AQUATIC HEALTH PROGRAM LABORATORY
SCHOOL OF VETERINARY MEDICINE
UNIVERSITY OF CALIFORNIA, DAVIS

FINAL REPORT

WATER QUALITY MONITORING AT A DELTA INTEGRATOR SITE: FISH HEALTH AND BEHAVIOR

Marie Stillway and Swee Teh

October 1, 2020

Contents

Tables

Figures

List of Acronyms

Abbreviation	**Meaning**
AChE	Acetylcholinesterase
ANOVA	Analysis of variance
BPA	Bisphenol A
C18	Liquid-Solid Extraction Disk
CSULB	California State University, Long Beach
DEET	N,N-diethyl-meta-toluamide; Active ingredient in repellent products
DO	Dissolved oxygen
DOC	Dissolved organic carbon
DWR	Department of Water Resources
EC	Electrical conductivity
EPA	Environmental Protection Agency
GCMS	Gas Chromatography combined with Mass Spectrometry
H. azteca	*Hyalella azteca*
HLB	Hydrophilic-Lipophilic-Balanced
LC/MS/MS	Liquid Chromatography combined with Mass Spectrometry
LC50	Median (50%) lethal concentration
MeOH	Methanol
MP4	Multimedia format in video and audio recordings
NOEC	No effect concentration
NSAID	Nonsteroidal anti-inflammatory drugs
NTU	Nephelometric Turbidity Units
O. mykiss	Oncorhynchus mykiss
PBDEs	Polybrominated Diphenyl Ethers
PFAS	Polyfluoroalkyl Substances
PPCP	Pharmaceuticals and personal care products
PVC	Polyvinyl chloride
RBT	Rainbow Trout
RT	Reference toxicant tests

Abbreviation	Meaning
SD	Standard deviation
SE	Standard error
SSRI	Selective Serotonin Reuptake Inhibitor
TCCP	Tris (1-chloro-2-propyl) phosphate; Flame retardant
TCEP	Tri(2-chloroethyl)-phosphate; reducing agent
TDCPP	Tris(1,3-dichloro-2-propyl)phosphate; Additive flame retardant
TOC	Total organic carbon
Tukey HSD	Tukey's honestly significant difference
UCD AHPL	University of California, Davis Aquatic Health Program Lab
YCT	Mixture of yeast, organic alfalfa, and trout chow

Acknowledgements

Funding for this project was provided by the State Water Contractors, Grant Agreement No. 19-0. Chemical analyses were funded in part by the State Water Resources Control Board. All ex-situ testing followed protocols and standards outlined by the Institute of Animal Welfare, Animal Use Protocol 19872. The authors declare no conflict of interest.

Executive Summary

The objective of this project was to look at a key indicator site that represents the integration of the Sacramento River watershed prior to entering the Delta, the Sacramento River at Hood DWR real-time monitoring station. The Sacramento River at Hood DWR Station was selected as a study site because of its history in long-term monitoring projects, as well as the number of special studies that have used this site as an integrator. The goal of this study was to use three categories of ecological indicators (water quality and fish/food web health) to evaluate the water quality of the Sacramento-San Joaquin Delta. To achieve this goal, we tested the following hypotheses:

1. Fish and amphipods exposed to contaminants in Sacramento River water will exhibit acute toxicity (e.g. mortality) and sub-lethal toxicity such as reduced growth and erratic swimming behavior.
2. Exposure of fish to contaminants enhances energy consumption, and because of the reallocation of energy resources on toxicants depuration and adaptation, fish will exhibit diminished growth as well as poor health.

The ex-situ system was designed as a flow-through device, using existing plumbing at the DWR station to pump water from the Sacramento River into test replicate chambers. Chemical analyses were included in this project in order to determine the type and number of contaminants that were present in the Sacramento River water during the time of the ex-situ exposures. Chemical analyses had two main components: 1) one-time grab samples collected on Day 0 of each exposure period, and 2) a Chemcatcher® passive sampler apparatus that was deployed for the entire duration of each exposure period. At the termination of each ex-situ exposure, Rainbow Trout and *H. azteca* from the Hood and Control exposures were analyzed for the swimming behavior endpoint in order to determine sub-lethal effects of ambient water exposure.

Event 1: First Flush

First Exposure Period, initiated November 28, 2018

No mortality was observed with the Rainbow Trout, and a significant reduction in survival was observed with the *H. azteca (*P=0.00669)*,* likely due to a pathogen. There were no differences in distance traveled or velocity with the *H. azteca* or Rainbow Trout.

Second Exposure Period, initiated December 14, 2018

No mortality was observed with the Rainbow Trout, and a significant reduction in survival was observed with the *H. azteca* (P=0.00344). There were no significant differences in distance traveled (P=0.0799) or velocity (P=0.09326) with *H. azteca*. Although there was a considerable difference between distance traveled of Rainbow Trout exposed to Hood water when compared to those in the Control, fish in one replicate swam slower than the rest of the replicates, leading to higher variability and therefore, a higher p-value (P=0.42827).

Event 2: Snowmelt

First Exposure Period, initiated June 21, 2019

Rainbow Trout in the First Exposure Period of the Snowmelt Event had a bad reaction to the food, which resulted in high mortality in both the control and Hood treatments for the First Exposure Period. We did not meet test acceptability criteria for the survival endpoint in this exposure period. *H. azteca* did not exhibit significant mortality during this exposure period and met all test acceptability criteria. We are unable to provide swimming behavior analyses for this exposure period, as the videos were erased prior to analysis.

Second Exposure Period, initiated July 9, 2019

As mentioned previously, we observed high mortality in the Rainbow Trout Control on Day 11 of the Second Exposure Period. All fish were alive and healthy on Day 10, however when technicians arrived at the field station on Day 11, half of the control replicates exhibited almost 100% mortality, reducing overall survival in the control to 50%. There were no outliers in the water quality parameters measured for that day. Calculated total ammonia and unionized ammonia values were 0.83 and 0.009 mg/L, respectively. The cause of this fish mortality is unknown at this time. In the same event, *H. azteca* met test acceptability criteria, and those *H. azteca* exposed to Sacramento River at Hood water exhibited a significant reduction in weight compared to the control (P=0.0070). There were no significant differences observed in swimming behavior in either species.

Event 3: Summer Irrigation

First Exposure Period, initiated August 14, 2019

The third project event was initiated on August 14, 2019, for the Summer Irrigation period. All organisms in this test met test acceptability criteria, and there were no confounding factors observed in this test. There were no statistically significant differences observed in either survival or weight in either species during this first exposure period. For *H. azteca*, significant reductions in distance traveled (P=0.03032) and velocity (P=0.03967) were observed during this exposure period.

Second Exposure Period, initiated August 30, 2019

The Second Exposure Period for the Summer Irrigation Event was initiated on August 30, 2019. *H. azteca* deployed in the field did not exhibit any statistically significant reductions in survival or weight during this exposure period. The Rainbow Trout performed well for the first 13 days of the field exposure. When technicians arrived at the field station on Day 14 for termination, one replicate in the control exhibited 100% mortality. All other replicates were performing normally. All water quality parameters were in range during this timeframe. Flow was present in the control tank, and water was flowing through the replicate chambers when the technicians arrived on Day 14, so it is unlikely that stagnant water, lack of dissolved oxygen, or any other abiotic factor related to water quality was the cause of mortality. At this time, the cause is unknown. This replicate mortality resulted in overall control survival to fall below the test acceptability criterion. There were no significant differences observed in swimming behavior in either species.

Event 4: Fall Event

First Exposure Period, initiated October 16, 2019

The first exposure period was initiated on October 16, 2019. Both Rainbow Trout and *Hyalella azteca* exhibited high survival and there were no significant differences observed in survival, weight, distance travelled or velocity. However, Control *H. azteca* did not increase in weight during the exposure period, as test organisms weighed as much as they did at the beginning of the test.

Second Exposure Period, initiated November 1, 2019

The second exposure period was initiated on November 1, 2019. All organisms in this test met test acceptability criteria. *H. azteca* exposed to Hood water exhibited a significant reduction in weight compared to the control (P=0.0133). There were no significant differences in swimming behavior for either species.

Exposure to the Sacramento River water at Hood, California, did not elicit any acutely negative effects in the Rainbow Trout over the course of the study. In comparison, *Hyalella azteca* did exhibit both acute and sub-lethal negative effects at various time points across the study period, namely during the second First Flush Event, the second Snowmelt Event, the first Summer Irrigation Event, as well as the second Fall Event. Contaminants were present in all events. There were several compounds that were consistently detected across the study period, most notably BPA and gemfibrozil, which were detected 10 times across all events and water types (grab vs. passive sampler). Testosterone, salicylic acid, amoxicillin, caffeine, carbamazepine, DEET, TCPP and TDCPP were all detected six times across the study period. Most chemical detections were in the ng/L range, with the exception of TDCPP, which was detected in the low µg/L in the Second Snowmelt and First Summer Irrigation events. The Chemcatcher® passive sampler was successful in detecting a variety of contaminants across the study period and was comparable to those analytes detected in the one-time grab samples, even with the differences in concentration and number of contaminants detected.

It is possible that the *H. azteca* were exposed to additional hydrophobic contaminants bound to the sediment fraction in the water column, as the amphipods spent more time in the settled sediment in the replicate test chambers when compared to the Rainbow Trout. This coupled with the amphipod's general sensitivity to contaminants may account for the higher number of instances where negative effects were observed. It is also possible that the Rainbow Trout used in this study were too old to be sensitive enough to elicit lethal and sub-lethal effects when exposed to Sacramento River water, based solely on swimming behavior and weight determinations that were observed in the current project. The original goal of this study was to include biomarker analyses on surviving Rainbow Trout; thus, we selected fish that were old enough where we could observe changes in endocrine function. This age group may have exhibited less sensitivity to the compounds present during these selected time points at the detected concentrations. Because we were unable to procure funding for this element, we are unable to determine if molecular, enzymatic, or other sub-lethal responses were taking place in the fish during the ex-situ exposures. This highlights the importance of the inclusion of biomarkers when conducting field studies, as biomarker analyses may help explain the mode of action and can provide a weight of evidence approach when evaluating sub-lethal toxicity.

Introduction

The Sacramento-San Joaquin Delta, together with the San Francisco Bay, is the largest estuary on the west coast (NAWQA 2010). As an integral part of California, it provides abundant wildlife habitat for hundreds of native species, nutrient-rich land for agriculture, and half of the state's municipal and drinking water needs (SRWP 2017). Many indigenous fish species, once abundant to the Delta, are now classified as threatened or endangered. The causes of species declines have been attributed to a number of factors, including but not limited to: habitat loss and degradation, food limitations, invasive species, and toxic contaminants from agriculture and urban inputs (Cloern and Jassby, 2012; Sommer et al. 2007).

Anadromous fishes are widely distributed in California, including the Sacramento-San Joaquin River Delta, and rivers and streams of the Central Valley (SWAP, 2015). The Sacramento River supports one of the most important salmon fisheries in California, with four separate runs of Chinook salmon (fall-run, late fall-run, winter-run, and spring-run (SRWP, 2017). These sensitive anadromous fishes are threatened by the decrease and degradation of freshwater and estuarine ecosystems due to massive water development (SRWP, 2017). Additionally, impacts on aquatic invertebrates as primary consumers, often the most sensitive group to pesticides and contaminants can cause an imbalance in the predator-prey ratio in the aquatic community. Disruptions in the food web and trophic level dynamics can be detrimental to whole populations and ecosystems. Moreover, a decline in food organisms can affect the survival of fish larvae due to starvation and increased vulnerability to predation (Bennett et al., 1995).

The Sacramento River at Hood DWR Station was selected as a study site because of its history in long-term monitoring projects, as well as the number of special studies that have used this site as an integrator. In addition, this site is of interest due to its water quality. The Hood location represents downstream input from four significant areas in the Sacramento River Watershed: the Colusa Basin Drain (comprised primarily of agricultural runoff); 2) the Sacramento metropolitan area (urban storm water); 3) the Feather River (primarily agricultural influences), and 4) discharge from the Sacramento Regional Water Treatment Plant.

The objective of this project was to look at a key indicator site that represents the integration of the Sacramento River watershed prior to entering the Delta, the Sacramento River at Hood DWR real-time monitoring station. The DWR station can house flow-through exposure tanks that integrate toxicity in real time, and therefore, expose the test species to river water constantly over the course of three sensitive time periods (e.g., first flush, spring runoff and summer irrigation). The goal of this study was to use three categories of ecological indicators (water quality and fish/food web health) to evaluate the water quality of the Sacramento-San Joaquin Delta. However, we were only able to procure funding for two of the three tasks. To achieve this goal, we tested the following hypotheses:

1. Fish and amphipods exposed to contaminants in Sacramento River water will exhibit acute toxicity (e.g. mortality) and sub-lethal toxicity such as reduced growth and erratic swimming behavior.
2. Exposure of fish to contaminants enhances energy consumption, and because of the reallocation of energy resources on toxicants depuration and adaptation, fish will exhibit diminished growth as well as poor health.

Based on the sensitive time points of first flush, spring runoff, and summer irrigation periods, we exposed in real-time flow-through exposures, juvenile Rainbow Trout (*Oncorhynchus mykiss*) and the amphipod *Hyalella azteca*, to water from the Sacramento River at Hood. Water pumped from the Sacramento River

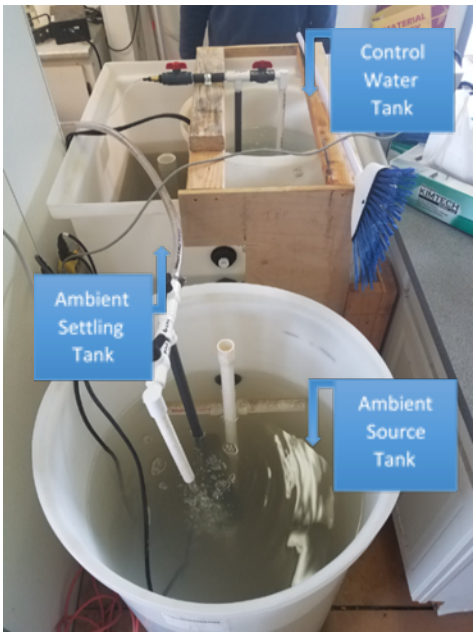
was used in flow-through testing with *O. mykiss* and *H. azteca*, where we analyzed survival and sub-lethal endpoints such as growth and swimming behavior. Although we were unable to obtain funding for this task, we preserved surviving *O. mykiss* at test termination for future biomarker analyses. Chemical analyses were also included in this project, with a combination of a passive sampler apparatus, deployed for the duration of the exposure and one-time grab samples that were collected at the beginning of each event.

Materials and Methods

Ex-situ System

The ex-situ system was designed as a flow-through device, using existing plumbing at the DWR station to pump water from the Sacramento River into test replicate chambers. In previous projects conducted at the DWR station, excessive sedimentation and high turbidity was a confounding variable, therefore this system was designed with additional settling tanks to reduce this variable under the current test conditions (Figure 1).

