DELTA REGIONAL MONITORING PROGRAM

End of Year Summary Report for FY 2018-2019

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.

We had several issues with *C. dilutus* which resulted in not meeting the ash-free dry weight endpoint in most tests, therefore we launched an investigation early in 2019 to determine why we had such a high frequency of pupation during the test, especially considering that the December 2018 *C. dilutus* test was successful. By width of the head capsule, we determined that the midges obtained from the vendor were closer to 3rd instar than 2nd, with an approximate age range of 10-14 days. This apparent difference in age and instar explains why the organisms pupated into flies during the test when they should not have. Statistically, we considered *C. dilutus* that have pupated from midge to fly 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 and it affected the *C. dilutus* completeness factor for the project by invalidating some tests after the project period.

Ambient water samples were collected from eight sites four times from December 18, 2018 to June 18, 2019. There were 34 sampling events for each species. Of the 170 sample comparisons made, 140 of them met test acceptability criteria and were considered valid. Not including field duplicate results, there were 27 instances of significant reductions in toxicity endpoints which occurred in 7 tests (*C. dilutus* toxicity is not included in this count because these tests were later invalidated). There were no reductions in any endpoints observed with field duplicates.

The *S. capricornutum* growth endpoint had the highest frequency of statistically significant reductions, which was observed in 13 instances. *C. dubia* reproduction was the second most frequent endpoint impacted, which was observed 12 times. *C. dubia* survival and *P. promelas* biomass were each significantly impacted once. *H. azteca* was not negatively impacted by any of the sites collected, and *C. dilutus* are not included in this tally. We did observe four instances of potential pathogen-related toxicity (not included in the above counts), which were followed up in PRT-protocol style tests. Of these instances, one sample had significantly reduced biomass when compared to the control in the PRT-follow up test. Samples collected from the Yolo Bypass-Cache Slough subregion made up 74% of the instances of significantly reduced endpoints, with 19% coming from sites collected from the Sacramento subregion, and 2% from the Central Delta subregion, i.e., San Joaquin River at Buckley Cove.

Samples which exhibit a 50% reduction in an endpoint compared to the appropriate control were initiated in a Toxicity Identification Evaluation (TIE). In the February 2019 *S. capricornutum* test, 511ULCABR and YOLO-007 met the TIE trigger with significantly reduced algal cell growth that was 16% and 21%, respectively, of the control performance. These samples were initiated in a follow-up TIE test on February 12, 2019, and in addition to a retest of the ambient samples, included C18 column manipulations and Chelex additions. Toxicity was alleviated in both ambient samples with the addition of Chelex 100, indicating metals as the likely cause of toxicity in these sites.

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 13, 2016 to June 13, 2017.

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 2017-2018 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-001	38.16498	-121.62099	Sacramento Subregion
SACR-002	38.26207	-121.65129	Sacramento Subregion
SACR-003	38.23917	-121.52149	Sacramento Subregion
SACR-005	38.18899	-121.64127	Sacramento Subregion
SACR-006	38.24024	-121.60198	Sacramento Subregion
SACR-007	38.47372	-121.52027	Sacramento Subregion
SACR-008	38.19473	-121.61907	Sacramento Subregion
SACR-025	38.29400	-121.58244	Sac-Ovr-025; Sac. R. Oversample Point #1
YOLO-001	38.27952	-121.66100	Yolo Bypass - Cache Slough
YOLO-002	38.26919	-121.69239	Yolo Bypass - Cache Slough
YOLO-003	38.26105	-121.74786	Yolo Bypass - Cache Slough
YOLO-004	38.31957	-121.69276	Yolo Bypass - Cache Slough

Site	Latitude	Longitude	Description
YOLO-005	38.25905	-121.66765	Yolo Bypass - Cache Slough
YOLO-006	38.25214	-121.67558	Yolo Bypass - Cache Slough
YOLO-007	38.27122	-121.70283	Yolo Bypass - Cache Slough
YOLO-008	38.27430	-121.67392	Yolo Bypass - Cache Slough
YOLO-009	38.24957	-121.67482	Yolo Bypass - Cache Slough
YOLO-010	38.46178	-121.58863	Yolo Bypass - Cache Slough
YOLO-011	38.30568	-121.65721	Yolo Bypass - Cache Slough
YOLO-012	38.28241	-121.68100	Yolo Bypass - Cache Slough
YOLO-013	38.20820	-121.66306	Yolo Bypass - Cache Slough
YOLO-014	38.38195	-121.62601	Yolo Bypass - Cache Slough
YOLO-015	38.26789	-121.66321	Yolo Bypass - Cache Slough
YOLO-016	38.25806	-121.72580	Yolo Bypass - Cache Slough

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 and it affected the *C. dilutus* completeness factor for the project by invalidating some tests after the completion of the project period.

