DELTA REGIONAL MONITORING PROGRAM

End of Year Summary Report for FY 2019-2020

UC DAVIS AQUATIC HEALTH PROGRAM LABORATORY

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Glossary of Terms and Acronyms

µg/L	Micrograms per liter
µm	Micrometer
ASTM	American Society for Testing and Materials
$CaCO_3$	Calcium carbonate
CV	Coefficient of variation
DO	Dissolved oxygen
EC	Electrical conductivity
EC_{25}	Effect concentration at which a toxicant causes an adverse effect on a quantal (all or nothing) response in 25% of the organisms (US EPA 2002)
EC_{50}	Effect concentration at which a toxicant causes an adverse effect on a quantal (all or nothing) response in 50% of the organisms (US EPA 2002)
GF/A	Whatman Glass Fiber filter, Grade A. Referred to as grade GF/A.
g/L	Grams per liter
IC_{25}	Inhibition concentration at which a toxicant causes an adverse effect on a non-quantal response in 25% of the organisms (US EPA 2002)
IC_{50}	Inhibition concentration at which a toxicant causes an adverse effect on a non-quantal response in 50% of the organisms (US EPA 2002)
LC_{50}	Lethal concentration at which a toxicant causes death in 50% of the organisms (US EPA 2002)
L1650%	50% L16 media and water amended to a hardness of 80-100 mg/L as $CaCO_3$ used with *Ceriodaphnia dubia*
mg	Milligrams
mg/L	Milligrams per liter
mL	Milliliter
MS-222	Tricaine methanesulfonate, fish anesthetic
QAPP	Quality Assurance Project Plan
Delta RMP	Delta Regional Monitoring Program
ROEPAMH	Reverse-Osmosis water amended to a hardness of 80-100 mg/L as $CaCO_3$ used with fathead minnow
ROEPAMHR	Reverse-Osmosis reconstituted water amended to a hardness of 80-100 as $CaCO_3$ used with *Hyalella azteca* and *Chironomus dilutus*
SE	Standard error
SWAMP	Surface Water Ambient Monitoring Program
SWRCB	State Water Resources Control Board
TIE	Toxicity Identification Evaluation
TIE Trigger	50% or greater mortality and statistical differences from the control for *Ceriodaphnia dubia*, *Pimephales promelas*, and *Hyalella azteca*, and a 50% or greater reduction in cell growth for *Selenastrum capricornutum*
UCD AHPL	University of California Davis, Aquatic Health Program Laboratory
USEPA	United States Environmental Protection Agency
USGS	United States Geological Survey
$\dot{X}$	Mean
YCT	*Ceriodaphnia dubia* food consisting of yeast, organic alfalfa, and trout chow

Executive Summary

The Delta Regional Monitoring Program conducted water sampling with the primary goal of tracking and documenting the effectiveness of beneficial use protection and restoration efforts through comprehensive monitoring of water quality constituents and their effects in the Delta through the use of toxicity testing and analytical chemistry. Toxicity tests were performed on samples collected by the United States Geological Survey. Samples were initiated with *Pimephales promelas*, *Ceriodaphnia dubia*, *Selenastrum capricornutum, Hyalella azteca*, and *Chironomus dilutus*, employing toxicity testing methods based on protocols developed by USEPA, SWAMP, and UCD AHP SOPs. Low conductivity controls were included to match the conductivity of ambient samples at or below 100 µS/cm. PRT-style test protocols were used as follow-up tests for ambient samples exhibiting pathogen interference during the initial screening test.

Issues with obtaining known-age *C. dilutus* from vendors, which resulted in several deviations and corrective actions in the 2018-2019 reporting period have been resolved. After the investigation we opted to raise *C. dilutus* from egg cases whenever possible, and during storm events, we ensured to order the youngest age midges available for shipping. These corrective actions proved successful, as we met all test acceptability criteria for the *C. dilutus* tests during this project period. Although there were still some isolated incidences where organisms pupated out during the test, variability was reduced and we met the AFDM endpoint in all tests.

Ambient water samples were collected from eight sites four times from September 2019 to February 2020. There were 34 sampling events for each species. Of the 170 sample comparisons made, all 170 of them met test acceptability criteria and were considered valid. Not including field duplicate results, there were 16 instances of significant reductions in toxicity endpoints which occurred in 15 tests. There was one reduction in algal cell growth in the field duplicate of SACR-011 collected in September 2019.

