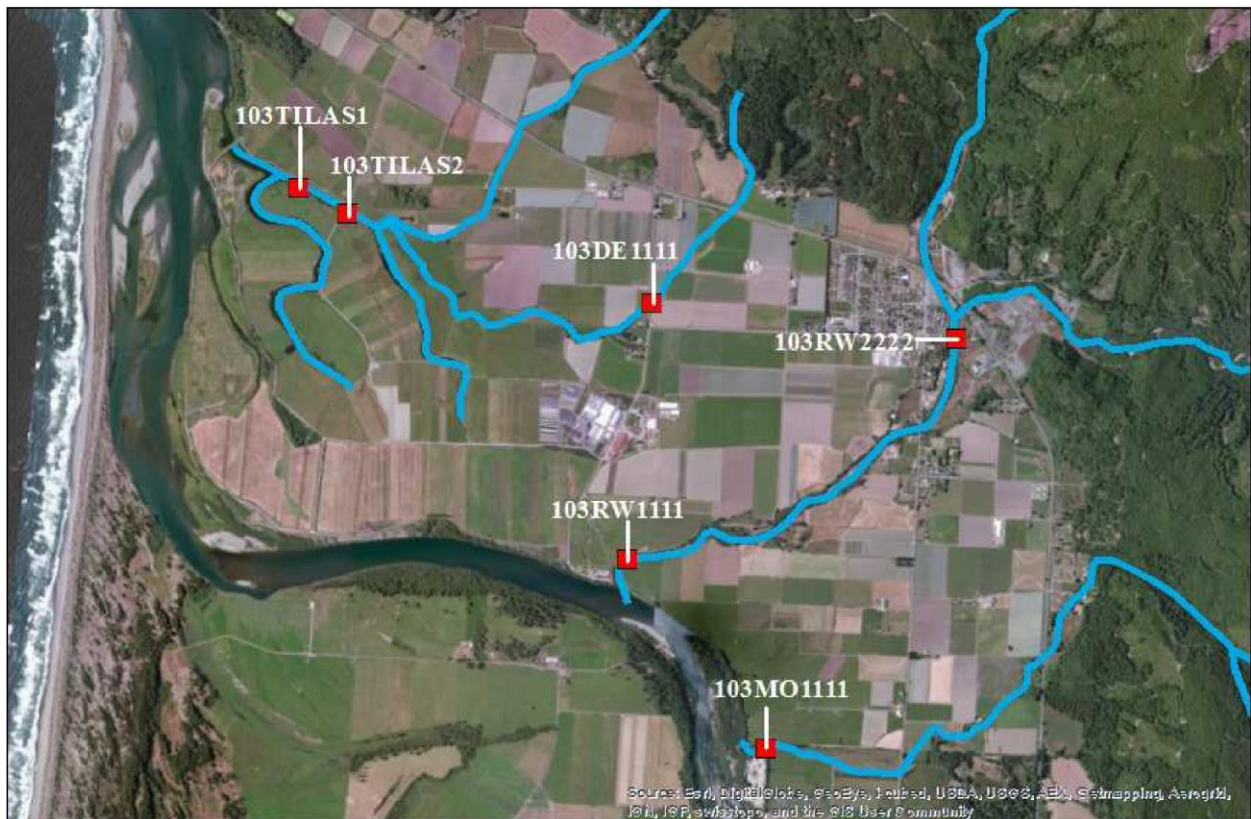
**UC Davis Aquatic Health Program Laboratory**

## Introduction/Background

Staff of the North Coast Regional Water Quality Control Board (NCRWQCB) are currently developing an Agricultural Lands Discharge Program to address water quality impacts associated with irrigated agricultural lands in the North Coast Region. The overall goal of this monitoring project is to develop baseline data from which the NCRWQCB management can evaluate the effectiveness of, and adaptively manage the implementation of the NCRWQCB's Irrigated Agriculture Discharge Program.<sup>1</sup>

Six sites were selected for testing with *Ceriodaphnia dubia*. Sites in which conductivities exceeded *C. dubia* tolerance levels were tested with *Hyalella azteca*. In some cases, designated site locations were dry upon arrival for sample collection. In these instances, alternative sites were collected at either upstream or downstream locations where water was present. These locations will have different site codes than what are indicated below in Figure 1.

This report discusses the results of toxicity tests conducted with *Ceriodaphnia dubia* and *Hyalella azteca*, on samples collected in 2013 and 2015.



**Figure 1. Agricultural Lands Monitoring Program station locations**

## Activities Undertaken

### Summary of Completed Milestones

The following tasks were completed during this reporting period:

- Nine *C. dubia* initial screening toxicity tests
- One *C. dubia* dilution series test on Delilah Creek
- Three *C. dubia* Toxicity Identification Evaluations follow-up (Delilah Creek and Upper Rowdy Creek)
- Two *H. azteca* initial toxicity tests (on samples with conductivities greater than 2500  $\mu\text{S}/\text{cm}$ )

### Summary: Project Year 2013

Samples were collected at designated sites five times during this project year, as outlined in Table 1.

Table 1. Summary of sites collected in 2013

Site Code	Collection Date	Species Tested	Result
Lower Rowdy Creek	8/7/13	<i>C. dubia</i>	SL: Survival SL: Reproduction
Delilah Creek	8/7/13		
Tilas Slough	8/8/13		
Delilah Creek	10/1/13	<i>C. dubia</i>	SL: Reproduction
Upper Rowdy Creek	10/1/13		SL: Reproduction
FIELDQA (Upper Rowdy Creek)	10/1/13		
Morrison Creek	10/1/13		SL: Reproduction
Tilas Slough	10/2/13		
Delilah Creek	11/5/13	<i>C. dubia</i>	
Lower Rowdy Creek	11/5/13		
Upper Rowdy Creek	11/5/13		
Morrison Creek	11/6/13		
Tilas Slough	11/5/13		

Toxicity test data submitted to the SWAMP database are examined in two ways. Each sample is compared to the concurrently performed test acceptability control (TAC) by a t-test, and each sample is marked to indicate whether test organism performance fell below the performance threshold of 80% of the performance of the TAC control. The type of t-test used is a one-tailed test that does not assume that the variances of the control and sample data are equal. For the purposes of this document, samples causing organism performance to be both significantly lower than the method control and lower than the 80% performance threshold are referred to as toxic, and are indicated with the SWAMP Significance Code of 'SL' for a particular endpoint.

### Summary: Project Year 2015

Samples were collected at designated sites three times during this project year, as outlined in Table 2. Table 2. Summary of sites collected in 2015

Site Code	Collection Date	Species Tested	Result
Delilah Creek	3/11/15	<i>C. dubia</i>	SL: Survival* SL: Reproduction
Tilas Slough	3/12/15		
Upper Rowdy Creek	3/12/15		
Morrison Creek	3/12/15		
Lower Rowdy Creek	3/11/15		
FIELDQA (Morrison Creek	3/12/15		
Delilah Creek	3/23/15	<i>C. dubia</i>	
Lower Rowdy Creek	3/23/15		SL: Reproduction
Upper Rowdy Creek	3/23/15		SL: Reproduction
Morrison Creek	3/23/15		
FIELDQA (Delilah Creek	3/23/15		
Delilah Creek roadside ditch (	3/23/15		
Tilas Slough	3/23/15	<i>H. azteca</i>	
Lower Rowdy Creek	6/23/15	<i>C. dubia</i>	SL: Reproduction
Delilah Creek	6/24/15		SL: Reproduction*
Upper Rowdy Creek	6/25/15		SL: Reproduction*
Tilas Slough	6/23/15	<i>H. azteca</i>	

\* Initiated in follow-up TIE procedures

## Materials and Methods

### Water Sample Collection

Staff from the NCRWQCB collected water samples as subsurface grabs in clean 1-gal amber glass bottles. Water samples were transported, stored and preserved following protocols outlined in the UC Davis-Aquatic Health Program Laboratory (UCD AHP) and SWAMP standard operating procedures. All containers used for water collections were labeled with the site ID, collection date and time, initials of the sampler and then rinsed three times with ambient water prior to filling. Up to 6 gallons were collected from each site. All samples were placed on wet ice for transport to the UCD AHP and kept between 0-6°C<sup>2</sup>. Upon receipt, samples were stored in the dark in an environmental chamber maintained between 0-4°C until their use in a test.

### Water Quality

Field water quality measurements included salinity and were recorded for each sampling time on SWAMP sample chain of custody sheets by NCRWCQB field staff. Ammonia-nitrogen was measured at UCD AHP within 24 hours of sample receipt using a HACH DR-890 portable colorimeter and a HACH AmVer Low-Range Ammonia Test'N Tube Reagent Set. Ammonia measurements of 0.06 mg/L and below are reported herein as Non-Detects (ND). Hardness and alkalinity were measured on all ambient samples (titrimetric methods) within 48-hours of sample receipt.

### **Toxicity Testing Methods**

UCD AHP toxicity testing methods are based on protocols developed by USEPA<sup>2</sup>, SWAMP QAPrP<sup>3</sup>, and UCD AHP SOPs<sup>4</sup>. Chronic toxicity testing for *Ceriodaphnia dubia* followed protocols outlined in *Short term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*<sup>2</sup>. Acute, 10-day *Hyalella azteca* water column toxicity tests were employed with samples that had conductivities greater than 2500  $\mu\text{S}/\text{cm}$  and were based on protocols outlined in SWAMP Quality Assurance Project Plan<sup>3</sup> as well as protocols outlined in *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates*<sup>5</sup>.

Before test initiation and water renewals, water samples were shaken thoroughly in their original sample containers for 60 seconds and sub-samples were filtered through a 53- $\mu\text{m}$  screen to remove debris and other organisms. Prior to test initiation and renewals, waters were warmed to test temperature ( $25 \pm 1^\circ\text{C}$  for *C. dubia*;  $23 \pm 1^\circ\text{C}$  for *H. azteca*) using a water bath maintained at  $25 \pm 2^\circ\text{C}$  and aerated at a rate of 100 bubbles per minute until the DO concentration fell below saturation. Water quality measurements including pH, EC, DO and temperature were recorded for all treatments at test initiation and termination. DO and pH was measured on fresh sample water prior to renewals; pH, DO and temperature were measured on 24-hr (or 48-hr for *H. azteca*) waste water.

### ***Ceriodaphnia dubia***

*C. dubia* were cultured in-house, following methods outlined in USEPA<sup>2</sup> and in UCD AHP SOPs<sup>4</sup>. Cultures originally obtained from Aquatic Research Organisms (Hampton, NH) and AQUA Science (Davis, CA) were kept in an environmental chamber maintained at  $25 \pm 2^\circ\text{C}$ . Test organisms employed in toxicity testing were derived asexually. Nutrient-rich Sierra Springs<sup>TM</sup> water amended to USEPA moderately hard standards (L1650: hardness: 80-100 mg/L  $\text{CaCO}_3$ , alkalinity: 57-64 mg/L  $\text{CaCO}_3$ , EC 250-300  $\mu\text{S}/\text{cm}$ , pH, 7.8-8.2)<sup>2</sup> was used as the TAC control.

Tests were initiated using blocking by known parentage with less than 24-hr old *C. dubia*, born within an 8-hr period. Each of ten replicate 20 mL glass vials contained 15 mL of sample water and one test organism. *C. dubia* were transferred into a vial of fresh solution and fed YCT and *S. capricornutum* daily. Tests were conducted at  $25 \pm 1^\circ\text{C}$  with a 16-hr light: 8-hr dark photoperiod under fluorescent light. Mortality and reproduction were assessed daily and at termination.

### ***Hyalella azteca***

*H. azteca* were obtained from Aquatic Research Organisms (Hampton, NH) and were acclimated to laboratory conditions for 48-h prior to test initiation. Acute 10-d toxicity tests consisted of five 250 mL replicate glass beakers with 100 mL of sample, 10 organisms and a one square inch piece of nitex screen as artificial substrate. Reverse-Osmosis water amended to USEPA moderately hard standards (ROEPAMHR: hardness: 80-100 mg/L  $\text{CaCO}_3$ , alkalinity: 57-64 mg/L  $\text{CaCO}_3$ , EC 250-300  $\mu\text{S}/\text{cm}$ , pH, 7.88.2)<sup>5</sup> was used as the control. Eighty percent of the test solution was renewed every 48-hrs. Organisms were fed 1 mL of YCT (yeast, organic alfalfa and trout chow) at test initiation and after water renewals.

Tests were conducted at  $23 \pm 1^\circ\text{C}$  with a 16-hr light: 8-hr dark photoperiod under fluorescent light. Mortality was scored daily; at this time dead organisms and detritus were removed from test chambers if present.

### **Statistics**

This project was designed to create data comparable with data contained in the database of California's Surface Water Ambient Monitoring Program. To this end, test organism performance (control v. ambient sample) in ambient toxicity tests was evaluated using SWAMP standard statistical protocols. The

SWAMP protocol involves the examination of significant differences in test organism performance by a one-tailed heteroschedastic t-test ( $\alpha = 0.05$ ) and a categorization of the performance of organisms exposed to the ambient sample as either greater or less than 80% of the control performance<sup>3</sup>. For the purposes of this report, samples were considered toxic only when both a significant t-test result and performance below 80% of the control was observed (SWAMP Significance Code of SL). All analyses were performed using custom Excel spreadsheets created by the SWAMP Database Management Team at Moss Landing Marine Laboratories (Office Excel 2007 (v. 12), Microsoft Inc., USA). Toxicity tests may include conductivity controls when one or more ambient samples have a lower or higher specific conductance than SWAMP's species specific thresholds. Low Conductivity Controls were included with *C. dubia* tests during the 2015 project year to match the conductivity of samples collected. A Low Conductivity Control is first statistically compared to the standard Test Acceptability Criteria control (TAC) to determine whether low conductivity has a negative impact on the test organism. In instances where the Low Conductivity Control impairs a particular endpoint (e.g. *C. dubia* reproduction), the ambient sample with the lower conductivity is statistically compared to the Low Conductivity Control, rather than the standard TAC control, to determine whether the ambient sample is toxic.

In reference toxicant tests, lethal and sub-lethal effect concentrations were calculated using CETIS v. 1.8.7.2 (Tidepool Scientific Software, McKinleyville, CA, USA). NOEC and LOEC values were calculated using USEPA standard statistical protocols<sup>2</sup>. LC<sub>50</sub>s and EC<sub>25</sub>s were calculated using linear regression, nonlinear regression, or linear interpolation methods.

## Quality Assurance

### *Test Acceptability Criteria*

Test acceptability criteria for laboratory analyses included minimum control organism survival and sublethal fitness requirements. Tests where organisms did not meet these minimum requirements were repeated.

- Chronic *C. dubia* toxicity tests require 80% or greater average control survival, with at least 60% of the surviving females having an average of 15 neonates and three broods
- Acute 10-day *H. azteca* toxicity tests require 80% or greater average control survival

### *Field Duplicates*

For SWAMP projects, field duplicates are collected at a rate of 5% or one sample per every 20 samples collected to assess precision. Field duplicate samples were collected three times during this reporting period (October 1, 2013 at site Upper Rowdy Creek; March 12, 2015 at site Morrison Creek; and on March 23, 2015 at site Delilah Creek). With respect to biological endpoints, field duplicate samples are in agreement when the primary sample and its duplicate are either statistically similar or statistically different from the control. Samples collected on October 1, 2013 at Upper Rowdy Creek were not in agreement in the *C. dubia* reproduction endpoint. Reproduction in the primary sample was significantly reduced compared to the control, while the field duplicate was not. Primary samples and their duplicates were all in agreement in the 2015 project year.



## Precision

Precision is the degree to which the primary sample agrees with its duplicate. Precision is measured by calculating the Relative Percent Difference (RPD) between sample measurements. The RPD between a sample and its duplicate is calculated using the following equation:

RPDs were calculated on water chemistry measurements of DO, pH, EC, hardness, alkalinity and ammonia, as well as the biological endpoints of survival and reproduction. Individual RPDs for water quality measurements are outlined in Table A-1 in the Appendix. SWAMP Measurement Quality Objectives (MQOs) for precision require duplicate RPDs to be equal to or less than 20%. During this reporting period, there were six instances where the RPDs exceeded the SWAMP criterion:

1. The final electrical conductivity (EC) reading at test termination for Morrison Creek and its duplicate, collected on March 12, 2015, exceeded the RPD criterion with a value of 21.12%. Site Morrison Creek had an EC measurement of 116 and its duplicate had an EC measurement of 94  $\mu\text{S}/\text{cm}$ .
2. The ammonia-nitrogen reading for Delilah Creek and its duplicate, collected on March 23, 2015, exceeded the RPD criterion with a value of 28.57%. Site Delilah Creek had an ammonia-nitrogen reading of 0.06 (ND), while its duplicate had a reading of 0.08 mg/L. In this instance, this is a case not of poor precision, but is an artifact of very low concentrations of ammonia-nitrogen being measured. We believe this data is reliable.
3. The reproduction endpoint for Upper Rowdy Creek and its duplicate tested on October 2, 2013, exceeded the RPD criterion, with a value of 30.8%. The primary sample had an average of 11 neonates and its duplicate had 15 neonates.
4. The reproduction endpoint for Morrison Creek and its duplicate tested on March 13, 2015, exceeded the RPD criterion, with a value of 46.2%. The primary sample had an average of 18.5 neonates, and its duplicate had 11.5 neonates.
5. The reproduction endpoint for Delilah Creek and its duplicate tested on March 24, 2015, exceeded the RPD criterion, with a value of 26.1%. The primary sample had an average of 19.5 neonates and its duplicate had 15.
6. The survival endpoint for Delilah Creek and its duplicate tested on March 24, 2015, exceeded the RPD criterion, with a value of 22.2%. Average survival for Delilah Creek was 100%, and survival in the duplicate sample was 80%.