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 and nutrient add-back 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, *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. 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. Tests were conducted with the addition of EDTA. 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°C. 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 Weight.

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 weight of >0.60 mg/individual.

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

1. Site 511ULCABR, which was initiated in a *C. dilutus* toxicity test in the February 2019 collection date, did not meet TAC after the October 2019 recalculation of missing vs. dead organisms in the toxicity test.
2. In February 2019, all sites initiated in the *S. capricornutum* toxicity test did not meet TAC, due to not meeting control growth requirements. A retest was initiated on February 12, 2019 for 511ULCABR and YOLO-007 and on February 22, 2019 for sites 544LSAC13, SACR-003, SACR-025 and its duplicate. The retests were flagged for missing the holding time for test initiation.
3. In April 2019, all sites initiated in the *S. capricornutum* toxicity test did not meet TAC, due to a bad algal slant that resulted in no control growth. We could not repeat this experiment.
4. In April 2019, all sites initiated in the *C. dilutus* toxicity test did not meet TAC after the October 2019 recalculation of missing vs. dead organisms in the toxicity test.

5. In June 2019, 544LSAC13, 511ULCABR, SACR-007 and its duplicate initiated in the *S. capricornutum* toxicity test did not meet TAC, due to low control growth. This test was repeated on July 2, 2019, which did not include the addition of EDTA per request of the Technical Advisory Committee. The retest was flagged for missing the holding time for test initiation.

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. There are no *S. capricornutum* toxicity data for the 8 sites collected in April 2019. We had several issues with *C. dilutus* (see Deviations section), which resulted in not meeting the ash-free dry weight endpoint in most tests. Additionally, 511ULCABR in the February *C. dilutus* event and 8 sites in the April *C. dilutus* toxicity event did not meet survival TAC after the October 2019 recalculation, which invalidated these tests (see Statistics section above). This leaves 140 samples that met all test acceptability criteria. We therefore consider the overall project completeness to be 82%. Project completeness is 95% without the inclusion of *C. dilutus*.

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	26	76
H. azteca	34	34	100
C. dilutus	34	12	35

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 February (SACR-025) and June (511ULCABR) 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 and no RPD exceeded 25%. The RPD in the fathead minnow test conducted in February 2019 had an RPD of 23% for survival and 24% for the biomass endpoint, which was due to pathogen-related toxicity.

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, 2) temperature deviations, and 3) deviations related to *C dilutus*.

1. Holding times were missed for samples initiated in retests. Retests were conducted for all sites collected for the February 2019 *S. capricornutum* test, and for sites 544LSAC13, 511ULCABR, SACR-007 and its duplicate in the June 2019 *S. capricornutum* test.
2. Hardness and alkalinity were analyzed outside of holding time for 511ULCABR collected in December 2018. This occurred due to technician oversight.
3. Minor deviations in temperature occurred in December 2018 and in June 2019:
    a. Temperatures for 511ULCABR and YOLO-002 in the December 2018 fathead minnow test were 0.6°C and 0.1°C, respectively, outside of the 3° range as dictated by US EPA.
    b. Temperatures for June 2019 *S. capricornutum* retest which included sites 544LSAC13, SACR-007, 511ULCABR and its duplicate, exceeded the US EPA 3° range, varying from 0.6°C - 1.2°C.
    c. A broken heater resulted in a low temperature in the June 2019 *C. dubia* test on Day 3. Low temperatures ranged from 20.1° - 22.5°C, resulting in an exceedance of the US EPA 3°C range by 1.7°C - 2.8°C.
4. We had a number of issues with obtaining known-age *C. dilutus* from vendors, which resulted in several deviations.
    a. In the December 2018 toxicity event, the vendor did not send enough organisms. This resulted in our inability to have pre-weight measurements or to conduct a concurrent RT test.

b. In the February 2019 toxicity event, the vendor sent organisms older than 2nd instar. These organisms pupated into flies and escaped test replicate chambers, resulting in an uneven number of organisms at the end of the test and we did not meet the AFDW endpoint.

c. In the April 2019 toxicity event, the vendor sent unhealthy organisms that were older than 2nd instar. Due to poor health we were unable to load 12 organisms per replicate and instead loaded 10 animals per replicate. These organisms were larger than normal and became aggressive in the replicate beakers. Cannibalism was observed. These animals also pupated into flies and escaped the test replicate chambers, resulting in an uneven number of organisms at the end of the test. Sites 544LSAC13, YOLO-009, YOLO-010, YOLO-011, YOLO-012, and SACR-006 met the AFDW endpoint, but this test was invalidated after the October 2019 recalculation.

d. In the June 2019 toxicity event, we were unable to obtain an accurate AFDW endpoint because organisms were muffle-furnaced prior to obtaining tare boat weights; this was due to technician error.