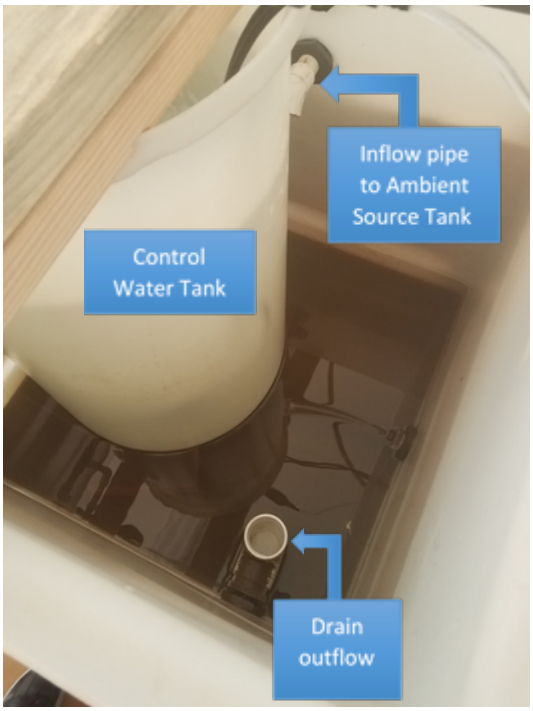
FIGURE 1: EX-SITU FLOW-THROUGH SYSTEM. CONSISTS OF CONTROL WATER TANK, AMBIENT SETTLING TANK, AND AMBIENT SOURCE TANK.



Ambient water from the Sacramento River is first pumped into the Ambient Settling Tank. We placed the Control Water Tank inside of the Ambient Settling Tank to match the water temperature to that of the Sacramento River. Ambient water filled the Ambient Settling Tank until it reached the inflow pipe into the Ambient Source Tank. The inflow pipe was placed high in the Ambient Settling Tank so that suspended sediments would settle down to the bottom of the tank, and the water that crested the inflow pipe into the Ambient Source Tank would be less turbid. This ambient water was then pumped into the replicate tanks.

During our daily visits to the DWR station, we topped off water in the Control Tank (also flow-through designed) and drained the Ambient Settling Tank, leaving behind the accumulated sediment. We then used a Shop-Vac to remove the sediment from the bottom of the Ambient Settling Tank before refilling the system (Figure 2).

FIGURE 2: AMBIENT SETTLING TANK AFTER BEING DRAINED. THIS SEDIMENT WAS REMOVED DAILY VIA SHOP VAC.



After completion of Event 1, we decided to include an additional head tank to the ex-situ system, in order to more evenly distribute flow among replicates and to decrease the amount of sediment entering the replicate test chambers. We included two additional head tanks, one for the control water, and one for the ambient water pumped from the Sacramento River (Figure 3A). The head tanks were plumbed to have the overflow pumped back into the Control and Ambient Source tanks (Figures 3B and 3C). The addition of these head tanks increased total volume of control water by an additional 10 gallons, allowing for a slight increase in flow to all replicates (Figure 3D).

Ambient and control water was pumped via the Ambient Source and Control Tanks into the Ambient and Control Head Tanks, and from there the water was gravity-fed into the individual test replicates. Test replicate chambers were designed with drilled holes that acted as outflows. This outflow water drained into the water bath, which was used to maintain ambient water temperature of the test replicates. To maintain appropriate water bath volume, excess water drained out through an outlet and passed through a UV filter (to kill any pathogens) before being released back into the Sacramento River.

FIGURE 3: EX-SITU IMPROVEMENTS, INCLUDING: A) HEAD TANKS ADDED TO THE EX-SITU SYSTEM; B) RETURN FLOW TO THE CONTROL WATER TANK; C) RETURN TO THE AMBIENT SOURCE TANK; D) OVERVIEW OF THE EX-SITU SYSTEM WITH ADDITIONAL HEAD TANKS INSTALLED.

Water Quality

Field water quality measurements were recorded continuously from the DWR water quality monitoring station for the duration of the exposures. These measurements included DOC, TOC, pH, DO, EC, turbidity, temperature, and chlorophyll, in real-time using automated systems. These real-time water quality measurements are presented in Appendix I for all Events. In addition, aliquots from the Control Tank and the Ambient Settling Tank were collected daily for conductivity, pH, nitrate, nitrites, ammonia-nitrogen, hardness and alkalinity. These same water quality parameters were measured daily from composite samples collected from the Rainbow Trout replicates for final water quality.

Chemical Analyses

Chemical analyses were included in this project in order to determine the type and amount of contaminants that were present in the Sacramento River water during the time of the ex-situ exposures. In addition, we included two different types of sample collection methods: one-time grab samples and passive sampler apparatus, with the goal of comparing the two methods. Chemical analyses had two main components: 1) one-time grab samples collected on Day 0 of each exposure period to represent a snapshot in time and to mirror the types of sample collection typically used in toxicity studies, and 2) a Chemcatcher® passive sampler apparatus that was deployed for the entire duration of each exposure period. Both grab and passive sampler filter extracts were analyzed for the constituents outlined in Table 1.

TABLE 1 COMPOUND GROUPS AND ANALYTICAL METHODS

Compound group	Method
1-4-Dioxane	GCMS by EPA 8270M
Alkyl Phenols	GCMS D7065 by ASTM D7065
OPP low-level	EPA 525.2 Mod QQQ
Polyfluoroalkyl Substances (PFAS)	EPA 537M
Neonicotinoids	LC/MS/MS by EPA 538
Semivolatile Organic Compounds	EPA 625
Polybrominated Diphenyl Ethers (PBDEs)	EPA 1614M by GC/MS SIM
PPCP - Hormones	LC/MS/MS-APCI+ by EPA 1694M-APCI
PPCP – Pharmaceuticals (ESI-)	LC/MS/MS-ESI- by EPA 1694M-ESI-
PPCP – Pharmaceuticals (ESI+)	LC/MS/MS-ESI+ by EPA 1694M-ESI+
Pyrethroid Pesticides	GC/MS/MS by EPA 8270M
Tributyltin	GC/MS by Krone, et al, 1989

For the Chemcatcher® passive sampler, both HLB and C18 filter disks were deployed during the First Flush event, to determine the efficacy of each filter type. However, after receiving the results, there was a considerable discrepancy between the analytes captured by the C18 filters when compared to the HLB filters (see results section). Based on these results, we chose to only use C18 filters for the remainder of the project. C18 filter disks were pre-conditioned with methanol prior to insertion into the filter disk holder (figure 4A). Once the filter disks were attached to the holders, the apparatus was fully assembled (Figure 4B), and deployed into the Ambient Source Tank (Figure 4C) for the duration of each 14-day exposure period. HLB disks used in the First Flush did not require preconditioning before use.

Filter disks were collected at the cessation of each exposure period, wrapped in foil, and kept frozen at -20°C. Frozen filter disks were shipped on ice to California State University Long Beach (CSULB), to the Stream Ecology and Assessment Laboratory, for filter extraction. Filters were extracted by filtering 20 mL of HPLC grade methanol through each disk. For the First Flush Event, three disks each of HLB and C18 were extracted using 20 mL methanol per disk. The eluates for each disk were sent and the extracts were analyzed separately. We averaged the results of the three disks (per type) to provide analytical chemistry results for that exposure period. For the remainder of the project, two C18 disks in total were used per exposure period. 20 mL of methanol was run through each disk and then the elutriates were combined for analysis. After extraction, 20 mL of elutriate was sent to Weck Laboratories (Hacienda Heights, CA) for

analysis. Grab samples were kept between 0-4°C overnight after collection. The following day, samples were shipped on ice directly to Weck Laboratories for analysis. One-time grab sample results were calculated based on the volume of water collected and are presented herein as ng/L.

Passive samplers can be used to determine time-weighted average (TWA) concentrations of a substance, or the equilibrium concentration in the sampler; however, the sampler must be calibrated prior to deployment to determine the uptake rate (R_s) of a specific analyte. In these cases, R_s is used as a surrogate for the volume of water that is passed through the passive sampler over a unit of time, typically L/day (Townsend et al. 2018 and references within). We did not have the capability to perform the passive sampler calibrations for the number of analytes that were being screened for in this study and thus we cannot provide TWA or specific analyte concentrations (e.g., ng/L/day). Because there was no calibration of the passive sampler, we are unable to determine R_s (Folsvik et al. 2000; Charriau et al. 2016); therefore, passive sampler chemical analyses results are provided in ng/L of methanol (MeOH), based on the results provided by Weck Laboratories. For compounds reported by Weck in µg/L, these values have been converted into ng/L or ng/L/MeOH. Whole water samples are reported herein as ng/L.

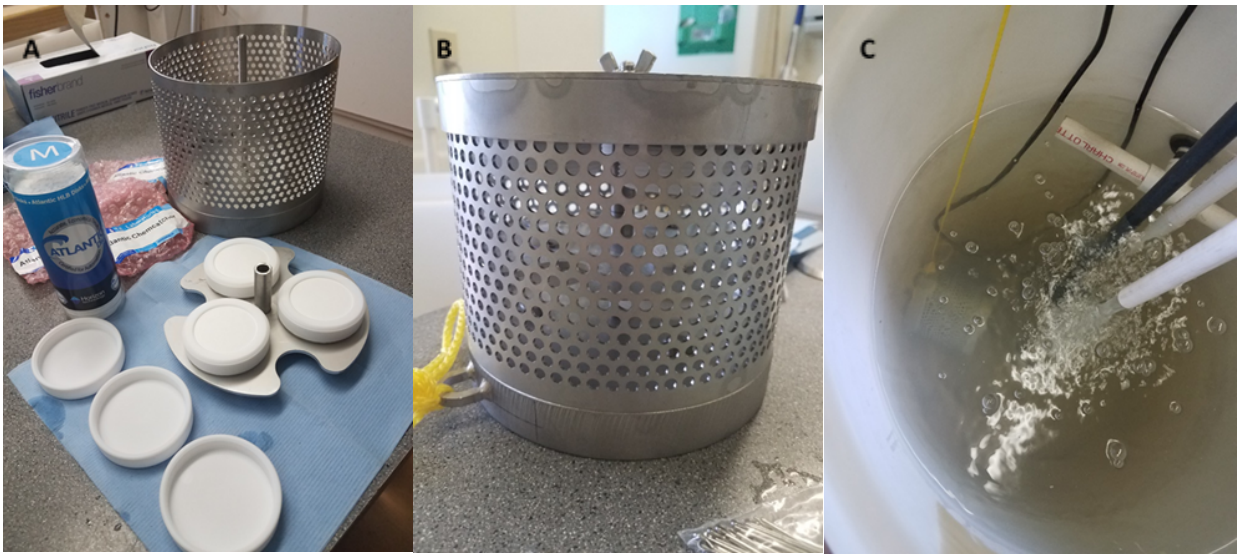


FIGURE 4: CHEMCATCHER® PREPARATION AND DEPLOYMENT: A) CHEMCATCHER® PASSIVE SAMPLER APPARATUS LOADED WITH HLB DISKS PRIOR TO DEPLOYMENT. B) CHEMCATCHER® FULLY ASSEMBLED. C) CHEMCATCHER® DEPLOYED IN AMBIENT SOURCE TANK.

Test Organisms

Hyalella azteca

H. azteca were obtained from Aquatic Research Organisms (Hampton, NH). Upon receipt, *H. azteca* were split into two sub-cultures: one at 23°C for the in-house concurrent reference toxicant test, and the other acclimated to 12°C for use at the DWR station. Organisms at 23°C were fed YCT (a mixture of yeast, organic alfalfa, and trout chow); whereas *H. azteca* at 12°C were fed Tetramin flakes. Juvenile *H. azteca* were approximately 4-6 weeks old at test initiation.

In-house, 96-hr water only non-renewal reference toxicant (RT) tests were initiated the same day as field organism deployment. Each of four replicate 250 mL glass beakers contained 100 mL sample, 1 piece of Nitex screen as artificial substrate, and 10 *H. azteca* each. Tests were conducted at 23 ± 1°C with a 16-hr

light: 8-hr dark photoperiod under fluorescent and ambient light. Tests were scored daily, where organisms were counted and dead *H. azteca* removed. Replicates were fed daily with 500 µL YCT. Mortality was assessed daily and at test termination. Test acceptability criterion is 90% control survival.

Field replicate test chambers consisted of two square plastic boxes connected with a bulkhead (Figure 5). The first chamber acted as a settling tank to reduce turbidity and included a tea strainer (Republic of Tea, Novato, CA) at the inflow to reduce sedimentation. The second chamber acted as the test replicate and contained the test organisms. Test chambers were flow-through with screened outflow holes on the second test-chamber side; therefore, there were no water renewals during this event. Organisms were scored daily with extra sediment and dead organisms removed. Ten *H. azteca* were included per replicate and were fed a mixture of Tetramin flakes, *Selenastrum*, and water, after daily replicate cleaning and scoring. Mortality was assessed daily and at test termination. At termination, *H. azteca* were placed under video surveillance, using a GoPro, for 5-minute intervals in order to determine swimming behavior effects. Afterwards, these *H. azteca* were transported back to the UCD AHPL and placed on boats to obtain dry weights. Test acceptability criteria included 80% control survival and measurable growth in organisms compared to those at the start of the exposure.

FIGURE 5: *HYALELLA AZTECA* TEST REPLICATE CHAMBERS.

Rainbow Trout

Rainbow trout were purchased from Thomas Fish Company (Anderson, CA). Upon receipt, fish were fed and acclimated to laboratory conditions until their use. Fish were kept at 12°C for both field and in-house RT testing. Rainbow trout were fed Trout Chow Crumble #1 ad libitum while in culture.

For ex-situ exposures that took place over the summer, Rainbow Trout were received at the laboratory approximately 7-10 days prior to the event to acclimate to the increased ambient temperatures. Upon arrival to the lab, Rainbow Trout were split into two cultures: 1) temperature-acclimated for use in the field, and 2) fish kept at 12°C for use in in-lab reference toxicant tests. Temperature acclimation occurred with 1-3°C increases daily until the target temperature was met. Water temperatures at Hood during July and August (Snowmelt and Summer Irrigation exposure periods) ranged from 20-22°C.

In-house RT tests were initiated using fish approximately 30 days old. Each of four 5L plastic buckets contained 4 L of test solution and 10 trout. Eighty percent of the test solution was renewed at the 48-hr time point, at which time debris and dead fish were removed from the test chambers. Fish were fed Trout Chow Crumble #1 three times daily. Tests were conducted at 12 ± 1°C with a 16-hr light: 8-hr dark photoperiod under fluorescent and ambient light. Mortality was assessed daily and at test termination.

Field replicate test chambers consisted of two 5L plastic buckets connected with a bulkhead (Figure 6). The first chamber acted as a settling tank to reduce turbidity and included a tea strainer (Republic of Tea, Novato, CA) at the inflow to reduce sedimentation. The second chamber acted as the test replicate and contained the test organisms. Test chambers were flow-through with drilled outflow holes on the second test-chamber side; therefore, there were no water renewals during this event. Fish were scored daily with extra sediment and dead organisms removed daily.