RPDs for all field duplicate samples collected during this project period did not meet the SWAMP MQO criterion of  $\leq 20\%$  for the reproduction endpoint. One possibility for this variation could be the number of replicate animals which had three broods at test termination. Part of USEPA test acceptability criteria mandates that 60% of surviving females have three broods in the control. This means at a minimum, six out of ten replicate organisms (assuming 100% survival in the treatment) need to have three broods in order for the test to be considered valid. While not a requirement for ambient treatments, the number of broods test organisms can have in a test treatment may have an effect on variability among

$$RPD = \left( \frac{2|Dup_1 - Dup_2|}{Dup_1 + Dup_2} \right) \cdot 100\%$$

replicates, and therefore increase RPDs. Primary and duplicate samples can have smaller RPDs, provided that each treatment has the same number of organisms which have three broods prior to test termination.



The primary and field duplicate samples collected during this project period differed in the number of replicates which had three broods, and thus may account for the exceeded RPD values in the reproduction endpoint:

- In the October 1, 2013 sample, 70% of test replicates had a third brood in Upper Rowdy Creek, whereas 100% of test organisms had three broods in the duplicate.
- In the March 12, 2015 sample, 30% of test replicates had a third brood in Morrison Creek, whereas 80% of test organisms had three broods in the duplicate.
- In the March 23, 2015 sample, 100% of test replicate organisms had a third brood in Delilah Creek, whereas 50% of test organisms had three broods in the duplicate.

In terms of field duplicate precision, the majority of field duplicate samples were in agreement with their primary sample counterparts in matching toxicity, with the exception of the field duplicate of Upper Rowdy Creek collected October 1, 2013, as previously stated above. We are currently evaluating ways to reduce variability among replicates with respect to the third brood in *C. dubia* toxicity tests.

### **Deviations**

There were two deviations which occurred during this reporting period:

1. Samples initiated in a *C. dubia* test from the August 7-8, 2013 sample collection date, did not meet test acceptability criteria. These samples were initiated in a retest on August 20, 2013. The 48-hour holding time was missed for this retest.
2. Sample Tilas Slough, collected on March 23, 2015, missed the 48-hour holding time for test initiation. This test was initiated on March 27, 2015, as the original organisms were lost in transit and a replacement set had to be procured prior to initiation.

### **Deviation follow-up**

SWAMP protocols require a 48-hr holding time for test initiation. Although all initial screening tests are typically initiated by that holding time criterion (with some exceptions), a retest almost always takes place after the initial 48-hrs have passed. An extended holding time can possibly reduce the presence of a toxicant, as labile chemicals can degrade over time. Water samples are stored in amber glass containers and kept in the dark (to reduce photo-degradation) between 0-6°C, so extreme toxicant degradation for most chemicals is unlikely. Additionally, *C. dubia* in the samples with an extended holding time still demonstrated an adverse response, as site Lower Rowdy Creek exhibited reduced survival and reproduction compared to the control; therefore we consider the effect of this extended holding time to be negligible on the potential loss of toxicity of the samples.

### **Completeness**

UCD AHP strives for a minimum of 90% completeness of work performed in accordance with SWAMP guidelines. With the exception of the aforementioned test listed above, all other bioassays met test acceptability criteria. The *C. dubia* test which did not meet TAC was repeated. We therefore consider completeness for this project 100%.

### **Reference Toxicant Tests**

Reference Toxicant (RT) tests were conducted to ascertain whether organism responses fell within the acceptable range as dictated by USEPA. The LC<sub>50</sub>/EC<sub>25</sub> for each RT endpoint was plotted to determine whether it fell within the 95% confidence interval (CI) of the running mean. If an effect concentration, LC<sub>50</sub> or EC<sub>25</sub> is outside of the 95% CI, test organism sensitivity can be considered atypical and it's possible that organisms used in ambient toxicity tests during that month could be either more or less sensitive than normal. RT tests with *C. dubia* and *H. azteca* were performed using sodium chloride. One RT test

was performed per sampling event for all testing species and was conducted concurrently with project tests if organisms were purchased from an outside vendor (*H. azteca*). Tests where in-house cultures were utilized were performed monthly (*C. dubia*). Reference toxicant test chemicals were obtained from Fisher Scientific (Pittsburgh, PA).

With the exception of two data points in the *H. azteca* LC<sub>50</sub> for survival endpoint, no other effect concentration data (LC<sub>50</sub>s, EC<sub>25</sub>s) fell outside of 2 SDs of the running mean for all species endpoints. For the *H. azteca* LC<sub>50</sub> endpoints out of range, they did not occur during the months in which there were *H. azteca* toxicity tests for this project; thus the test organisms were considered to be within their normal range of sensitivity for all collection dates. RT control charts are presented in Figures A-1 to A-9 in the Appendix.

## Results

All toxicity and water quality summaries are outlined in Tables A2-A29 in the Appendix.

### *August 7-8, 2013 collection date*

Sites Lower Rowdy Creek, Delilah Creek and Tilas Slough were initiated in a *C. dubia* toxicity test on August 9, 2013. This test did not meet TAC (see section above), and these sites were initiated in a retest on August 20, 2013. Sample Lower Rowdy Creek exhibited significantly reduced survival (70%) and reproduction (20 neonates) compared to the control (100% survival and 28 neonates) in the August 20, 2013 retest.

### *October 1-2, 2013 collection date*

Sites Delilah Creek, Upper Rowdy Creek, FIELDQA (Upper Rowdy Creek duplicate), Morrison Creek and Tilas Slough, were initiated in a *C. dubia* test on October 3, 2013. Samples Delilah Creek, Upper Rowdy Creek, and Morrison Creek exhibited significantly reduced reproduction (12, 11, and 12 neonates, respectively) compared to the control (17 neonates).

### *November 5-6, 2013 collection date*

Sites Delilah Creek, Lower Rowdy Creek, Upper Rowdy Creek, Morrison Creek, and Tilas Slough, were initiated in a *C. dubia* test on November 7, 2013. There were no significant reductions in survival or reproduction endpoints for these sites during this testing period.

### *March 11-12, 2015 collection date*

Sites Delilah Creek, Tilas Slough, Upper Rowdy Creek, Morrison Creek, Lower Rowdy Creek, and FIELDQA (Morrison Creek duplicate), were initiated in a *C. dubia* test on March 13, 2015. Site Delilah Creek had significantly reduced survival (0%) and reproduction (0 neonates) compared to the control (100% survival, 23 neonates). A Low Conductivity Control was included in this test to match the conductivities of Delilah Creek,

Upper Rowdy Creek, Morrison Creek and Lower Rowdy Creek and results were not significantly different when compared to the TAC Control, therefore conductivity may not be a factor in the toxicity exhibited in site Delilah Creek.

This site was initiated in a *C. dubia* dilution series test on March 18, 2015, in order to determine the magnitude of toxicity in this sample. Based on the results of this test (outlined in more detail in Table A10 of the Appendix), we determined there were 4 Toxic Units present in this sample. For the purposes of this report, a Toxic Unit is calculated by dividing 100% by the percent dilution of the sample causing 50% mortality in 96 hours.

We initiated a Phase I Toxicity Identification Evaluation (TIE) test on March 20, 2015, in order to elucidate the cause of toxicity in Delilah Creek. In a Phase I TIE, non-statistical comparisons are made between an unmanipulated sample and individual sample manipulations to provide information on the physical and/or chemical characteristics of the contaminant in a toxic sample (Table 3). Additionally, the toxic sample is retested to confirm toxicity. The manipulations used in this test are described below. Solid Phase Extraction (SPE) columns primarily remove non-polar organic chemicals from ambient samples. A toxic sample is passed through an SPE column and the through-column “rinsate” is tested along with the unmanipulated sample. Control water is also passed through the SPE column and serves as one of the method controls (method blank). The adsorbate is then eluted with methanol and the “eluate” is added to control water and tested along with the appropriate method control(s). If the toxicant is a non-polar organic chemical, the ambient sample and the control water amended with methanol eluate exhibits mortality while the ambient sample passed through the SPE column (rinsate) will result in reduced or alleviated mortality. Typically the ‘eluate’ is added back at 3x the original concentration, because some compounds adhere to the column resin, resulting in lower recovery. Adding the eluate back at 3x ensures an organism response in the TIE, however this manipulation has the potential to evoke toxicity that did not exist in the original ambient sample.

Toxic samples are amended with Piperonyl Butoxide (PBO) to inhibit or reduce toxicity caused by a metabolically activated organophosphorus (OP) insecticide such as diazinon, chlorpyrifos and Malathion.<sup>6</sup> However if the contaminant is a pyrethroid insecticide, such as lambda-cyhalothrin or permethrin, the addition of PBO can synergize or increase toxicity in the PBO-manipulated sample. The unmanipulated sample and the sample amended with PBO are tested along with the appropriate method blanks. If the contaminant is a metabolically activated OP insecticide, the unmanipulated test sample will exhibit high mortality while the test sample amended with PBO will result in reduced or alleviated mortality. In contrast, mortality will be accelerated in a sample with PBO when pyrethroids are present.

Heavy metals can be toxic to aquatic species if concentrations exceed threshold levels. EDTA binds to various metals (e.g. copper, cadmium, zinc, manganese, nickel and lead), making them unavailable to biota. Three concentrations of EDTA are added to toxic samples and tested along with the appropriate controls. If the contaminant is a metal(s) the unmanipulated sample will exhibit high mortality while the sample amended with EDTA results in reduced or alleviated toxicity.

Details of the Phase I TIE conducted on the sample collected from Delilah Creek are presented in Table A-12 in the Appendix. It is important to recognize that TIEs are somewhat subjective; each lab may arbitrarily determine thresholds that warrant further investigation (i.e. chemical analyses or proceeding to a Phase II TIE). Differences in the performance between one manipulation and its appropriate comparison that are large and prolonged provide the most reliable TIE signals. For example, in this TIE, the addition of EDTA to Delilah Creek increased survival by a minimum of 75% for all four days relative to the unmanipulated Delilah Creek. This is a robust TIE signal (large survival difference for a prolonged period) that strongly suggests that a metal(s) is driving the toxicity of this sample.

Other signals were far less apparent, either in terms of percent difference or duration, and thus only hint at the potential for other contaminants. Briefly, the “rinsate” of Delilah Creek (or the sample after being passed through the SPE column) exhibited a slight delay in toxicity, compared to the unmanipulated ambient sample, indicating that toxicity may, to a much lesser extent, have been caused by a non-polar

organic compound. The sample “eluate” (or the adsorbate pulled from the SPE column), when added back at 3x, caused acute toxicity, indicating the presence of a non-polar organic compound. PBO additions in the Phase I TIE exhibited a very weak TIE signal, and these additions were retested in order to determine if a pyrethroid(s) was contributing to the toxicity of this sample. A secondary follow-up *C. dubia* test was initiated with a 50% dilution of Delilah Creek and the addition of PBO, on March 26, 2015. All treatments exhibited good survival ( $\geq 85\%$ ). Results of this test are outlined in Table A-14 in the Appendix.

#### **March 23, 2015 collection date**

Sites Delilah Creek, Lower Rowdy Creek, Upper Rowdy Creek, Morrison Creek, FIELDQA (Delilah Creek duplicate), and Delilah Creek roadside ditch were initiated in a *C. dubia* test on March 25, 2015. A Low Conductivity Control was included with this test to match the conductivities of Lower Rowdy Creek and Upper Rowdy Creek. Both sites exhibited significantly reduced reproduction (11 neonates each) compared to both the Low Conductivity Control (22 neonates) and the TAC Control (19 neonates). Because the Low Conductivity Control outperformed the TAC control in the reproduction endpoint, conductivity was not considered a contributor in the exhibited toxicity.

Site Tilas Slough was initiated in a *H. azteca* test on March 27, 2015. This site was not significantly different than the control.

#### **June 23, 2015 collection date**

Site Lower Rowdy Creek was initiated in a *C. dubia* test on June 24, 2015. A Low Conductivity Control was included to match this site’s conductivity. Both Lower Rowdy Creek and the Low Conductivity Control exhibited significantly reduced reproduction (18 and 25 neonates, respectively) compared to the TAC Control (31 neonates). Following SWAMP statistical protocols, site Lower Rowdy Creek was then statistically compared to the Low Conductivity Control, and was still significant. Based on these results, a combination of low conductivity as well as a contaminant(s) most likely contributed to the reduced reproduction in site Lower Rowdy Creek.

Site Tilas Slough was initiated in a *H. azteca* test on June 25, 2015. This site was not significantly different than the control.

#### **June 23-24, 2015 collection date**

Sites Delilah Creek and Upper Rowdy Creek were initiated in a *C. dubia* test on June 25, 2015. A Low Conductivity Control was included in this test to match the conductivity of the collected samples, but was not statistically different from the TAC Control (26 neonates). Both Delilah Creek and Upper Rowdy Creek exhibited statistically reduced reproduction (6 and 16 neonates, respectively) compared to the TAC Control.

#### **Delilah Creek follow-up**

Site Delilah Creek was initiated in a chronic Phase I TIE on July 2, 2015, in order to determine the cause of the exhibited reproductive toxicity observed in the initial screening test (Table A-24). This 7-day chronic *C. dubia* test consisted of a retest of the original toxic sample, with the additions of the SPE column manipulations, PBO and EDTA. One manipulation was added to evaluate whether increasing the hardness and conductivity of site Delilah Creek would improve reproduction, as samples with conductivities below 100  $\mu\text{S}/\text{cm}$  can sometimes cause impairment in the absence of other contaminants. Unlike the original screening test, the sample collected from Delilah Creek also affected survival in the TIE (60% survival). The organisms in the TAC control met test acceptability criteria, but had relatively few

offspring (average of 15 neonates), suggesting that the organisms were less robust than usual and may be considered more sensitive to stressors. In general, the ambient sample and its associated manipulations were more impaired than the method blanks for both survival and reproduction endpoints. Overall, this test produced very weak TIE signals, with the greatest difference in survival being a 30% improvement in the Rinsate and Hardness-adjusted (up) Delilah Creek treatments. The increased hardness/conductivity manipulation improved survival and reproduction by 30 and 51%, respectively. This was the strongest TIE signal within the test, which suggests that low hardness/conductivity may be a stressor for *C. dubia*. Given the weakness of TIE signals in this test, we were unable to identify any chemical groups that might be contributing to the low reproduction.

### ***Upper Rowdy Creek follow-up***

With the reduced reproduction observed in the initial screening test, Upper Rowdy Creek was also initiated in a follow-up *C. dubia* test on July 2, 2015. This follow-up test investigated the role of low conductivity and low hardness on the results of the reproduction endpoint. Manipulations included a hardness-adjusted (down) control, a hardness-adjusted (up) control, as well as the original unmanipulated ambient sample, and a hardness-adjusted (up) Upper Rowdy Creek. Unmanipulated Upper Rowdy Creek had an average of 13 neonates, while in comparison the hardness-adjusted (up) Upper Rowdy Creek had an average of 20 neonates. All controls had an average of 18-21 neonates. These results indicate that low conductivity/hardness may have had a role in the reproductive toxicity observed at this site.

### ***Low Hardness/Low Conductivity in Region 1***

In general, sample hardness and conductivity in Region 1 sites, especially with respect to those collected during the 2015 project year, had some of the lowest values we have tested in the laboratory. As such, hardness/conductivity interferences although not consistent, need to be considered. This variability could be due to the event-based nature of the project, where water samples were collected during or directly after a significant storm event. Depending on the size of the storm, runoff could have more or less of an impact on the low hardness and/or low conductivities observed with these samples. With this in mind, care should be used when interpreting data results. Including Low Conductivity Controls in initial screening tests can help elucidate conductivity interferences; however in some cases the combination of low hardness/conductivity and a contaminant(s), appeared to be a major contributor of some of the observed impairment in this data set. Moreover, TIE manipulations, originally aimed for use in moderately hard waters, may not be suitable for very soft waters, such as those at Region 1 sites. For instance, method blanks included in the chronic *C. dubia* reproductive TIE for site Delilah Creek, typically do not negatively affect the reproduction endpoint; yet the majority of these method blanks had reduced reproduction when compared to the un-manipulated controls. In that test specifically, only the TAC Control, the C8 Blank, and the Hardness-adjusted (up) Control, did not exhibit reduced reproduction, and were the only waters which had higher hardness/conductivity. While the Hardness-adjusted (down) Control method blank did exhibit relatively robust fecundity, low hardness/conductivity alone may not have an effect on test organisms, but the combination of low hardness/conductivity and an added manipulation (such as EDTA) could cause stress to the organism and elicit a negative effect in very soft waters. Due to water limitations, we were unable to investigate this further; however it may be prudent to reevaluate the concentrations of TIE manipulations used in chronic follow-up tests in very soft waters, given the results presented herein. Additionally, including hardness-adjusted controls/samples in initial screening tests may be helpful in interpreting data in the first phase of testing.