5. Miscellaneous deviations include feeding the *H. azteca* test after test initiation rather than before, and the use of EDTA in *S. capricornutum* tests during this reporting period.

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).

Given the number of issues we experienced with the *C. dilutus* test we launched an investigation early in 2019 to determine why we had such a high frequency of pupation during the test, especially considering that the December 2018 *C. dilutus* test was successful. The results of the investigation were presented to the Technical Advisory Committee during the June 2019 TAC meeting. With the uncertain nature of sampling storm events for toxicity testing, we are unable to raise *C. dilutus* from egg cases, as we need at a minimum a 10-day lead for the eggs to mature, hatch, and for the organisms to grow to the proper life stage for use in a toxicity test. Thus, we typically ordered the organisms from a vendor to arrive when the midges are at 2nd instar, which under SWAMP MQOs, ranges from 7-10 days old. To our knowledge there are three vendors which supply *C. dilutus*. For the purposes of this report, these vendors will be referenced herein as Vendors A, B, and C. Midges obtained from Vendor C were always received dead on arrival. Vendor B had healthy midges that were the proper age, but didn't have enough stock on hand to supply the numbers of organisms we needed to initiate our toxicity tests. In those cases, Vendor B would use Vendor C or Vendor A as a back-up. Vendor A became our main source of

organisms, as they were the only one who could supply the numbers we needed. Organisms which pupated during the test were obtained from Vendor A.

As part of our investigation we obtained *C. dilutus* egg cases and raised them in culture, obtaining morphometric data during the process. We then obtained 2nd instar *C. dilutus* from Vendor A and compared them against our in-house culture and the available literature. We compared the width of the midge head capsule and found that 2nd instar organisms obtained from Vendor A exceeded the width of midges that are considered 2nd instar in the literature. By width of the head capsule, we determined that the midges obtained from Vendor A were closer to 3rd instar than 2nd, with an approximate age range of 10-14 days. This apparent difference in age and instar explains why the organisms pupated into flies during the test when they should not have. Additionally, the majority of the growth that takes place with the *C. dilutus* test occurs during 2nd and 4th instars. When animals arrive close to the 3rd instar, these organisms are larger and have a slower growth at this larval stage, which also resulted in us not meeting the AFDM endpoint in some cases.

With this knowledge, we attempted to order younger organisms to use in the tests, however, the vendor would not ship out midges younger than 7 days because of their sensitivity to transport. Therefore, when we order organisms, we request the youngest age possible that can be shipped. In addition, we planned on using midges raised in-house from egg-cases for future toxicity events that were not storm event-based. Younger organisms were obtained for the June 2019 toxicity event and we did not observe any midges pupate into flies, although we did still observe aggressive behavior. Additional details of this investigation can be found in Appendix E.

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 December 2018 to June 2019. There were 34 sampling events for each species. Of the 170 sample comparisons made, 136 of them met test acceptability criteria and were considered valid (*C. dilutus* toxicity is not included in this count because these tests were later invalidated). Not including field duplicate results, there were 27 instances of significant reductions in toxicity endpoints which occurred in 7 tests. There were no reductions in any endpoints observed with field duplicates.

The *S. capricornutum* growth endpoint had the highest frequency of statistically significant reductions, which was observed in 13 instances. *C. dubia* reproduction was the second most frequent endpoint impacted, which was observed 12 times. *C. dubia* survival and *P. promelas* biomass were each significantly impacted once. *H. azteca* were not negatively impacted by any of the sites collected, and *C. dilutus* are not included in this tally. We did observe four instances of potential pathogen-related toxicity (not included in the above counts), which were followed up in PRT-protocol style tests. Of these instances, one sample had significantly reduced biomass when compared to the control in the PRT-follow up test (Table 3). Samples collected from the Yolo Bypass-Cache Slough subregion made up 74% of the instances of significantly reduced endpoints, with 19% coming from sites collected from the Sacramento subregion, and 2% from the Central Delta subregion, i.e., San Joaquin River at Buckley Cove.