The *S. capricornutum* growth endpoint had the highest frequency of statistically significant reductions, which was observed in 7 instances. *C. dubia* reproduction was impacted 3 times as was *P. promelas* biomass. *C. dubia, P. promelas,* and *H. azteca* survival were each negatively impacted once. There were no reductions in survival or AFDM in the *C. dilutus* tests during this reporting period. We did observe five (5) instances of potential pathogen-related toxicity (two of which were included in the above counts as they were statistically significant), which were followed up in PRT-protocol style tests. Samples collected from the Sacramento subregion made up 63% of the instances of significantly reduced endpoints, with 31% coming from sites collected from the Yolo Bypass-Cache Slough subregion, and 6% from the Central Delta subregion, i.e., San Joaquin River at Buckley Cove. There were no significant impairments of endpoints in organisms exposed to samples collected from the Northeast Delta subregion.

Samples which exhibit a 50% reduction in an endpoint compared to the appropriate control were initiated in a Toxicity Identification Evaluation (TIE). Six TIEs were conducted on five samples. We were unable to recover the toxicity in algal and *C. dubia* TIEs conducted on 544LSAC13 and SACR-010 collected July 29, 2019, SACR-011 and SACR-012, collected September 16 and 17, 2019. The results from the TIE conducted on the December 2 2019 collection of 511ULCABR indicate that water column toxicity to *H. azteca* was likely caused by a low concentration of pyrethroid(s).

Introduction

The Delta Regional Monitoring Program (DRMP) conducted water sampling monthly with the primary goal of tracking and documenting the effectiveness of beneficial use protection and restoration efforts through comprehensive monitoring of water quality constituents and their effects in the Delta through the use of toxicity testing and analytical chemistry. This end of year report summarizes the results of toxicity tests and water quality parameters conducted on samples collected from July 29, 2019 to February 13, 2020. We were unable to conduct the March 2020 sampling event due to the University shutdown from the COVID-19 pandemic.

Materials and Methods

Sample collection

Staff from United States Geological Survey (USGS) collected water samples as sub-surface grabs in clean 1-gal amber glass bottles. Water samples were transported, stored, and preserved following protocols outlined in the University of California Davis, Aquatic Health Program Laboratory (UCD AHPL) and the Surface Water Ambient Monitoring Program (SWAMP) Standard Operating Procedures (SWAMP, 2008; UCD AHPL 2018). Site IDs, sample descriptions and locations are outlined in Table 1. Rather than collecting from the same sites throughout the project, a rotating basin probabilistic monitoring design was implemented with sites designated in different Delta subregions (ASC 2018). Ulatis Creek at Brown Road and the San Joaquin River at Buckley Cove were fixed stations that were collected with every event. Chain of Custody and field data sheets for the 2019-2020 project year are presented in Appendix A.

Table 1. Summary of sample sites and locations

Site	Latitude	Longitude	Description
511ULCABR	38.30700	-121.79420	Ulatis Creek at Brown Road
544LSAC13	37.97183	-121.37362	San Joaquin River at Buckley Cove
SACR-009	38.31436	-121.57723	Sacramento Subregion
SACR-010	38.45881	-121.50240	Sacramento Subregion
SACR-011	38.51454	-121.54563	Sacramento Subregion
SACR-012	38.19272	-121.56752	Sacramento Subregion
SACR-013	38.33821	-121.56530	Sacramento Subregion
SACR-014	38.37770	-121.54217	Sacramento Subregion
SACR-015	38.53481	-121.51925	Sacramento Subregion
SACR-016	38.17289	-121.64852	Sacramento Subregion
YOLO-017	38.28330	-121.68577	Yolo Bypass - Cache Slough Subregion
YOLO-018	38.26025	-121.67886	Yolo Bypass - Cache Slough Subregion
YOLO-019	38.43301	-121.60288	Yolo Bypass - Cache Slough Subregion
YOLO-020	38.27881	-121.67780	Yolo Bypass - Cache Slough Subregion
YOLO-021	38.30108	-121.72977	Yolo Bypass - Cache Slough Subregion
YOLO-022	38.31798	-121.65177	Yolo Bypass - Cache Slough Subregion
YOLO-023	38.27899	-121.68779	Yolo Bypass - Cache Slough Subregion
YOLO-024	38.18487	-121.66101	Yolo Bypass - Cache Slough Subregion
NORT-001	Not available	Not available	Northeast Delta Subregion
NORT-002	Not available	Not available	Northeast Delta Subregion
NORT-004	Not available	Not available	Northeast Delta Subregion
NORT-005	Not available	Not available	Northeast Delta Subregion