Table 3. Summary of non-statistical comparisons made between typical Toxicity Identification Evaluation Treatments and the general focus of manipulations.

Treatment Descriptions for Common Manipulations	Focus of Manipulations	Statistical Comparisons													
		EPAMH (HA) to match Ambient Sample	EPAMH (HA) + Mid Concentration EDTA	EPAMH (HA) + High Concentration EDTA	EPAMH (HA) + 50 ppb PBO	EPAMH (HA) + 100 ppb PBO	EPAMH C8 Blank	EPAMH (HA) + MeOH @ 0.6%	EPAMH (HA) + Eluate adback @ 3x	Ambient Sample	Ambient Sample + Low Concentration EDTA	Ambient Sample + Mid Concentration EDTA	Ambient Sample + High Concentration EDTA	Ambient Sample + 100 ppb PBO	Ambient Sample C8 Rinsate
EPAMH (Moderately Hard Control Water)	Test Acceptability Criteria	■													
EPAMH Hardness Adjusted (HA) to match Ambient Sample	Method Blanks to determine whether specific TIE manipulations have an effect on a sample		■							■					
EPAMH (HA) + Mid Concentration EDTA				■											
EPAMH (HA) + High Concentration EDTA					■										
EPAMH (HA) + 50 ppb PBO						■									
EPAMH (HA) + 100 ppb PBO							■								
EPAMH C8 Blank								■							
EPAMH (HA) + MeOH @ 0.5%									■						
EPAMH (HA) + Eluate adback @ 3x	Non-polar Organic Chemicals								■						
Ambient Sample	Toxicity Persistence									■					
Ambient Sample + Low Concentration EDTA	Metals such as Cooper, Cadmium, Zinc, Manganese, Nickel, and Lead										■				
Ambient Sample + Mid Concentration EDTA												■			
Ambient Sample + High Concentration EDTA													■		
Ambient Sample + 100 ppb PBO	Pyrethroid or OP Pesticides												■		
Ambient Sample C8 Rinsate	Non-polar Organic Chemicals													■	

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## APPENDIX A1

Table A-1. Summary of Relative Percent Differences for field duplicate samples collected during the 2013 and 2015 project years. Endpoints which exceed the SWAMP RPD criterion are highlighted in red font.

Site	Collection Date	Relative Percent Difference (%)						
		EC	DO		pH	Hardness	Alkalinity	Ammonia
<b>Upper Rowdy Creek</b>	October 1, 2013	0.61	3.51	0.00	1.30	9.52	5.13	0.00
		4.58	0.00	12.99	0.25			
			0.00	2.35	0.38			
			1.23	1.21	0.26			
			1.24	1.23	1.54			
			2.38	1.23	1.30			
			1.20	0.00	0.50			
					0.39			
<b>Morrison Creek</b>	March 12, 2015	2.10	1.18	1.27	1.41	13.33	0.00	0.00
		21.12	0.00	1.40	0.54			
			1.38	3.59	1.19			
			1.18	2.67	1.19			
			2.63	1.23	0.13			
			0.00	2.82	0.40			
			1.32	1.32	0.13			
					1.07			
<b>Delilah Creek</b>	March 23, 2015	0.52	0.00	1.21	0.94	0.00	9.52	28.57
		8.32	2.44	5.06	1.09			
			0.00	3.73	1.49			
			4.88	1.32	1.23			
			6.21	0.00	0.68			
			0.00	3.68	0.82			
			4.03	2.70	5.96			



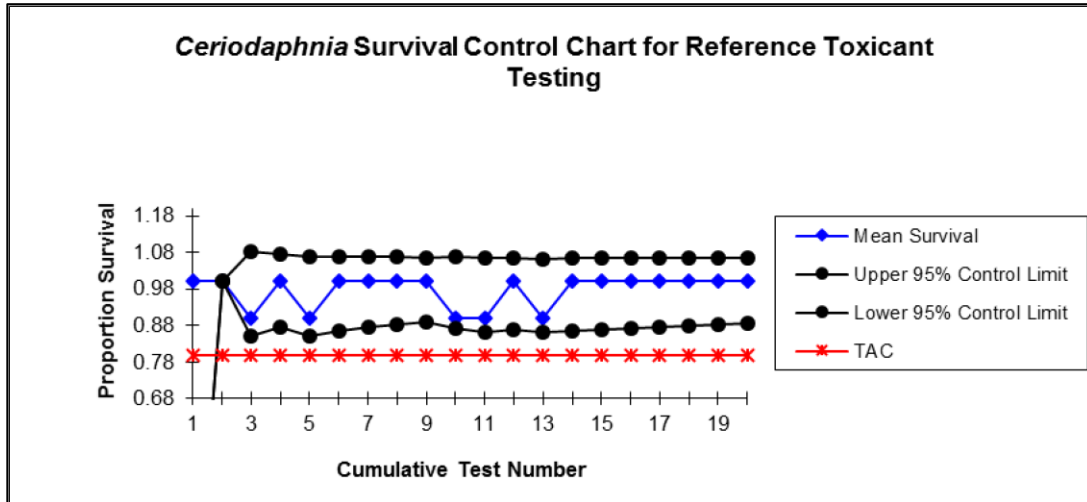


Figure A-1. RT control chart for *C. dubia* control survival spanning the 2013-2015 project years.

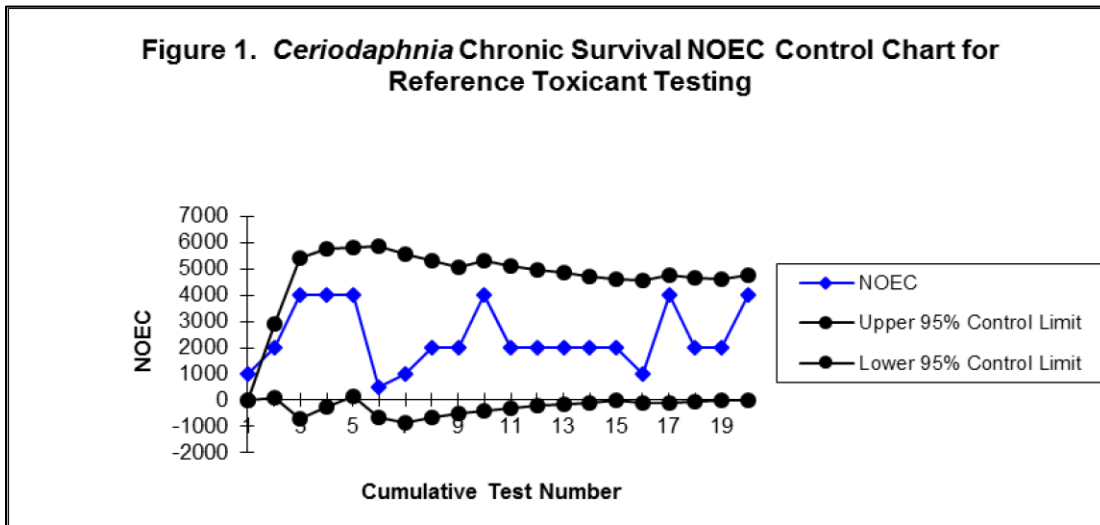


Figure A-2. RT control chart for *C. dubia* control survival NOEC, spanning the 2013-2015 project years.

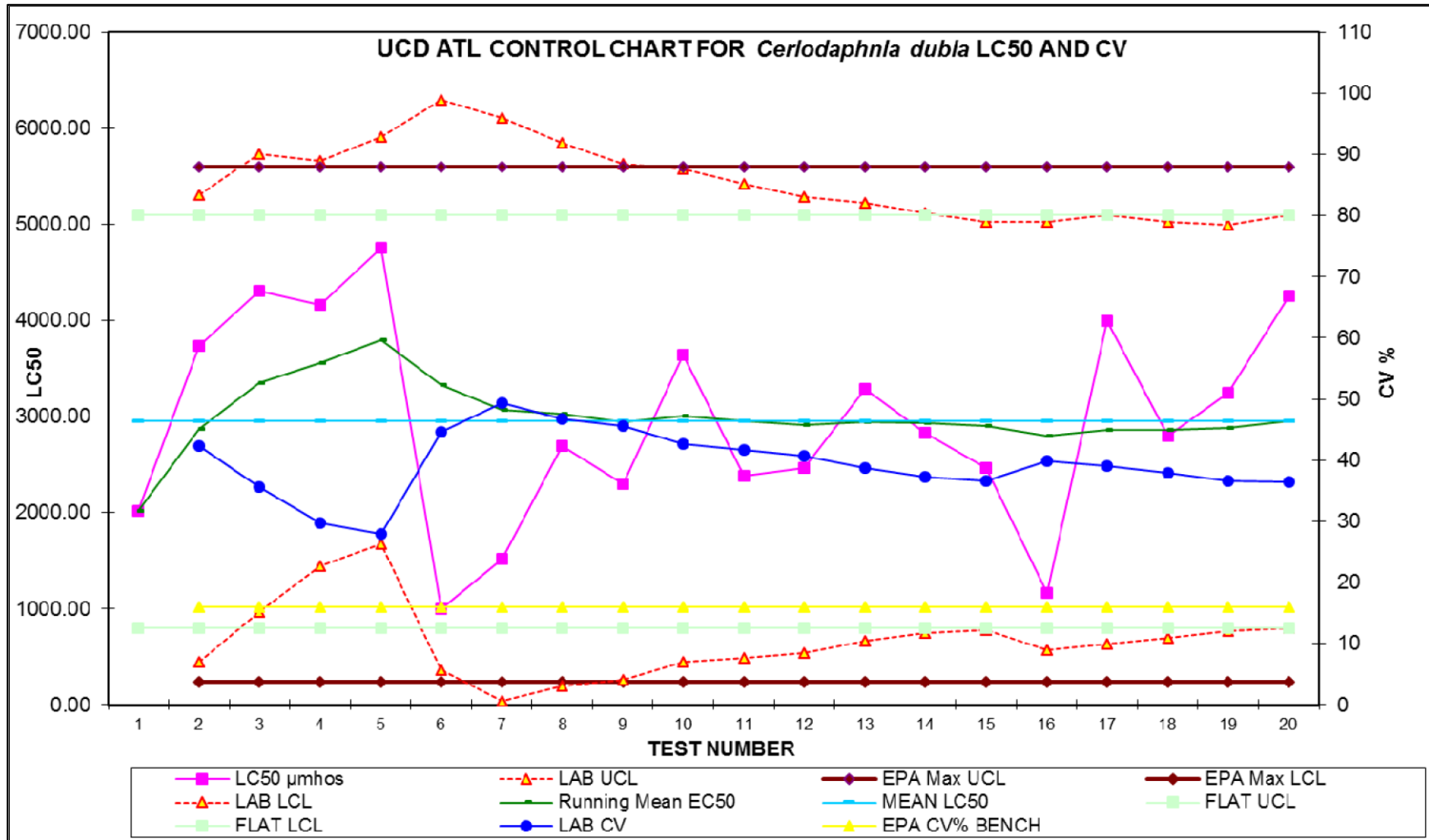


Figure A-3. RT control chart for *C. dubia* survival LC<sub>50</sub>s, spanning the 2013-2015 project years.

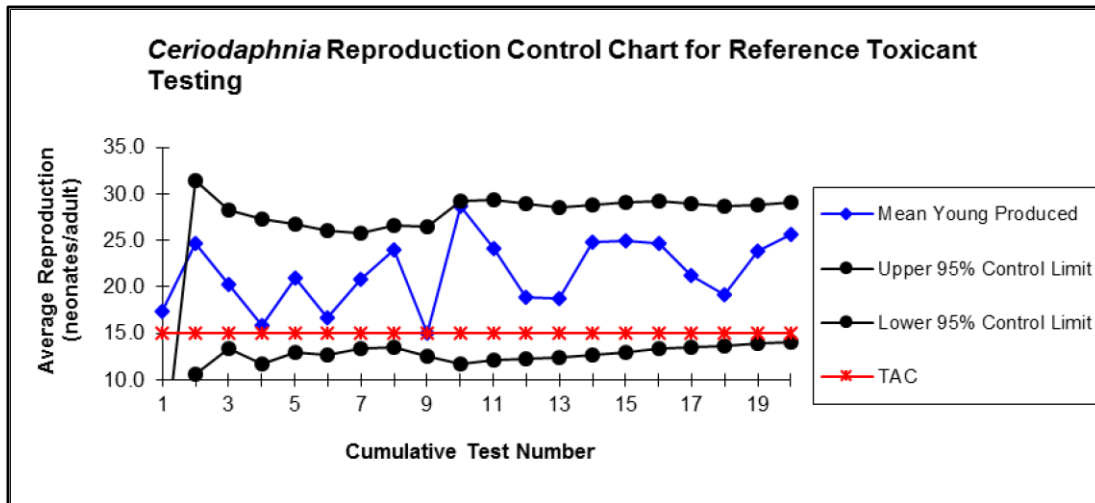


Figure A-4. RT control chart for *C. dubia* control reproduction, spanning the 2013-2015 project years.

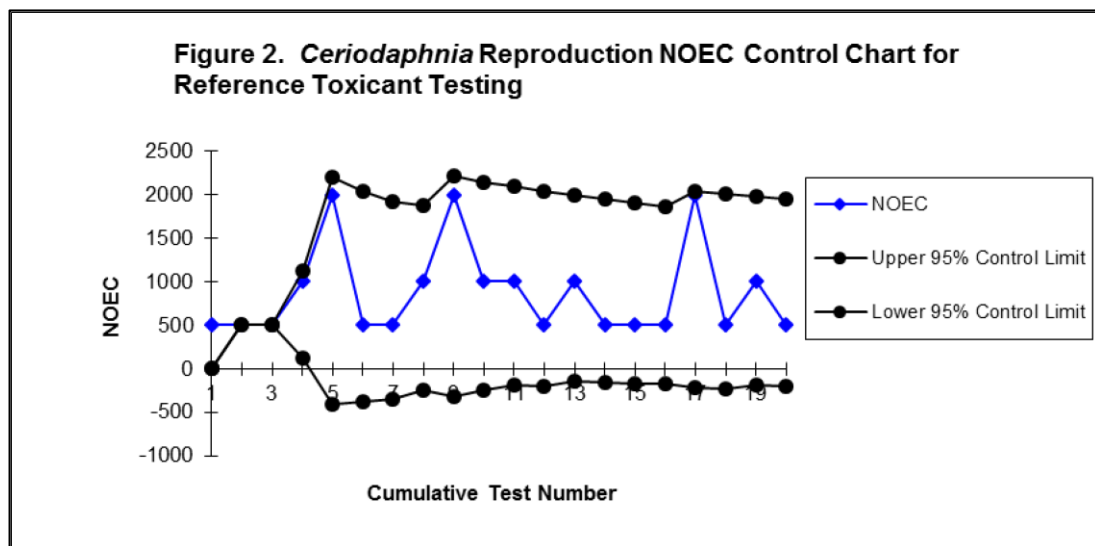


Figure A-5. RT control chart for *C. dubia* control reproduction NOEC, spanning the 2013-2015 project years.

