

WATER QUALITY, FISH COMMUNITY, AND EDNA MONITORING DURING 2021 DROUGHT LOWER AMERICAN RIVER, CALIFORNIA

Technical Memorandum



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Sacramento Water Forum

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INTRODUCTION

Severe Northern California drought conditions, compounded by water resource over-allocation (Sellheim et al. 2020), severely limited the quantity and quality of water available to the Lower American River (LAR) during summer 2021, resulting in curtailed discharge and a diminished cold-water pool. Steelhead/Rainbow Trout (*Oncorhynchus mykiss*) is a primary LAR management focus (listed as Threatened under US ESA); therefore, Reclamation and NMFS identified a maximum daily average water temperature threshold of 21° C for the river section between Nimbus Dam and the Hazel Avenue Bridge to provide a potential refuge for over-summering *O. mykiss* (rearing) within this reach. Secondarily, fall-run Chinook Salmon (*O. tshawytscha*), though not listed, are also a LAR management priority, and adults may enter and hold in the river weeks to months before fall and early winter spawning occurs.

Maintaining this fisheries temperature goal requires precise modeling and management of Folsom Reservoir operation associated with local climatic conditions. Therefore, monitoring management action success, including target species presence, under such dire environmental conditions is important for adaptive management (Murchie et al. 2008; Lennox et al. 2019). This includes determining whether specific habitat features (e.g., thermal stratification, hyporheic flow etc.) may support temperature refugia (Torgersen et al. 2012) for focal salmonid species. Understanding water quality dynamics, including both temperature and dissolved oxygen (DO), are crucial in order to improve management success under future climatic scenarios, including extreme drought.

In addition to water quality monitoring, documenting the presence, density, behavior, and survival of fish species of management concern under drought conditions provides the most direct means of determining focal species response to management actions. However, collecting the necessary data has become increasingly challenging due to an increased emphasis on reducing physical handling of rare endemic fishes, especially during stressful water quality conditions (Castañeda et al. 2020).

To address these data needs, we implemented a water quality survey, including water temperature and DO, within habitat features that were hypothesized to support temperature refugia under stressful conditions. We conducted a short-duration (2-day) fish community video survey within potential salmonid holding areas (Nimbus Basin to Watt Avenue; **Figure 1**). We also collected environmental DNA (eDNA) samples at 1-km intervals from Nimbus Basin to the Watt Avenue Bridge to visualize potential *O. mykiss* and *O. tshawytscha* eDNA hot spots.

Key objectives of this research are to:

- 1) Document presence of *O. mykiss* in the LAR
- 2) Document Chinook Salmon immigration and holding in late summer and fall
- 3) Determine whether temperature refugia exist in key LAR areas (from Nimbus Basin to Watt Avenue); and
- 4) Support data-driven adaptive management under future scenarios.

These data are intended to complement ongoing monitoring and modeling efforts on the LAR to support both short-term flow decisions and long-term adaptive management.

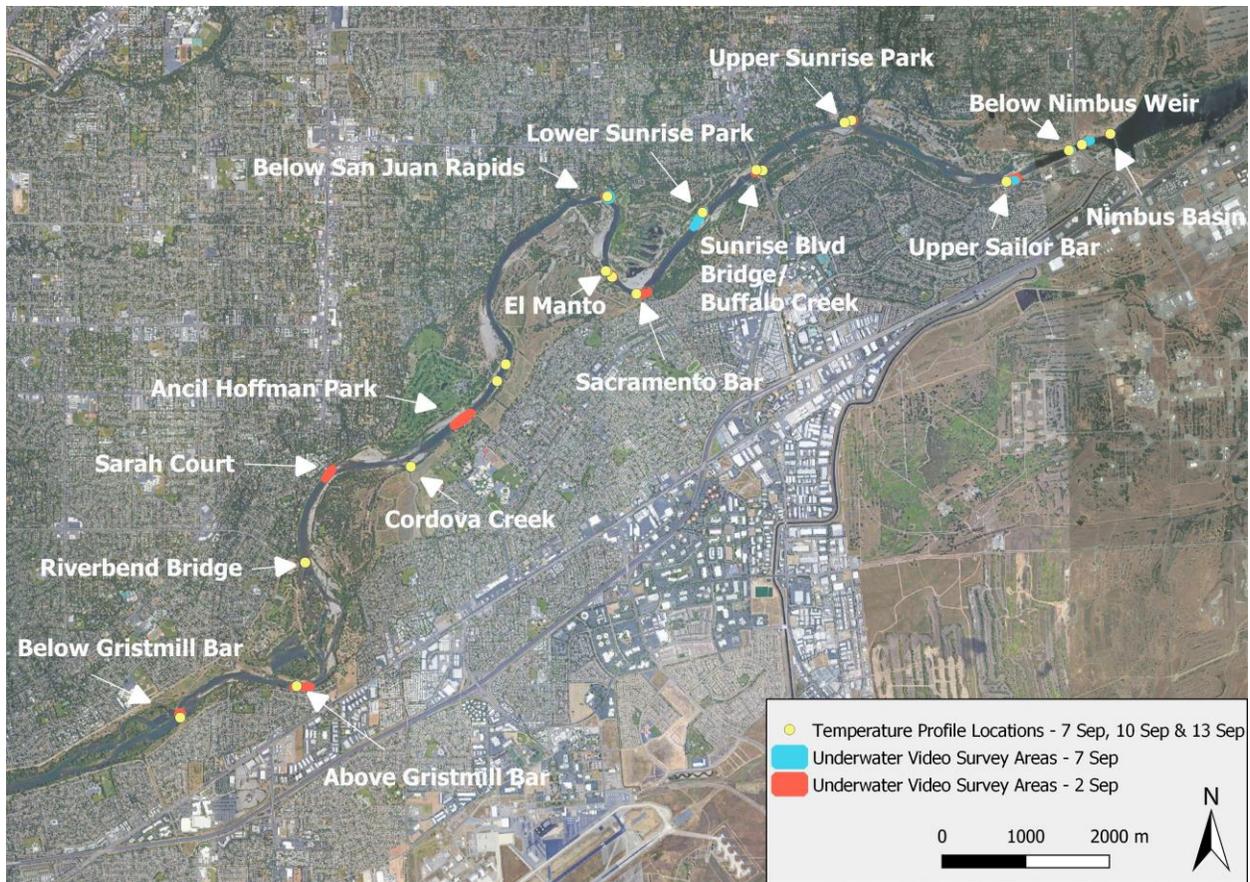


Figure 1. Lower American River temperature and video monitoring locations.

METHODS

Vertical Water Quality Profile Surveys

Study Locations

Eighteen main channel sites and two creek entrance sites were selected for water quality profile surveys on the LAR between Watt Avenue and Nimbus Dam, using the same site selection approach described under video surveys (see below). Water quality profiles were conducted from an inflatable raft within deeper pools (>2 m deep) that might support stratification under low flow conditions (Matthews and Berg 1997; Torgersen et al. 1999; Butler and Hunt 2013). We also surveyed pools downstream from specific features such as large gravel bars that might facilitate reach scale temperature reduction due to sub-surface intergravel flow (Johnson 2004), pools with pronounced bedrock features (Torgersen et al. 1999), and mixing zones of tributaries and mainstem (Brewitt and Danner 2014). All vertical profiles were conducted from an inflatable raft. Locations include San Juan Rapids, Sunrise Trussell, Sailor Bar, and Nimbus Dam Basin. Water quality sites included the 13 video survey sites (described below) and seven additional sites, for a total of 20 sites (**Figure 1**).

On 7 September, vertical water quality profiles were taken at two sites in the Nimbus Basin and one additional site just below the Nimbus Hatchery weir (a relatively deep area where salmonids historically congregated). On 10 September, vertical water quality profiles were taken upstream to downstream at 11 sites between Upper Sailor Bar and Rossmoor Bar including one site at the entrance of Buffalo Creek. On 13 September, vertical water quality profiles were taken upstream to downstream at six sites between Rossmoor Bar and Watt Avenue including one site at the entrance of Cordova Creek. Flows on the LAR ranged from 613 to 644 cubic feet per second (cfs) at the time of the vertical water quality profile surveys (**Figure 2**).