Site	Latitude	Longitude	Description
NORT-006	Not available	Not available	Northeast Delta Subregion
NORT-007	Not available	Not available	Northeast Delta Subregion
NORT-008	Not available	Not available	Northeast Delta Subregion
NORT-025	Not available	Not available	Northeast Delta Subregion

Water quality

Field water quality measurements included at a minimum salinity and specific conductance (SC), and were recorded for each sampling time on SWAMP sample Chain of Custody sheets by USGS field staff. Additional field water quality measurements of velocity, water temperature, pH, dissolved oxygen (DO), and turbidity were recorded on the SWAMP field data sheets. Meters were calibrated according to the manufacturers' specifications at the start of each field day. Ammonia-nitrogen was measured at UCD AHPL 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) and were determined by UCD AHPL internal testing procedures. 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, and UCD AHPL. 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* (USEPA, 2002). Acute 96-hour toxicity testing for *Hyalella azteca* followed acute protocols in the SWAMP Acute *H. azteca* SOP, and chronic 10-day toxicity tests with *Chironomus dilutus* followed chronic protocols in the SWAMP Chronic *C. dilutus* SOP, which are based on water column reference toxicant testing protocols outlined in *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates* (USEPA, 2000). Test protocols and Method Quality Objectives (MQOs) follow those provided in the Delta RMP QAPP (v4; 2018).

Test preparations

Before test initiation and water renewals, water samples were shaken thoroughly in their original sample containers for 60 seconds to disassociate loosely adsorbed pesticides. Sub-samples for *C. dubia* were filtered through a 53-µm screen to remove debris and other organisms. Sub-samples for *S. capricornutum* were passed through a Whatman grade GF/A glass fiber filter with a 0.45 µm particle size retention prior to testing. Sub-samples for *P. promelas, H. azteca,* and *C. dilutus* were not filtered. Once in their warming containers, prior to and after water quality measurements are taken, samples were stirred vigorously prior to being aliquoted into replicate test chambers. Water quality measurements including pH, specific conductance (SC), 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 old water.

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 heteroscedastic 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. Statistics were run through a SWAMP-provided toxicity transformer.

In *H. azteca* and *C. dilutus* tests, survival comparisons were calculated as [# surviving / (# surviving + # dead bodies found)]. Animals missing from the test vessels may have died because of exposure to test waters, and then disappeared due to rapid decomposition, but it is also possible that animals have died due to desiccation when individuals resting on the water surface leave the water or are washed out of the water and adhere to the side of the test vessel. In this vein, *C. dilutus* that have pupated from midge to fly were considered as missing. Thus, only animals whose remains are found submerged in the test vessels were included in the counts of animals that died in test replicates. This method of scoring can result in qualification of the test data due to an uneven number of organisms being reported that differ from the number of starting animals required by the method. In October 2019, we revised this method with SWAMP IQ to all missing organisms in a test are considered dead, with notes indicating whether these organisms were found outside the water column or had pupated to the next developmental stage (TMO). Organisms missing on Day 1 of a toxicity test are considered loading errors (TOQ). This change affected the organism survival data of some tests.

In following SWAMP statistical guidance, additional Low Conductivity Controls were included with the *C. dubia, P. promelas, H. azteca,* and *C. dilutus* tests when ambient sample conductivities were at or below 100 µS/cm. In some cases, multiple Low Conductivity Controls were included when multiple samples had different low conductivities. A Low Conductivity Control must meet test acceptability criteria in order to be statistically compared to its associated low conductivity ambient sample. Low conductivity ambient samples are statistically compared to the Low Conductivity Control regardless of whether there is impairment to an organism endpoint. 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).

Test Organisms

Ceriodaphnia dubia

C. dubia were cultured in-house, following methods outlined in USEPA and in UCD AHP SOPs. Cultures originally obtained from Aquatic Research Organisms (Hampton, NH) and AQUA Science (Davis, CA), were kept in an environmentally-controlled room maintained at 25 ± 2°C. Test organisms employed in toxicity testing were derived asexually. Prior to test initiation and renewals, waters were warmed to test temperature (25 ± 1°C) in 400 mL mason jars using a water bath maintained at 25 ± 2°C and aerated at a rate of 100 bubbles per minute until the DO concentration fell below saturation (about 8.6 mg/L). Sierra Springs™ water amended to USEPA moderately hard standards (hardness: 80-100 mg/L $CaCO_3$, alkalinity: 57-64 mg/L $CaCO_3$, EC 250-300 µS/cm, pH, 7.8-8.2; USEPA, 2002) was used as the control (L1650). Low Conductivity Controls were comprised of L1650 diluted with distilled water until the desired conductivity was met. After dilution, nutrients that did not affect water quality (e.g., hardness, alkalinity, conductivity) were added back to the Low Conductivity Control to match the concentration in the standard 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 organism. *C. dubia* were transferred into a vial of fresh solution and fed YCT and *S. capricornutum* daily. Tests were conducted at 25 ± 1°C with a 16-hr light: 8-hr dark photoperiod under fluorescent light. Mortality and reproduction were assessed daily and at termination.