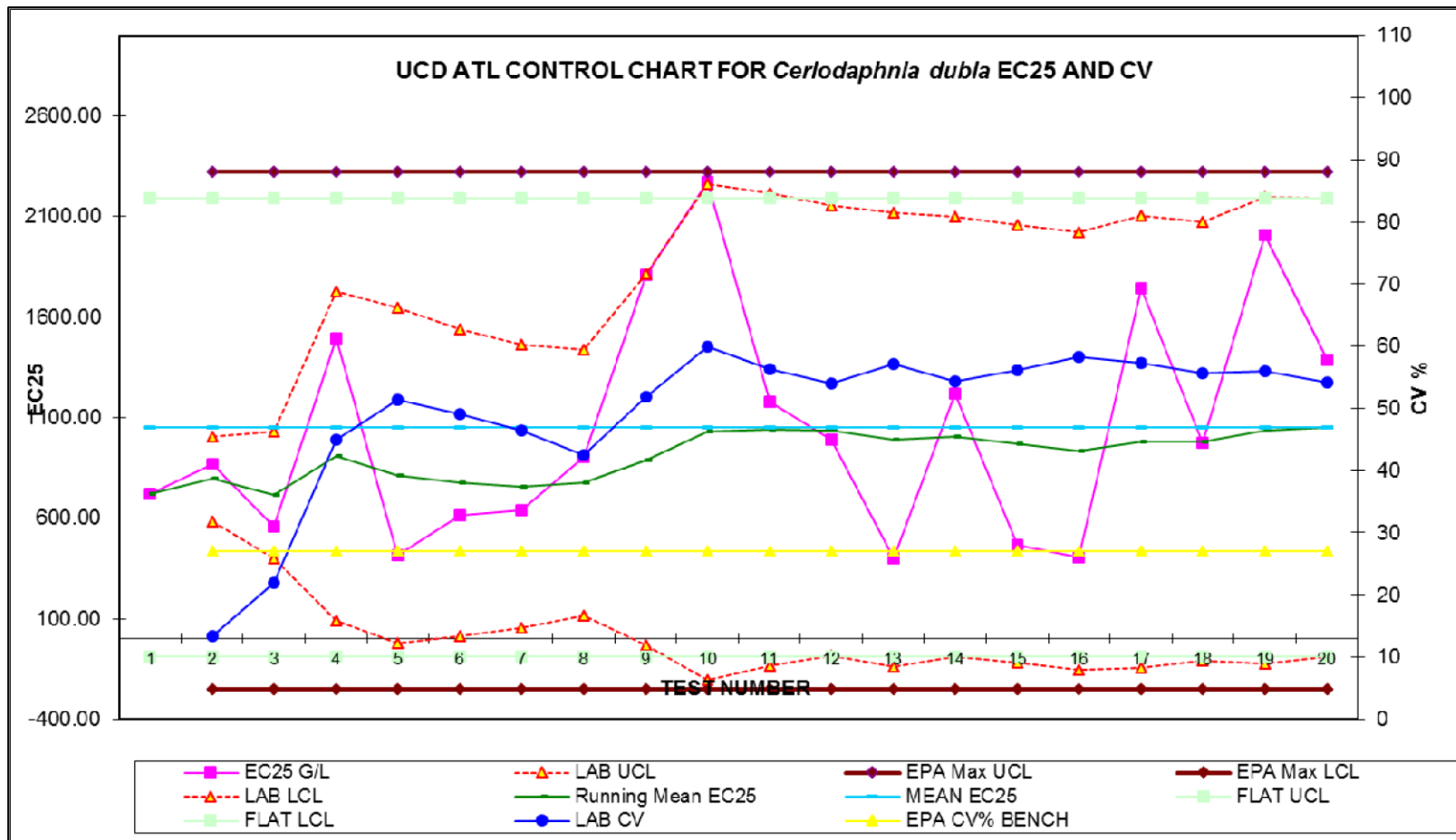


Figure A-6. RT control chart for *C. dubia* reproduction EC<sub>25</sub>s, spanning the 2013-2015 project years.

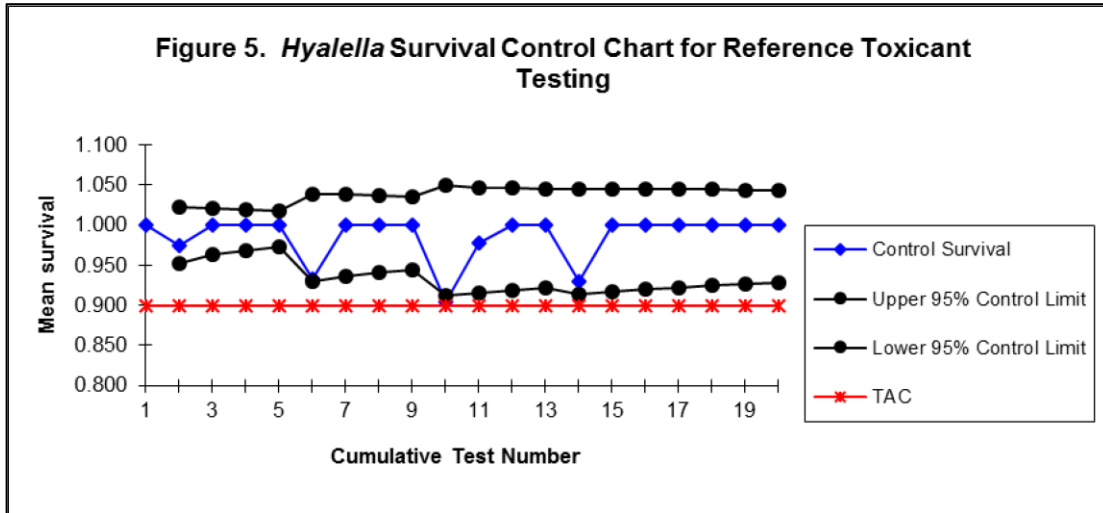


Figure A-7. RT control chart for *H. azteca* control survival, spanning the 2013-2015 project years.

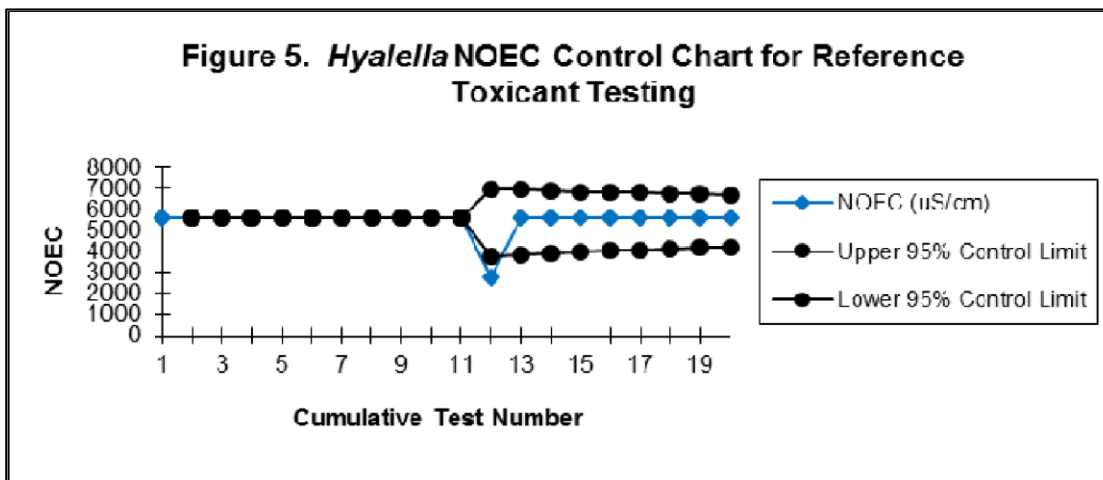


Figure A-8. RT control chart for *H. azteca* control survival NOEC, spanning the 2013-2015 project years.

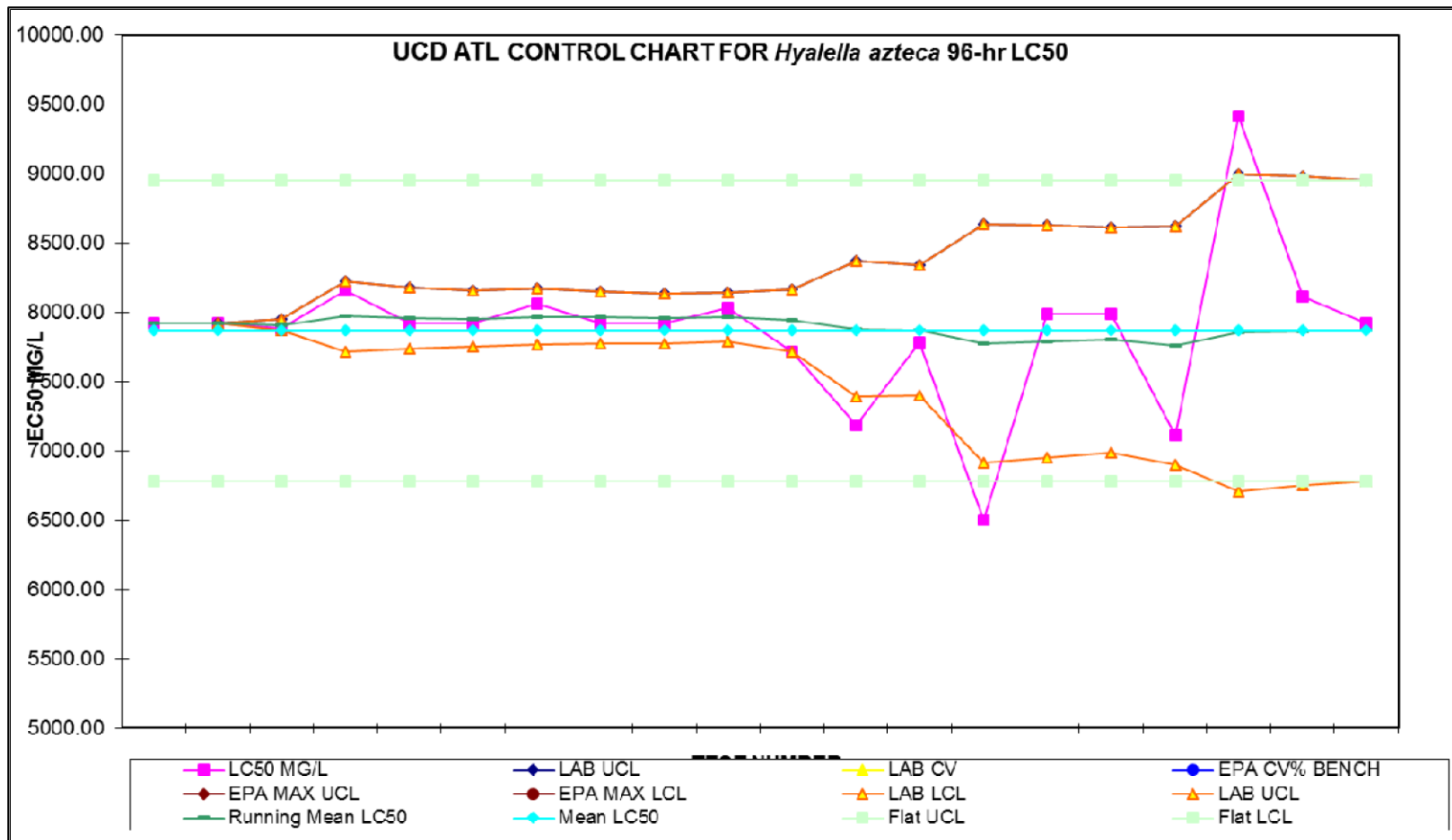


Figure A-9. RT control chart for *H. azteca* survival LC<sub>50</sub>S, spanning the 2013-2015 project years.

Table A-2. Summary of results of a *C. dubia* chronic toxicity test initiated on 8/20/13, evaluating the toxicity of ambient surface water samples collected on 8/7/13 and 8/8/13.

Treatment	Survival (%) <sup>1</sup>	Reproduction (offspring) <sup>1</sup>	
		Mean	SE
L1650	100	28.4	1.45
Lower Rowdy Creek	70	20.3	4.14
Delilah Creek	90	25.7	2.80
Tilas Slough	100	36.9	1.04

1. Highlighted cells indicate statistically significant reductions in survival or reproduction compared to the laboratory control. Data were analyzed using SWAMP standard statistical protocols.

Table A-3. Summary of water chemistry during a chronic *C. dubia* toxicity test initiated on 8/20/13, examining the toxicity of ambient surface water samples collected on 8/7/13 and 8/8/13.

Treatment	DO (mg/L)				pH			
	Initial	Final	Min	Max	Initial	Final	Min	Max
L1650	8.1	7.4	7.2	8.2	8.03	7.62	7.62	8.03
Lower Rowdy Creek	8.1	7.1	7.1	8.3	7.92	7.47	7.47	7.92
Delilah Creek	8.0	7.4	7.1	8.3	7.60	7.25	7.25	7.61
Tilas Slough	8.6	8.0	7.1	8.6	7.36	7.89	7.36	8.00
		EC	Total Ammonia	Unionized Ammonia <sup>1</sup>	Alkalinity (CaCO <sub>3</sub> )	Hardness (CaCO <sub>3</sub> )	Temperature (°C)	
		(µS/cm)	(mg/L)				Min	Max
L1650		272	NR	NR	58	88	23.1	24.3
Lower Rowdy Creek		106	ND	ND	38	32	23.1	24.3
Delilah Creek		136	ND	ND	20	36	22.9	24.2
Tilas Slough		205	0.29	0.003	78	76	22.8	24.4

1. This unionized ammonia reading is based off the total ammonia measured at sample receipt and upon the water chemistry measured at test initiation. ND: Non-Detect. NR: Not Reported

Table A-4. Summary of results of a *C. dubia* chronic toxicity test initiated on 10/3/13, evaluating the toxicity of ambient surface water samples collected on 10/1/13 and 10/2/13.

Treatment	Survival (%) <sup>1</sup>	Reproduction (offspring) <sup>1</sup>	
		Mean	SE
L1650	89	17.4	1.43
Delilah Creek	100	11.6	2.07
Upper Rowdy Creek	100	10.6	1.39
FIELDQA (Upper Rowdy Creek)	100	14.7	1.40
Morrison Creek	90	12.3	1.65
Tilas Slough	100	27.5	2.21

1. Highlighted cells indicate statistically significant reductions in survival or reproduction compared to the laboratory control. Data were analyzed using SWAMP standard statistical protocols.

Table A-5. Summary of water chemistry during a chronic *C. dubia* toxicity test initiated on 10/3/13, examining the toxicity of ambient surface water samples collected on 10/1/13 and 10/2/13.

Treatment	DO (mg/L)				pH			
	Initial	Final	Min	Max	Initial	Final	Min	Max
L1650	8.4	7.5	7.5	8.5	7.94	7.89	7.89	8.06
Delilah Creek	8.5	7.1	7.1	8.5	7.00	7.20	7.00	7.47
Upper Rowdy Creek	8.4	7.5	7.5	8.5	7.72	7.70	7.70	7.96
FIELDQA (Upper Rowdy Creek)	8.7	7.5	7.2	8.7	7.62	7.73	7.62	7.92
Morrison Creek	8.7	7.3	7.3	8.7	7.44	7.46	7.44	7.74
Tilas Slough	8.4	7.3	7.3	8.5	7.25	7.58	7.25	7.66
		EC	Total Ammonia	Unionized Ammonia <sup>1</sup>	Alkalinity (CaCO <sub>3</sub> )	Hardness (CaCO <sub>3</sub> )	Temperature (°C)	
		(µS/cm)	(mg/L)				Min	Max
L1650		267	ND	ND	54	84	23.8	24.5
Delilah Creek		204	ND	ND	14	72	23.9	24.3
Upper Rowdy Creek		98	ND	ND	40	44	23.8	24.1
FIELDQA (Upper Rowdy Creek)		98	ND	ND	38	40	23.6	24.3
Morrison Creek		92	ND	ND	20	24	23.6	24.1
Tilas Slough		305	0.50	0.004	30	80	23.7	24.2

1. This unionized ammonia reading is based off the total ammonia measured at sample receipt and upon the water chemistry measured at test initiation. ND: Non-Detect.





Table A-6. Summary of results of a *C. dubia* chronic toxicity test initiated on 11/7/13, evaluating the toxicity of ambient surface water samples collected on 11/5/13 and 11/6/13.

Treatment	Survival (%) <sup>1</sup>	Reproduction (offspring) <sup>1</sup>	
		Mean	SE
L1650	100	22.3	3.56
Delilah Creek	100	26.3	1.84
Lower Rowdy Creek	90	16.9	2.16
Upper Rowdy Creek	90	15.8	2.49
Morrison Creek	100	21.8	2.61
Tilas Slough	100	30.0	1.16

1. Highlighted cells indicate statistically significant reductions in survival or reproduction compared to the laboratory control. Data were analyzed using SWAMP standard statistical protocols.

Table A-7. Summary of water chemistry during a chronic *C. dubia* toxicity test initiated on 10/3/13, examining the toxicity of ambient surface water samples collected on 10/1/13 and 10/2/13.

Treatment	DO (mg/L)				pH				
	Initial	Final	Min	Max	Initial	Final	Min	Max	
L1650	8.1	7.8	7.3	8.2	7.98	7.91	7.75	7.98	
Delilah Creek	8.4	7.9	6.6	8.4	8.03	7.81	7.40	8.03	
Lower Rowdy Creek	8.4	7.8	7.4	8.4	8.04	7.72	7.43	8.04	
Upper Rowdy Creek	8.4	7.9	7.5	8.4	7.99	7.74	7.51	7.99	
Morrison Creek	8.4	7.6	7.3	8.4	7.62	7.72	7.52	7.79	
Tilas Slough	8.4	6.8	6.8	8.4	6.96	7.63	6.96	7.79	
		EC	Total Ammonia	Unionized Ammonia <sup>1</sup>	Alkalinity (CaCO <sub>3</sub> )	Hardness (CaCO <sub>3</sub> )	Temperature (°C)		
		(µS/cm)	(mg/L)					Min	Max
L1650		256	ND	ND	52	80	22.4	23.6	
Delilah Creek		114	0.10	0.005	18	32	23.5	23.7	
Lower Rowdy Creek		101	ND	ND	34	40	23.5	23.7	
Upper Rowdy Creek		98	ND	ND	36	32	23.6	23.6	
Morrison Creek		106	0.16	0.003	30	32	23.6	23.6	
Tilas Slough		295	0.41	0.002	44	72	23.4	23.6	

1. This unionized ammonia reading is based off the total ammonia measured at sample receipt and upon the water chemistry measured at test initiation. ND: Non-Detect



Table A-8. Summary of results of a *C. dubia* chronic toxicity test initiated on 3/13/15, evaluating the toxicity of ambient surface water samples collected on 3/11/15 and 3/12/15.