Field Methods

Vertical water quality profiles were conducted upstream to downstream using a raft in LAR areas encompassing habitat features hypothesized to influence stream water temperatures (see description above; **Figure 1**). Total water column depth (m) was measured using a Fishing Buddy II portable depth and fish finder, temperature (°C) and DO (mg/L) were measured using a weighted handheld Xylem brand YSI (YSI Inc.) unit with a 30 m cable attachment. Locations were marked with a Garmin GPS unit and classified by habitat type. Prior to each water quality measurement, total depth was recorded and the YSI cable was lowered to record water quality at approximate total depths. An additional measurement was recorded roughly 1 m below the water surface at each location. If tributary habitat was identified, measurements were taken where the tributary flowed into the LAR main channel (mixing area; Brewitt and Danner 2014) and a control measurement was taken ~ 2 m upstream from the tributary entrance.

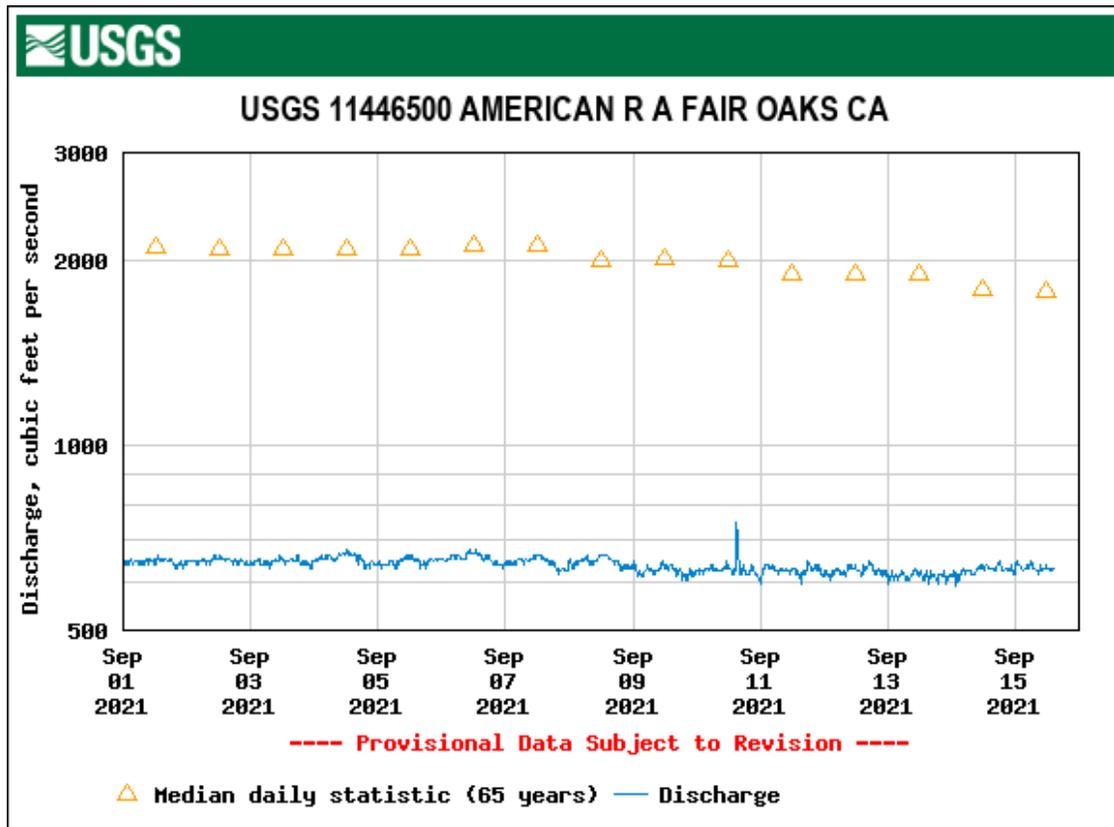


Figure 2. Lower American discharge at the USGS AFO gage at Fair Oaks 1 September – 16 September 2021.

Statistical Analysis

The LAR is generally a gaining (temperature) reach during the hot-dry period because of the insulatory effects from Folsom storage, and because air temperature is a major influence on water temperature released below Nimbus Dam. Therefore, we developed a generalized linear model (GLM) to assess the effect of date, time of day, distance from Nimbus Dam (kilometer; km), depth below water surface (m), and stream features (tributary entrance, clay/Mehrten formation, hyporheic flow through gravel riffles) on both temperature (°C) and DO (mg/L).

In general, water temperature is negatively correlated with DO because warmer water cannot contain as much oxygen as cooler water. However, chemical and biological demands and water exposure to the atmosphere also influence DO levels within the stream water column. To better understand the relative influence of these factors on DO in the LAR, a linear regression was developed to assess correlation between temperature and DO.

All statistical analyses were performed in JMP[®], Version 15.2.1 SAS Institute Inc., Cary, NC, 1989-2021.

Video Surveys

Underwater video and still images have been used as a non-invasive method to augment or validate seining and snorkel surveys of salmonids and other species that are relatively rare in the environment (Anderson et al. 2018; Bergman et al. 2011; Orell et al. 2011). We used an 18-ft jetboat with downrigger and HD video camera to perform transects at locations we assumed to have relatively high juvenile *O. mykiss* and adult Chinook Salmon holding habitat due to previously documented habitat preferences and features known to support relatively cold water during stressful summer and fall temperature periods (Thorpe 2020; Ebersole et al. 2015). Video transects were generally conducted through deeper pools (>2 m deep) that might support temperature stratification (see above) or swimming energy conservation by fish due to relatively low velocities (Torgersen et al. 1999; Butler and Hunt 2013). When possible, we surveyed the same sites as the water quality profiles, as these locations were predicted to have potential thermal refuge habitat. We also surveyed reaches with conditions under which salmonids would be predicted to occur based on other physical habitat features and previous observations.

Using the parameters described above, we selected thirteen sites along the LAR main channel between Watt Avenue and the Nimbus Dam (**Figure 1**). Locations include San Juan Rapids, Sunrise Trussell, Sailor Bar, and Nimbus Dam Basin. On 2 and 7 September, we collected underwater video using a GoPro Hero 5 underwater video camera¹. Flows ranged from 639 to 661 cfs at the time the video surveys were performed (**Figure 2**).

A GoPro HERO series camera was secured in a housing and attached to a downrigger on the sampling vessel (**Figure 3**). To reduce fish behavior effects caused by sampling equipment and

¹ Video was initially collected on 2 September 2021; videos were immediately reviewed for quality images (e.g., orientation, lighting, turbidity). It was determined that five sites required additional sampling. Those sites (Below San Juan Rapids, Lower Sunrise Park, Upper Sailor Bar, Below Nimbus Weir, and Nimbus Basin) were sampled a second time on 7 September 2021. Data from both sample dates are reported.

boat presence, a ten-foot aluminum extension was affixed to the downrigger to allow the camera to operate away from the boat. Coaxial antenna cable (RG 174) was connected between the GoPro and an iPad tablet to maintain Wi-Fi connection while the GoPro was underwater, allowing the crew to see real-time video. We oriented the boat upstream into the current to optimize image stability and clarity and to further reduce fish disturbance while recording video transects. During video collection, we adjusted the downrigger in response to depth changes, observed from the real-time video feed. A Samsung mobile phone recorded transects in tandem with the video using the GPS logger application for GPS coordinates.



Figure 3. A CFS Biologist operating camera system at San Juan Rapids (left). Camera and associated mount that attaches to downrigger (right).

Environmental DNA (eDNA) Transport Evaluation

To determine the optimal sampling interval, we first examined DNA transport using a controlled point source experiment (live car). For the DNA point source, two species that do not occur within the study area, Ballyhoo (*Hemiramphus brasiliensis*) and Northern Anchovy (*Engraulis mordax*), were tethered inside a mesh bag at a fixed location near the American River Hatchery (**Figure 4**). These species were purchased as frozen bait from commercial suppliers. Two biomasses were evaluated: high mass (3172 grams of Ballyhoo) and low mass (612 grams of Anchovy).