Low conductivity controls, nutrient add-back, and EDTA investigation

Between 2015 and 2017, *C. dubia* reproductive performance in low conductivity controls was often significantly lower than the standard control and moreover, we observed reproductive impairment in low conductivity controls up to approximately 130 µS/cm. Standard AHPL practice is to dilute the standard control water down to the lowest conductivity of the ambient samples collected, using distilled water, in order to make these low conductivity secondary controls. In the summer of 2018, before the study project began we determined that the low reproductive performance observed in the low conductivity controls was very likely due to the dilution of essential ions and nutrients that were present in the standard control water. In November 2018, the Delta RMP QAPP was updated with additional guidance for use of these low conductivity tolerance controls. Members of the pesticide subcommittee were concerned that adding back nutrients and ions to the low conductivity controls may artificially improve organism performance such that those organisms in low conductivity ambient samples could have a reduced performance during statistical comparison, and may increase the number of false positives in the study project period. Therefore, additional testing was added for research purposes with the intent of understanding if nutrient additions to low conductivity ambient samples would increase *C. dubia* reproduction. In 2019 and 2020, *C. dubia* tests that had low conductivity ambient samples ranging from less than 100 µS/cm up to 130 µS/cm would include additional nutrient add-back treatments. These additional treatments were tested concurrently with each batch of samples, and the results were analyzed to determine if *C. dubia* reproductive performance was affected. In August of 2019, this additional research plan was expanded to include the evaluation of EDTA in low conductivity controls and low conductivity ambient samples (Irvine and Mussen, 2019). The results of this investigation are provided in Appendix G.

Pimephales promelas

Fish were purchased from Aquatox Inc. (Hot Springs, AR). Upon receipt, fish were fed and acclimated to laboratory test conditions until their use in a test. Prior to test initiation and renewals, sample waters were warmed to test temperature (25 ± 1°C) in 1L glass beakers using a water bath maintained at 25 ± 2°C, and aerated at a rate of 100 bubbles per minute until DO the concentration fell below saturation (about 8.6 mg/L). Reverse-osmosis water amended with inorganic salts to USEPA moderately hard specifications (hardness: 80-100 mg/L $CaCO_3$, alkalinity: 57-64 mg/L $CaCO_3$, EC 250-300 µS/cm, pH, 7.8-8.2; USEPA, 2002) was used as the control (ROEPAMH). Low Conductivity controls were comprised of ROEPAMH diluted with reverse-osmosis water until the desired conductivity was met.

Tests were initiated using fish less than 48-hr old. Each of four replicate 600 mL beakers contained 250 mL of sample water and 10 minnows. Eighty percent of the test solution was renewed daily, at which time debris and dead fish were removed from the test chambers. Fish were fed *Artemia* nauplii twice daily. Tests were conducted at 25 ± 1°C with a 16-hr light: 8-hr dark photoperiod under fluorescent and ambient light. Mortality was assessed daily. At test termination, surviving fish were dried to a constant weight at 103-105°C, and weighed using a Mettler AE163 balance to determine dry biomass.

When *P. promelas* are considered to be infected by pathogens, called Pathogen-Related Toxicity (PRT), the Percent Coefficient of Variation (%CV) of survival among the four replicates of a treatment is greater than or equal to 40%, there is sporadic mortality observed in replicate test chambers, and the presence of fungus is observed on deceased fish. When these indicators occur in concert, the sample and its appropriate control(s) are retested with 20 replicates containing two fish each. This modified approach maintains the same number of fish per treatment and statistical power, while the reduced number of fish per replicate minimizes the spread of pathogens to other fish. At test termination, the 20 replicates are pooled in batches of five to provide four

survival and biomass replicates per sample. These four replicates are then statistically processed in the same fashion as the standard test method. This follow-up test occurs after the initial screening test, and therefore does not meet the 36-hour holding time for test initiation.