Originally, fifteen Rainbow trout were included per replicate to allow for enough tissue for biomarker archival, as well as for dry weight determinations. However, due to the number of fish per replicate, it was difficult to obtain viable swimming behavior. Additionally, there were concerns about dissolved oxygen concentrations not being sufficient during the summer months with this increased number of fish per replicate. Therefore, we decreased the number of Rainbow Trout to 10 fish per replicate for the remainder of the study period.

Fish were fed Trout Chow Crumble #1 after daily replicate cleaning and scoring. Mortality was assessed daily and at test termination. At termination, surviving Rainbow trout were placed under video surveillance for 5-minute intervals in order to determine swimming behavior effects. Afterwards, five of the surviving Rainbow trout were dissected and preserved for biomarker analyses, with the remaining five fish transported back to the UCD AHPL and placed on boats to obtain dry weights. Test acceptability criteria included 80% control survival and measurable growth in fish compared to those at the start of the exposure.

FIGURE 6: RAINBOW TROUT TEST REPLICATE CHAMBERS.

Biomarker Analyses

A sub-set of surviving Rainbow Trout were preserved for biomarker analyses. We were unable to procure funding for this task. However, Rainbow Trout samples have been archived if funding becomes available in the future.

Behavioral Analyses

At the termination of each ex-situ exposure, Rainbow Trout and *H. azteca* from the Hood and Control exposures were analyzed for the swimming behavior endpoint to determine sub-lethal effects of ambient water exposure. Swimming behavior was obtained via two GoPro Hero Black 6s (GoPro; Los Angeles, CA), which were installed above the Rainbow Trout replicate tanks in the ex-situ system. This allowed the fish to remain in their test replicates and negated the need for us to move them into separate video replicate tanks, which reduced acclimation variability. *H. azteca* were removed from their replicate test chambers and placed into individual wells to obtain swimming behavior videos. The amphipods were acclimated for two minutes prior to filming. Test organisms were recorded in MP4 format in 5-minute increments. Swimming behavior endpoints included distance traveled (cm) and velocity (cm/s). Swimming behavior was analyzed using Noldus EthosVision XT Animal Behavior Software (Wageningen, The Netherlands) using the EthoVision XT 11 Base Module together with the EthoVision XT 11 Social Interaction Module.

Statistics

Each sample was characterized by descriptive statistics, including the mean response and variation among replicates. For this project, toxicity is defined as a statistically significant reduction in test organism performance in the Hood sample compared to the control. Organism performance (control v. ambient sample) was evaluated using independent two-sample t-tests. The Student's t-test was used when data exhibited equal variance, and Welch's t-test was used when the data exhibited unequal variance.

In reference toxicant tests, 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. LC50s were calculated using linear regression.

Quality Assurance / Reference Toxicant Tests

In-lab reference toxicant tests (RT) were conducted on the same batch of organisms that were obtained for each ex-situ exposure period. RT tests were initiated on the same day as the ex-situ exposures, with the exception of the Snowmelt Event I. Rainbow Trout used for the first Snowmelt Event exhibited high mortality in the beginning of the ex-situ exposure because they were not properly acclimated for the higher water temperatures. We reinitiated the ex-situ exposure shortly thereafter; however, because of increased workload associated with other in-lab projects, we did not have the space to conduct a concurrent RT retest.

All test organism performance in RT tests conducted during this reporting period fell within the US EPA acceptable range of plus/minus two standard deviations from the running mean (US EPA 2002). *H. azteca* LC50 and NOEC data are provided below in Figures 7 and 8. Rainbow Trout LC50 and NOEC data are provided in Figures 9 and 10.

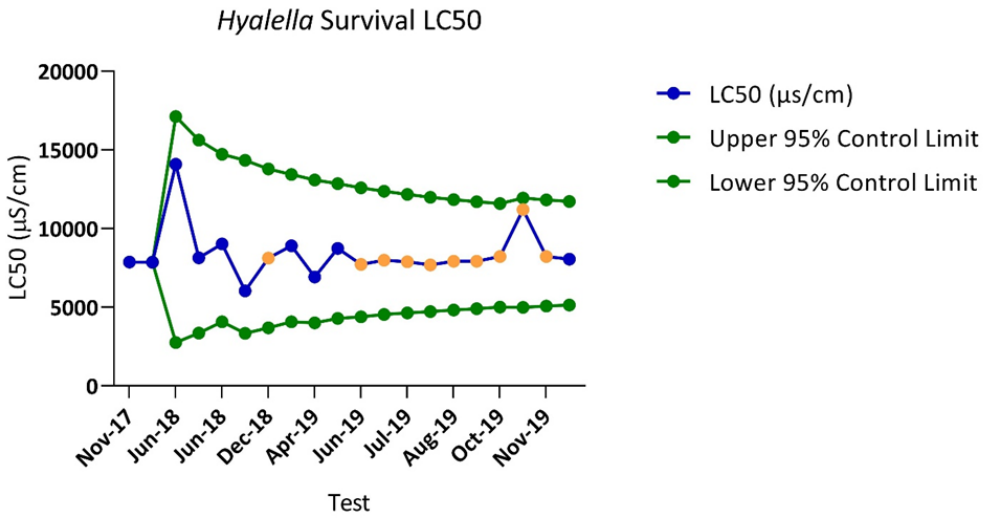


FIGURE 7: SUMMARY OF *HYALELLA AZTECA* LC50 FOR SURVIVAL. ORANGE DATA POINTS ARE THOSE REFERENCE TOXICANT TESTS CONDUCTED DURING THE PROJECT PERIOD.

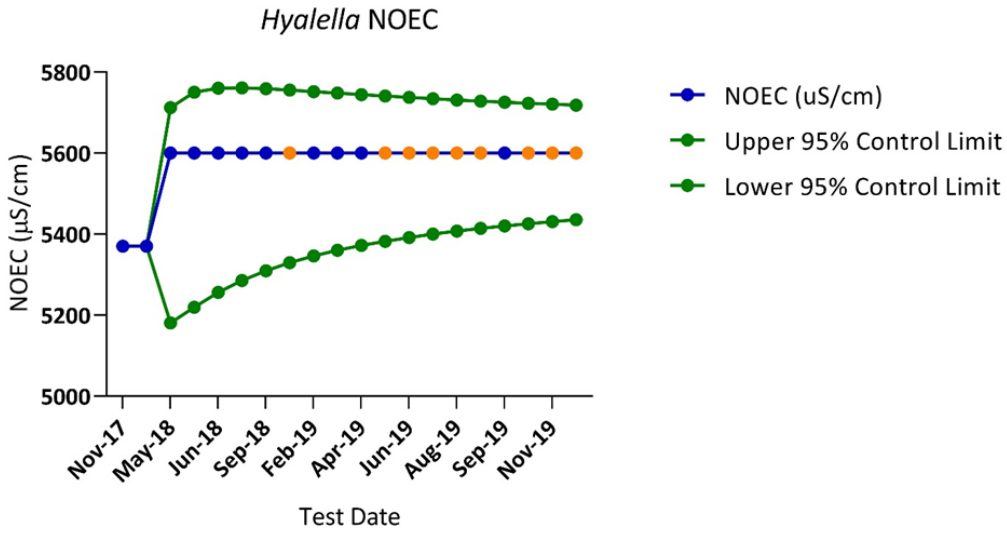


FIGURE 8: SUMMARY OF *HYALELLA AZTECA* NOEC FOR SURVIVAL. ORANGE DATA POINTS ARE THOSE REFERENCE TOXICANT TESTS CONDUCTED DURING THE PROJECT PERIOD.

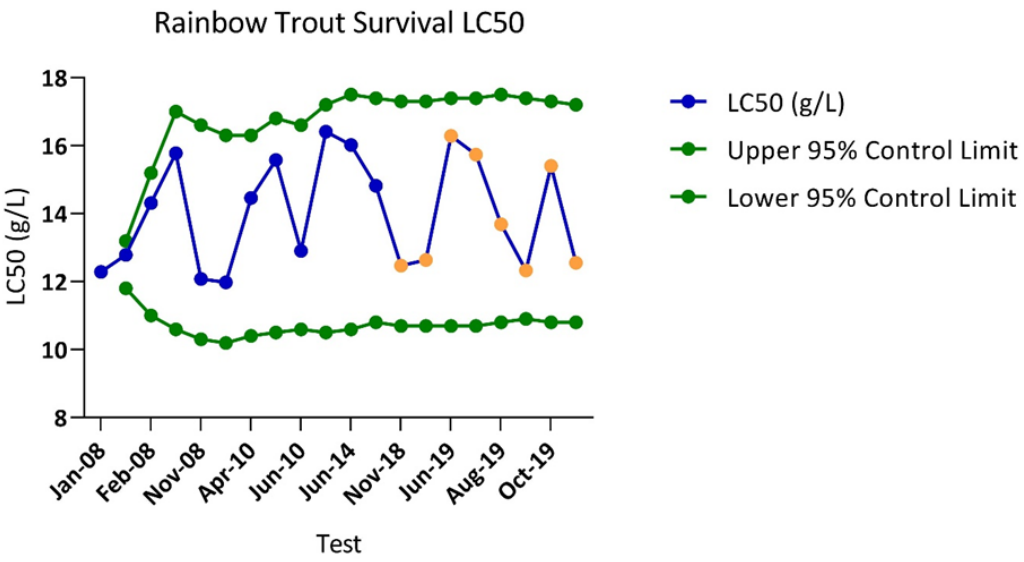


FIGURE 9: SUMMARY OF RAINBOW TROUT LC50 FOR SURVIVAL. ORANGE DATA POINTS ARE THOSE REFERENCE TOXICANT TESTS CONDUCTED DURING THE PROJECT PERIOD.

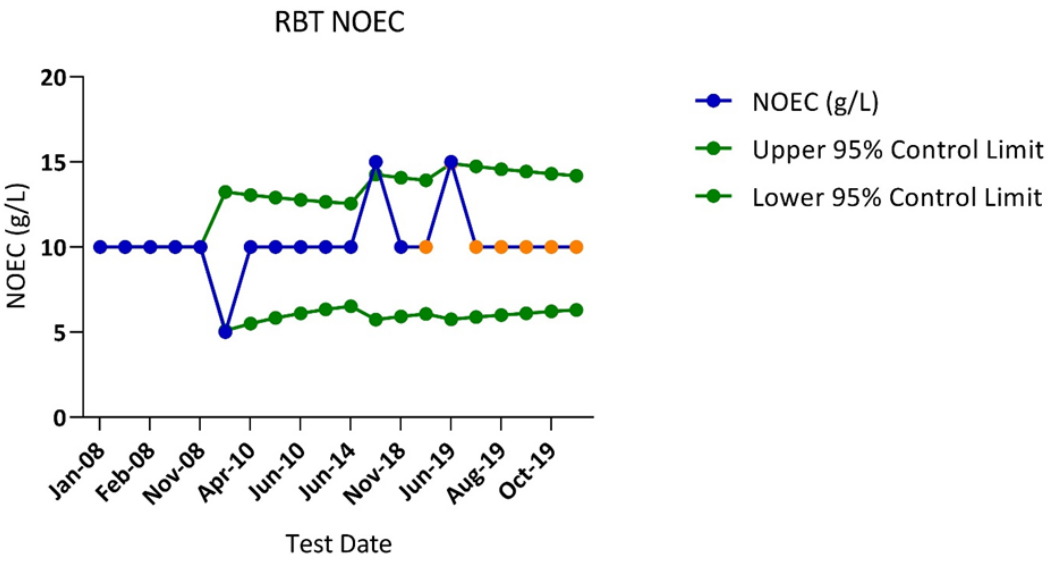


FIGURE 10: SUMMARY OF RAINBOW TROUT NOEC FOR SURVIVAL. ORANGE DATA POINTS ARE THOSE REFERENCE TOXICANT TESTS CONDUCTED DURING THE PROJECT PERIOD.

Project Challenges

Rainbow Trout Survival

We experienced intermittent mortality in control replicates in the Rainbow Trout ex-situ exposures for the following events: Snowmelt I, Snowmelt II, and Summer Irrigation II. The mortality observed during Snowmelt I was due to poor food quality. The cause of the mortality observed in Snowmelt II and Summer Irrigation II is unknown. Water quality parameters were within ranges, and there were no indications that Rainbow Trout were in poor health. In fact, in both instances, the observed mortality occurred during the last few days of the exposure, and quickly affected all fish in the specific replicate. It is possible that this unexplained mortality may have been due to a pathogen, given the swift time to death and that the mortality was observed in one or two replicates only; however, we do not have any evidence to confirm this hypothesis.

Snowmelt I

The first deployment for the Snowmelt Event was originally scheduled for June 5, 2019. We had set up the ex-situ exposure on that day, however we observed high mortality in all Rainbow Trout replicates because we had not adequately acclimated the fish for the higher ambient temperatures. We had to wait two weeks before reinitiating the ex-situ exposure in order to obtain the correct-aged Rainbow Trout.

After we had reinitiated the Snowmelt Event with properly acclimated fish, the Rainbow Trout in the First Exposure Period had a bad reaction to the food. Prior to deployment, the food used for the Rainbow Trout was switched from Trout Chow Crumble to a purified casein diet that we thought would be beneficial for fish growth. However, the fish were unable to digest the new food, which resulted in high mortality in both the control and Hood treatments. We did not meet the test acceptability criterion for survival.

Snowmelt II

High mortality was observed in the Rainbow Trout in two control replicates, which occurred on Day 11 of the exposure period. At this time, we cannot explain the observed mortality. We did not meet the test acceptability criterion for survival.

Summer Irrigation II

High mortality was observed in the Rainbow Trout in one of the control replicates, which occurred on Day 14 of the exposure period (termination). At this time, we cannot explain the observed mortality. We did not meet the test acceptability criterion for survival.

Analytical Chemistry

Not all targets were met with the analytical chemistry portion of this project. Some of these deviations occurred due to technician oversight, while others are due to circumstances beyond our control.

Data Gaps

First Flush I

For the First Flush I event, not all the passive sampler supplies had arrived at UCD AHPL in time for the Chemcatcher® to be deployed during the first event. We do not have passive sampler data for First Flush I.

Whole water samples were collected during the first exposure period and were sent to the intermediary laboratory in a timely manner. However, those water samples were never sent to the analysis laboratory and by the time this mistake was discovered, the holding time was considerably exceeded. The whole

water samples were too old to analyze. We therefore do not have whole water data for this exposure period.

First Flush II, Snowmelt I, II

Whole water samples were not collected during these exposure periods due to technician oversight. Technicians mistakenly thought that no grab samples were to be collected because we had deployed the passive sampler in these exposures. We do not have whole water data for these exposure periods.

Summer Irrigation I

Whole water samples were sent to the analysis laboratory for chemical analyses. The lab disposed of the samples prior to completion of the analyses. Therefore, we do not have pharmaceutical data for the whole water sample collected during the first exposure period of the Summer Irrigation Event.

Results

Event 1: First Flush

First Exposure Period, initiated November 28, 2018

Hyalella azteca and Rainbow Trout were deployed at the DWR field station on Wednesday November 28, 2018. During this first exposure period, approximately 5.5 inches of precipitation accumulated throughout the Sacramento Region, with the heaviest day of rain taking place on Day 1 of the exposure (11/29/18) where approximately 1.5 inches of rain fell in Sacramento. Almost a third of an inch of rain fell on Day 3 (12/1/18) and about a tenth of an inch of rain was observed on Day 7 (12/5/18). Average air temperatures during this exposure period reached a high of 58°F, and a low of 42°F. Average water temperatures ranged from 11-13°C in both the control and ambient systems. Precipitation levels during the First Exposure Period are outlined in Figure A2-1 and A2-2 in Appendix II.