The sampling intervals downstream from DNA source were 0, 25, 50, 100, 500, 1000, 1500 and 2000 meters (**Figure 4**). The placement of the 0 site coincided with the location of the American River Hatchery outflow. Three additional samples were collected at the Nimbus Basin (above hatchery outflow) to determine if the outflow was introducing exogenous *O. mykiss* eDNA into the study area. At each interval, eDNA was sampled by using a peristaltic pump to draw 500ml of river water through a Millipore Sterivex™ Filter Unit (0.45um PVDF membrane) (MilliporeSigma). Each interval was sampled with a total of three filters, for a total of 1500ml.

Environmental DNA was purified from filters using QIAamp DNA Mini Kit following a modified Miya et al. (2016) protocol, and then interrogated for the presence of target species DNA using fluorescence-based quantitative real-time Polymerase Chain Reaction (qPCR) which measures DNA with speed, specificity, and sensitivity (Wittwer et al. 1997; Bustin et al. 2009). For this study, qPCR was used to detect the presence of two control species (Ballyhoo and Anchovy) and two native target species (Chinook Salmon and *O. mykiss*) (Appendix B, **Table 1**).

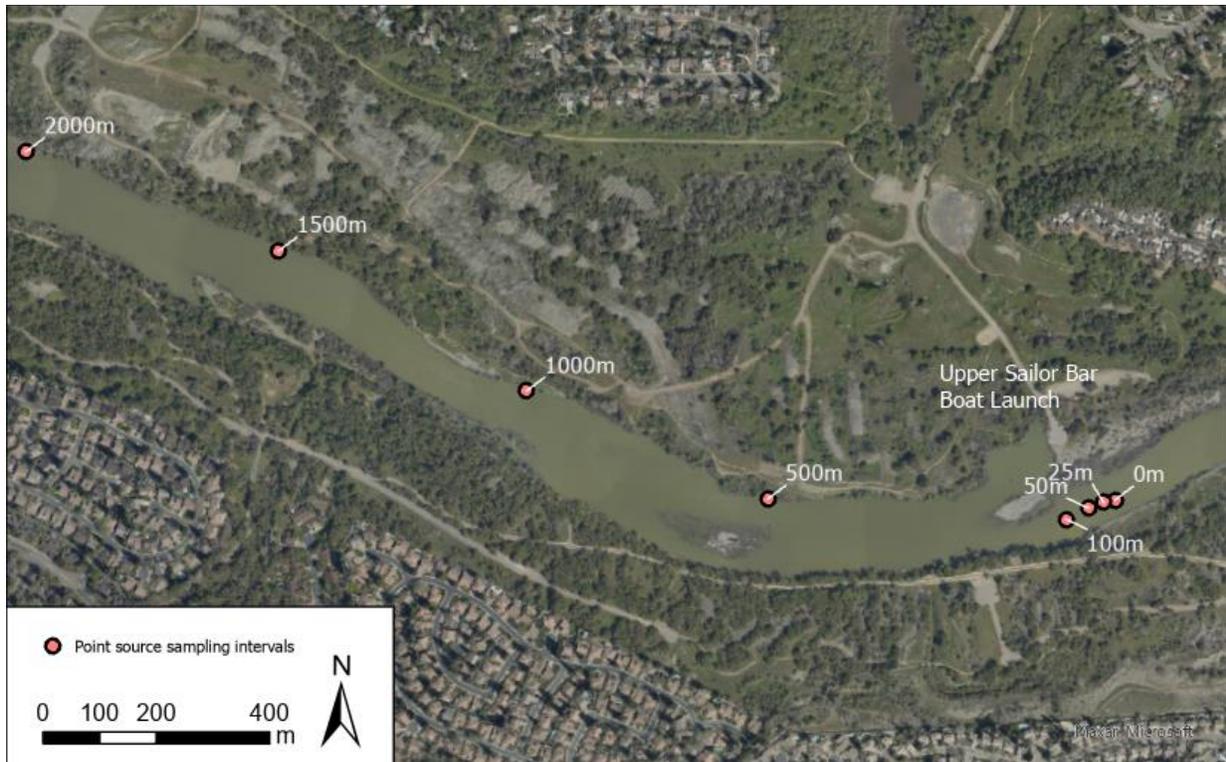


Figure 4. Point source eDNA experiment sampling interval locations. “0m” coincides with the placement of the mesh bag containing Ballyhoo and Anchovy.

Environmental DNA (eDNA) Survey

A 16-ft drift boat was used to collect eDNA samples from the middle of the river every 1000 meters along a 22-km section of the LAR from Nimbus Basin to the Watt Avenue Bridge (**Figure 5**), with the exception of sample “22” (Nimbus Basin), which was collected from shore due to underwater obstructions and lack of boat access beyond the Nimbus Hatchery weir. All samples were collected on September 23, following sampling protocols outlined in the previous section. Samples were analyzed for the presence of Chinook Salmon and *O. mykiss* eDNA.

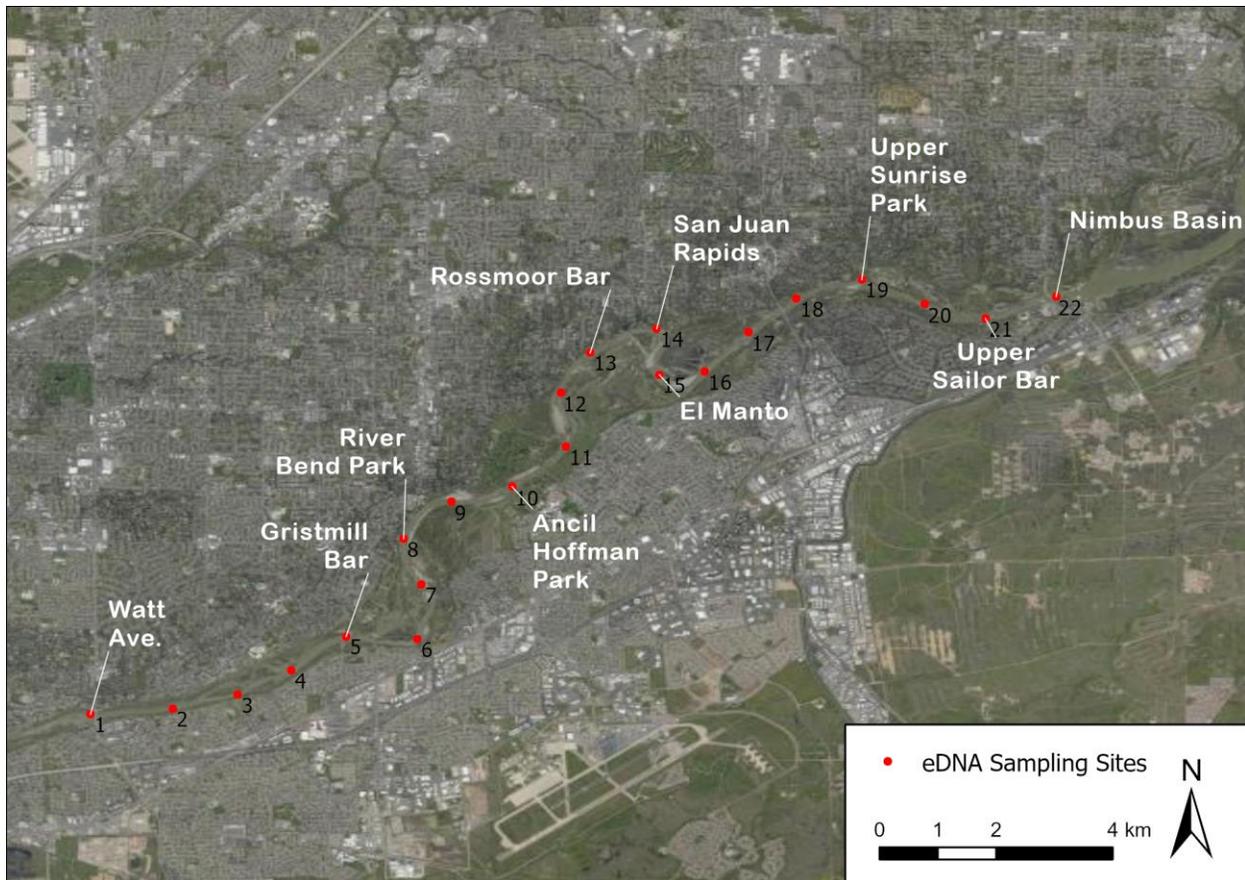


Figure 5. eDNA sampling locations in the Lower American River.