Treatment	Survival (%) <sup>1</sup>	Reproduction (offspring) <sup>1</sup>	
		Mean	SE
<b>L1650</b>	100	22.8	3.75
<b>Delilah Creek</b>	0	0.0	0.00
<b>Tilas Slough</b>	100	19.2	3.60
<b>Upper Rowdy Creek</b>	100	14.4	2.43
<b>Morrison Creek</b>	100	11.5	3.33
<b>Lower Rowdy Creek</b>	100	13.4	2.35
<b>FIELDQA (Morrison Creek)</b>	100	18.4	2.54
<b>Low Conductivity Control</b>	100	16.9	1.02

1. Highlighted cells indicate statistically significant reductions in survival or reproduction compared to the laboratory control. Data were analyzed using SWAMP standard statistical protocols.

Table A-9. Summary of water chemistry during a chronic *C. dubia* toxicity test initiated on 10/3/13, examining the toxicity of ambient surface water samples collected on 10/1/13 and 10/2/13.

Treatment	DO (mg/L)				pH			
	Initial	Final	Min	Max	Initial	Final	Min	Max
<b>L1650</b>	8.1	7.4	7.1	8.5	8.17	7.85	7.82	8.17
<b>Delilah Creek</b>	8.3	6.8	6.8	8.5	7.50	7.22	7.22	7.50
<b>Tilas Slough</b>	8.5	7.7	7.1	8.6	8.00	7.76	7.65	8.00
<b>Upper Rowdy Creek</b>	8.5	7.8	6.9	8.6	8.00	7.65	7.54	8.00
<b>Morrison Creek</b>	8.5	7.6	7.0	8.6	7.86	7.50	7.47	7.86
<b>Lower Rowdy Creek</b>	8.4	7.6	7.2	8.6	7.84	7.57	7.51	7.84
<b>FIELDQA (Morrison Creek)</b>	8.4	7.5	7.2	8.6	7.75	7.42	7.42	7.75
<b>Low Conductivity Control</b>	8.5	7.6	7.1	8.6	7.68	7.34	7.31	7.68
		<b>EC</b>	<b>Total Ammonia</b>	<b>Unionized Ammonia<sup>1</sup></b>	<b>Alkalinity (CaCO<sub>3</sub>)</b>	<b>Hardness (CaCO<sub>3</sub>)</b>	<b>Temperature (°C)</b>	
		(µS/cm)	(mg/L)				Min	Max
<b>L1650</b>		288	ND	ND	58	120	23.9	24.0
<b>Delilah Creek</b>		79	0.10	0.002	16	32	23.7	25.2
<b>Tilas Slough</b>		2625	ND	ND	58	308	23.6	24.2
<b>Upper Rowdy Creek</b>		93	ND	ND	32	28	23.8	24.0
<b>Morrison Creek</b>		75	ND	ND	26	32	23.9	24.1
<b>Lower Rowdy Creek</b>		78	ND	ND	30	28	23.8	24.3
<b>FIELDQA (Morrison Creek)</b>		77	ND	ND	26	28	23.7	24.2
<b>Low Conductivity Control</b>		75	NR	NR	NR	NR	23.4	24.0

1. This unionized ammonia reading is based off the total ammonia measured at sample receipt and upon the water chemistry measured at test initiation. ND: Non-Detect, NR: Not Recorded.

Table A-10. Summary of results of a 96-hr *C. dubia* dilution series test initiated on 3/18/15 for Delilah Creek collected on 3/11/15.

Treatment	24-hr Survival		48-hr Survival		72-hr Survival		96-hr Survival	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
<b>L1650</b>	95	5	90	6	90	6	90	6
<b>Delilah Creek @ 6.25%</b>	100	0	95	5	89	7	89	7
<b>Delilah Creek @ 12.5%</b>	80	8	75	5	75	5	75	5
<b>Delilah Creek @ 25%</b>	65	13	55	15	55	15	55	15
<b>Delilah Creek @ 50%</b>	80	0	50	17	25	15	5	5
<b>Delilah Creek @ 100%</b>	5	5	0	0	0	0	0	0

1. Highlighted cells indicate a significant reduction in survival compared to the control. Data were analyzed using CETIS statistical software.

Table A-11. Summary of water chemistry during a 96-hr *C. dubia* dilution series test initiated on 3/18/15 for Delilah Creek collected on 3/11/15.

Treatment	Initial EC	Temperature (°C)		DO (mg/L)		pH	
	(µS/cm)	Min	Max	Min	Max	Min	Max
<b>L1650</b>	284	23.6	24.6	7.0	9.8	7.90	8.17
<b>Delilah Creek @ 6.25%</b>	-	24.4	24.5	7.2	8.2	8.05	8.11
<b>Delilah Creek @ 12.5%</b>	-	24.5	24.7	7.3	8.1	8.03	8.08
<b>Delilah Creek @ 25%</b>	-	24.4	24.5	7.2	8.3	7.98	8.04
<b>Delilah Creek @ 50%</b>	-	23.8	24.5	7.3	8.5	7.87	7.96
<b>Delilah Creek @ 100%</b>	88	23.7	24.4	7.4	9.6	7.17	7.54

Table A-12. Summary of results from a 96-hr *C. dubia* Phase I Toxicity Identification Evaluation initiated on 3/20/15 for Delilah Creek collected on 3/11/15.

Treatment	24-hr Survival		48-hr Survival		72-hr Survival		96-hr Survival		Interpretation
	(%) <sup>1</sup>								
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
L1650	100	0.0	100	0.0	100	0.0	100	0.0	
L1650 Hardness Adj. (HA) @ 32 mg/L	100	0.0	100	0.0	95	5.0	95	5.0	
L1650 (HA) + MeOH @ 0.05%	100	0.0	100	0.0	100	0.0	100	0.0	
L1650 (HA) + Eluate @ 3x	60	14.1	0	0.0	0	0.0	0	0.0	Toxicity indicates the presence of a non-polar organic compound, although toxicity may be magnified by 3-fold concentration.
L1650 (HA) + 8 mg/L EDTA	100	0.0	100	0.0	100	0.0	100	0.0	
L1650 (HA) + 16 mg/L EDTA	100	0.0	100	0.0	100	0.0	100	0.0	
L1650 (HA) + 32 mg/L EDTA	95	5.0	95	5.0	95	5.0	95	5.0	
L1650 (HA) + 50 ppb PBO	95	5.0	95	5.0	95	5.0	95	5.0	
L1650 (HA) + 100 ppb PBO	100	0.0	100	0.0	100	0.0	100	0.0	
L1650 C8 Blank	100	0.0	100	0.0	95	5.0	95	5.0	
Delilah Creek	15	15.0	0	0.0	0	0.0	0	0.0	Sample is still acutely toxic.
Delilah Creek + 8 mg/L EDTA <sup>2</sup>	100	0.0	95	5.0	90	5.8	85	5.0	Near elimination of mortality indicates toxicity was primarily due to metals.
Delilah Creek + 16 mg/L EDTA <sup>2</sup>	100	0.0	95	5.0	95	5.0	95	5.0	
Delilah Creek + 32 mg/L EDTA <sup>2</sup>	100	0.0	85	5.0	75	9.6	75	9.6	
Delilah Creek + 50 ppb PBO <sup>2</sup>	0	0.0	0	0.0	0	0.0	0	0.0	
Delilah Creek + 100 PBO <sup>2</sup>	5	5.0	0	0.0	0	0.0	0	0.0	
Delilah Creek C8 Rinsate <sup>2</sup>	45	9.6	15	9.6	0	0.0	0	0.0	Slight delay in toxicity on Day 1 only indicates toxicity may in part have been caused by a non-polar organic compound (very minor contribution).

1. Highlighted areas indicate specific TIE signals and are compared to the appropriate control or method blank.

2. These treatments were compared to the unmanipulated ambient sample of Delilah Creek.

Table A-13. Summary of water chemistry during a 96-hr *C. dubia* Phase I Toxicity Identification Evaluation initiated on 3/20/15 for Delilah Creek collected on 3/11/15.

Treatment	DO (mg/L)		pH		Initial EC ( $\mu$ S/cm)	Final Temp ( $^{\circ}$ C)
	Min	Max	Min	Max		
L1650	6.4	9.1	7.80	7.92	269	24.7
L1650 Hardness Adj. (HA) @ 32 mg/L	6.6	9.2	7.46	7.62	96	24.9
L1650 (HA) + MeOH @ 0.05%	6.9	6.9	7.50	7.50	-	24.5
L1650 (HA) + Eluate @ 3x	6.9	6.9	7.44	7.44	-	24.5
L1650 (HA) + 8 mg/L EDTA	6.8	6.8	7.33	7.33	-	24.4
L1650 (HA) + 16 mg/L EDTA	6.9	6.9	7.34	7.34	-	24.3
L1650 (HA) + 32 mg/L EDTA	7.0	7.0	7.33	7.33	-	24.3
L1650 (HA) + 50 ppb PBO	6.8	6.8	7.35	7.35	-	24.5
L1650 (HA) + 100 ppb PBO	6.7	6.7	7.39	7.39	-	24.5
L1650 C8 Blank	6.7	6.7	7.81	7.81	-	24.5
Delilah Creek	6.9	9.4	7.24	7.33	79	24.6
Delilah Creek + 8 mg/L EDTA	7.1	7.1	7.19	7.19	-	24.6
Delilah Creek + 16 mg/L EDTA	7.2	7.2	7.16	7.16	-	24.6
Delilah Creek + 32 mg/L EDTA	7.2	7.2	7.11	7.11	-	24.0
Delilah Creek + 50 ppb PBO	8.4	8.4	7.59	7.59	-	23.8
Delilah Creek + 100 PBO	6.6	6.6	7.19	7.19	-	24.1
Delilah Creek C8 Rinsate	7.1	7.1	7.26	7.26	-	24.2



Table A-14. Summary of results of a 96-hr *C. dubia* follow-up test initiated on 3/26/15, examining the effects of PBO on a 50% dilution of Delilah Creek collected 3/11/15. Results indicate toxicity observed in the initial screening test was not due to a pyrethroid.

Treatment	24-hr Survival		48-hr Survival		72-hr Survival		96-hr Survival	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
<b>L1650</b>	100	0.0	100	0.0	100	0.0	100	0.0
<b>L1650 + 100 ppb PBO</b>	100	0.0	100	0.0	100	0.0	100	0.0
<b>Delilah Creek @ 50%</b>	100	0.0	100	0.0	85	9.6	85	10.0
<b>Delilah Creek @ 50% + 100 ppb PBO</b>	95	5.0	90	5.7	85	5.0	85	5.0

Table A-15. Summary of water chemistry during a 96-hr *C. dubia* follow-up test initiated on 3/26/15, examining the effects of PBO on a 50% dilution of Delilah Creek collected 3/11/15.

Treatment	Temp (°C)	EC (µS/cm)	DO (mg/L)	pH
	Average			
<b>L1650</b>	24.8	275	8.2	7.96
<b>L1650 + 100 ppb PBO</b>	24.4	271	8.0	7.87
<b>Delilah Creek @ 50%</b>	24.4	184	8.4	7.71
<b>Delilah Creek @ 50% + 100 ppb PBO</b>	24.5	181	8.0	7.66

Table A-16. Summary of results of a *C. dubia* chronic toxicity test initiated on 3/25/15, evaluating the toxicity of ambient surface water samples collected on 3/23/15.

Treatment	Survival (%) <sup>1</sup>	Reproduction (offspring) <sup>1</sup>	
		Mean	SE
<b>L1650</b>	100	18.8	1.60
<b>Low Conductivity Control</b>	100	22.0	1.16
<b>Delilah Creek</b>	100	19.5	1.09
<b>Lower Rowdy Creek<sup>2</sup></b>	100	11.1	1.22
<b>Upper Rowdy Creek<sup>2</sup></b>	100	10.6	0.75
<b>Morrison Creek</b>	90	19.6	1.83
<b>FIELDQA (Delilah Creek)</b>	80	15.0	2.44
<b>Delilah Creek roadside ditch</b>	100	17.5	0.85

1. Highlighted cells indicate statistically significant reductions in survival or reproduction compared to the laboratory control. Data were analyzed using SWAMP standard statistical protocols.
2. These samples were compared to the Low Conductivity Control.

Table A-17. Summary of water chemistry during a chronic *C. dubia* toxicity test initiated on 3/25/15, examining the toxicity of ambient surface water samples collected on 3/23/15.

Treatment	DO (mg/L)				pH			
	Initial	Final	Min	Max	Initial	Final	Min	Max
<b>L1650</b>	8.2	7.2	7.2	8.6	8.09	7.93	7.75	8.09
<b>Low Conductivity Control</b>	8.6	7.4	7.3	8.6	7.81	7.42	7.31	7.81
<b>Delilah Creek</b>	8.6	7.3	7.3	8.6	7.45	7.33	7.26	7.45
<b>Lower Rowdy Creek</b>	8.6	7.4	7.4	8.6	7.45	7.72	7.45	7.84
<b>Upper Rowdy Creek</b>	8.5	7.6	7.4	8.5	7.99	7.84	7.71	7.99
<b>Morrison Creek</b>	8.6	7.5	7.1	8.6	7.69	7.48	7.31	7.77
<b>FIELDQA (Delilah Creek)</b>	8.6	7.5	7.3	8.6	7.52	7.78	7.29	7.78
<b>Delilah Creek roadside ditch</b>	8.5	6.9	5.7	8.5	7.16	7.08	6.74	7.25
		<b>EC</b>	<b>Total Ammonia</b>	<b>Unionized Ammonia<sup>1</sup></b>	<b>Alkalinity (CaCO<sub>3</sub>)</b>	<b>Hardness (CaCO<sub>3</sub>)</b>	<b>Temperature (°C)</b>	
		(µS/cm)	(mg/L)				Min	Max
<b>L1650</b>		255	ND	ND	58	84	23.3	24.1
<b>Low Conductivity Control</b>		67	ND	ND	NR	NR	23.0	23.8
<b>Delilah Creek</b>		97	0.10	0.001	22	32	23.4	23.4
<b>Lower Rowdy Creek</b>		95	ND	ND	58	48	23.4	23.5
<b>Upper Rowdy Creek</b>		95	ND	ND	54	48	23.4	23.7
<b>Morrison Creek</b>		76	0.10	0.002	30	32	23.2	23.5
<b>FIELDQA (Delilah Creek)</b>		96	ND	ND	20	32	23.3	23.6
<b>Delilah Creek roadside ditch</b>		63	0.27	0.002	16	20	23.4	23.7

1. This unionized ammonia reading is based off the total ammonia measured at sample receipt and upon the water chemistry measured at test initiation. ND: Not Detected, NR: Not Recorded

Table A-18. Summary of results of an *H. azteca* acute 10-day water column toxicity test initiated on 3/27/15, evaluating the toxicity of ambient surface water samples collected on 3/23/15.

Treatment	Survival (%) <sup>1</sup>	
	Mean	SE
ROEPAMHR	94	2.4
Tilas Slough	100	0.0

1. Highlighted cells indicate a significant reduction in survival compared to the laboratory control. Data were analyzed using SWAMP standard statistical protocols.

Table A-19. Summary of water chemistry during an *H. azteca* 10-day water column toxicity test initiated on 3/27/15, examining the toxicity of ambient surface water samples collected on 3/23/15.

Treatment	DO (mg/L)				pH			
	Initial	Final	Min	Max	Initial	Final	Min	Max
ROEPAMHR	8.6	8.3	7.4	8.6	8.23	8.11	7.66	8.23
Tilas Slough	8.7	8.2	7.2	8.7	8.02	7.74	7.57	8.02
		EC	Total Ammonia	Unionized Ammonia <sup>1</sup>	Alkalinity (CaCO <sub>3</sub> )	Hardness (CaCO <sub>3</sub> )	Temperature (°C)	
		(µS/cm)	(mg/L)				Min	Max
ROEPAMHR		317	ND	ND	60	100	20.2	23.2
Tilas Slough		2813	0.31	0.013	60	320	20.3	23.7

1. This unionized ammonia reading is based off the total ammonia measured at sample receipt and upon the water chemistry measured at test initiation.

Table A-20. Summary of results of a *C. dubia* chronic toxicity test initiated on 6/24/15, evaluating the toxicity of ambient surface water samples collected on 6/23/15.

Treatment	Survival (%) <sup>1</sup>	Reproduction (offspring) <sup>1</sup>	
		Mean	SE
L1650	100	31.1	4.1
Lower Rowdy Creek	100	18.2	6.6
Low Conductivity Control	100	24.7	3.0

1. Highlighted cells indicate statistically significant reductions in survival or reproduction compared to the laboratory control. Data were analyzed using SWAMP standard statistical protocols.