As depicted above in Figure 6, Sacramento River water was clear on Day 0, however with the storms in the region, turbidity increased significantly, leading us to include additional sediment removal protocols, such as siphoning out the test replicate chambers. In addition, with the increased sedimentation and turbidity, we believe that one of the *H. azteca* replicates succumbed to a pathogen, as high mortality was observed within the first few days of the exposure in that single replicate, compared to the other three replicates. The deceased *H. azteca* in this replicate were covered in fungus. Real-time turbidity measurements from the DWR station are provided in Appendix I.

During the first few days of the exposure period, we lost several Rainbow Trout from the test replicates, as we believed they had jumped out of the replicate buckets. We had covered them with a screen to allow light to pass through and at the time, we believed that would be sufficient to keep the fish in the replicates. However, when we came in on Days 1 and 2 of the exposure, several fish were missing from the replicates and were swimming freely in the water bath. In response, we replaced the covers with plastic diffuser panels that had more weight, and from that point, no other fish were lost from the replicates. As a result, for this exposure period we did not have 15 fish in each replicate bucket.

No mortality was observed with the Rainbow Trout, and a significant reduction in survival was observed with the *H. azteca (*P=0.00669)*,* likely due to a pathogen (Table 2). There were no differences in distance traveled or velocity with the *H. azteca* or Rainbow Trout. Water quality measurements from sub-samples

collected from the Control and Ambient Source Tanks measured by UCD AHPL are outlined in Tables A3-1 and A3-2 in Appendix III.

TABLE 2. SUMMARY OF SURVIVAL AND WEIGHT RESULTS FROM EVENT 1: FIRST FLUSH, FIRST EXPOSURE PERIOD, INITIATED ON NOVEMBER 28, 2018.

Species	Mean Survival (%)[1]	SD Survival (%)	SE Survival (%)	Mean Weight (mg)	SD Weight (mg)	SE Weight (mg)
Hyalella azteca: Control	84.4	12.6	6.3	0.361	0.154	0.077
Hyalella azteca: Hood	35.0	20.8	10.4	0.217	0.176	0.088
Rainbow Trout: Control	100.0	0.0	0.0	132.3	8.3	4.8
Rainbow Trout: Hood	100.0	0.0	0.0	120.7	12.9	7.4

1. Highlighted cells indicates a statistically significant reduction in survival compared to the control (P=0.00669).

TABLE 3 SUMMARY OF SWIMMING BEHAVIOR RESULTS FROM EVENT 1: FIRST FLUSH, FIRST EXPOSURE PERIOD, INITIATED ON NOVEMBER 28, 2018.

Species	Mean Distance Traveled (cm)	SD Distance Traveled (cm)	SE Distance Traveled (cm)	Mean Velocity (cm/s)	SD Velocity (cm/s)	SE Velocity (cm/s)
Hyalella azteca: Control	51.21	32.24	16.12	0.243	0.152	0.076
Hyalella azteca: Hood	42.26	56.50	28.25	0.191	0.187	0.093
Rainbow Trout: Control	1,397.35	27.57	13.78	4.739	1.339	0.669
Rainbow Trout: Hood	1,185.34	182.38	91.19	4.235	0.739	0.369

Chemical Analyses

Not all of the filters and supplies had arrived at UCD AHPL prior to deployment during this First Exposure Period for use with the passive sampler device. Instead, we collected approximately 4 gallons per day from the Ambient Source Tank as one-time grab samples for use in chemical analyses. These whole water samples were shipped to CSULB laboratory for analysis from Weck Laboratories. Partway through the project period, it was discovered that these whole water samples were never shipped to Weck, and that the holding time had been exceeded such that it was too late to analyze once it was discovered. Therefore, there are no whole water sample analyses for this Exposure Period.

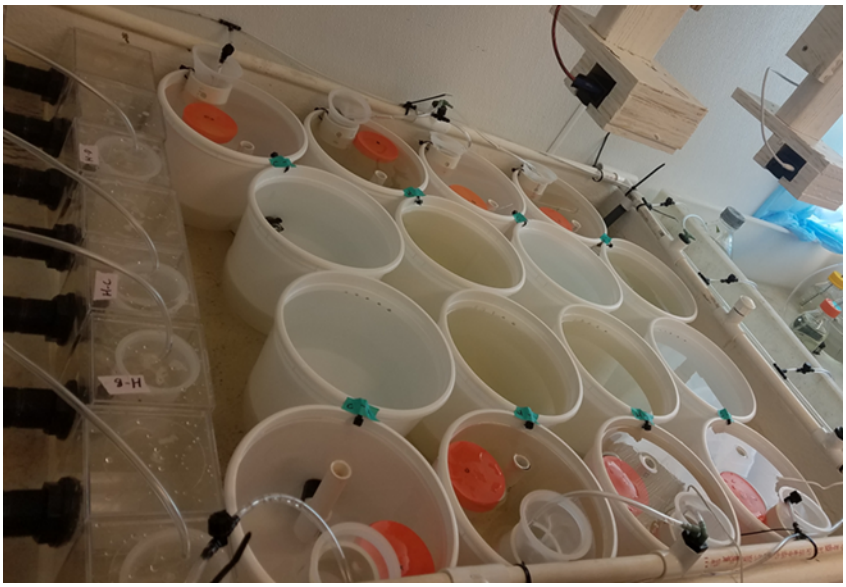
Second Exposure Period, initiated December 14, 2018

Hyalella azteca and Rainbow Trout were deployed at the DWR field station on Friday December 14, 2018. During this second exposure period, approximately 5.9 inches of precipitation accumulated throughout the Sacramento Region, with the heaviest day of rain taking place on Day 3 of the exposure (12/17/18) where a little more than one inch of rain fell in Sacramento. Almost a tenth of an inch of rain fell on Day 7 (12/21/18) and Day 10 (12/24/18), and a little more than a quarter of an inch of rain was observed on Day 11 (12/25/18). Average air temperatures during this exposure period were 58°F for the high, and 43°F for

the low. Precipitation levels for the Second Exposure Period are outlined in Figure A2-2 in Appendix II. Average water temperatures ranged from 11-15°C in both the control and ambient systems.

Based on the turbidity and sedimentation issues we observed with *H. azteca* during the First Exposure Period, we included a second set of replicates for *H. azteca*, in floating cages. We believed that this would keep the amphipods out of the sediment and reduce the potential for pathogen interference. These additional replicate cages consisted of the same tea strainers used for sedimentation removal (Republic of Tea, Novato, CA) and a plastic PVC pipe cap as a cover. We deployed these floating cages into the settling tank side of the Rainbow Trout replicates (Figure 11). Protocols for organism loading, daily mortality counts and feeding followed those used during the previous exposure period. Interestingly, survival in the floating *H. azteca* cages placed in the Hood replicates were similar to those in the square replicate chambers. However, organisms in the control replicates exhibited significantly less survival than that of the control square replicate cages, we believe due to the constant handling and removal from the water to obtain daily mortality counts. Unlike the replicate squares, the cages had to be pulled from the water column, gently opened, and the organisms examined. These steps were performed as quickly and gently as possible as to not harm the organisms; however, it would appear that this constant handling negatively affected the organisms in these replicates. Therefore, we did not measure weight or swimming behavior with *H. azteca* from the floating cages.

FIGURE 11: SECONDARY *H. AZTECA* REPLICATE CAGES (ORANGE CAPS) WERE INCLUDED DURING THE SECOND EXPOSURE PERIOD AND PLACED IN THE SETTLING TANK PORTION OF THE RAINBOW TROUT REPLICATES.



In this Second Exposure Period, we again lost a significant number of Rainbow Trout during the first few days of the exposure period from the test replicates and found more than half of the Rainbow Trout swimming freely in the water bath. We observed that this batch of fish used for test initiation were small enough to fit through the outflow holes in the replicate tanks, leading us to infer that this was the cause of the missing trout in the prior exposure period. In response, we covered the outflow holes with screen. However, due to the high number of Rainbow Trout missing from the test replicates, we did not have

enough tissue for biomarkers and for the weight endpoint. Therefore, we replaced the missing Rainbow Trout with additional fish on Day 3 of the test, after screening off the outflow holes.

No mortality was observed with the Rainbow Trout, and a significant reduction in survival was observed with the *H. azteca* (P=0.00344; Table 4). There were no significant differences in distance traveled (P=0.0799) or velocity (P=0.09326) with *H. azteca* (Table 5). Although there was an increase of distance traveled of the Rainbow Trout exposed to Hood water when compared to those in the Control, one replicate of fish exposed to Hood water consistently swam slower than the rest of the replicates. This replicate swam slower, and did not cover as much distance as the fish in the other three replicates in the Hood water, which lead to higher variability and therefore, a higher p-value (P=0.42827; Table 5). This variability precluded our ability to see a statistically significant difference in this instance of Rainbow Trout swimming behavior for fish exposed to Hood ambient water. Water quality measurements from sub-samples collected from the Control and Ambient Source Tanks measured by UCD AHPL are outlined in Tables A3-3 and A3-4 in Appendix III.

TABLE 4. SUMMARY OF SURVIVAL AND WEIGHT RESULTS FROM EVENT 1: FIRST FLUSH, SECOND EXPOSURE PERIOD, INITIATED ON DECEMBER 14, 2018.

Species	Mean Survival (%)[1]	SD Survival (%)	SE Survival (%)	Mean Weight (mg)	SD Weight (mg)	SE Weight (mg)
Hyalella azteca: Control - Squares	95.0	5.8	2.9	0.413	0.051	0.026
Hyalella azteca: Hood - Squares	57.5	15.0	7.5	0.421	0.095	0.048
Hyalella azteca: Control - Cage	52.5	20.6	10.3	-	-	-
Hyalella azteca: Hood - Cage	60.0	18.3	9.1	-	-	-
Rainbow Trout: Control	100.0	0.0	0.0	97.766	15.428	7.714
Rainbow Trout: Hood	100.0	0.0	0.0	111.849	6.165	3.083

TABLE 5. SUMMARY OF SWIMMING BEHAVIOR RESULTS FROM EVENT 1: FIRST FLUSH, SECOND EXPOSURE PERIOD, INITIATED ON DECEMBER 14, 2018.

Species	Mean Distance Traveled (cm)	SD Distance Traveled (cm)	SE Distance Traveled (cm)	Mean Velocity (cm/s)	SD Velocity (cm/s)	SE Velocity (cm/s)
Hyalella azteca: Control	77.02	35.67	17.83	0.306	0.147	0.073
Hyalella azteca: Hood	36.81	13.68	6.84	0.153	0.045	0.023
Rainbow Trout: Control	1,540.85	106.96	53.48	5.456	0.371	0.186
Rainbow Trout: Hood	2,014.41	1,109.97	554.98	7.621	3.344	1.672

Chemical Analyses

All the filters and supplies had arrived at UCD AHPL prior to deployment during this Second Exposure Period and we were able to deploy the passive sampler apparatus into the ambient source tank. The passive sampler apparatus was initiated with three C18 filters and three HLB filters to do a comparison between filter matrices. Only those analytes that were detected are outlined in Table 6. Detections presented in Table 6 are the detected concentrations that were averaged across the three replicates. Whole water samples were not collected for this exposure due to technician error.

TABLE 6. C18 VS HLB COMPARISON ON EVENT 1: FIRST FLUSH, SECOND EXPOSURE PERIOD, INITIATED ON DECEMBER 14, 2018. CONCENTRATIONS PROVIDED HAVE BEEN AVERAGED ACROSS THE THREE FILTERS.

Analyte	Average concentration C18 detections (ng/L of methanol)	Average concentration HLB Detections (ng/L of methanol)	Chemical Class
Bisphenol A	2,467	1,170	Bisphenol
Gemfibrozil	760	-	Cholesterol medication
Caffeine	1,433	657	PPCP
DEET	353	-	Pesticide
TCPP	12,333	2,700	Chlorinated organophosphate flame retardant
TDCPP	927	-	Chlorinated organophosphate flame retardant
Atenolol	330	-	Beta blocker
Azinphos methyl	7	-	Organophosphate
Stirophos	7	-	Organophosphate
Carbamazepine	92	-	Anticonvulsant
Butyl-benzyl phthalate	-	350,000	Phthalate (plasticizer for PVC)

Event 2: Snowmelt

Timing of the Snowmelt Event

Due to unusually cool temperatures (Figures A2-3 and A2-4 in Appendix II) and high flows (Figures A2-5 to A2-7 in Appendix II), we were not able to initiate the Snowmelt event until June 2019. We had originally planned to initiate the First Exposure Period on June 5; however, inadequate temperature acclimation procedures resulted in poor survival with the Rainbow Trout. Ambient river temperatures were approximately 20°C and we did not allow sufficient temperature acclimation prior to organism deployment. High mortality was observed within the first few days of exposure in both Control and Ambient treatments. We terminated the field exposure tests after seven days and rescheduled the event to begin on June 21, 2019, as that was the earliest time point, we could obtain fish within the proper age range. We had already conducted concurrent in-house RT tests the week of June 5. Due to additional toxicity tests in the lab requiring temperature-controlled water bath space during the week of June 21, we were unable to include

concurrent RT tests with the June 21 deployment. For the First Exposure Period, RT information is from the tests initiated on June 5, 2019. However, concurrent in-house RT tests were conducted with the Second Exposure Period, which was initiated on July 9, 2019.

First Exposure Period, initiated June 21, 2019

As mentioned above, Rainbow Trout in the First Exposure Period of the Snowmelt Event had a bad reaction to the food, which resulted in high mortality in both the control and Hood treatments for the First Exposure Period. We did not meet test acceptability criteria for the survival endpoint in this exposure period (Table 7). *H. azteca* did not exhibit significant mortality during this exposure period and met all test acceptability criteria.

We are unable to provide swimming behavior analyses for this exposure period, as the videos were erased prior to analysis. Water quality measurements from sub-samples collected from the Control and Ambient Source Tanks measured by UCD AHPL are outlined in Tables A3-5 to A3-7 in Appendix III.

TABLE 7. SUMMARY OF SURVIVAL AND WEIGHT RESULTS FROM EVENT 2: SNOWMELT EVENT, FIRST EXPOSURE PERIOD, INITIATED ON JUNE 21, 2019.

Species	Mean Survival (%)	SD Survival (%)	SE Survival (%)	Mean Weight (mg)	**SD Weight (mg)**	**SE Weight (mg)**
Hyalella azteca: Control	90.0	0.0	0.0	1.006	0.131	0.065
Hyalella azteca: Hood	92.5	9.6	4.8	0.948	0.199	0.099
Rainbow Trout: Control	55.0	52.6	26.3	129.720	0.020	0.014
Rainbow Trout: Hood	42.5	50.6	25.3	81.424	66.901	47.306

Chemical Analyses

C18 filters were deployed in the passive sampler for the duration of the study. The filters were sent to CSULB for extraction and analysis by Weck laboratories. Results of the C18 filter analyses are outlined in Table 8 below. Whole water samples were not collected for this exposure due to technician error.