RESULTS

Vertical Water Quality Profile Surveys

Over the three monitoring days (7, 10 and 13 September 2021), measurements were taken near maximum channel depths at all locations except in Nimbus Basin and below Nimbus weir (old hatchery weir), where maximum channel depths were unknown (**Figure 6; Table 1**). Maximum recorded depths were at Nimbus Basin (sluice outflow; maximum depth 10 m), El Manto (8 m) and below Riverbend Bridge (6.1 m) (**Table 1**). Average temperature recorded between Nimbus Dam (river kilometer; rkm 35.4) and just below Gristmill Bar (rkm 17.7) was 21.6 °C (**Figure 1**). Lowest recorded temperatures were at the entrances of Buffalo Creek (20.4 °C) and Cordova Creek (19.2 °C) and maximum recorded temperature was 23.3 °C just below Gristmill Bar, the downstream-most survey location (**Figure 6**).

Table 1. Sites where water quality measurements were taken during each collection date, including, river kilometer (river mile), distance from the dam and maximum depths at which measurements were recorded. All maximum depth measurements were taken at the maximum channel depth, except at Nimbus Basin below the dam and below Nimbus weir, where maximum channel depths were unknown.

Date	Time	Site	rkm (RM)	Distance from dam (km)	Maximum measurement depth (m)
9/7/2021	10:00	Nimbus Basin*	35.4 (22)	0	10
9/7/2021	10:00	Nimbus Basin*	35.4 (22)	0.4	1
9/7/2021	10:15	Below Nimbus Weir*	35.4 (22)	0.6	5
9/10/2021	09:30	Upper Sailor Bar	35.4 (22)	1.4	2.5
9/10/2021	10:24	Upper Sunrise Park	32.2 (21)	3.4	1.8
9/10/2021	10:24	Upper Sunrise Park	32.2 (21)	3.4	3
9/10/2021	10:51	Buffalo Creek	30.6 (19)	4.6	0.3
9/10/2021	11:15	Sunrise Blvd Bridge	30.6 (19)	4.6	3
9/10/2021	11:34	Lower Sunrise Park	30.6 (19)	5.4	1.5
9/10/2021	12:35	Sacramento Bar	29 (18)	6.7	4
9/10/2021	12:53	El Manto	30 (18)	7.1	2.1
9/10/2021	13:12	El Manto	31 (18)	7.2	8
9/10/2021	13:12	El Manto	32 (18)	7.2	0.7
9/10/2021	13:31	Below San Juan Rapids	27.4 (17)	8.1	3.5
9/13/2021	09:05	Ancil Hoffman Park	25.9 (15)	10.9	3
9/13/2021	09:17	Ancil Hoffman Park	24.1 (15)	11.1	3.7
9/13/2021	09:54	Cordova Creek	23.5 (15)	12.5	0.3
9/13/2021	10:47	Riverbend Bridge	20.9 (13)	14.6	6.1
9/13/2021	11:22	Above Gristmill Bar	19.3 (12)	16.5	2.5
9/13/2021	12:21	Below Gristmill Bar	17.7 (11)	18.1	3.7

* Maximum channel depth unknown

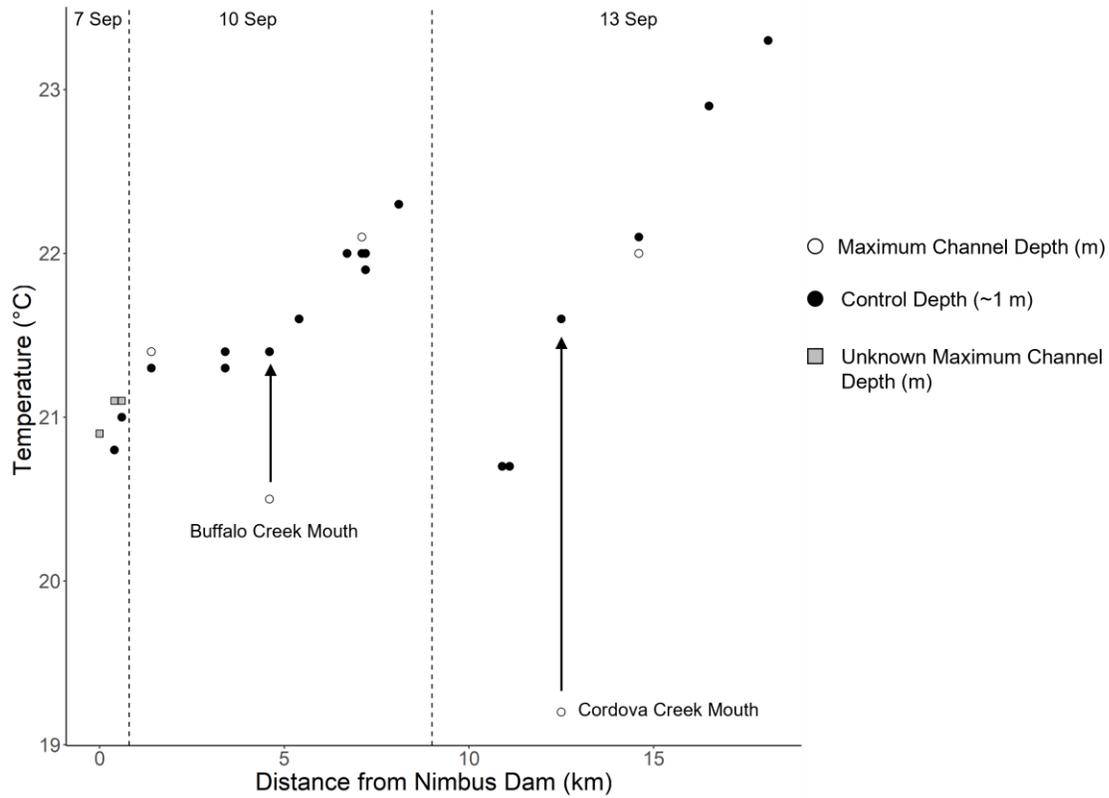


Figure 6. Temperature (°C) measured by kilometer (km) below Nimbus Dam at maximum channel depths and depths ~1 m below surface measured between 7, 10 and 13 September 2021. Measurements near Buffalo and Cordova creeks were taken at entrance into main channel of the Lower American River (open circles) and ambient control temperatures (closed circles) were taken ~2 m upstream of each creek entrance into the main channel (indicated by black arrows).

Average recorded DO between Nimbus Dam and just below Gristmill Bar was 7.8 mg/l. The lowest recorded DO was measured just below Nimbus Dam (5.5 mg/L) and the highest recorded DO was measured below Gristmill Bar (10.2 mg/L) (**Figure 7**).