Table A-21. Summary of water chemistry during a chronic *C. dubia* toxicity test initiated on 6/24/15, examining the toxicity of ambient surface water samples collected on 6/23/15.

Treatment	DO (mg/L)				pH			
	Initial	Final	Min	Max	Initial	Final	Min	Max
<b>L1650</b>	8.20	7.80	7.20	8.30	8.21	7.91	7.91	8.23
<b>Lower Rowdy Creek</b>	8.30	7.60	7.30	8.40	8.10	7.64	7.64	8.10
<b>Low Conductivity Control</b>	8.30	7.70	7.30	8.30	7.94	7.50	7.50	7.94
		<b>EC</b>	<b>Total Ammonia</b>	<b>Unionized Ammonia<sup>1</sup></b>	<b>Alkalinity (CaCO<sub>3</sub>)</b>	<b>Hardness (CaCO<sub>3</sub>)</b>	<b>Temperature (°C)</b>	
		(µS/cm)	(mg/L)				Min	Max
<b>L1650</b>		259	ND	ND	60	92	24.1	25.0
<b>Lower Rowdy Creek</b>		89	ND	ND	36	40	24.2	25.0
<b>Low Conductivity Control</b>		94	ND	ND	20	36	24.0	25.0

1. This unionized ammonia reading is based off the total ammonia measured at sample receipt and upon the water chemistry measured at test initiation.

Table A-22. Summary of results of a *C. dubia* chronic toxicity test initiated on 6/25/15, evaluating the toxicity of ambient surface water samples collected on 6/24/15 and 6/25/15.

Treatment	Survival (%) <sup>1</sup>	Reproduction (offspring) <sup>1</sup>	
		Mean	SE
<b>L1650</b>	100	25.9	6.2
<b>Low Conductivity Control</b>	100	21.7	8.5
<b>Delilah Creek</b>	90	5.8	5.8
<b>Upper Rowdy Creek</b>	80	16.1	10.8

1. Highlighted cells indicate statistically significant reductions in survival or reproduction compared to the laboratory control. Data were analyzed using SWAMP standard statistical protocols.

Table A-23. Summary of water chemistry during a chronic *C. dubia* toxicity test initiated on 6/25/15, examining the toxicity of ambient surface water samples collected on 6/24/15 and 6/25/15.

Treatment	DO (mg/L)				pH			
	Initial	Final	Min	Max	Initial	Final	Min	Max
<b>L1650</b>	7.6	7.4	7.3	8.6	8.21	7.83	7.83	8.21
<b>Low Conductivity Control</b>	7.6	7.2	7.2	8.6	7.91	7.45	7.44	7.91
<b>Delilah Creek</b>	7.7	7.6	7.5	8.6	7.85	7.44	7.35	8.03
<b>Upper Rowdy Creek</b>	7.6	7.4	7.4	8.6	8.09	7.65	7.64	8.16
		<b>EC</b>	<b>Total Ammonia</b>	<b>Unionized Ammonia<sup>1</sup></b>	<b>Alkalinity (CaCO<sub>3</sub>)</b>	<b>Hardness (CaCO<sub>3</sub>)</b>	<b>Temperature (°C)</b>	
		(µS/cm)	(mg/L)				Min	Max
<b>L1650</b>		255	ND	ND	60	92	23.9	24.6
<b>Low Conductivity Control</b>		76	ND	ND	18	24	24.2	24.7
<b>Delilah Creek</b>		80	ND	ND	16	16	24.1	24.5
<b>Upper Rowdy Creek</b>		120	ND	ND	34	36	24.1	24.7

1. This unionized ammonia reading is based off the total ammonia measured at sample receipt and upon the water chemistry measured at test initiation. ND: Non-Detect

Table A-24. Summary of results from a 7-day *C. dubia* Phase I Toxicity Identification Evaluation initiated on 7/2/15 for Delilah Creek collected on 6/24/15.

Treatment	7-day Survival	Reproduction (offspring)	
	(%)	Mean	SE
<b>L1650</b>	100	15.1	2.6
<b>L1650 Hardness Adj. (HA) @ 18 mg/L</b>	100	14.9	1.4
<b>L1650 (HA) + MeOH @ 0.33%</b>	100	8.7	1.4
<b>L1650 (HA) + Eluate @ 2x</b>	100	11.8	1.2
<b>L1650 (HA) + 3 mg/L EDTA</b>	100	9.0	1.6
<b>L1650 (HA) + 8 mg/L EDTA</b>	100	1.9	1.0
<b>L1650 (HA) + 25 ppb PBO</b>	100	8.5	1.2
<b>L1650 (HA) + 50 ppb PBO</b>	90	9.2	1.9
<b>L1650 C8 Blank</b>	100	17.6	0.8
<b>L1650 (HA) Hardness-Adj. Up</b>	100	17.4	1.2
<b>Delilah Creek</b>	60	5.7	2.1
<b>Delilah Creek + 3 mg/L EDTA<sup>2</sup></b>	78	5.1	2.1
<b>Delilah Creek + 8 mg/L EDTA<sup>2</sup></b>	80	0.0	0.0
<b>Delilah Creek + 25 ppb PBO<sup>2</sup></b>	80	6.5	2.4
<b>Delilah Creek + 50 PBO<sup>2</sup></b>	40	1.8	1.6
<b>Delilah Creek C8 Rinsate<sup>2</sup></b>	90	5.7	1.4
<b>Delilah Creek Hardness-Adj. Up<sup>2</sup></b>	90	8.6	1.6

1. Highlighted areas indicate specific TIE signals and are compared to the appropriate control or method blank.

2. These treatments were compared to the unmanipulated ambient sample of Delilah Creek.

Table A-25. Summary of water chemistry from a 7-day *C. dubia* Phase I Toxicity Identification Evaluation initiated on 7/2/15 for Delilah Creek collected on 6/24/15.

Treatment	DO (mg/L)		pH		Initial EC (µS/cm)	Final Temp (°C)
	Min	Max	Min	Max		
L1650	7.5	7.9	8.00	8.13	261	24.2
L1650 Hardness Adj. (HA) @ 18 mg/L	7.1	8.0	7.50	7.74	67	24.1
L1650 (HA) + MeOH @ 0.33%	7.4	8.3	7.47	7.64	66	24.1
L1650 (HA) + Eluate @ 2x	7.2	8.2	7.42	7.61	66	24.1
L1650 (HA) + 3 mg/L EDTA	7.4	8.2	7.42	7.48	66	24.2
L1650 (HA) + 8 mg/L EDTA	7.6	8.3	7.47	7.52	67	24.0
L1650 (HA) + 25 ppb PBO	7.5	8.2	7.48	7.70	66	24.1
L1650 (HA) + 50 ppb PBO	7.3	8.0	7.42	7.63	66	24.1
L1650 C8 Blank	7.4	8.0	7.96	8.12	262	24.0
L1650 (HA) Hardness-Adj. Up	7.8	7.9	7.77	7.90	167	24.0
Delilah Creek	8.0	8.1	7.51	7.61	76	24.1
Delilah Creek + 3 mg/L EDTA <sup>2</sup>	8.0	8.2	7.51	7.54	76	24.0
Delilah Creek + 8 mg/L EDTA <sup>2</sup>	7.8	8.0	7.49	7.59	77	24.0
Delilah Creek + 25 ppb PBO <sup>2</sup>	7.9	8.3	7.48	7.51	78	24.0
Delilah Creek + 50 PBO <sup>2</sup>	8.0	8.4	7.50	7.58	76	24.0
Delilah Creek C8 Rinsate <sup>2</sup>	8.1	8.2	7.63	7.66	77	24.1
Delilah Creek Hardness-Adj. Up	8.0	8.1	7.87	7.93	174	24.0

Table A-26. Summary of results from a 7-day *C. dubia* Phase I Toxicity Identification Evaluation initiated on 7/2/15 for Upper Rowdy Creek collected on 6/25/15.

Treatment	7-day Survival	Reproduction (offspring)	
	(%)	Mean	SE
<b>L1650</b>	90	18.0	2.2
<b>L1650 Hardness Adj. (HA) @ 34 mg/L</b>	100	18.2	1.3
<b>L1650 Hardness Adj. Up</b>	100	20.9	0.6
<b>Upper Rowdy Creek</b>	90	13.3	2.4
<b>Upper Rowdy Creek Hardness Adj. Up</b>	90	20.4	2.7

Table A-27. Summary of water chemistry from a 7-day *C. dubia* Phase I Toxicity Identification Evaluation initiated on 7/2/15 for Upper Rowdy Creek collected on 6/25/15.

Treatment	DO (mg/L)		pH		Initial EC (μS/cm)	Final Temp (°C)
	Min	Max	Min	Max		
<b>L1650</b>	7.6	8.3	7.99	8.07	264	24.0
<b>L1650 Hardness Adj. (HA) @ 34 mg/L</b>	7.5	8.3	7.63	7.82	113	23.6
<b>L1650 Hardness Adj. Up</b>	7.7	8.1	7.75	7.94	159	23.7
<b>Upper Rowdy Creek</b>	7.7	8.2	7.77	7.97	87	24.0
<b>Upper Rowdy Creek Hardness Adj. Up</b>	7.8	8.2	7.83	7.98	131	23.9

Table A-28. Summary of results of an *H. azteca* acute 10-day water column toxicity test initiated on 6/25/15, evaluating the toxicity of ambient surface water samples collected on 6/23/15.

Treatment	Survival (%) <sup>1</sup>	
	Mean	SE
<b>ROEPAMHR</b>	98	4.4
<b>Tilas Slough</b>	100	0.0

1. Highlighted cells indicate a significant reduction in survival compared to the laboratory control. Data were analyzed using SWAMP standard statistical protocols.



Table A-29. Summary of water chemistry during an *H. azteca* 10-day water column toxicity test initiated on 6/25/15, examining the toxicity of ambient surface water samples collected on 6/23/15.

Treatment	DO (mg/L)				pH	
	Initial	Final	Min	Max	Initial	Final
<b>ROEPAMHR</b>	7.8	8.1	7.8	8.3	8.14	8.07
<b>Tilas Slough</b>	8.3	8.2	8.1	8.4	7.48	7.88
	EC	Total Ammonia	Unionized Ammonia <sup>1</sup>	Alkalinity (CaCO <sub>3</sub> )	Hardness (CaCO <sub>3</sub> )	Temperature (°C)
	(µS/cm)	(mg/L)				Initial
<b>ROEPAMHR</b>	320	ND	ND	58	108	23.3
<b>Tilas Slough</b>	2400	0.07	0.001	58	118	22.4

1. This unionized ammonia reading is based off the total ammonia measured at sample receipt and upon the water chemistry measured at test initiation.

## APPENDIX B – ORGANIC CHEMICAL ANALYTES LIST (2013-2015)

1,1,1,2-Tetrachloroethane	Bensulide	Diafenthuron
1,1,1-Trichloroethane	Benzene	Diazinon
1,1,2,2-Tetrachloroethane	Benzoximate	Dibromochloromethane
1,1,2-Trichloroethane	Bifenazate	Dibromomethane
1,1-Dichloroethane	Bitertanol	Dichlorodifluoromethane
1,1-Dichloroethene	Boscalid	Dichlorvos
1,1-Dichloropropene	Bromobenzene	Diclobutrazol
1,2,3-Trichlorobenzene	Bromochloromethane	Dicrotophos
1,2,3-Trichloropropane	Bromodichloromethane	Diethofencarb
1,2,4-Trichlorobenzene	Bromoform	Difenoconazole
1,2,4-Trimethylbenzene	Bromomethane	Difflubenzuron
1,2-Dibromo-3-chloropropane	Bromuconazole (isomer)	Dimethenamide
1,2-Dibromoethane (EDB)	Bupirimate	Dimethoate
1,2-Dichlorobenzene	Buprofezin	Dimethomorph
1,2-Dichloroethane	Butafenacil	Dimoxystrobin
cis-1,2-Dichloroethene	Butocarboxim	Diniconazole
trans-1,2-Dichloroethene	Carbaryl	Dioxacarb
1,2-Dichloropropane	Carbaryl	Diuron
1,3-Dichlorobenzene	Carbendazim	DMST
1,3-Dichloropropane	Carbetamide	DNOC
cis-1,3-Dichloropropene	Carbofuran	Dodine
trans-1,3-Dichloropropene	Carbofuran	Doramectin
1,4-Dichlorobenzene	Carbon disulfide	Emamectin Ba
1-Naphthol	Carbon tetrachloride	Epoxiconazole
2,2-Dichloropropane	Carbosulfan	Eprinomectin
2-Butanone	Carboxine	Esprocarb
2-Chlorotoluene	Carfentrazone-ethyl	Ethidimuron
2-Hexanone	Chlorantraniliprole	Ethiofencarb
3-Hydroxycarbofuran	Chlordimeform	Ethiofencarb-sulfoxide
3-Hydroxycarofuran	Chlorfenvinphos	Ethion
4-Chlorotoluene	Chlorfluazuron	Ethiprole
4-Isopropyltoluene	Chlorobenzene	Ethirimol
4-Methyl-2-pentanone	Chloroethane	Ethofumesate
Acephate	Chloroform	Ethoprop
Acetamiprid	Chloromethane	Ethylbenzene
Acetochlor	Chloroxuron	Etobenzanid
Acetone	Chlorpropham (CIPC)	Etoxazole
Acibenzolar-S-methyl	Chlorpyrifos	Famoxadone
Alanycarb	Chlorpyrifos-methyl	Fenamidone
Aldicarb	Chlortoluron	Fenarimol
Aldicarb sulfone	Clethodim	Fenazaquin
Aldicarb sulfoxide	Clofentezine	Fenbuconazole
Aldicarb-sulfone	Clothianidin	Fenbutatin Oxide
Aldicarb-sulfoxide	Coumaphos	Fenhexamid
Ametryn	Cumyluron	Fenobucarb
Amicarbazone	Cyanazine	Fenpropimorph
Aminocarb	Cyazofamid	Fenpuroximate
Aspon	Cycluron	Fenuron
Atrazine	Cyflufenamid	Feoxycarb
Avermectin Ba	Cyhexatin	Flonicamid
Azinphos-methyl	Cymoxanil	Fluazifop-butyl
Azoxystrobin	Cyproconazole (isomer)	Flubendiamide
Benalaxyl	Cyprodinil	Fludioxinil
Bendiocarb	Cyromazine	Flufenacet
Benfuracarb	Daimuron	Flufenoxuron

Fluometuron	Methylene chloride	Pyriproxyfen
Fluopicolide	Metobromuron	Quinoxifen
Fluoxastrobin	Metolachlor	Sebuthylazine
Fluquinconazole	Metoxuron	Secbumeton
Flusilazole	Metribuzin	sec-Butylbenzene
Flutolanil	Metrofenone	Sethoxydim
Flutrialfol	Metsulfuron-methyl	Siduron
Forchlorfenuron	Mevinphos	Simazine
Formesafen	Mexacarbate	Simetryn
Formetanate	MITC	Spinetoram-J+L
Fuberidazole	Molinate	Spinosad
Furalaxyl	Monocrotophos	Spirodiclofen
Halofenozide	Monolinuron	Spiromecifen
Hexachlorobutadiene	Moxidectin	Spirotetramat
Hexaflumuron	Myclobutanil	Spiroxamine
Hexazinone	Naphthalene	Sulfentrazone
Hexythiazox	Napropamide	Tebuconazole
Hydramethylnon	n-Butylbenzene	Tebufenozide
Imazalil	Neburon	Tebuthiuron
Imazapyr	Nitenpyram	Tefufenpyrad
Imazethapyr	Novaluron	Temephos
Imibenconazole	n-Propylbenzene	Tempraloxymid
Imidacloprid	Omethoate	Terbumeton
Indoxacarb	Oxadixyl	Terbutryn
Iodomethane	Oxamyl	tert-Butylbenzene
Ipconazole	Oxamyl	Tertraconazole
Iprovalicarb	o-Xylene	Tetrachloroethene
Isocarbamid	Pencycuron	Thiabendazole
Isofenphos	Pendimethalin	Thiacloprid
Isoprocarb	Phorate	Thiamethoxam
Isoprofuron	Phosmet	Thidiazuron
Isopropylbenzene	Phoxim	Thiobencarb
Isoprothiolane	Picoxystrobin	Thiofanox
Ivermectin	Piperonyl butoxide	Thiophanate-methyl
Kresoxim-methyl	Pirimicarb	Toluene
Lactofen	Pirimiphos-methyl	Triadimefon
Linuron	Proamocarb	Triadimenol
m,p-Xylene	Prochloraz	Triazophos
Malathion	Promecarb	Trichloroethene
Mandipropamid	Prometon	Trichlorofluoromethane
Mefenacet	Prometryn	Trichlorotrifluoroethane
Mepanipyrim	Propachlor	Tricyclazole
Mepronil	Propargite	Tridemorph
Metaflumizone	Propazine	Trifloxystrobin
Metalaxyl	Propham	Triflumizole
Metconazole	Propiconazole	Triflumuron
Methamidophos	Propoxur	Triflusulfuron-methyl
Methfuroxam	Propoxur	Triphenyl Phosphate
Methidathion	Propyzamide	Triticonazole
Methiocarb	Pryaclostrobin	Uniconazole
Methiocarb	Pymetrozin	Vamidotion
Methomyl	Pyracarbolid	Xylenes, total
Methomyl	Pyridaben	Zoxamide
Methoprotryne	Pyridaphenthion	
Methoxyfenozide	Pyrimethanil	

## APPENDIX C – FOLLOW-UP RUNOFF SAMPLE (JUNE 8, 2017)

### Purpose

In 2013 and 2015 the Regional Water Board implemented a monitoring program to further our understanding of water and sediment quality conditions in the tributaries to the Smith River that flow through the Smith River Plain and to evaluate if the application of agricultural pesticides are impacting the aquatic environment. The monitoring program analyzed surface water samples collected during both wet and dry seasons focusing on standard water quality measures (temperature, dissolved oxygen, conductivity, and pH), nutrients, various pesticides, dissolved copper and zinc, and toxicity.