TABLE 8. SUMMARY OF ANALYTICAL CHEMISTRY DETECTIONS FROM C18 FILTERS DEPLOYED IN THE CHEMCATCHER PASSIVE SAMPLER DURING THE FIRST EXPOSURE PERIOD OF THE SNOWMELT EVENT.

Chemical	Concentration (ng/L of methanol)
Testosterone	930
BPA	4,300
Gemfibrozil	710
Salicylic acid	1,500
Amoxicillin	2,900
Caffeine	500
Carbamazepine	120
DEET	1,600
TCPP	5,300
TDCPP	10,000

Second Exposure Period, initiated July 9, 2019

As mentioned previously, we observed high mortality in the Rainbow Trout Control on Day 11 of the Second Exposure Period. All fish were alive and healthy on Day 10, however when technicians arrived at the field station on Day 11, half of the control replicates exhibited almost 100% mortality, reducing overall survival in the control to 50% (Table 9). There were no outliers in the water quality parameters measured for that day (Table A3-8 in Appendix III). Calculated total ammonia and unionized ammonia values were 0.83 and 0.009 mg/L, respectively. The cause of this fish mortality is unknown at this time. In the same event, *H. azteca* met test acceptability criteria, and those *H. azteca* exposed to Sacramento River at Hood water exhibited a significant reduction in weight compared to the control (P=0.0070). There were no significant differences observed in swimming behavior in either species (Table 10). Water quality measurements from sub-samples collected from the Control and Ambient Source Tanks measured by UCD AHPL are outlined in Tables A3-8 to A3-10 in Appendix III.

TABLE 9. SUMMARY OF SURVIVAL AND WEIGHT RESULTS FROM EVENT 2: SNOWMELT EVENT, SECOND EXPOSURE PERIOD, INITIATED ON JULY 9, 2019.

Species	Mean Survival (%)	SD Survival (%)	SE Survival (%)	Mean Weight (mg) [1]	SD Weight (mg)	SE Weight (mg)
Hyalella azteca: Control	90.0	11.5	5.8	0.938	0.075	0.037
Hyalella azteca: Hood	85.0	12.9	6.5	0.750	0.057	0.028
Rainbow Trout: Control	50.0	57.7	28.9	119.126	17.089	12.084
Rainbow Trout: Hood	100.0	0.0	0.0	118.999	7.442	3.721

1. Highlighted cells indicate a statistically significant reduction in weight compared to the control (P=0.0070).

TABLE 10. SUMMARY OF SWIMMING BEHAVIOR RESULTS FROM EVENT 2: SNOWMELT EVENT, SECOND EXPOSURE PERIOD, INITIATED ON JULY 9, 2019.

Species	Mean Distance Traveled (cm)	SD Distance Traveled (cm)	SE Distance Traveled (cm)	Mean Velocity (cm/s)	SD Velocity (cm/s)	SE Velocity (cm/s)
Hyalella azteca: Control	141.89	67.01	33.50	0.492	0.238	0.119
Hyalella azteca: Hood	192.43	61.03	30.52	0.656	0.198	0.099
Rainbow Trout: Control	3,012.01	321.22	227.14	8.379	0.575	0.407
Rainbow Trout: Hood	2,925.75	800.45	400.22	8.180	2.792	1.396

Chemical Analyses

C18 filters were deployed in the passive sampler for the duration of the study. The filters were sent to CSULB for extraction and analysis by Weck laboratories. Results of the C18 filter analyses are outlined in Table 11 below. Whole water samples were not collected for this exposure due to technician error.

TABLE 11. SUMMARY OF ANALYTICAL CHEMISTRY DETECTIONS FROM C18 FILTERS DEPLOYED ON THE CHEMCATCHER PASSIVE SAMPLER DURING THE SECOND EXPOSURE PERIOD OF THE SNOWMELT EVENT.

Chemical	Concentration (ng/L of methanol)
Testosterone	300
BPA	2,800
Gemfibrozil	580
Salicylic acid	1,400
Amoxicillin	3,200
Caffeine	560
Carbamazepine	220
DEET	1,400
TCPP	5,500
TDCPP	18,000

Event 3: Summer Irrigation

First Exposure Period, initiated August 14, 2019

The third project event was initiated on August 14, 2019, for the Summer Irrigation period. All organisms in this test met test acceptability criteria, and there were no confounding factors observed in this test. There were no statistically significant differences observed in either survival or weight in either species during this first exposure period (Table 12). For *H. azteca*, significant reductions in distance traveled (P=0.03032) and velocity (P=0.03967) were observed during this exposure period (Table 13). Water quality measurements from sub-samples collected from the Control and Ambient Source Tanks measured by UCD AHPL are outlined in Tables A3-11 to A3-13 in Appendix III.

TABLE 12. SUMMARY OF SURVIVAL AND WEIGHT RESULTS FROM EVENT 3: SUMMER IRRIGATION, FIRST EXPOSURE PERIOD, INITIATED ON AUGUST 14, 2019.

Species	Mean Survival (%)	SD Survival (%)	SE Survival (%)	Mean Weight (mg)	SD Weight (mg)	SE Weight (mg)
Hyalella azteca: Control	95.0	5.8	2.9	0.596	0.105	0.053
Hyalella azteca: Hood	90.0	8.2	4.1	0.424	0.134	0.067
Rainbow Trout: Control	100.0	0.0	0.0	101.739	17.760	8.880
Rainbow Trout: Hood	100.0	0.0	0.0	99.833	15.177	7.589

TABLE 13. SUMMARY OF SWIMMING BEHAVIOR RESULTS FROM EVENT 3: SUMMER IRRIGATION, FIRST EXPOSURE PERIOD, INITIATED ON AUGUST 14, 2019.

Species	Mean Distance Traveled (cm)[1]	SD Distance Traveled (cm)	SE Distance Traveled (cm)	Mean Velocity (cm/s)[1]	SD Velocity (cm/s)	SE Velocity (cm/s)
Hyalella azteca: Control	182.03	19.52	9.76	0.572	0.057	0.028
Hyalella azteca: Hood	92.75	60.22	30.11	0.309	0.193	0.096
Rainbow Trout: Control	3,130.83	630.51	315.25	5.882	1.365	0.682
Rainbow Trout: Hood	3,506.73	330.21	165.11	7.066	0.711	0.356

1. Highlighted cells indicate a significant reduction in distance traveled (P=0.03032) and velocity (P=0.03967).

Chemical Analyses

C18 filters were deployed in the passive sampler for the duration of the study. The filters were sent to CSULB for extraction and analysis by Weck laboratories. Results of the C18 filter analyses are outlined in Table 14 below. Whole water samples were collected and sent to Weck for analysis. No analytes were detected in this water sample. Whole water sample results for this event do not include pharmaceutical analyses, as the sample was disposed of by Weck before the analyses were complete.

TABLE 14. SUMMARY OF ANALYTICAL CHEMISTRY DETECTIONS FROM C18 FILTERS DEPLOYED IN THE CHEMCATCHER PASSIVE SAMPLER DURING THE FIRST EXPOSURE PERIOD OF THE SUMMER IRRIGATION EVENT.

Chemical	Concentration (ng/L of methanol)
Testosterone	620
BPA	26,000
Gemfibrozil	420
Salicylic acid	1,600
Amoxicillin	2,800
Caffeine	520
Carbamazepine	180
DEET	2,300
TCPP	9,100
TDCPP	16,000

Second Exposure Period, initiated August 30, 2019

The Second Exposure Period for the Summer Irrigation Event was initiated on August 30, 2019. *H. azteca* deployed in the field did not exhibit any statistically significant reductions in survival or weight during this exposure period (Table 15). The Rainbow Trout performed well for the first 13 days of the field exposure. When technicians arrived at the field station on Day 14 for termination, one replicate in the control exhibited 100% mortality. All other replicates were performing normally. All water quality parameters were in range during this timeframe (Table A3-14 in Appendix III). Flow was present in the control tank, and water was flowing through the replicate chambers when the technicians arrived on Day 14, so it is unlikely that stagnant water, lack of dissolved oxygen, or any other abiotic factor related to water quality was the

cause of mortality. At this time, the cause is unknown. This replicate mortality resulted in overall control survival to fall below the test acceptability criterion. There were no significant differences observed in swimming behavior in either species (Table 16). Water quality measurements from sub-samples collected from the Control and Ambient Source Tanks measured by UCD AHPL are outlined in Tables A3-14 to A3-16 in Appendix III.

TABLE 15. SUMMARY OF SURVIVAL AND WEIGHT RESULTS FROM EVENT 3: SUMMER IRRIGATION, SECOND EXPOSURE PERIOD, INITIATED ON AUGUST 30, 2019.

Species	Mean Survival (%)	SD Survival (%)	SE Survival (%)	Mean Weight (mg)	SD Weight (mg)	SE Weight (mg)
Hyalella azteca: Control	97.5	5.0	2.5	0.488	0.073	0.037
Hyalella azteca: Hood	92.5	9.6	4.8	0.427	0.056	0.028
Rainbow Trout: Control	75.0	50.0	25.0	94.406	3.828	2.210
Rainbow Trout: Hood	97.5	5.0	2.5	89.460	6.896	3.448

TABLE 16. SUMMARY OF SWIMMING BEHAVIOR RESULTS FROM EVENT 3: SUMMER IRRIGATION, SECOND EXPOSURE PERIOD, INITIATED ON AUGUST 30, 2019.

Species	Mean Distance Traveled (cm)	SD Distance Traveled (cm)	SE Distance Traveled (cm)	Mean Velocity (cm/s)	SD Velocity (cm/s)	SE Velocity (cm/s)
Hyalella azteca: Control	88.03	41.94	20.97	0.289	0.137	0.068
Hyalella azteca: Hood	88.71	37.95	18.98	0.286	0.132	0.066
Rainbow Trout: Control	2,054.26	52.62	30.38	6.502	0.084	0.048
Rainbow Trout: Hood	2,157.78	252.57	126.28	6.908	0.781	0.391

Chemical Analyses

C18 filters were deployed in the passive sampler for the duration of the study. These filters were sent to CSULB for extraction and analysis by Weck laboratories. Results of the C18 filter analyses are outlined in Table 17 below. Whole water samples were collected and sent to Weck for analysis. Detected compounds from the whole water sample are outlined below in Table 18.

TABLE 17. SUMMARY OF ANALYTICAL CHEMISTRY DETECTIONS FROM C18 FILTERS DEPLOYED IN THE CHEMCATCHER PASSIVE SAMPLER DURING THE SECOND EXPOSURE PERIOD OF THE SUMMER IRRIGATION EVENT.

Chemical	Concentration (ng/L of methanol)
Testosterone	790
BPA	2,200
Gemfibrozil	380
Salicylic acid	1,300

Chemical	Concentration (ng/L of methanol)
Amoxicillin	2,800
Caffeine	1,900
Naproxen	250
DEET	1,300
TCPP	4,300
TDCPP	11,000

TABLE 18. SUMMARY OF ANALYTICAL CHEMISTRY RESULTS FROM THE WHOLE WATER SAMPLE COLLECTED ON DAY 0 OF EVENT 3: SUMMER IRRIGATION, SECOND EXPOSURE PERIOD, ON AUGUST 30, 2019.

Analyte	Concentration (ng/L)	Chemical Class
Bisphenol A	15.0	Bisphenol
Gemfibrozil	14.0	Cholesterol medication
Ibuprofen	1.6	NSAID
Naproxen	5.6	NSAID
Atenolol	5.4	Beta blocker
Caffeine	3.8	PPCP
Carbamazepine	2.4	Anticonvulsant
Cotinine	11.0	Alkaloid (main metabolite of nicotine)
DEET	7.0	Pesticide
Meprobamate	3.7	Anxiolytic
Primidone	2.4	Anticonvulsant
Sulfamethoxazole	8.1	Antibiotic
TCEP tri(2-chloroethyl)-phosphate	1.5	Chlorinated organophosphate flame retardant
TCPP	51.0	Chlorinated organophosphate flame retardant
TDCPP	18	Chlorinated organophosphate flame retardant
Trimethoprim	7.1	Antibiotic

Event 4: Fall Event

Considering some of the quality assurance issues that occurred during this project period (e.g., sporadic Rainbow Trout control mortality, missing swimming behavior and chemical analyses), we included an additional fourth event before the cessation of the study. This event was timed to take place in the fall of 2019, with the goal of catching a First Flush event; however, it was dry across both exposure periods.

First Exposure Period, initiated October 16, 2019

The first exposure period was initiated on October 16, 2019. Both Rainbow Trout and *Hyalella azteca* exhibited high survival and there were no significant differences observed in survival, weight (Table 19), distance travelled or velocity (Table 20). However, Control *H. azteca* did not increase in weight during the exposure period, as test organisms weighed as much as they did at the beginning of the test. Water quality

measurements from sub-samples collected from the Control and Ambient Source Tanks measured by UCD AHPL are outlined in Tables A3-17 to A3-19 in Appendix III.

TABLE 19. SUMMARY OF SURVIVAL AND WEIGHT RESULTS FROM EVENT 4: FALL EVENT, FIRST EXPOSURE PERIOD, INITIATED ON OCTOBER 16, 2019.

Species	Mean Survival (%)	SD Survival (%)	SE Survival (%)	Mean Weight (mg)	SD Weight (mg)	SE Weight (mg)
Hyalella azteca: Control	95.0	5.8	2.9	0.656	0.046	0.023
Hyalella azteca: Hood	95.0	5.8	2.9	0.553	0.075	0.038
Rainbow Trout: Control	100.0	0.0	0.0	91.59	14.39	7.197
Rainbow Trout: Hood	100.0	0.0	0.0	96.84	8.37	4.183

TABLE 20. SUMMARY OF SWIMMING BEHAVIOR RESULTS FROM EVENT 4: FALL EVENT, FIRST EXPOSURE PERIOD, INITIATED ON OCTOBER 16, 2019.

Species	Mean Distance Traveled (cm)	SD Distance Traveled (cm)	SE Distance Traveled (cm)	Mean Velocity (cm/s)	SD Velocity (cm/s)	SE Velocity (cm/s)
Hyalella azteca: Control	56.61	16.02	8.01	0.438	0.114	0.057
Hyalella azteca: Hood	46.25	13.76	6.88	0.402	0.103	0.052
Rainbow Trout: Control	1905.37	130.98	75.62	6.529	0.433	0.250
Rainbow Trout: Hood	1647.74	259.99	130.00	5.755	0.909	0.454

Chemical Analyses

C18 filters were deployed in the passive sampler for the duration of the study. These filters were sent to CSULB for extraction and analysis by Weck laboratories. Results of the C18 filter analyses are outlined in Table 21 below. Whole water samples were collected and sent to Weck for analysis. Detected compounds from the whole water sample are outlined below in Table 22.

TABLE 21. SUMMARY OF ANALYTICAL CHEMISTRY DETECTIONS FROM C18 FILTERS DEPLOYED IN THE CHEMCATCHER PASSIVE SAMPLER DURING THE FIRST EXPOSURE PERIOD OF THE FALL EVENT.