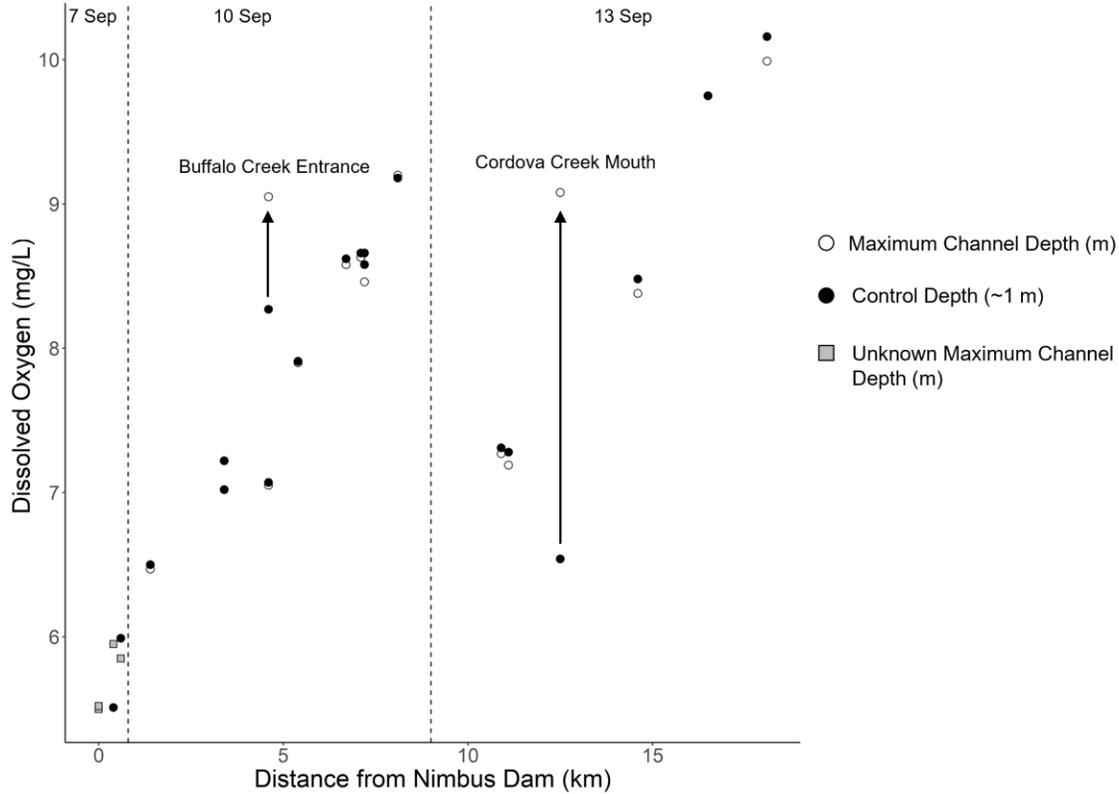


Figure 7. Dissolved Oxygen (mg/L) measured by kilometer (km) below Nimbus Dam at maximum channel depths and depths ~1 m below surface measured between 7, 10, and 13 September. Measurements near Buffalo and Cordova creeks were taken at entrance into the Lower American River main channel (open circles) and ambient control temperatures (closed circles) were taken ~2 m upstream of creek entrance into the main channel (indicated by black arrows).

Statistical Analysis

The GLM results comparing temperature to environmental factors and time indicated that date, time of day and distance below dam (km) were significant predictors of temperature (**Table 2**). Tributary mixing zone was the only habitat feature that had a significant temperature effect. Clay/Mehrtens formations, hyporheic flow, and depth below surface did not demonstrate a significant temperature relationship (**Table 2**).

Table 2. General Linear Model results predicting the effects of date, time, distance downstream from Nimbus dam (km), depth below water surface (m), and habitat type (hyporheic flow, creek and clay/mehrten formations) on temperature (°C).

Term	Estimate	Std Error	ChiSquare	Prob> ChiSquare
Intercept	23.963	0.853	121.266	<.001*
Distance from dam (km)	0.645	0.065	50.219	<.001*
Depth below surface (m)	0.010	0.017	0.308	0.579
Hyporheic flow (Y/N)	0.075	0.083	0.800	0.371
Creek (Y/N)	0.833	0.078	53.819	<.001*
Clay/Mehrten formations (Y/N)	-0.090	0.048	3.355	0.067
Date (9/7/2021)	3.062	0.340	44.355	<.001*
Date (9/10/2021)	1.604	0.188	41.472	<.001*
Time	0.000	0.000	25.840	<.001*

The GLM results using DO as a response variable and date, distance from dam, depth below water surface, and time of day indicate that time of day and distance below dam (km) had a significant positive effect on DO, similar to temperature (**Table 3**). Depth below water surface (m) had a significant negative effect on DO with higher DO measured closer to the water surface (~1 m).

In addition, temperature and DO were positively correlated, particularly when the two outlier temperature measurements taken at creek inlets were excluded from the regression ($r^2 = 0.37$ including outliers, $r^2 = 0.77$ excluding outliers) (**Figure 8**). Distance from Nimbus Dam was positively correlated with both temperature and DO suggesting that water quality originating from Lake Natoma has a greater effect on DO than temperature (**Figure 8**).

Table 3. GLM results predicting the effects of time, distance below Nimbus dam (km), and depth below water surface (m) on DO (mg/l).

Term	Estimate	Std Error	ChiSquare	Prob>ChiSquare
Intercept	1.089	0.676	2.513	0.113
Distance from dam (km)	0.154	0.016	47.859	<0.001*
Depth below surface (m)	-0.082	0.038	4.350	0.037*
Time	0.000	0.000	40.993	<0.001*

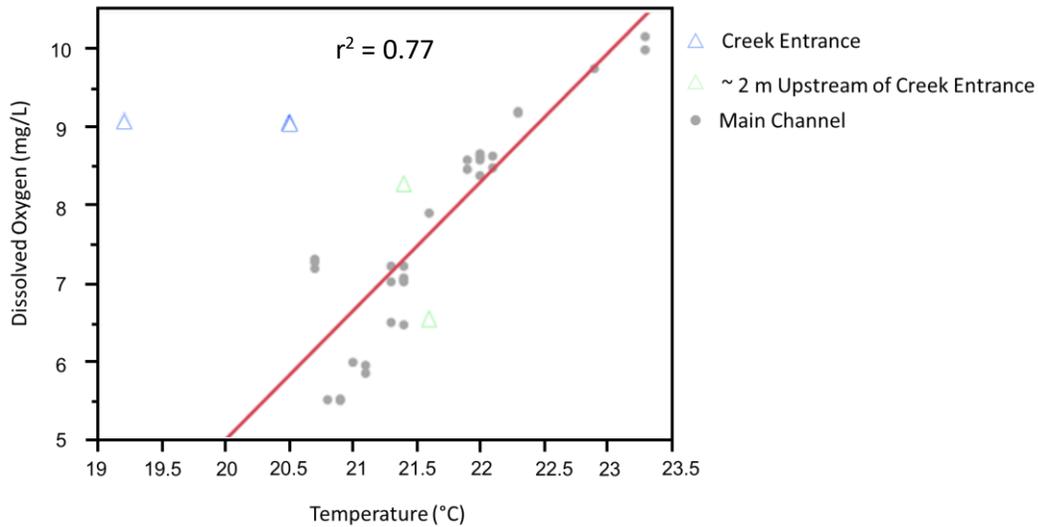


Figure 8. Correlation between temperature (°C) and dissolved oxygen (mg/L). Blue triangles represent measurements taken at creek entrances (Buffalo Creek and Cordova Creek) and green triangles represent measurements taken ~2 m upstream of creek entrances in the main channel. Grey dots represent all additional points measured in the main channel. Red line indicates best fit line for the data points. R² value excludes blue triangles representing creek entrances, as they are outliers.

Video Surveys

Videos were initially reviewed by technicians and any fish observations difficult to identify were reviewed a second time by a senior scientist. Fish were identified to species when possible; when not possible, due to image quality (e.g., partial image; turbidity, lighting, etc.), fish were identified to the next possible taxonomic level. A total of 138.4 minutes (2.31 hours) of video was reviewed and 212 total fish observations made. Catch per unit effort (CPUE), including all taxa observed, was calculated for each site (**Table 4; Figures 9 and 10**) using the following formula: $CPUE = \# \text{ fish} / \text{video review time in minutes}$.

Table 4. Total CPUE for all species at each site.