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
12/19/2018	YOLO-003	Cerio	Reproduction	37.7%, average of 11.2 neonates
		Algae	Growth	70.5%, average of 0.931 x 10^6 cells/mL
	YOLO-004	Cerio	Reproduction	72.8%, average of 21.7 neonates
		Algae	Growth	66.9%, average of 0.883 x 10^6 cells/mL
	YOLO-001	Cerio	Reproduction	74.5%, average of 22.2 neonates
		Algae	Growth	68.7%, average of 0.907 x 10^6 cells/mL
	511ULCABR	Algae	Growth	52.6%, average of 0.699 x 10^6 cells/mL
	544LSAC13	Algae	Growth	58.2%, average of 0.769 x 10^6 cells/mL
	YOLO-002	Algae	Growth	69.4%, average of 0.916 x 10^6 cells/mL
	SACR-002	Algae	Growth	75.2%, average of 0.993 x 10^6 cells/mL
	SACR-001	Algae	Growth	57.3%, average of 0.757 x 10^6 cells/mL
2/4/2019	511ULCABR	Algae	Growth	15.8%; average of 0.324 x 10^6 cells/mL, TIE
2/5/2019	YOLO-007	Algae	Growth	21.2%, average of 0.435 x 10^6 cells/mL, TIE
	YOLO-005	Algae	Growth	67.5%, average of 1.34 x 10^6 cells/mL

Collection date	Site ID	Species	Endpoint	Organism performance as percent of control
	YOLO-006	Algae	Growth	64.4%, average of 1.28 x 10^6 cells/mL
	YOLO-007	Algae	Growth	72.9%, average of 1.45 x 10^6 cells/mL
4/29/2019	511ULCABR	Cerio	Survival	70.0%; average survival 70%
			Reproduction	8.2%, average of 2.4 neonates
4/30/2019	544LSAC13	Cerio	Reproduction	68.4%, average of 25.5 neonates
	YOLO-009	Cerio	Reproduction	76.9%, average of 28.7 neonates
	YOLO-010	Cerio	Reproduction	37.3%, average of 13.9 neonates
	YOLO-011	Cerio	Reproduction	36.5%, average of 23.7 neonates
	YOLO-012	Cerio	Reproduction	57.2%, average of 21.3 neonates
	SACR-005	Cerio	Reproduction	78.5%, average of 25.6 neonates
	SACR-006	Cerio	Reproduction	55.8%, average of 18.2 neonates
6/18/2019	YOLO-014	Cerio	Reproduction	65.6%, average of 14.5 neonates
	SACR-008	FHM	Biomass[1]	71.5%, average of 0.311 mg/individual

1. This reduction was observed in the PRT-follow up test.

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). In the February 2019 *S. capricornutum* test, 511ULCABR and YOLO-007 met the TIE trigger with significantly reduced algal cell growth that was 16% and 21%, respectively, of the control performance. These samples were initiated in a follow-up TIE test on February 12, 2019, and in addition to a retest of the ambient samples, included C18 column manipulations and Chelex additions (Table 4). Toxicity was alleviated in both ambient samples with the addition of Chelex 100, indicating metals as the likely cause of toxicity in these sites. Additional details can be found in the TIE summary report in Appendix F.

Table 4. TIE summary for 511ULCABR and YOLO-007 conducted February 12, 2019.

Treatment	Cell Growth (x10^6)			Interpretation
	Mean	Stdev	%CV	
Glass Distilled	1.649	0.477	28.9	Standard control
ROEPAMH	2.052	0.167	8.1	Control water method blank
ROEPAMH C18 Blank	1.254	0.080	6.4	C18 method blank (non-polar organics)
ROEPAMH + Chelex 100	1.384	0.091	6.6	Chelex 100 method blank (metals)
511ULCABR	0.321	0.038	11.9	Still acutely toxic
511ULCABR + Chelex 100	1.234	0.107	8.7	Removal of toxicity indicates likely metal toxicity
511ULCABR C18 Rinsate	0.827	0.209	25.3	Toxicity not alleviated by C18 treatment, likely not a non-polar organic compound
YOLO-007	0.435	0.063	14.4	Still acutely toxic
YOLO-007+ Chelex 100	1.292	0.147	11.4	Removal of toxicity indicates likely metal toxicity
YOLO-007 C18 Rinsate	0.638	0.394	61.9	Toxicity not alleviated by C18 treatment, likely not a non-polar organic compound

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