Selenastrum capricornutum

S. capricornutum were cultured and maintained in-house at UCD AHP from cultures originally obtained from Star Culturing, University of Texas (Austin, TX). Axenic algal cells were placed in media for 4-7 days prior to test initiation to ensure cells were in exponential growth.

The *S. capricornutum* 96-hr chronic tests consisted of four replicate 250 mL glass flasks with 100 mL of sample and 1 mL of 1.0 x 10^6 cells/mL of *S. capricornutum*. A fifth replicate flask was inoculated and used for daily chemistry measurements. EDTA was not included in the tests for this reporting period. Test chambers were incubated in a temperature-controlled environmental chamber maintained at 25 ± 2°C under constant cool white fluorescent light. Flasks were kept in random placement in a mechanical shaker in constant orbital motion at 100 cycles per minute and were randomized twice daily. Distilled water amended with nutrients (Hardness: 0 mg/L, Alkalinity: 0-4 mg/L, EC: 95-105 µS/cm, pH 7.8-8.2; USEPA, 2002) was used as the control (Glass Distilled). As the distilled water control already has a conductivity at or below 100 µS/cm, additional Low Conductivity Controls were not included with this test species. Cell growth was measured at test termination with a Coulter Counter Z1 particle counter (Beckman Coulter, Pasadena CA).

Hyalella azteca

H. azteca were obtained from Aquatic Research Organisms (Hampton, NH), and were acclimated to laboratory conditions for 48 hours. Prior to test initiation and renewals, sample waters were warmed to test temperature (23 ± 1°C) in 600 mL glass beakers using a water bath maintained at 25 ± 2°C, and aerated at a rate of 100 bubbles per minute until DO the concentration fell below saturation (about 8.9 mg/L). The 96-hr acute water column 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 reconstituted to moderately hard standards using inorganic salts (Hardness 90-100 mg/L, Alkalinity 50-70 mg/L, EC: 330-360, pH 7.8-8.2; US EPA, 2000) was used as the control (ROEPAMHR). Low Conductivity Controls were comprised of ROEPAMHR diluted with reverse-osmosis water until the desired conductivity was met.

Tests were conducted at 23 ± 1°C with a 16-hr light: 8-hr dark photoperiod under fluorescent and ambient light. Mortality was assessed daily. Eighty percent of the test solution was renewed at the 48-hr time point, when debris and dead organisms were removed from the test chambers. *H. azteca* were fed 1.5 mL of YCT (yeast, organic alfalfa and trout chow) prior to test initiation and 2 hours prior to water renewal at 48-hr.

Chironomus dilutus

C. dilutus were obtained from Aquatic Biosystems (Fort Collins, CO) or Aquatic Research Organisms (Hampton, NH). These organisms were generally ordered to arrive at the second instar (7-10 days old), but occasionally *C. dilutus* egg cases were ordered ahead of time and raised in culture at the AHPL until their use in a test. Prior to test initiation and renewals, sample waters were warmed to test temperature (23 ± 1°C) in 1L glass beakers using a water bath maintained at 25 ± 2°C, and aerated at a rate of 100 bubbles per minute until DO the concentration fell below saturation (about 8.9 mg/L). The 10-day chronic water column toxicity tests consisted of four 250 mL replicate beakers with 5 mL of autoclaved control sand, 200 mL of sample water, and 12 dilutus each. Reverse-osmosis water reconstituted to moderately hard standards using inorganic salts (Hardness 90-100 mg/L, Alkalinity 50-70 mg/L, EC: 330-360, pH 7.8-8.2; US EPA, 2000) was used as the control (ROEPAMHR). Low

Conductivity Controls were comprised of ROEPAMHR diluted with reverse-osmosis water until the desired conductivity was met.

Tests were conducted at 23 ± 1°C with a 16-hr light: 8-hr dark photoperiod under fluorescent and ambient light. Mortality was assessed daily, with dead organisms removed when observed. Eighty percent of the test solution was renewed every 48 hours. *C. dilutus* were fed 250 µL of Tetramin slurry (Tetramin, *Selenastrum* and water) daily. At test termination, surviving *C. dilutus* were ash dried with a muffler furnace at 550°C to obtain Ash Free Dry Mass.