Throughout the study period, standard water quality measures were observed to be in compliance with water quality objectives, and within acceptable limits for a healthy aquatic ecosystem. While nutrient analysis documented exceedances of the USEPA criteria in a number of instances, the concentrations were consistent with similar locations and settings, (i.e. alluvial flood plain and agricultural environment).

The chemical analysis of surface water samples documented the presence of several legacy (used exclusively before 2000) and current use pesticides in the tributaries of the Smith River Plain. In some cases the concentrations of these pesticides exceeded the lowest USEPA 2014 Aquatic Life Benchmarks for fish and invertebrates. Additionally, dissolved copper (used as a fungicide) was detected in every surface water sample with 6 of 27 samples exceeding the California Toxics Rule (CTR) criteria to protect freshwater for chronic toxicity.

Toxicity testing documenting the survival (acute toxicity) and reproductive capacity (chronic toxicity) of the test species *Ceriodaphnia dubia* in surface water samples was performed on samples collected from five locations in the Smith River Plain to evaluate if there were any observed negative impacts to the aquatic environment. In 8 of 27 samples, these tests demonstrated statistically significant reductions in reproductivity (positive for chronic toxicity), including three tests in which the “control” location (Upper Rowdy Creek) was positive for chronic toxicity. In another 2 samples, a positive acute toxic response was documented, with 1 of the samples demonstrating no test species survival.

To determine the cause of the observed toxic responses in 2015, three samples that exhibited chronic or acute toxicity were further tested utilizing a toxic identification evaluation (TIE). The TIE results identified three factors responsible for the positive toxic test results; low conductivity and the presence of both a metal and a non-polar organic compound.

In an effort to gain further understanding on the cause of the large number of chronic toxicity responses that were documented in 2013 and 2015, Regional Water Board staff conducted additional sampling at three locations (Delilah Creek, Morrison Creek, and Upper Rowdy Creek) during a 1 ½ inch rain storm on June 8, 2017. These samples were evaluated for pesticides, metals (copper and zinc), and water column toxicity.

## Analytical Results

### Physico-chemical Field Measurements

Physico-chemical measurements obtained in the field during the sample event are presented in Table 1.

**Table 1.** Physico-chemical field measurements at surface water sampling sites.

Sample Site	Dissolved Oxygen, mg/L	pH	Specific Conductance, uS/CM	Water Temperature, °C
Delilah Creek	10.03	8.29	69	13.15
Morrison Creek	10.46	8.08	58	12.56
Upper Rowdy Creek	11.16	8.07	64	12.42

uS/cm = microSiemens per centimeter

The Regional Water Board's Basin Plan Objectives are presented in Table 4 (see page 9) of the main report for reference. All of the parameters were within expected ranges.

### Pesticides

Samples were analyzed for a suite of 206 pesticides (including isomers and degradants, see Appendix E). Two current use pesticides were detected in samples from Delilah Creek (Table 2). Diuron was detected at 4.5 ug/L, below the EPA Aquatic Life Benchmark concentration of 26.4 ug/L. Chlorpropham does not have an established benchmark or threshold by which to determine if the detected concentration of 8.1 ug/L would be considered toxic.

**Table 2.** Concentrations of 2 detected pesticides and associated thresholds (criteria or standards) at Delilah Creek from June 8, 2017.

Analyte, ug/L	Last Use per CaDPR*	Delilah Creek	Threshold, (ug/L)	Reference
Chlorpropham	2015	8.1	No Threshold	
Diuron	2015	4.5	26.4	1

\* "Last use per CaDPR" - 2015 is the most recently available information.

Criteria and threshold references, per Marshack (2016)

1: USEPA 2017 Aquatic Life Benchmarks (lowest value, see Table 7 main report page 13)

### Metals

Surface water samples were analyzed for two metals: copper and zinc (total and dissolved fractions). These metals are naturally prevalent in the Smith River Watershed, but are relevant to this study since pesticide compounds that include copper and zinc are also applied to the agricultural fields of the Smith River Plain at various times throughout the year.

The toxicity to aquatic organisms by copper and zinc in surface water is dependent upon the concentration of each metal and the hardness of the surface water. Metals toxicity increases as water hardness decreases, which means at a given concentration, copper or zinc will have a more pronounced negative affect on aquatic life at a lower water hardness level.

Total zinc was detected in samples from Delilah and Morrison Creek in concentrations below the CTR criteria to protect freshwater aquatic life for acute and chronic toxicity. Copper (total and dissolved fractions) was detected in every sample collected (see Table 3). The total and dissolved copper concentration/hardness pairs at Delilah Creek exceeded both the CCC and CMC of the CTR Freshwater Aquatic Life Criteria, (see figures 1 and 2 below). As discussed below, these exceedances alone are not indicative of an environment that may lead to reduced reproduction or survival. However, chronic toxicity was observed in the sample from Delilah Creek.

Table 3. Copper, zinc, and hardness concentrations in samples from June 8, 2017.

Station	Hardness (mg/L)	Copper (ug/L)		Zinc (ug/L)	
		Total	Dissolved	Total	Dissolved
Morrison Creek	28	1.21	0.49	1.26	ND
Delilah Creek	24	<b>21.8</b>	<b>8</b>	5.36	ND
Upper Rowdy Creek	32	0.6	0.23	ND	ND

**Red text** indicates exceedance of the CTR Freshwater Aquatic Life Criteria.

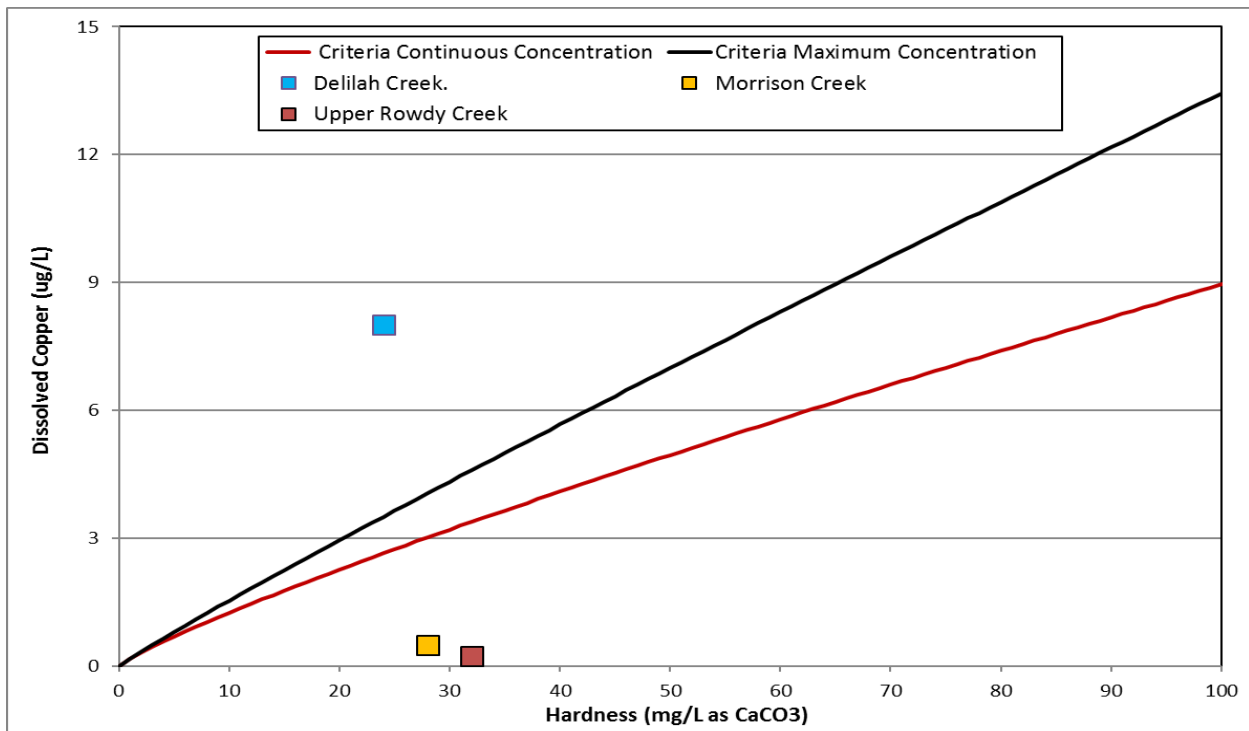


Figure 1. Dissolved copper concentration and water hardness in surface water samples collected on June 8, 2017 in comparison to the CCC and CMC of the CTR Freshwater Aquatic Life Criteria.



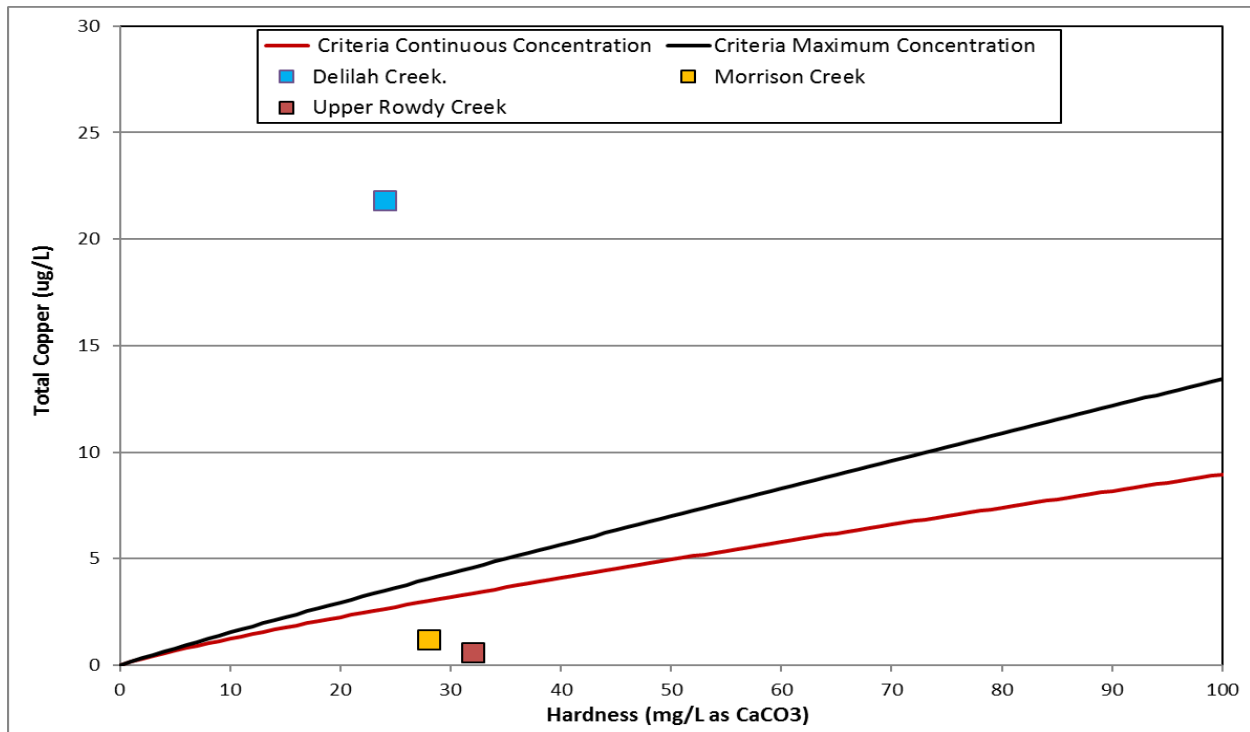


Figure 2. Total copper concentration and water hardness in surface water samples collected on June 8, 2017 in comparison to the CCC and CMC of the CTR Freshwater Aquatic Life Criteria.

## Toxicity Results

One sample from each sample site was analyzed for aquatic toxicity using three separate test species; the water flea *Ceriodaphnia dubia*, the fathead minnow *Pimephales promelas*, and the green alga *Selenastrum capricornutum*. The conductivity of the sample water was below 100µs/cm for all three sites. Therefore, in addition to conducting routine toxicity testing, a corresponding Low Conductivity Control test was conducted on the *C. dubia* and *P. promelas* in order to rule out low conductivity as a confounding factor in any toxicity results. The test control used in *S. capricornutum* tests already has a low conductivity, therefore it was not necessary to include a Low Conductivity Control for this species.

The *C. dubia* and *P. promelas* toxicity tests did not exhibit any statistically significant reductions in survival or reproduction when compared to the Low Conductivity Control populations. In the *S. capricornutum* toxicity test, the sample collected from Delilah Creek exhibited a significant reduction in algal cell growth when compared to the control. A follow-up Toxic Identification Evaluation (TIE) was initiated on this sample, which indicated that a non-polar organic compound (i.e. a pesticide) and a metal(s) were the main contributors to the toxicity observed in the initial screening toxicity test.

## Discussion

It has been documented through extensive toxicity testing in 2013, 2015, and 2017 that the extremely low conductivity and hardness of the source waters flowing through the Smith River Plain can have a negative impact on the reproductive capabilities of the toxicity test species *C. dubia*. This negative impact manifests itself in lower reproduction endpoints when compared to the toxicity test control populations, which in turn suggests that toxicity effect exists in the sample when there is no toxicity present producing a false positive result. This was reflected in the results for Morrison and Upper

Rowdy Creeks, where there was no statistically significant reduction in survival of *C. dubia* between the standard toxicity test results and the low conductivity control results. Toxicity testing procedures for evaluating the toxicity of surface water must take into consideration the conductivity and hardness of the source water.

The result from Delilah Creek also demonstrated a significant reduction in *S. capricornutum* cell growth when compared to the control results, and TIE results strongly suggest that metal(s) and a non-polar organic compound (i.e. pesticides) were the main contributors to the toxicity observed. Chemical analysis of the sample from Delilah Creek demonstrated exceedances of CTR Freshwater Aquatic Life Criteria for the metal copper.

The results of the main study and this sample event demonstrate that chemicals and metals used as pesticides in agricultural activities are being found in low level concentrations in surface waters of the Smith River Plain, and can affect the water quality of the tributaries by contributing to the observed toxicity. Individually the chemicals may not be in concentrations that would produce a toxic response or be directly harmful, but the extremely low hardness and conductivity may act to increase the sensitivity of aquatic life and the associated response to these low level concentrations of contaminants that may be present in the water column.



**APPENDIX D - TOXICITY FINAL REPORT (JUNE 8, 2017)**

**Toxicity Final Report**

**UC Davis Aquatic Health Program Laboratory**

### ***Introduction/Background***

Three sites were selected for testing with the cladoceran *Ceriodaphnia dubia*, the fathead minnow *Pimephales promelas*, and the green alga *Selenastrum capricornutum*. This report discusses the results of toxicity tests conducted on samples collected on June 8, 2017.

### ***Activities Undertaken***

The following tasks were completed during this sampling event:

- One (each) *C. dubia*, *P. promelas*, and *S. capricornutum* initial screening toxicity test
- One *S. capricornutum* Toxicity Identification Evaluation follow-up test examining the toxicity observed with site Delilah Creek

### ***Materials and Methods***

Staff from the NCRWQCB collected water samples on June 8, 2017 as subsurface grabs in clean 1-gal amber glass bottles. Water samples were transported, stored and preserved following protocols outlined in the UC Davis-Aquatic Health Program Laboratory (UCD AHP) and SWAMP standard operating procedures.

### ***Water Quality***

Field water quality measurements included salinity and were recorded for each sampling time on SWAMP sample chain of custody sheets by NCRWQCB field staff. Ammonia-nitrogen was measured at UCD AHP within 24 hours of sample receipt using a HACH DR-890 portable colorimeter and a HACH Am-Ver Low-Range Ammonia Test'N Tube Reagent Set. Ammonia measurements of 0.06 mg/L and below are reported herein as Non-Detects (ND). Hardness and alkalinity were measured on all ambient samples (titrimetric methods) within 48-hours of sample receipt.