Site	CPUE
Below Gristmill Bar	0.97
Above Gristmill Bar	1.68
Sarah Ct	4.38
Ancil Hoffman	2.75
Below San Juan Rapids	0.70
El Manto	1.38
Sacramento Bar	1.21
Lower Sunrise Park	0.94
Sunrise Blvd Bridge	0.84
Upper Sunrise Park	1.11
Upper Sailor Bar	0.07
Below Nimbus Weir	3.38
Nimbus Basin	2.85

Native fish species CPUE was higher further downstream, with the exception of Chinook Salmon, which were mostly seen holding at Nimbus Basin (**Figure 9**). This relationship was driven by Sacramento Sucker, which were the most common native species observed. The opposite relationship was observed for non-native species which had a greater CPUE further upstream (**Figure 10**). This pattern was driven by Striped Bass (*Morone saxatilis*), the most common non-native species observed. No *O. mykiss* were observed in any of the transects. Adult Chinook Salmon were observed holding in the Nimbus Basin, at the sluice outflow, and one was observed above Gristmill Bar. Other species occasionally observed include native Sacramento Pikeminnow (*Ptychocheilus grandis*) and non-native Largemouth Bass (*Micropterus salmoides*) (**Table 4**). Eleven fish could not be identified to species (**Table 5**). Examples of underwater photos captured during surveys are shown in Appendix A.

We also observed three salmon carcasses during the surveys. One was observed on river right just upstream of the 2021 habitat restoration project at Ancil Hoffman. Three Turkey Vultures were actively feeding on what little remained of this carcass. Two more carcasses were observed within a slow glide (center channel) upstream of the Banister Ponds (rkm 29).

Table 5. Total species observed per site.

Date	Site	Chinook Salmon	Sacramento Sucker	Sacramento Pikeminnow	Striped Bass	Largemouth Bass	Unidentified fish
9/2/2021	Below Gristmill Bar		5				
	Above Gristmill Bar	1	12				
	Sarah Ct		23		1		
	Ancil Hoffman Park		14				1
	Below San Juan Rapids		7	2	2		
	El Manto		5				
	Sacramento Bar		10	3		1	
	Lower Sunrise Park		3				
	Sunrise Blvd Bridge		2		2		
	Upper Sunrise Park		3		5		
	Upper Sailor Bar		1				
	Below Nimbus Weir			1		5	4
	Nimbus Basin	4				11	2
	9/7/2021	Below San Juan Rapids		3	5		
Lower Sunrise Park			12				
Upper Sailor Bar				<i>No fish observed</i>			
Below Nimbus Weir				4	21		1
Nimbus Basin		18			15		2

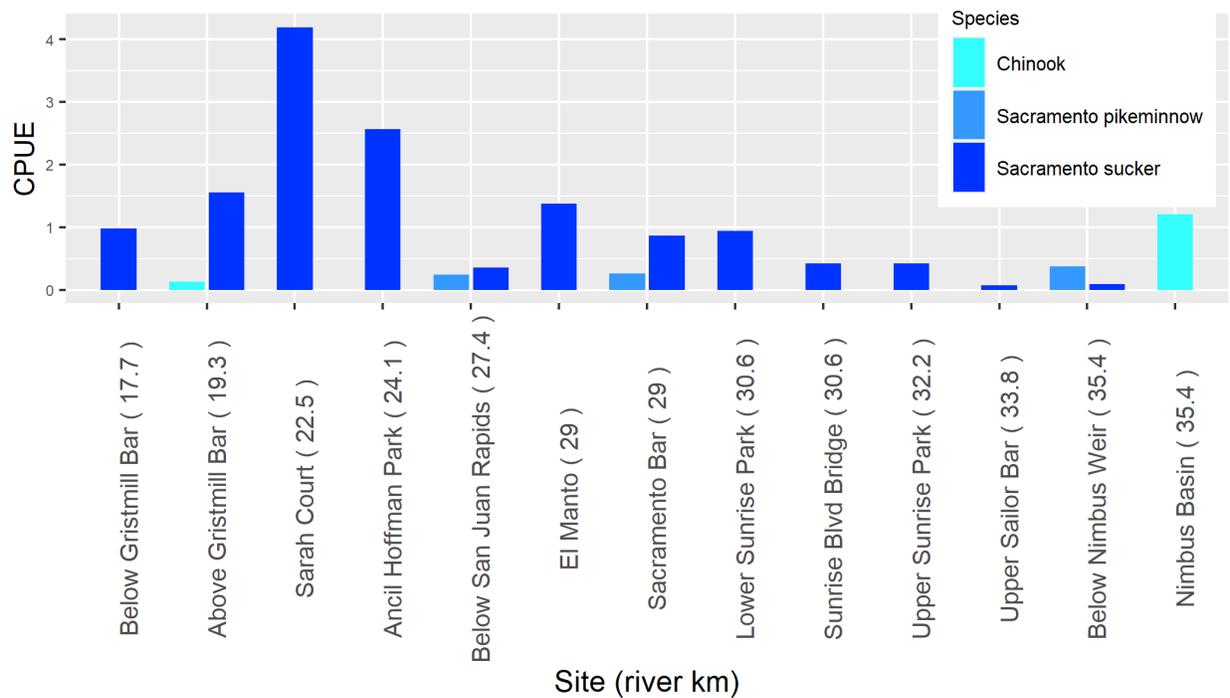


Figure 9. Native species density at each site. Catch per unit effort (CPUE) is the number of fish observed per minute of video review.

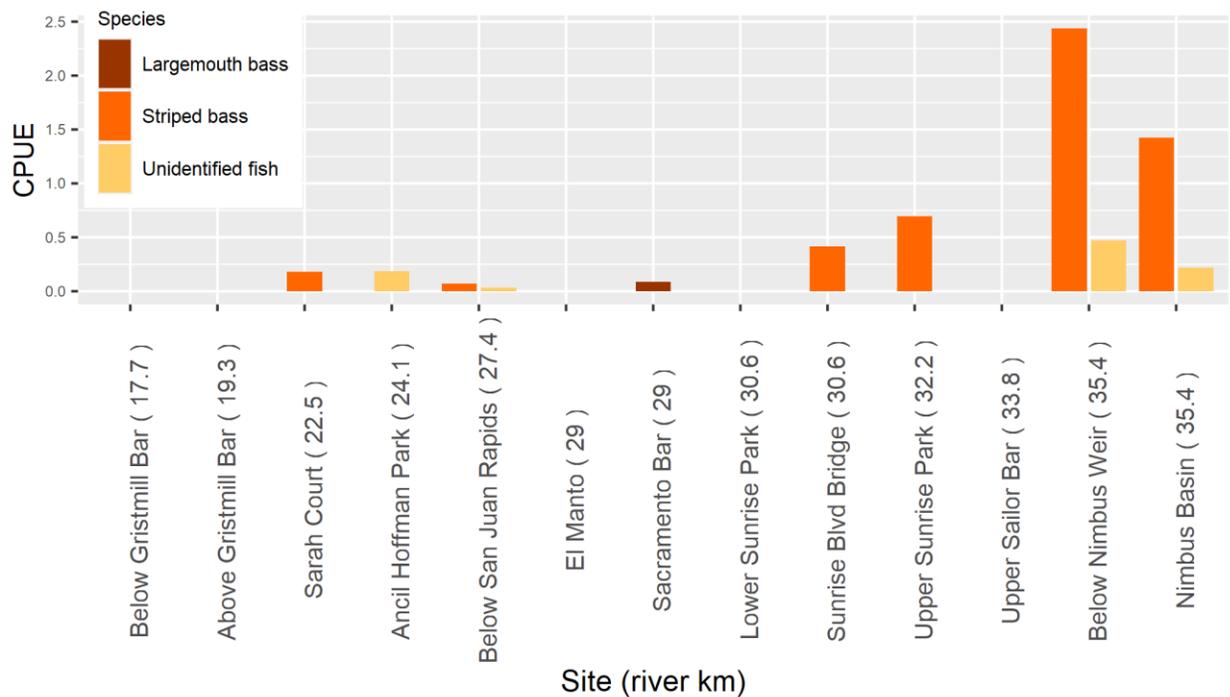


Figure 10. CPUE for non-native and unidentified fish at each site. Catch per unit effort (CPUE) is the number of fish observed per minute of video review.