Chemical	Concentration (ng/L of methanol)
Testosterone	670
BPA	3,400
Gemfibrozil	1,200
Salicylic acid	1,600
Amoxicillin	2,800
Caffeine	590
Carbamazepine	84

Chemical	Concentration (ng/L of methanol)
DEET	100
Phenytoin (Dilantin)	390
TCPP	3,100
TDCPP	9,400

TABLE 22. SUMMARY OF ANALYTICAL CHEMISTRY RESULTS FROM THE WHOLE WATER SAMPLE COLLECTED ON DAY 0 OF EVENT 4: FALL EVENT, FIRST EXPOSURE PERIOD, ON OCTOBER 16, 2019.

Analyte	Concentration (ng/L)	Chemical Class
Bisphenol A	10.0	Bisphenol
Gemfibrozil	20.0	Cholesterol medication
Ibuprofen	2.0	NSAID
Naproxen	1.7	NSAID
Diclofenac	1.6	NSAID
Caffeine	2.2	PPCP
DEET	2.8	Pesticide
Primidone	1.7	Anticonvulsant
Sulfamethoxazole	1.8	Antibiotic
TCPP	24.0	Chlorinated organophosphate flame retardant
TDCPP	4.9	Chlorinated organophosphate flame retardant

Second Exposure Period, initiated November 1, 2019

The second exposure period was initiated on November 1, 2019. All organisms in this test met test acceptability criteria. *H. azteca* exposed to Hood water exhibited a significant reduction in weight compared to the control (P=0.0133; Table 23). There were no significant differences in swimming behavior for either species (Table 24). Water quality measurements from sub-samples collected from the Control and Ambient Source Tanks measured by UCD AHPL are outlined in Tables A3-20 to A3-22 in Appendix III.

TABLE 23. SUMMARY OF SURVIVAL AND WEIGHT RESULTS FROM EVENT 4: FALL EVENT, SECOND EXPOSURE PERIOD, INITIATED ON NOVEMBER 1, 2019.

Species	Mean Survival (%)	SD Survival (%)	SE Survival (%)	Mean Weight (mg)[1]	SD Weight (mg)	SE Weight (mg)
Hyalella azteca: Control	97.5	5.0	2.5	0.308	0.020	0.010
Hyalella azteca: Hood	100.0	0.0	0.0	0.204	0.056	0.028
Rainbow Trout: Control	100.0	0.0	0.0	117.73	5.922	2.961
Rainbow Trout: Hood	100.0	0.0	0.0	128.73	8.59	4.30

1. Highlighted cells indicate a significant reduction in weight compared to the control (P=0.0133).

TABLE 24. SUMMARY OF SWIMMING BEHAVIOR RESULTS FROM EVENT 4: FALL EVENT, SECOND EXPOSURE PERIOD, INITIATED ON NOVEMBER 1, 2019.

Species	Mean Distance Traveled (cm)	SD Distance Traveled (cm)	SE Distance Traveled (cm)	Mean Velocity (cm/s)	SD Velocity (cm/s)	SE Velocity (cm/s)
Hyalella azteca: Control	63.04	22.06	11.03	0.832	0.600	0.300
Hyalella azteca: Hood	59.48	30.71	15.36	0.822	0.282	0.141
Rainbow Trout: Control	1,844.39	198.01	114.32	6.174	0.677	0.391
Rainbow Trout: Hood	2,192.82	243.01	121.51	7.474	0.992	0.496

Chemical Analyses

C18 filters were deployed in the passive sampler for the duration of the study. These filters were sent to CSULB for extraction and analysis by Weck laboratories. Results of the C18 filter analyses are outlined in Table 25 below. Whole water samples were collected and sent to Weck for analysis. Detected compounds from the whole water sample are outlined below in Table 26.

TABLE 25. SUMMARY OF ANALYTICAL CHEMISTRY DETECTIONS FROM C18 FILTERS DEPLOYED IN THE CHEMCATCHER PASSIVE SAMPLER DURING THE SECOND EXPOSURE PERIOD OF THE FALL EVENT.

Chemical	Concentration (ng/L of methanol)
Testosterone	870
BPA	1,300
Gemfibrozil	1,600
Salicylic acid	1,600
Amoxicillin	3,300
Carbamazepine	230
DEET	1,100
TCPP	5,400
TDCPP	12,000

TABLE 26. SUMMARY OF ANALYTICAL CHEMISTRY RESULTS FROM THE WHOLE WATER SAMPLE COLLECTED ON DAY 0 OF EVENT 4: FALL EVENT, SECOND EXPOSURE PERIOD, ON NOVEMBER 1, 2019.

Analyte	Concentration (ng/L)	Chemical Class
Atenolol	12.0	Beta blocker
Caffeine	5.4	PPCP
Carbamazepine	5.3	Anticonvulsant
Cotinine	6.0	Alkaloid (main metabolite of nicotine)
DEET	7.1	Pesticide
Meprobamate	1.1	Anxiolytic

Analyte	Concentration (ng/L)	Chemical Class
Primidone	3.3	Anticonvulsant
Sulfamethoxazole	9.2	Antibiotic
TCEP tri(2-chloroethyl)-phosphate	1.2	Chlorinated organophosphate flame retardant
TCPP	48.0	Chlorinated organophosphate flame retardant
TDCPP	25.0	Chlorinated organophosphate flame retardant
Trimethoprim	8.4	Antibiotic
Atorvastatin	2.1	Statin (cholesterol medicine)
Azithromycin	18.0	Antibiotic
Ciprofloxacin	14.0	Antibiotic
Fluoxetine	1.0	SSRI
Phenytoin (Dilantin)	1.9	Anticonvulsant

Discussion

Trends across events

Hyalella azteca

Hyalella azteca exposed to Hood river water exhibited reductions in organism fitness intermittently across the duration of the project, with no apparent seasonal or temporal trend (Table 27). In general, *H. azteca* survival was consistently robust (Figure 12) across the study period, with some exceptions. Reductions in *H. azteca* endpoints were generally sub-lethal, with the exception of the First Flush Event. Amphipods in the First Exposure Period succumbed to a pathogen, which prompted us to include an additional head tank in the system, as well as to include additional daily replicate chamber cleaning.

TABLE 27. OVERALL SUMMARY OF *HYALELLA AZTECA* PERFORMANCE ACROSS THE PROJECT PERIOD. [1]

Event	Survival (%)	Weight (mg/surv. Ind)	Distance Traveled (cm)	Velocity (cm/s)
First Flush I	35	0.217	42.26	0.191
First Flush II	58	0.421	36.81	0.153
Snowmelt I	93	0.948	No video data	
Snowmelt II	85	0.750	192.43	0.656
Summer Irrigation I	90	0.424	92.75	0.309
Summer Irrigation II	98	0.427	88.71	0.286
Fall Event I	95	0.553	46.25	0.402
Fall Event II	100	0.204	59.48	0.822

1. Highlighted cells indicate a significant reduction in survival, weight, distance traveled, or velocity, compared to the control. Organism responses are those exposed to Hood ambient water.

H. azteca exhibited a significant reduction in survival in the Second Exposure Period during the First Flush Event, where we detected a variety of contaminants from the passive sampler filters. These contaminants ranged from pesticides, to flame retardants, and beta-blocker medicines (see Table 6 above). Those

contaminants with the highest concentrations were the organophosphorus flame retardant TCPP (12,333 ng/L MeOH), BPA (2,467 ng/L MeOH) and caffeine (1,433 ng/L MeOH). There is a distinct lack of literature examining the toxicity of these analytes to aquatic invertebrates, and those that do exist observed negative organism responses to concentrations higher than what was seen in the present study. Mihaich et al. (2019) evaluated BPA with several aquatic species, including *H. azteca*. *H. azteca* were exposed to concentrations of BPA ranging from 0.12 to 2.2 mg/L in a 42-d life-stage test. There were no negative effects on *H. azteca* growth at 1.1 mg/L BPA and below, and they observed a reproduction NOEC of 490 µg/L with a LOEC of 1.1 mg/L. The 42-d LC50 was 780 µg/L (Mihaich et al. 2019). These BPA concentrations are higher than what was detected during the First Flush Second Exposure Period. Kuster et al. (2009) evaluated the toxicity of the beta-blocker atenolol with a 14-d *H. azteca* exposure and found no negative effects up to 8.82 mg/L, which was the highest concentration tested. Atenolol was detected on the passive sampler C18 filters during the First Flush Second Exposure Period with an average of 220 ng/L/MeOH.

We observed significant reductions in the weight endpoint in the Second Exposure Period in the Snowmelt Event, as well as the Second Exposure Period in the Fall Event. It is interesting that a negative impact was observed during the Snowmelt Event in the weight endpoint, as these amphipods were some of the largest across all Events (Figure 13) and exhibited the farthest distance travelled (Table 10; Figure 14). Total concentrations of the chemicals detected by the Chemcatcher® passive sampler were low (Table 11), ranging from 220 ng/L/MeOH (carbamazepine) to 18,000 ng/L/MeOH with TDCPP having the highest total concentration for this event. Testosterone (300 ng/L/MeOH), caffeine (560 ng/L/MeOH), and gemfibrozil (580 ng/L/MeOH) were those concentrations on the lower end of the range, whereas TCPP (5,500 ng/L/MeOH), amoxicillin (3,200 ng/L/MeOH), and BPA (2,800 ng/L/MeOH) were those chemicals that had higher concentrations during this event period.

For the Second Exposure Period in the Fall Event, analytical chemistry results of the whole water sample collected on Day 0 of the exposure had 17 pharmaceutical and personal care products in concentrations in the ng/L range (Table 26). The highest detected concentrations in this event were the chlorinated organophosphate flame-retardants TCPP (48 ng/L) and TDCPP (25 ng/L), followed by the antibiotics azithromycin (18 ng/L) and ciprofloxacin (14 ng/L), and the beta-blocker atenolol (12 ng/L). Results from the passive sampler deployed during this exposure period included those analytes detected in the grab sample and the additional chemicals testosterone (870 ng/L/MeOH), BPA (1,300 ng/L/MeOH), gemfibrozil (1,600 ng/L/MeOH), salicylic acid (1,600 ng/L/MeOH), and amoxicillin (3,300 ng/L/MeOH).

There is little information regarding aquatic invertebrate sensitivity to these specific compounds in the literature. However, Dussalt et al. (2018) evaluated the toxicity of atorvastatin and carbamazepine to *H. azteca* in 10-d survival and growth tests and observed negative growth effects in the mg/L range of both compounds. *H. azteca* weight effects ranged from 1.4 mg/L for EC10, to 2.4 mg/L EC50 for atorvastatin, and ranged from 2.4 mg/L (EC10) to 15 mg/L (EC50) for carbamazepine (Dussalt et al. 2018). Atorvastatin was detected in this Second Fall Event Exposure Period at 2.1 ng/L, and carbamazepine was detected at 5.3 ng/L in the whole water sample, and at 230 ng/L/MeOH from the passive sampler; thus, these concentrations are magnitudes lower than what caused the effects observed by Dussalt and co-workers. However, the concentrations detected during this exposure period align with those found in surface waters in Canada for atorvastatin and in Europe and North America for carbamazepine (Dussalt et al. 2008 and citations referenced within).

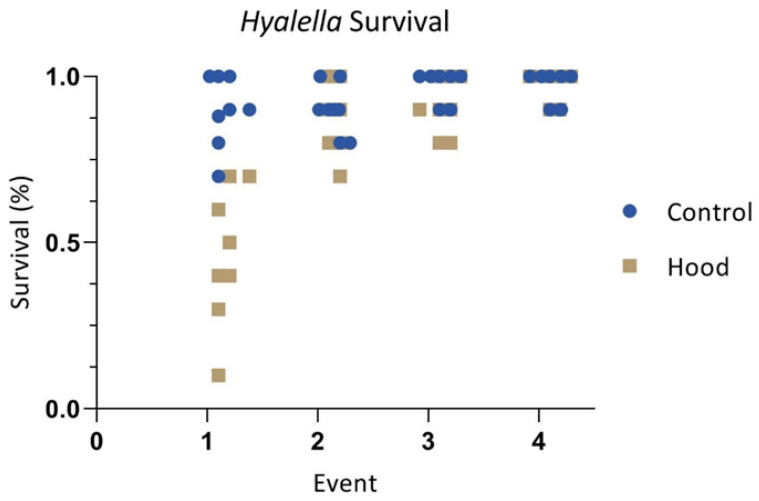


FIGURE 12: SUMMARY OF *HYALELLA AZTECA* SURVIVAL, ACROSS ALL EVENTS DURING THE PROJECT PERIOD. DATA POINTS REPRESENTED ON THIS FIGURE ARE THE INDIVIDUAL REPLICATES PER TREATMENT.

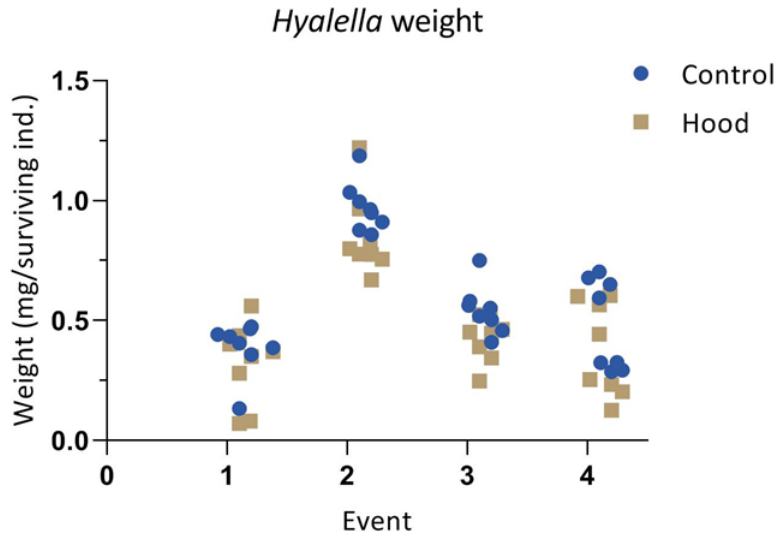


FIGURE 13: SUMMARY OF *HYALELLA AZTECA* WEIGHT, ACROSS ALL EVENTS DURING THE PROJECT PERIOD. DATA POINTS REPRESENTED ON THIS FIGURE ARE THE INDIVIDUAL REPLICATES PER TREATMENT.

With regards to swimming behavior, we observed a significant reduction in swimming behavior in the First Exposure Period in the Summer Irrigation Event, where *H. azteca* swam significantly slower (Figure 15) and covered less distance when compared to the Control. We do not have PPCP chemistry data for the whole water sample, and no other compounds were detected with the other chemical classes. With the passive sampler, similar chemicals were detected during this exposure period as in the others during this project period. Lower masses per disk were detected for carbamazepine (180 ng/L/MeOH), gemfibrozil (420

ng/L/MeOH), caffeine (520 ng/L/MeOH), and testosterone (620 ng/L/MeOH). In comparison, chemicals with higher mass per disk detections during this exposure period included salicylic acid (1,600 ng/L/MeOH), DEET (2,300 ng/L/MeOH), amoxicillin (2,800 ng/L/MeOH), TCPP (9,100 ng/L/MeOH), TDCPP (16,000 ng/L/MeOH), and BPA (26,000 ng/L/MeOH).