### ***Toxicity Testing Methods***

UCD AHP toxicity testing methods are based on protocols developed by USEPA, SWAMP QAPrP, and UCD AHP SOPs. Chronic toxicity testing for *Ceriodaphnia dubia*, *Pimephales promelas*, and *Selenastrum capricornutum* followed protocols outlined in *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*.

### ***Statistics***

This project was designed to create data comparable with data contained in the database of California's Surface Water Ambient Monitoring Program. The SWAMP protocol involves the examination of significant differences in test organism performance by a one-tailed heteroschedastic t-test ( $\alpha = 0.05$ ) and a categorization of the performance of organisms exposed to the ambient sample as either greater or less than 80% of the control performance. Therefore samples were considered toxic only when both a significant t-test result and performance below 80% of the control was observed. Additionally, Low Conductivity Controls were included with the *C. dubia* and *P. promelas* tests. A Low Conductivity Control is first statistically compared to the standard Test Acceptability Criteria control (TAC) to determine whether low conductivity has a negative impact on the test organism. In instances where the Low Conductivity Control impairs a particular endpoint (e.g. *C. dubia* reproduction), the ambient sample with the lower conductivity is statistically compared to the Low Conductivity Control, rather than the standard TAC control, to determine whether the ambient sample is toxic. All analyses were performed using custom Excel spreadsheets created by the SWAMP Database Management Team at Moss Landing Marine Laboratories (Office Excel 2007 (v. 12), Microsoft Inc, USA).

Sample conductivities were below 100  $\mu\text{S}/\text{cm}$  for all three sites (Delilah Creek, Morrison Creek at Fred Haight Road, and Upper Rowdy Creek); thus we included corresponding Low Conductivity Controls with the *C. dubia* and *P. promelas* tests in order to rule out conductivity as a confounding factor. The Test Acceptability Control used in *S. capricornutum* tests inherently has a low conductivity, therefore we did not include a separate Low Conductivity Control with this species.

### Results

In the *C. dubia* toxicity test, organisms exposed to sites Morrison Creek at Fred Haight Road and Upper Rowdy Creek exhibited a statistically significant reduction in reproduction compared to the Test Acceptability Control. The Low Conductivity Control also exhibited reduced reproduction (Table 1). Following SWAMP statistical protocols, these sites were then statistically compared to the Low Conductivity Control. In conducting this analysis, reproduction was no longer statistically significantly reduced. Therefore we believe that low conductivity was the main factor in the toxicity observed in these samples.

Table 1. Summary of results of a chronic *C. dubia* toxicity test initiated on June 9, 2017, examining the toxicity of ambient samples collected on June 8, 2017 by NCRWQCB staff. Highlighted cells indicate statistically significant reductions in reproduction compared to the laboratory control.

Sample ID	Survival		Reproduction	
	Mean	SE	Mean	SE
<b>L1650 – Test Acceptability Control</b>	100	0.00	17	1.43
<b>Delilah Creek</b>	90	0.10	21	2.11
<b>Morrison Creek at Fred Haight Road</b>	100	0.00	7	2.78
<b>Upper Rowdy Creek</b>	100	0.00	3	1.23
<b>Low Conductivity Control</b>	100	0.00	4	0.98

In the *P. promelas* toxicity test, we observed a slight pathogen interference in fish exposed to site Delilah Creek, as fungus was observed on and around deceased fish, and the coefficient of variation was 42% in this treatment (Table 2). However, survival in this site was 80% and biomass was 0.400 mg/individual; neither of these endpoints were reduced when compared to the Test Acceptability or Low Conductivity Controls, therefore no further follow-up was conducted.

Table 2. Summary of results of a chronic *P. promelas* toxicity test initiated on June 9, 2017, examining the toxicity of ambient samples collected on June 8, 2017 by NCRWQCB staff.

Sample ID	Survival		Biomass	
	Mean	SE	Mean	SE
<b>ROEPAMH – Test Acceptability Control</b>	98	2.50	0.489	0.012
<b>Delilah Creek</b>	80	16.83	0.440	0.071
<b>Morrison Creek at Fred Haight Road</b>	93	4.79	0.481	0.040
<b>Upper Rowdy Creek</b>	98	2.50	0.456	0.015
<b>Low Conductivity Control</b>	93	2.50	0.437	0.026

In the *S. capricornutum* toxicity test, site Delilah Creek exhibited a significant reduction in algal cell growth when compared to the Test Acceptability Control (Table 3). This reduction met the TIE follow-up trigger ( $\geq 50\%$  reduction in an endpoint in 96-hours). We conducted a follow-up Toxicity Identification Evaluation in order to determine the class of chemical(s) causing toxicity.

Table 3. Summary of results of a chronic *S. capricornutum* toxicity test initiated on June 9, 2017, examining the toxicity of ambient samples collected on June 8, 2017 by NCRWQCB staff. Highlighted cells indicate statistically significant reductions in cell growth compared to the laboratory control.

Sample ID	Cell Density (10 <sup>6</sup> )		%CV
	Mean	SE	
<b>Glass Distilled – Test Acceptability Control</b>	1.416	0.048	6.81
<b>Delilah Creek</b>	<b>0.428</b>	<b>0.023</b>	10.83
<b>Morrison Creek at Fred Haight Road</b>	1.535	0.064	8.32
<b>Upper Rowdy Creek</b>	1.585	0.038	4.76

In a Phase I TIE, non-statistical comparisons are made between an unmanipulated sample and individual sample manipulations to provide information on the physical and/or chemical characteristics of the contaminant in a toxic sample. Additionally, the toxic sample is retested to confirm toxicity. The manipulations used in this test are described below.

Solid Phase Extraction (SPE) columns primarily remove non-polar organic chemicals from ambient samples. A toxic sample is passed through an SPE column and the through-column “rinsate” is tested along with the unmanipulated sample. Control water is also passed through the SPE column and serves as one of the method controls (method blank). If the toxicant is a non-polar organic chemical, the ambient sample exhibits a reduced endpoint (in this case algal cell growth) while the ambient sample passed through the SPE column (rinsate) will result in higher algal cell growth. The methanol ‘eluate’ is not added-back in *S. capricornutum* tests, due to the algae’s sensitivity to solvents.

Heavy metals can be toxic to aquatic species if concentrations exceed threshold levels. Chelex100 is a chelating ion exchange resin which has a high preference for copper, iron, and other heavy metals over monovalent cations such as sodium and potassium. Chelex resin is added to the ambient sample, binds the metal(s) present in the sample, and then is filtered out prior to testing. If the contaminant is a metal(s) the unmanipulated sample will exhibit reduced algal cell growth while the sample amended with Chelex100 results in higher algal cell growth. Because this manipulation method blank requires a synthetic water that has measurable hardness and alkalinity concentrations, ROEPAMH is used as the Test Acceptability Control in the algal TIE. Addition of the Chelex100 resin pulls out polyvalent metal ions, however it also pulls out those ions such as calcium and magnesium, which account for a sample’s hardness. Also, the protonation of the metal ions results in a pH that falls out of the physiological range of the organism. We therefore adjust the hardness and pH back to the sample’s original parameters after the Chelex 100 manipulation for use in the TIE, and as such these water quality values may differ from those measured in the initial screening test.

In the algal TIE, site Delilah Creek exhibited a statistically significant reduction in cell growth when retested. Site water passed through the C18 SPE column and site water treated with Chelex100 both had robust cell growth which outperformed that of the Test Acceptability Control. Results of these manipulations indicate that a non-polar organic compound and a metal(s) were the main contributors to the toxicity observed in the initial screening toxicity test (Table 4). There was a statistically significant reduction in the C18 column blank, however this result is considered normal.

Table 4. Summary of results of a chronic *S. capricornutum* Toxicity Identification Evaluation examining the toxicity of site Delilah Creek. Highlighted cells indicate statistically significant reductions in cell growth compared to the laboratory control.

Sample ID	Cell Density ( $10^6$ )		%CV
	Mean	SE	
ROEPAMH – Test Acceptability Control	1.522	0.151	19.84
ROEPAMH C18 Blank	0.488	0.031	12.58
ROEPAMH + Chelex100	1.689	0.036	4.29
Delilah Creek	0.541	0.048	18.08
Delilah Creek + Chelex100	1.517	0.038	5.01
Delilah Creek C18 Rinsate	1.969	0.066	6.74

### Water Quality

Table 5. Summary of water quality measurements taken upon sample receipt. ND: Non-Detect.

Sample ID	Total Ammonia	Unionized Ammonia	Alkalinity	Hardness
	mg/L		mg/L as CaCO <sub>3</sub>	
Delilah Creek	0.07	0.003	12	24
Morrison Creek at Fred Haight Road	ND	ND	18	28
Upper Rowdy Creek	ND	ND	24	32
Low Conductivity Control – Cerio	ND	ND	22	30
Low Conductivity Control – Fish	ND	ND	22	20

Table 6. Summary of water quality measurements taken during the chronic *C. dubia* toxicity test.

Sample ID	Initial EC ( $\mu$ S/cm)	DO (mg/L)		pH		Temperature	
		Min	Max	Min	Max	Min	Max
L1650	284	6.97	8.29	7.63	8.34	24.0	26.5
Delilah Creek	84	6.88	8.31	6.85	8.11	24.3	26.5
Morrison Creek at Fred Haight Road	65	6.91	8.32	6.94	8.07	24.2	26.4
Upper Rowdy Creek	72	6.99	8.31	7.07	8.19	24.1	26.3
Low Conductivity Control	83	6.83	8.33	6.99	8.13	24.1	26.3

Table 7. Summary of water quality measurements taken during the chronic *P. promelas* toxicity test.

Sample ID	Initial EC ( $\mu$ S/cm)	DO (mg/L)		pH		Temperature	
		Min	Max	Min	Max	Min	Max
ROEPAMH	306	7.03	8.25	7.66	8.41	21.9	25.2
Delilah Creek	90	6.99	8.20	6.77	8.09	23.8	26.3
Morrison Creek at Fred Haight Road	68	6.85	8.47	6.82	8.03	23.8	25.3
Upper Rowdy Creek	78	6.97	8.36	7.06	8.10	23.8	25.2
Low Conductivity Control	85	7.05	8.26	6.85	8.12	23.3	25.2

Table 8. Summary of water quality measurements taken during the chronic *S. capricornutum* toxicity test.

Sample ID	Initial EC ( $\mu\text{S}/\text{cm}$ )	DO (mg/L)		pH		Temperature	
		Min	Max	Min	Max	Min	Max
Glass Distilled	85	8.46	8.55	7.24	8.15	21.9	22.6
Delilah Creek	130	8.25	8.31	7.54	8.03	19.7	22.7
Morrison Creek at Fred Haight Road	124	8.40	9.33	7.65	8.17	19.1	22.7
Upper Rowdy Creek	131	8.87	9.17	7.77	8.55	18.9	22.6

Table 9. Summary of water quality measurements taken during the chronic *S. capricornutum* Toxicity Identification Evaluation test.

Sample ID	Initial EC ( $\mu\text{S}/\text{cm}$ )	DO (mg/L)		pH		Temperature	
		Min	Max	Min	Max	Min	Max
ROEPAMH	270	7.87	8.80	7.94	9.06	19.7	24.9
ROEPAMH C18 Blank	150	8.02	8.45	7.89	9.07	19.8	25.6
ROEPAMH + Chelex100	640	7.91	8.37	8.06	8.85	20.3	25.8
Delilah Creek	1017	7.97	8.41	8.17	9.10	20.8	25.8
Delilah Creek + Chelex100	1175	8.01	8.75	8.38	8.95	21.1	25.5
Delilah Creek C18 Rinsate	93	7.88	8.47	7.77	8.24	21.4	24.9

## APPENDIX E – ORGANIC CHEMICAL ANALYTES LIST (JUNE 8, 2017)

1,1,1,2-Tetrachloroethane	beta-BHC	Endrin	PCB 1016
1,1,1-Trichloroethane (TCA)	Bifenthrin	Endrin aldehyde	PCB 1221
1,1,2,2-Tetrachloroethane	bis(2-Chloroethyl) ether	Endrin ketone	PCB 1232
1,1,2-Trichloroethane	Bolstar (Sulprofos)	EPN	PCB 1242
1,1-Dichloroethane	Bromacil	EPTC	PCB 1248
1,1-Dichloroethene	Bromobenzene	Esfenvalerate:Fenvalerate	PCB 1254
1,1-Dichloropropene	Bromochloromethane	Esfenvalerate-d6 (SS)	PCB 1260
1,2,3-Trichlorobenzene	Bromodichloromethane	Ethion	Pendimethalin
1,2,3-Trichloropropane	Bromoform	Ethoprop	Permethrin
1,2,4-Trichlorobenzene	Bromomethane (Methyl Bromide)	Ethyl tert-butyl ether (ETBE)	Phorate (Thimet)
1,2,4-Trimethylbenzene	Carbaryl (Sevin)	Ethylbenzene	Phosmet
1,2-Dibromo-3-chloropropane	Carbofuran	Fenpropathrin	Prometon
1,2-Dibromoethane (EDB)	Carbon disulfide	Fensulfothion	Prometryn
1,2-Dichlorobenzene	Carbon tetrachloride	Fenthion	Propachlor
1,2-Dichlorobenzene-d4 (SS)	Chlordane	Fenuron	Propazine
1,2-Dichloroethane (EDC)	Chlorobenzene	Fipronil	Propham
1,2-Dichloroethane-d4 (SS)	Chloroethane (Ethyl Chloride)	Fipronil Desulfinyl	Propoxur
1,2-Dichloroethene, total	Chloroform	Fipronil Sulfide	Ronnel (Trolene)
1,2-Dichloropropane	Chloromethane(Methyl Chloride)	Fipronil Sulfone	Secbumeton
1,3,5-Trimethylbenzene	Chloroxuron (SS)	Fluometuron	sec-Butylbenzene
1,3-Dichlorobenzene	Chlorpropham	gamma-BHC (Lindane)	Siduron
1,3-Dichloropropane	Chlorpyrifos	gamma-Chlordane (trans)	Simazine (Princep)
1,3-Dichloropropene, total	cis-1,2-Dichloroethene	Heptachlor	Simetryn
1,4-Dichlorobenzene	cis-1,3-Dichloropropene	Heptachlor epoxide	Styrene
2,2-Dichloropropane	cis-Tetrachlorvinphos	Hexachlorobutadiene	Sweep
2-Butanone (MEK)	Coumaphos (Co-Ral)	Imidacloprid	Tau-Fluvalinate
2-Chloroethyl vinyl ether	Cyanazine	Iodomethane	Tebuthiuron (SS)
2-Chlorotoluene	Cyfluthrin	Isopropylbenzene (Cumene)	Terbutylazine
2-Hexanone	Cypermethrin	Kepone	Terbutryn
2-Methyl-2-propanol (TBA)	Decachlorobiphenyl (SS)	Lambda-Cyhalothrin	tert-Amyl-Methyl ether (TAME)
4,4'-DDD	delta-BHC	Linuron	tert-Butylbenzene
4,4'-DDE	Deltamethrin:Tralomethrin	m+p-Xylene	Tetrachloroethene (PCE)
4,4'-DDT	Demeton -O and -S	Malathion	Tetrachloro-m-xylene (SS)
4-Bromofluorobenzene (SS)	Demeton-O	Merphos	Tetramethrin
4-Chlorotoluene	Demeton-S	Methidathion	Thiobencarb
4-Isopropyltoluene	Diazinon	Methiocarb	Tokuthion (Prothiofos)
4-Methyl-2-pentanone (MIBK)	Dibromochloromethane	Methomyl	Toluene
Acetone	Dibromofluoromethane (SS)	Methoxychlor	Toluene-d8 (SS)
Acrolein	Dibromomethane	Methyl tert-butyl ether (MTBE)	Total Trihalomethanes
Acrylonitrile	Dichlofenthion (SS)	Methylene chloride	Toxaphene
Aldicarb	Dichlorodifluoromethane (F-12)	Mevinphos (Phosdrin)	trans-1,2-Dichloroethene
Aldrin	Dichlorotrifluoroethane (F123)	Mirex	trans-1,3-Dichloropropene
Allethrin	Dichlorvos (DDVS)	Monuron	Trichloroethene (TCE)
alpha-BHC	Dieldrin	Naled (Dibrom)	Trichlorofluoromethane (F-11)
alpha-Chlordane (cis)	Diisopropyl ether (DIPE)	Naphthalene	Trichloronate
Ametryn	Dimethoate (Cygon)	n-Butylbenzene	Trichlorotrifluoroethane F-113
Aminocarb	Disulfoton (Di-Syston)	Neburon	Trifluralin (Treflan)
Atraton	Diuron	n-Propylbenzene	Triphenylphosphate (SS)
Atrazine (Aatrex)	Endosulfan I	Oxamyl	Vinyl acetate
Azinphos methyl (Guthion)	Endosulfan II	o-Xylene	Vinyl chloride
Barban	Endosulfan sulfate	Parathion (Parathion ethyl)	Xylenes, total
Benzene		Parathion methyl	