Quality Assurance

Test Acceptability Criteria

Test acceptability criteria (TAC) for laboratory analyses included minimum control organism survival and sub-lethal 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.
- Chronic *P. promelas* toxicity tests require 80% or greater control survival and an average biomass of ≥ 0.25 mg/individual.
- Chronic 96-hr *S. capricornutum* toxicity tests with EDTA require an average cell growth of 1 x 10^6 cells/mL and a coefficient of variation less than or equal to 20% among control replicates.
- Acute 96-hr *H. azteca* toxicity tests require 90% survival or greater in the control.
- Chronic 10-day *C. dilutus* toxicity tests require 80% or greater survival in the control and an average ash-free dry mass of >0.60 mg/individual.

One toxicity test failed to meet test acceptability criteria during this reporting period:

1. Sites 511ULCABR, YOLO-019, YOLO-017, YOLO-018, and YOLO-020 which were initiated in an *S. capricornutum* toxicity test in the July 2019 collection date, did not meet TAC due to a high %CV. These sites were initiated in a retest.

Completeness

UCD AHP strives for a minimum of 90% completeness of work performed in accordance with SWAMP guidelines. For the purposes of this project, completeness was determined by considering the number of statistical analyses that could be made between ambient samples and their appropriate control(s) over the entire project. On a per-species basis, total number of events was determined by multiplying the number of sample collections (4) by the number of sites collected (8) with the addition of field duplicates (2), which equals 34 events (Table 2).

These events, multiplied by the number of species tested in each event brings the total number of sample comparisons to 170 (not including controls) during this project period. All tests were completed during this reporting period. We therefore consider the overall project completeness to be 100%.

Table 2. Project completeness broken down by species

Species	Expected # Samples	Completed # Samples	Completeness (%)
C. dubia	34	34	100
P. promelas	34	34	100
S. capricornutum	34	34	100
H. azteca	34	34	100
C. dilutus	34	34	100

Field duplicates and precision

A field duplicate is a second sample collected in a separate container, immediately after the initial/primary test sample. Field duplicates are tested concurrently with its primary sample and the results are evaluated to determine precision of field variability and laboratory staff. For the DRMP Project, field duplicates were collected at a rate of 5%. Field duplicate samples are in agreement when the primary sample and its duplicate are either both statistically similar or both statistically different from the control. Field duplicate samples were collected twice during this reporting period, in September 2019 (SACR-011) and February 2020 (NORT-006) events.

Precision is a measure of the degree to which multiple independent analyses of a given sample agree with one another; it is the reproducibility and consistency of results. In toxicity testing, we determine precision by the degree to which the primary sample agrees with its duplicate. Precision is measured by calculating the Relative Percent Difference (RPD) between sample measurements. RPDs were calculated on laboratory water chemistry measurements of DO, pH, SC, hardness, alkalinity and ammonia, as well as on toxicity testing endpoints such as survival, cell growth, reproduction, and biomass. While there are no RPD requirements for toxicity outlined in the DRMP QAPP (2018), <25% RPD is listed in Table 14.2 for conventional analyses. The RPD between a sample and its duplicate was calculated by using the following equation:

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

Accuracy refers to the degree of agreement between a measured value and the expected value. Accuracy criteria are not applicable to toxicity testing responses (endpoints) because there are no standard (absolute) organisms against which to compare test results. Toxicity is a relative rather than absolute concept. Nonetheless, the approach to accuracy is enhanced with test replication; the mean response (mortality, reproduction, growth and etc.) approaches the “true” value with multiple trials. Data should be both accurate and precise. Data can be accurate, but imprecise, or be precise, but not accurate, neither, or both.

During this reporting period all field duplicates and their primary samples shared equivalent results. The RPD between the primary sample and field duplicate in algal cell growth exceeded 25% in both the September 2019 and February 2020 field duplicate pairs. In September 2019, the RPD was 30%, likely due to low cell growth, as the primary sample and its duplicate had 4.9% and 3.6% of control growth, respectively, and was initiated in a TIE follow-up. In the February 2020 test, the RPD for cell growth was 36%, with 1.670 x 10^6 cell/mL growth in the primary sample and 2.393 x 10^6 cells/mL growth in the duplicate, and these results were equivalent (i.e., non-

toxic). This may have been due to spiking of the nutrients and/or algal cells added to the test replicates. Additionally, the RPD for alkalinity in the September 2019 SACR-011 pair was 39% due to technician error.

Deviations from QAPP protocols

Protocol deviations occurred during this reporting period. These deviations generally fell into three major categories and consisted of 1) holding time exceedances and 2) temperature deviations.