Although there were not many instances where we observed significant differences in swimming behavior between *H. azteca* exposed to Hood River water and those exposed to the control, there were significant differences in *H. azteca* behavior across events (Table 28). For instance, amphipods in the First Flush Event swam significantly less distance than those in the Snowmelt Second Exposure Period. *H. azteca* in the Snowmelt Second Exposure Period swam significantly farther than those in the Summer Irrigation First Exposure Period and in both Fall Events. In terms of velocity, *H. azteca* in the First Flush First Exposure Period swam significantly slower than those in the Second Exposure Periods of the Snowmelt and Fall Events. *Hyalella* in the Second Exposure of the Fall Event were the fastest swimmers overall, with significant increases observed in five of the seven exposure periods.

TABLE 28. SUMMARY OF *H. AZTECA* SWIMMING BEHAVIOR ACROSS EVENTS. EVENTS WERE ANALYZED USING A ONE-WAY ANOVA WITH A POST-HOC TUKEY HSD.

Event	Designation	Significant P-value Distance Traveled	Comparison Change Distance Traveled	Significant P-value Velocity	Comparison Change Velocity
First Flush I	A	P=0.0014	Less than C	P=0.0192	Less than C
				P=0.0010	Less than G
First Flush II	B	P=0.0010	Less than C	P=0.0097	Less than C
				P=0.0010	Less than G
Snowmelt II	C	P=0.0394	More than E		
		P=0.0018	More than F		
		P=0.0048	More than G		
Summer Irrigation I	D			P=0.0081	Less than G
Summer Irrigation II	E			P=0.0054	Less than G
Fall Event I	F			P=0.0412	Less than G
Fall Event II	G				

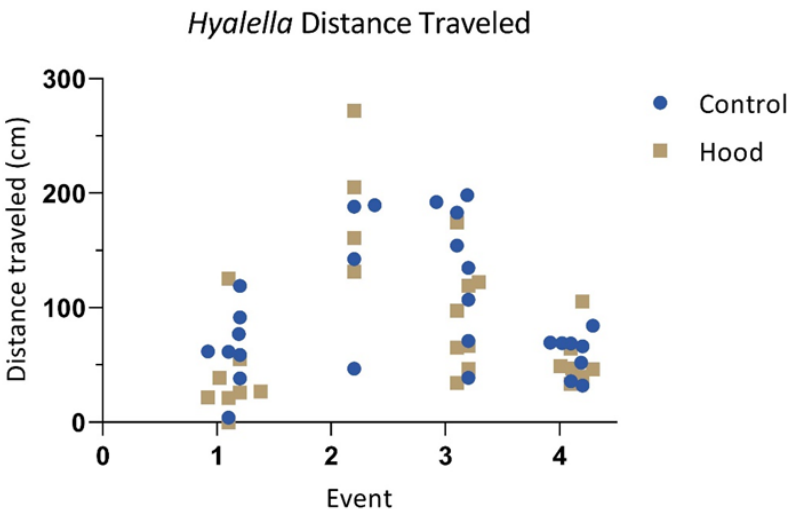


FIGURE 14: SUMMARY OF *HYALELLA AZTECA* DISTANCE TRAVELED, ACROSS ALL EVENTS DURING THE PROJECT PERIOD. DATA POINTS REPRESENTED ON THIS FIGURE ARE THE INDIVIDUAL REPLICATES PER TREATMENT.

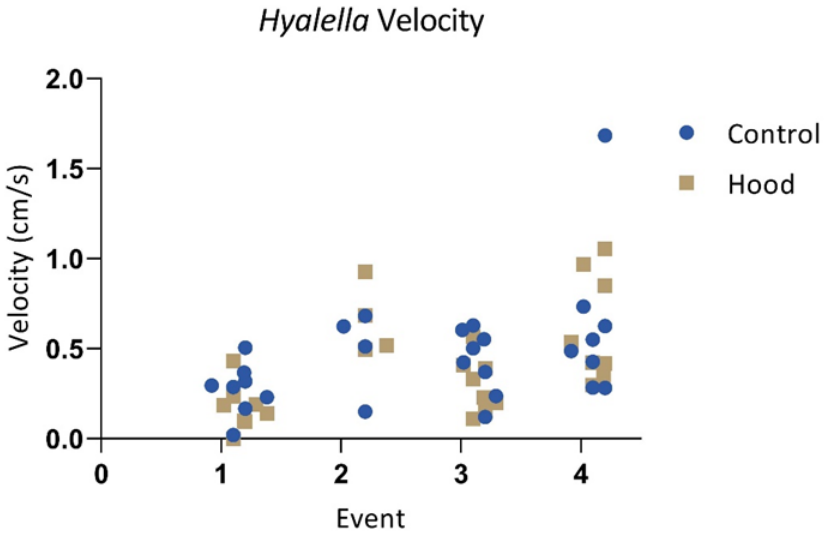


FIGURE 15: SUMMARY OF *HYALELLA AZTECA* VELOCITY, ACROSS ALL EVENTS DURING THE PROJECT PERIOD. DATA POINTS REPRESENTED ON THIS FIGURE ARE THE INDIVIDUAL REPLICATES PER TREATMENT.

Rainbow Trout

With the exceptions noted herein, Rainbow Trout exhibited robust survival over the study period (Figure 16), and the weight endpoint was never affected by exposure to Sacramento River water at the Hood location (Figure 17). There were no significant differences in Rainbow Trout body length observed over the course of the project, although fish length did appear to be relatively variable (Figure 18). An overall summary of Rainbow Trout performance is outlined below in Table 29.

TABLE 29. OVERALL SUMMARY OF RAINBOW TROUT PERFORMANCE ACROSS THE PROJECT PERIOD. [1]

Event	Survival (%)	Weight (mg/surv. Ind)	Distance Traveled (cm)	Velocity (cm/s)
First Flush I	100	120.7	1,185	4.2
First Flush II	100	111.8	2,014	7.6
Snowmelt I	43	81.4	No video data	
Snowmelt II	100	119.0	2,925	8.2
Summer Irrigation I	100	99.8	3,507	7.1
Summer Irrigation II	98	89.5	2,158	6.9
Fall Event I	100	96.8	1,648	5.8
Fall Event II	100	128.7	2,193	7.5

1. Organism responses are those exposed to Hood ambient water.

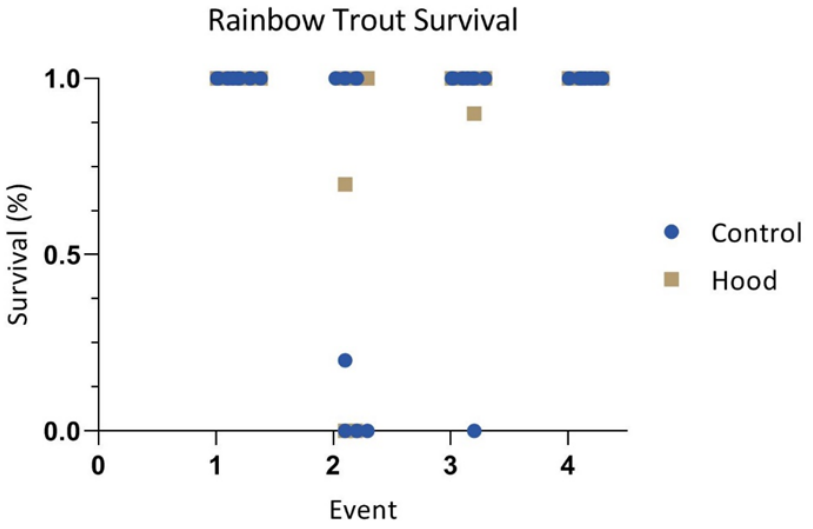


FIGURE 16: SUMMARY OF RAINBOW TROUT SURVIVAL, ACROSS ALL EVENTS DURING THE PROJECT PERIOD. DATA POINTS REPRESENTED ON THIS FIGURE ARE THE INDIVIDUAL REPLICATES PER TREATMENT.

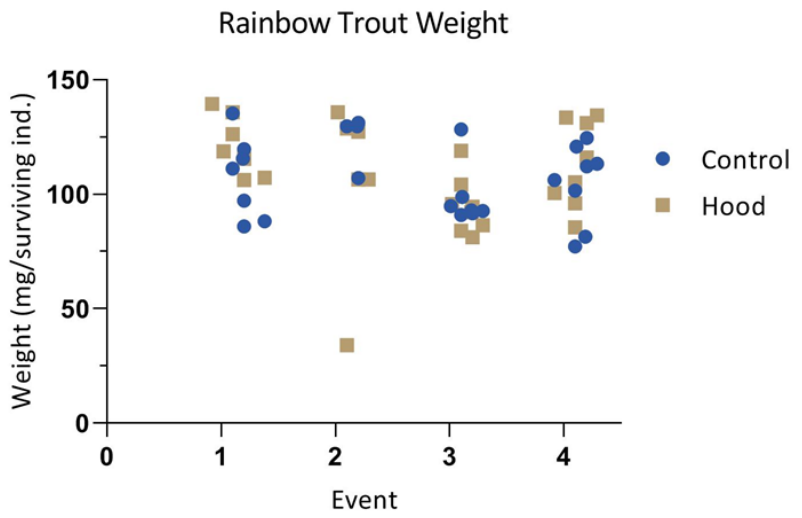


FIGURE 17: SUMMARY OF RAINBOW WEIGHT, ACROSS ALL EVENTS DURING THE PROJECT PERIOD. DATA POINTS REPRESENTED ON THIS FIGURE ARE THE INDIVIDUAL REPLICATES PER TREATMENT.

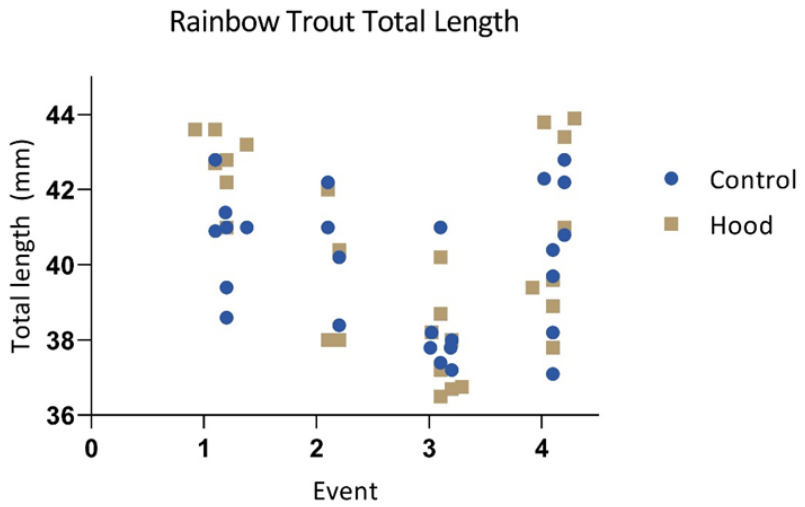


FIGURE 18: SUMMARY OF RAINBOW TROUT TOTAL LENGTH, ACROSS ALL EVENTS DURING THE PROJECT PERIOD. DATA POINTS REPRESENTED ON THIS FIGURE ARE THE INDIVIDUAL REPLICATES PER TREATMENT.

In terms of swimming behavior of the Rainbow Trout, fish were fairly consistent over the project period in terms of distance traveled (Figure 19) or velocity (Figure 20). Although there were no statistically significant differences in swimming behavior between Rainbow Trout exposed to Hood River water and those in the control, there were some significant differences observed in the distance traveled when compared across events (Table 30). For instance, Rainbow Trout in the First Flush Event swam significantly less distance than those in the Snowmelt or Summer Irrigation events, and fish in the First Exposure Period of the Summer Irrigation Event swam farther than those in Summer Irrigation Second Exposure Period, and both exposure periods in the Fall Event.

TABLE 30. SUMMARY OF RAINBOW TROUT SWIMMING BEHAVIOR - DISTANCE TRAVELED ACROSS EVENTS. EVENTS WERE ANALYZED WITH A ONE-WAY ANOVA WITH A POST-HOC TUKEY HSD.

Event	Designation	Significant P-value Distance Traveled	Comparison Change Distance Traveled
First Flush I	A	P=0.0041	Less than C
		P=0.0010	Less than D
First Flush II	B	P=0.0168	Less than D
Snowmelt II	C		
Summer Irrigation I	D	P=0.0367	More than E
		P=0.0021	More than F
		P=0.0442	More than G
Summer Irrigation II	E		
Fall Event I	F		
Fall Event II	G		

For the First Flush, First Exposure Period the reduction in distance swam compared to the other events may have been due to turbidity. There was a significant winter storm during this event, where we observed a considerable influx of sediment in the early stages of the exposure. Turbidity ranged from 10.0 to 7.5 NTU over the course of the exposure period, with the highest measured NTU of 45.77 on November 30 (Day 2 of the exposure), falling to 32.5 NTU on December 3 (Day 5), before gradually decreasing to 7.5 NTU on Day 14, test termination day (Figure A1-3 in Appendix III). Fine sediment associated with turbidity has been demonstrated to cause stress to fish (Newcombe 2003; Michiel et al. 2013, referenced in and demonstrated by Berli et al. 2014), resulting in reduced swimming behavior. Contaminants associated with a reduction in acetylcholinesterase (AChE) activity have also been demonstrated to reduce swimming behavior, such as with the organophosphate pesticides diazinon and malathion (Beauvais et al. 2000). It is possible that Rainbow Trout exposed to Hood water during the First Flush, First Exposure Period were exposed to a hydrophobic contaminant associated with the high turbidity, which caused an increase in erratic swimming behavior. However, we cannot confirm this as there is no analytical chemistry data for this event, nor were any biomarkers analyzed.

In comparison, Rainbow Trout in the First Exposure Period of the Summer Irrigation Event swam significantly farther than those in the Second Exposure Period, and both Fall Exposures. This is interesting, as *H. azteca* exhibited significant reductions in velocity and distance traveled during this exposure period. Whole water sample analytical results indicated very low concentrations of all detected analytes, at 15 ng/L or below, with the exception of TCPP, which was detected at 51 ng/L (Table 18 above). With the passive sampler, lower concentrations were detected for carbamazepine (180 ng/L/MeOH), gemfibrozil (420 ng/L/MeOH), caffeine (520 ng/L/MeOH), and testosterone (620 ng/L/MeOH). In comparison, chemicals with higher concentrations during this exposure period included salicylic acid (1,600 ng/L/MeOH), DEET (2,300 ng/L/MeOH), amoxicillin (2,800 ng/L/MeOH), TCPP (9,100 ng/L/MeOH), TDCPP (16,000 ng/L/MeOH), and BPA (26,000 ng/L/MeOH). The observed concentrations are those low enough to not cause acute effects, as corroborated by Jarmea et al. (2015), who didn't see any negative effects of TDCPP on zebrafish

at concentrations 1 mg/L and above, and by Nassef et al. (2009), where they determined the *Oryzias latipes* (medaka) NOEC for carbamazepine to be 61.5 µg/L.