Environmental DNA (eDNA) Transport Evaluation

DNA was detectable up to 1000 meters from the point source from the high biomass treatment (3172 grams) (red, **Figure 11**), but only 100 meters for the low biomass treatment (data not shown). Samples collected during the point source study were further analyzed for *O. mykiss* eDNA along with three additional samples that were collected in the Nimbus Basin (above the hatchery outflow) to determine if the hatchery outflow contributed exogenous *O. mykiss* eDNA into the environment. *O. mykiss* eDNA was detected at all locations, including above the hatchery outflow (blue, **Figure 11**).

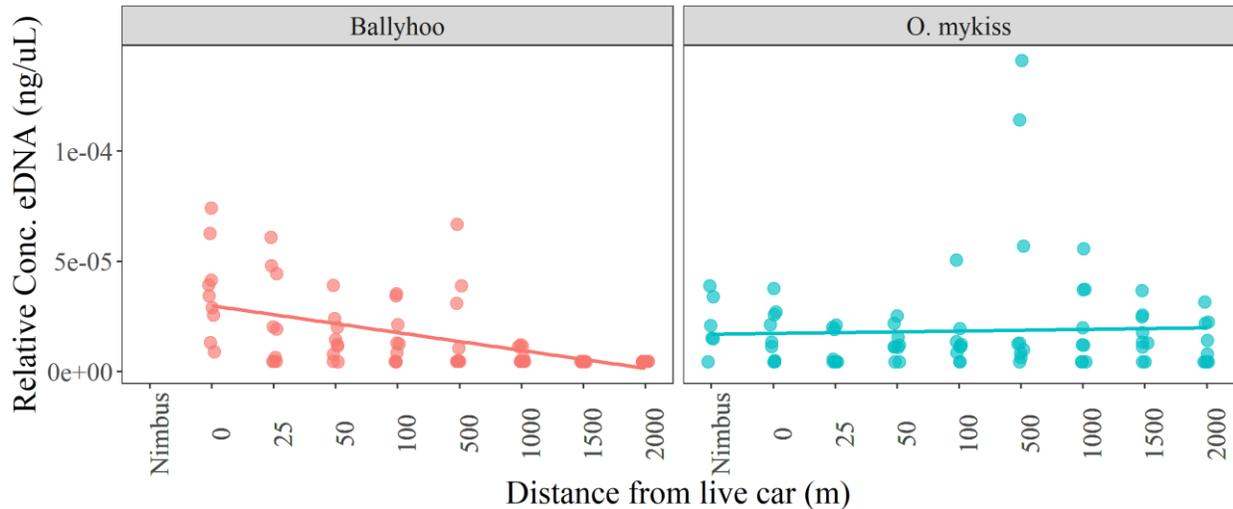


Figure 11. Relative concentration of eDNA (ng/uL) for Ballyhoo (live car) and *O. mykiss* (ambient) at given distance.

Environmental DNA (eDNA) Survey

Chinook Salmon and *O. mykiss* eDNA was detected at all sites that were sampled during the 22-km eDNA survey. However, eDNA signal intensity was not uniform throughout the survey area. Chinook Salmon eDNA concentrations were determined to be the highest in the reach between Upper Sunrise Park and El Manto, while *O. mykiss* eDNA concentrations peaked at the Upper Sunrise sampling location, with slight “hotspots” (sites with higher concentrations of eDNA) near El Manto, San Juan Rapids, and Gristmill Bar.

Relative concentrations of Chinook Salmon eDNA were higher compared to those observed for *O. mykiss* during the survey period (**Figure 12**).

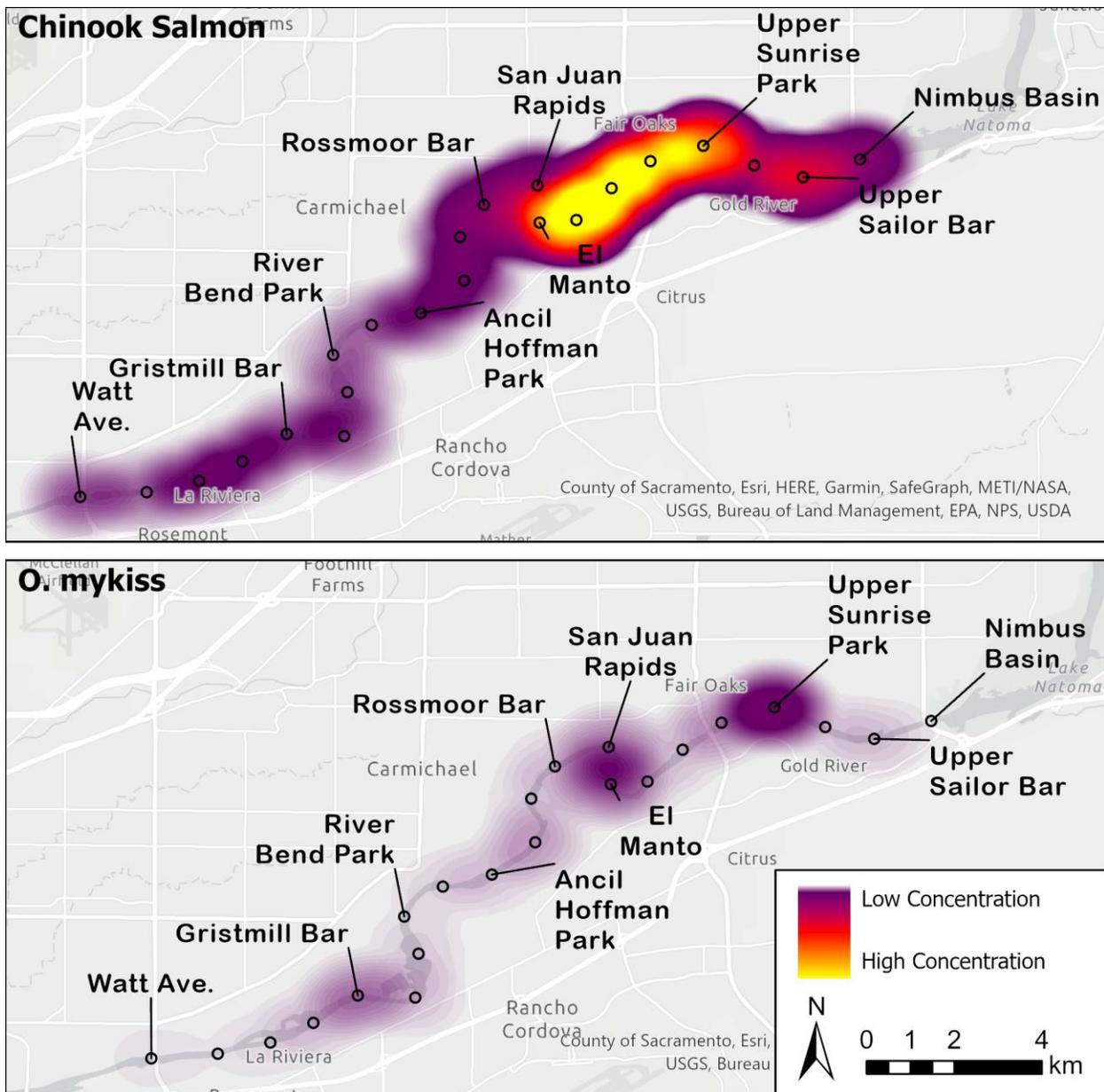


Figure 12. Relative eDNA concentration and distribution for Chinook Salmon (top) and *O. mykiss* (bottom).