1. Holding times were missed for several samples initiated in July 2019 toxicity tests:
    a. A retest was conducted for sites 511ULCABR, YOLO-019, YOLO-017, YOLO-018, and YOLO-020 for *S. capricornutum*, which did not meet TAC due to a high %CV.
    b. These same sites missed the holding time for the *P. promelas* toxicity test, due to the fish vendor not sending out the fish on the appropriate day.
    c. These same sites plus 544LSAC13, SACR-009, and SACR-010 missed the holding time for the *C. dilutus* test because the organisms received from the vendor were in poor health and could not be used, and we had to order another batch.

2. Holding time for *C. dubia* in the December 2019 toxicity tests missed the holding time for test initiation. Our *C. dubia* cultures were not performing well enough to conduct the toxicity test; we had to ship samples to a third-party subcontractor lab, which resulted in the missed holding time.

3. Minor deviations in temperature occurred in December 2019:
    a. Temperatures in the fathead minnow test were 0.8°C to 1.1°C outside of the 3° range as dictated by US EPA, due to cold ambient temperatures during the termination. Minimum temperatures ranged from 20.7-20.9°C.
    b. Temperatures in the *S. capricornutum* test exceeded the US EPA 3° range, varying from 0.6°C - 1.2°C. Minimum temperatures ranged from 21.3-21.4°C.

Corrective Actions

Depending on parameter, failure to meet QA criteria can have several outcomes. These outcomes are generally dictated by project-specific QAPP criteria. In some cases, corrective action can occur and in other cases it cannot. For example, if toxicity test acceptability criteria are not met with a sample, corrective action could be a re-test of the sample or substitution of a sample collected at the same site at a later date. Conversely, if samples arrive at UCD AHPL at a temperature far exceeding that specified in the project QAPP, or if testing cannot be initiated within the maximum sample holding time designated in the project QAPP, those samples will not be tested. In such cases, corrective action would be an alteration of procedures that ensure the arrival of future samples below the specified temperature (e.g., adding additional ice to transport coolers), and so that sample holding times are not exceeded (e.g., changing shipping methods or hand delivery).

As mentioned in the previous report, we had experienced a number of issues with obtaining known-age *C. dilutus* from vendors, which resulted in several deviations and corrective actions in the 2018-2019 reporting period. After the investigation (see Appendix E) we opted to raise *C. dilutus* from egg cases whenever possible,

and during storm events, we ensured to order the youngest age midges available for shipping. These corrective actions proved successful, as we met all test acceptability criteria for the *C. dilutus* tests during this project period. Although there were still some isolated incidences where organisms pupated out during the test, variability was reduced and we met the AFDM endpoint in all tests.

Reference toxicant tests

In lieu of an absolute measurement of toxicity test accuracy, Reference Toxicant (RT) tests are conducted to assess whether organisms are responding within prescribed limits. Reference toxicant tests were included in this project to assess changes of organism sensitivity over time. These tests included the laboratory control and a dilution series of a chemical in laboratory control water. The LC_{50}/EC_{25} 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_{50} or EC_{25} was outside of the 95% CI, test organism sensitivity can be considered atypical and results of tests conducted during the month of an RT outlier could be considered suspect.

The method UCD AHPL uses to calculate the acceptable range of variation differs from that recommended by USEPA. USEPA recommends that acceptable data should fall within two standard deviations of the mean for the total project data set. UCD AHPL accepts data that falls within two standard deviations from the running mean. These standard deviations represent the standard deviation for the last data point and nineteen previous points.

Changes in organism sensitivity may indicate problems with organism health, technician-handling techniques, and/or organism genetic variations. USEPA (2002) suggests that one outlying data value may be expected to occur by chance when 20 or more data points are plotted. UCD AHPL evaluates patterns of outlying values. When more than one outlier occurs, corrective actions will be taken. For instance, when two consecutive data points exceed the upper two-standard deviation line on an LC_{50} control chart, this may indicate that the test organisms are becoming less sensitive to reference toxicants.

RT tests were conducted concurrently with each test initiation. Sodium chloride was the toxicant used in *C. dubia*, *P. promelas*, *H. azteca,* and *C. dilutus* species; zinc chloride was the toxicant used with *S. capricornutum*. Testing organisms were considered to be within their normal ranges of sensitivity throughout the reporting period. There were a few instances where one data point fell outside of the two standard deviations (SD) of the running effect concentration mean. Although outside of the prescribed organism sensitivity range as per USEPA guidance, a single data point is not necessarily considered a qualification in terms of organism sensitivity. There were no second outliers that occurred during this reporting period. RT control charts are presented in Appendix B.