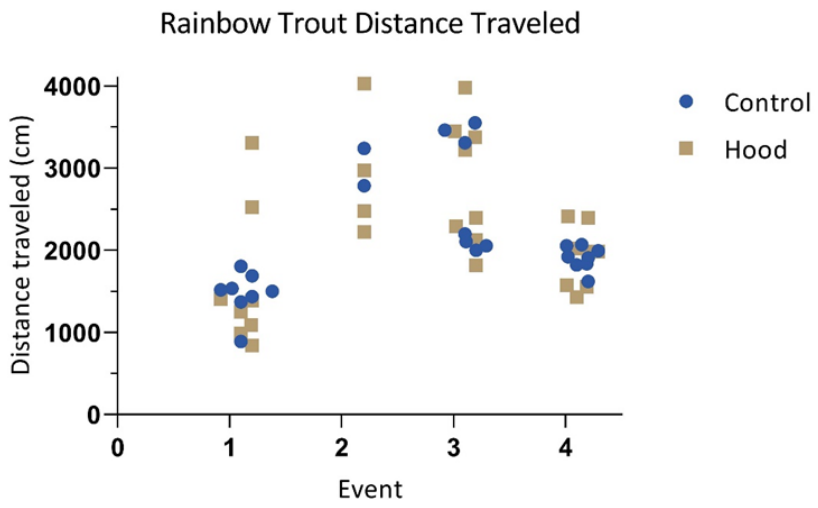


FIGURE 19: SUMMARY OF RAINBOW TROUT DISTANCE TRAVELED, ACROSS ALL EVENTS DURING THE PROJECT PERIOD. DATA POINTS REPRESENTED ON THIS FIGURE ARE THE INDIVIDUAL REPLICATES PER TREATMENT.

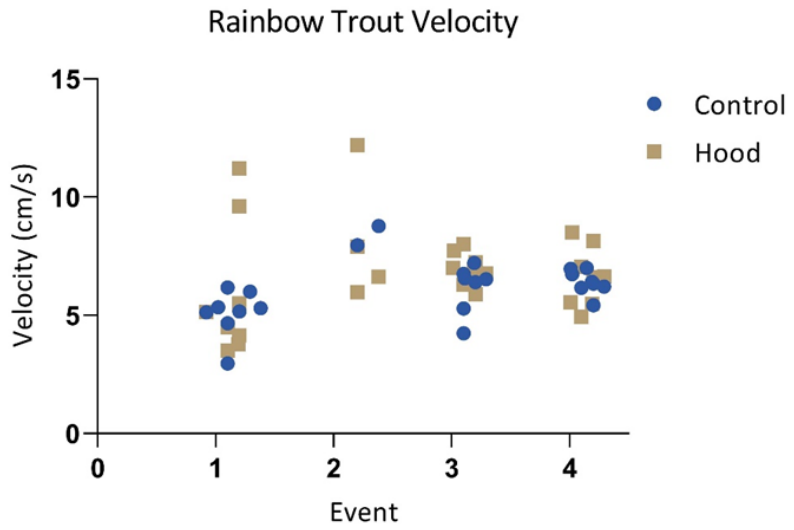


FIGURE 20: SUMMARY OF RAINBOW TROUT VELOCITY, ACROSS ALL EVENTS DURING THE PROJECT PERIOD. DATA POINTS REPRESENTED ON THIS FIGURE ARE THE INDIVIDUAL REPLICATES PER TREATMENT.

Chemcatcher® passive sampler vs grab samples

We included two types of sample collection methods for the chemical analysis portion of this study, in order to compare the efficacy of the two sampling types and to compare the concentrations of analytes detected. Typical sample collection methods involve a one-time sub-surface grab sample, which represents a snapshot in time of the water quality at the time of collection. This method is typically used for in-lab toxicity testing, as the same grab sample is used for the entire toxicity test, and thus the results of the chemical analyses can be directly compared to organism responses in the toxicity test. However, for field studies, a one-time grab sample doesn't capture the changing concentrations of chemical contaminants that may be present in the water body in real-time. A passive sampler such as the Chemcatcher® is deployed for the entire duration of a field study, can capture contaminant fluctuations across the time deployed, and can allow for better comparisons to organism responses out in the field. In addition, the extended deployment time of the passive sampler can allow for the detection of contaminants present in the waterbody at low concentrations, such that wouldn't be detected in a one-time grab sample.

One of the benefits of using a passive sampler apparatus such as the Chemcatcher® is the ability to provide time weighted average concentrations of contaminants in a waterbody over time. The contaminant masses collected on the disk must be converted to water concentrations, which requires knowledge of the time-integrated uptake of the analyte in question, water flow velocity, duration of exposure time, and the hydrophobicity of the analyte(s) being measured (Townsend et al. 2018). Uptake values (e.g., R_s) can be determined through calibration of the passive sampler. Because passive samplers use kinetics of accumulation for contaminants to sorb to the filter medium (e.g., C18 resin), these mechanisms are determined through experiments that evaluate sampler-water partition coefficients and kinetic constants for analyte uptake and elimination (Charriau et al. 2016).

There are a number of calibration mechanisms that can be used, but it is important to note that the conditions under which the passive sampler is calibrated may differ greatly from field conditions, i.e., a lab calibration may provide different uptake values in the absence of environmental conditions such as fluctuating temperature, pH, flow, etc. (Charriau et al. 2016). Charriau and coworkers evaluated a number of these calibration mechanisms in their review of the Chemcatcher® passive sampler use (2016), and determined that the passive sampler calibration should match the environmental conditions as closely as possible in order to provide the most accurate uptake values. Moreover, the authors note that there is a lack of a standardized calibration protocol that exists for the Chemcatcher®. Several researchers have determined the R_s for a number of different analytes; yet because of the difference in calibration protocols, these sampling rates cannot necessarily be compared to each other or used in other monitoring programs due to the difference in applications (Charriau et al. 2016).

As we did not have the capability to calibrate the Chemcatcher® for the variety of chemical classes that were investigated through analytical chemistry, we are unable to provide time-weighted average concentrations of the analytes that were detected with the passive sampler. As such, we can only provide the overall concentration per disk, in ng/L of methanol (ng/L/MeOH), of the variety of chemicals that were detected over the project period. This makes it difficult to compare the filter disk results to the one-time grab samples that were collected. Moreover, there is a lack of consistency between the collection of grab samples and use of the passive sampler during the project period that also precludes an accurate comparison of the two methods.

With that in mind, there were some noted trends observed between the two sample collection methods. For instance, pharmaceuticals and personal care products (PPCPs) were consistently detected across the project period for both collection types, and in particular some analytes in other chemical classes were detected in both grab and passive sampler filters, such as DEET, carbamazepine and BPA. It was interesting to see that some contaminants were detected in the grab sample, such as Ibuprofen, diclofenac, and sulfamethoxazole, but not in the passive sampler. It is possible that the log Kow of these chemicals may not be ideal for binding to the C18 filter disk that was used in the passive sampler which may be attributed for the differences in detections. It was also interesting that no pyrethroids were detected with either sample collection method, even with the passive sampler's ability to capture low concentrations of chemical pollutants.

Although the concentrations detected between the two sample collection methods varied and cannot be directly compared, the types of contaminants that were detected were consistent between the two methods. Due to budgetary restraints we were only able to collect one grab sample at Day-0. An ideal side-by-side comparison would include a calibrated passive sampler and daily one-time grab samples for the duration of the exposure period, in order to accurately compare the difference in concentrations detected, and to determine which is the better sample collection method in terms of concentrations and frequency of detected analytes with time-weighted averages. Future experiments that use passive samplers with a goal of obtaining time-weighted average concentrations should carefully consider their environmental application and the time needed prior to the start of the study in order to properly calibrate the sampler. It is unclear at this time whether there will be a point where sampling and uptake rates can be standardized and used across various experimental applications. This would increase the Chemcatcher's usability for researchers who do not have the analytical capability to conduct their own calibrations and sampling rates and would increase the applicability of this sample collection method in the future for state and federal agencies interested in applying this method to their regulatory decisions.

Conclusion

Exposure to the Sacramento River water at Hood, California, did not elicit any acutely negative effects in the Rainbow Trout over the course of the study. In comparison, *Hyalella azteca* did exhibit both acute and sub-lethal negative effects at various time points across the study period, namely during the second First Flush Event, the second Snowmelt Event, the first Summer Irrigation Event, as well as the second Fall Event. Contaminants were present in all events. There were several compounds that were consistently detected across the study period, most notably BPA and gemfibrozil, which were detected 10 times across all events and water types (grab vs. passive sampler). Testosterone, salicylic acid, amoxicillin, caffeine, carbamazepine, DEET, TCPP and TDCPP were all detected six times across the study period. Most chemical detections were in the ng/L range, with the exception of TDCPP, which was detected in the low µg/L in the Second Snowmelt and First Summer Irrigation events. The Chemcatcher® passive sampler was successful in detecting a variety of contaminants across the study period and was comparable to those analytes detected in the one-time grab samples, even with the differences in concentration and number of contaminants detected.

It is possible that the *H. azteca* were exposed to additional hydrophobic contaminants bound to the sediment fraction in the water column, as the amphipods spent more time in the settled sediment in the replicate test chambers when compared to the Rainbow Trout. This, coupled with the amphipod's general

sensitivity to contaminants may account for the higher number of instances where negative effects were observed. It is also possible that the Rainbow Trout used in this study were too old to be sensitive enough to elicit lethal and sub-lethal effects when exposed to Sacramento River water, based solely on swimming behavior and weight determinations that were observed in the current project. The original goal of this study was to include biomarker analyses on surviving Rainbow Trout; thus we selected fish that were old enough where we could observe changes in endocrine function. This age group may have exhibited less sensitivity to the compounds present during these selected time points at the detected concentrations. Because we were unable to procure funding for this element, we are unable to determine if molecular, enzymatic, or other sub-lethal responses were taking place in the fish during the ex-situ exposures. This highlights the importance of the inclusion of biomarkers when conducting field studies, as biomarker analyses may help explain the mode of action and can provide a weight of evidence approach when evaluating sub-lethal toxicity.

Literature Cited

Beauvais, S.L., S.B. Jones, S.K. Brewer, E.E. Little. 2000. Physiological measures of neurotoxicity of diazinon and malathion to larval rainbow trout (*Oncorhynchus mykiss*) and their correlation with behavior measures. *Environ. Toxicol. Chem*. 19(7): 1875-1880.

Berli, B.I., M.J.H. Gilbert, A.L. Ralph, K.B. Tierney, P. Burkhardt-Holm. 2014. Acute exposure to a common suspended sediment affects the swimming performance and physiology of juvenile salmonids. *Compar. Biochem. Phys.* Part A. 176: 1-10. Link to publication.

Charriau, A., Lissalde, S., Poulier, G., Mazzella, N., Buzier, R., Guibaud, G. 2016. Overview of the Chemcatcher® for the passive sampling of various pollutants in aquatic environments Part A: Principles, calibration, preparation and analysis of the sampler. Talanta. 148: 556-571.

Folsvik, N., Brevik, E.M., Berge, J.A. 2000. Monitoring of organotin compounds in seawater using semipermeable membrane devices (SPMDs) – tentative results. *J. Environ. Mont.* 2: 281-284.

Jassby, A. D., J. E. Cloern, and B. E. Cole. 2002. Annual primary production: Patterns and mechanisms of change in a nutrient-rich tidal ecosystem. *Limnology and Oceanography* 47: 698-712.

Jarema, K.A., Hunter, D.L., Shaffer, R.M., Behl, M., Padilla, S. 2015. Acute and developmental behavior effects of flame retardants and related chemicals in zebrafish. *Neurotoxicology and Teratology*. 52: 194-209.

Kuster, A., A.C. Alder, B.I. Escher, K. Duis, K. Fenner, J. Garric, T.H. Hutchinson, D.R. Lapen, A. Pery, J. Rombke, J. Snape, T. Ternes, E. Topp, A. Wehrhan, T. Knacker. 2009. Environmental Risk Assessment of Human Pharmaceuticals in the European Union: A Case Study with the β-Blocker Atenolol. *Int. Environ. Assess. Mange.* 6(1): 514-523. DOI: 10.1897/IEAM_2009-050.1.

Michel, C., Schmidt-Posthaus, H., Burkhardt-Holm, P., 2013. Suspended sediment pulse effects in rainbow trout *Oncorhynchus mykiss*— relating apical and systemic responses. *Can. J. Fish. Aquat. Sci*. 70, 630–641.

Mihaich, E.M., U. Friederich, N. Caspers, A. Tilghman Hall, G.M. Klecka, S.S. Dimond, C.A. Staples, L.S. Ortego, S.G. Hentges. Acute and chronic toxicity testing of bisphenol A with aquatic invertebrates and plants. *Ecotoxicol. Environ. Safety*. 72: 1392-1399. doi:10.1016/j.ecoenv.2009.02.005.

Nassef, M., Matsumoto, S., Seki, M., Kang, I.J., Moroishi, J., Shimasaki, Y., Oshima, Y. 2009. Pharmaceuticals and Personal Care Products Toxicity to Japanese Medaka Fish (*Oryzias latipes*). *Journal of the Faculty of Agriculture*, Kyushu University. 54(2): 407-411. Link to publication.

NAWQA 2010. "Study Unit Description". Sacramento River Basin NAWQA Program. U.S. Geological Survey. Retrieved 2010-08-28.

Newcombe, C.P., 2003. Impact assessment model for clear water fishes exposed to excessively cloudy water. *J. Am. Water Resourc. Assoc.* 39, 529–544.

Rogers-Bennett, L., Bennett, W.A., Fastenau, H.C., and C.M. Dewees 1995. Spatial variation in red sea urchin reproduction and morphology: implications for harvest refugia. *Ecological Applications* 5:1171-1180.

Sommer, T., Armor, C., Baxter, R., Breuer, R., Brown, L., Chotkowski, Kimmerer, W. (2007). The Collapse of Pelagic Fishes in the Upper San Francisco Estuary: El Colapso de los Peces Pelagicos en La Cabecera del Estuario San Francisco. *Fisheries*, 32(6), 270-277. doi:10.1577/1548-8446(2007)32[270:tcopfi]2.0.co;2.

SRWP 2017. Sacramento River Watershed Program (2017). Sacramento River Basin, 5–18. Sacramento, CA

SWAP 2015. California State Wildlife Action Plan (2015). Central Valley and Sierra Nevada Province. California Department of Fish and Wildlife, Vol 1, Chp, 5.4, pg 1-93. w.nrm.dfg.ca.gov/FileHandler.ashx?DocumentID=109211&inline.

Townsend, I., Jones, L., Broom, M., Gravell, A., Schumacher, M., Fones, G.R., Greenwood, R., Mills, G.A. 2018. Calibration and application of the Chemcatcher® passive sampler for monitoring acidic herbicides in the River Exe, UK catchment. *Environ. Sci. Poll. Res*. 25: 25130-25142. https://doi.org/10.1007/s11356-018-2556-3.

USEPA. 2002. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Fifth Edition. Office of Water, Washington, DC. EPA/821/R-02/012.