Results

Summary tables for all species and individual test results, including water quality measurements, are provided in Appendix C. These summary tables include the toxicity and chemistry data that was entered into the SWAMP database.

Ambient water samples were collected from eight sites four times from September 2019 to February 2020. There were 34 sampling events for each species. Of the 170 sample comparisons made, all 170 of them met test acceptability criteria and were considered valid. Not including field duplicate results, there were 16 instances of significant reductions in toxicity endpoints which occurred in 15 tests. There was one reduction in algal cell growth in the field duplicate of SACR-011 collected in September 2019.

The *S. capricornutum* growth endpoint had the highest frequency of statistically significant reductions, which was observed in 7 instances. *C. dubia* reproduction was impacted 3 times as was *P. promelas* biomass. *C. dubia, P. promelas* and *H. azteca* survival were each negatively impacted once (Table 3). There were no reductions in survival or AFDM in the *C. dilutus* tests during this reporting period. We did observe five (5) instances of potential pathogen-related toxicity (two of which were included in the above counts as they were statistically significant), which were followed up in PRT-protocol style tests. Samples collected from the Sacramento subregion made up 63% of the instances of significantly reduced endpoints, with 31% coming from sites collected from the Yolo Bypass-Cache Slough subregion, and 6% from the Central Delta subregion, i.e., San Joaquin River at Buckley Cove. There were no significant impairments of endpoints in organisms exposed to samples collected from the Northeast Delta subregion.

Table 3. Summary of instances where statistically significant reductions in organism fitness endpoints were observed during the project period.

Collection date	Site ID	Species	Endpoint	Organism performance as percent of control
7/29/2019	544LSAC13	Algae	Growth	33.3%; average of 0.151 x 10^6 cells/mL; TIE
	SACR-009	Algae	Growth	64.1%; average of 0.367 x 10^6 cells/mL
	SACR-010	Cerio	Survival	0%; 0% survival; TIE
		Cerio	Reproduction	0%; 0% reproduction; TIE
		Algae	Growth	24%; average of 0.115 x 10^6 cells/mL; TIE
7/30/2019	YOLO-018	Cerio	Reproduction	63.1%; average of 16.6 neonates
	YOLO-020	Cerio	Reproduction	62%; average of 16.3 neonates
9/16/2019	SACR-011	Algae	Growth	4.9%; average 0.017 x 10^6 cells/mL; TIE
9/17/2019	YOLO-023	FHM	Survival	65%; average 65% survival, likely PRT
	YOLO-024	Algae	Growth	73.9%; average of 0.262 x 10^6 cells/mL
	SACR-012	FHM	Biomass	72.8%; average 0.274 mg/individual
		Algae	Growth	39.8%; average of 0.184 x 10^6 cells/mL; TIE
12/2/2019	SACR-013	FHM	Biomass	53%; average 0.159 mg/individual; likely PRT
	511ULCABR	Algae	Growth	59.3%; average 0.285 x 10^6 cells/mL
		Hyalella	Survival	38.7%; average 38.7% survival; TIE
2/12/2020	SACR-015	FHM	Biomass	73.9%; average 0.327 mg/individual

Toxicity Identification Evaluations

Samples which exhibit a 50% reduction in an endpoint compared to the appropriate control were initiated in a Toxicity Identification Evaluation (TIE). Six TIEs were conducted on five samples. Details of these TIEs can be found in Appendix F.

1. 544LSAC13 collected July 29, 2019, was initiated in an *S. capricornutum* TIE.
    - Results of this TIE are inconclusive, as we were unable to recover the toxicity observed in the initial screening test.
2. SACR-010 collected July 29, 2019, was initiated in a *C. dubia* and *S. capricornutum* TIE.
    - Results of this TIE are inconclusive, as we were unable to recover the toxicity observed in the initial screening test.
3. SACR-011 collected September 16, 2019, was initiated in an *S. capricornutum* TIE.

- Results of this TIE are inconclusive, as we were unable to recover the toxicity observed in the initial screening test.

4. SACR-012 collected September 17, 2019, was initiated in an *S. capricornutum* TIE.

    - Results of this TIE are inconclusive, as we were unable to recover the toxicity observed in the initial screening test.

5. 511ULCABR collected December 2, 2019, was initiated in an *H. azteca* TIE.

    - Results of this TIE indicate that water column toxicity was likely caused by a low concentration of pyrethroid(s).

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