DISCUSSION

The Mediterranean climate of California is characterized by a distinct cool-wet season followed by a warm-dry season. Rivers within this region, and the plant and animal communities that evolved within them, are influenced by a sequence of regular and often extreme flooding and drying periods (Gasith and Resh 1999; He et al. 2017; Hershkovitz and Gasith 2013). However, annual precipitation variability can lead to extreme flooding and drying conditions, and this has been intensified in recent years due to climate change (Mann and Gleick 2015; McCabe et al. 2018). Drought conditions exacerbate challenges to maintain healthy salmonid streams that are

already heavily impacted by habitat loss due to passage barriers, mining, urban and agricultural expansion, and invasive predatory or competitive species suited to the conditions present in a flow-regulated stream (Taylor et al. 2014; Katz et al 2012; Zeug et al 2011; Schick et al 2005; Yoshiyama et al 1998). In addition, flow regulations and dam operations dampen peak winter and spring stream flows compromising migration timing and predation risks for sensitive aquatic species including salmonids within remaining habitats downstream (Hunter 1992; Yoshiyama et al. 1998; Sellheim et al. 2020). Inadequate streamflow resulting from drought conditions and flow regulations impact multiple salmonid freshwater life stages (Sellheim et al. 2020).

Balancing freshwater ecosystem integrity with societal water needs is extremely challenging during severe dry periods due to unpredictable annual precipitation, limited water availability, population needs, and economy-driven subsidized irrigation transfers (Sellheim et al. 2020). Adaptive management is widely recommended by restoration practitioners, including measuring ecosystem response to management actions to inform future decisions (Lennox et al. 2019; Murchie et al. 2008). However, this is difficult to accomplish in practice, in part because streams that support rare and protected species require state and/or federal permits in order to monitor using conventional invasive methods, which may require several months for review and approval. Because precipitation and subsequent management actions are not predictable within the timeframe required for permit acquisition, many study methods are not practical for collecting data to inform adaptive management under extreme conditions.

The primary objectives of this short-term study were to determine if specific habitat features influence LAR water quality, including temperature and DO, under severe drought conditions and to assess habitat occupation of *O. mykiss* and Chinook Salmon under these conditions. To collect the data within the timeframe that salmonids were experiencing stressful conditions and in which flow management decisions were being made, we utilized a non-invasive method that did not require permits. Below, we discuss these results in the context of previous studies in the California Central Valley and available scientific literature.

Water Quality

Water Temperature

Water temperatures between Nimbus Dam outflow downstream to the Hazel Avenue bridge fluctuated at or below 21°C during the single morning sampling period. It is important to note that we did not record temperatures outside of the main thalweg but would assume the longer residence time of water near the Aquatic Center and the fish ladder would be warmer (river left) due to lower velocities. We also sampled during the morning (before 10am), when cooler temperatures would be expected due to cooler evening air temperatures. Note that while temperatures as high as 23°C were observed in the LAR further downstream, temperatures below 21°C were observed outside the target area when taken in the early morning. Brewitt and Danner (2014) found that mainstem temperature was the strongest predictor of fish use of thermal refugia for juvenile *O. mykiss* in the Klamath River; the majority (>80%) of juveniles moved into thermal refuges when mainstem temperatures reached 22-23°C, and all fish moved into refuge areas by 25°C. Torgersen et al. (2012) considered a 2°C differential as a thermal refuge, although this appears an arbitrary threshold.

On the LAR, Buffalo and Cordova creek mixing zones had significantly cooler temperatures compared to measurements observed in the main channel immediately upstream (as much as 2°C in this short duration experiment). Buffalo and Cordova creeks are unique to the LAR in that both are fed with discharges from treated groundwater, storm water runoff and residential irrigation runoff. Brewitt and Danner (2014) showed that tributary mouths can provide *O. mykiss* temperature refugia during periods when temperatures surpass 22-23°C. This was the only LAR habitat feature surveyed that supported the tested hypotheses, and this result is in agreement with previous temperature measurements recorded in the LAR by cbec several years prior to this study (cbec, personal communication). In the Brewitt and Danner (2014) study mainstem temperature, time of day, and their interactions with body size were the most important predictors of *O. mykiss* thermal refuge use. Unfortunately, our equipment could not survey for fish beyond the immediate mixing zone of these two creeks due to shallow water depth. Buffalo Creek enters the LAR through a drop structure which we assume is a fish barrier, but Cordova Creek does support fish access during some flow conditions, and continued habitat restoration is planned at Cordova Creek to improve fish passage. This should be studied further, as Cordova Creek has the potential to provide important thermal refuge for salmonids that could improve following restoration, particularly given the lack of thermal refuge habitat observed in other LAR habitats.

Note that these results should be analyzed with caution given the short duration of this study and the limited number of samples taken at each of the features hypothesized to support thermal refuge habitat. For example, in other large salmon-bearing river systems, long stretches of deep alluvium have demonstrated water temperature influence (Burkholder et al. 2008). This is an avenue for future research.

Dissolved Oxygen

Dissolved oxygen is one of the most important indicators of biological health of rivers, and can exhibit large fluctuations over a wide range of spatial and temporal scales. It is primarily dependent upon water temperature; however, this relationship can be altered by changes in hydro-meteorological conditions (Rajwa et al., 2014) and biological processes such as photosynthesis, respiration and decomposition of organic matter (Rajwa-Kuligiewicz et al. 2015). The strong positive correlation we observed between DO and distance from Nimbus Dam, coupled with a corresponding temperature increase, indicate relatively poor water quality released from Nimbus Dam. All measurements recorded within the focal temperature management area (upstream of Hazel Avenue) were below 6 mg/L. At oxygen levels of 6.5 mg/L most salmonid species exhibit symptoms of oxygen distress (Davis 1975, as cited in Carter et al 2005a). DO values above 8 mg/L, which were not observed until 5 km downstream of Nimbus Basin, are considered optimal for salmonids (Carter et al. 2005a).

While we did not specifically measure turbidity or indications of primary productivity, relatively low visibility at the basin, coupled with visibly high algal levels (see Appendix A **Figures A4** and **A5**) suggest that relatively high primary productivity within upstream reservoirs may have reduced DO levels. This is further supported by the positive correlation between DO and time of day, suggesting respiration driving DO levels down in the evening and photosynthesis increasing DO levels throughout the day.

Video surveys

The lack of *O. mykiss* observations, minimal observations of Chinook Salmon and relative prevalence of other fish species are indicative of the stressful water quality conditions observed over the short monitoring duration. At temperatures ranging between 18-22°C, a transition in dominance from salmonids to other species occurs is articulated for the Pacific Northwest (USEPA 1999, as cited in Carter 2005b). All of the 19 measurements recorded during this short survey were within this range. USEPA considers 22-24°C to be a temperature range in Pacific Northwest streams that eliminates salmonids from an area [USEPA 2001; USEPA (McCullough) 1999, as cited in Carter 2005b]. Forty-seven percent (9) of the 19 temperature measurements were within this range.

Over-summer rearing *O. mykiss*

No *O. mykiss* were observed in over 2.5 hours of video recorded at 13 locations that were considered to be the most likely locations to support them based on habitat parameters, including potential temperature refuge habitat.

Nielsen et al. (1994) studied thermally stratified pools and their use by juvenile steelhead in three California North Coast rivers including the Middle Fork Eel River, Redwood Creek at Redwood National Park, and Rancheria Creek, located in the Navarro River watershed. In detailed observations of juvenile steelhead behavior in and near thermally stratified pools in Rancheria Creek, Nielsen et al. (1994) noted behavioral changes including decreased foraging and increased aggressive behavior as pool temperature reached approximately 22°C. As pool temperature increased above 22°C, juveniles left the observation pools and moved into stratified pools where temperatures were lower.

In laboratory conditions, Age-0 steelhead acclimated to 11°C exhibited a critical thermal maximum of 27.5°C, while Age-0 steelhead acclimated to 19°C exhibited a critical thermal maximum of 29.6°C (Myrick and Cech 2005). Reese and Harvey (2002) found that while interspecies competition had negligible effects on *O. mykiss* growth at temperatures of 15-18°C, growth was severely curtailed by pikeminnow interactions at temperatures of 20-23°C.

Hypoxia has been shown to influence behavior related to predator vulnerability in *O. mykiss* (Frost et al. 2013). As described in the above section, DO levels of 6.5 mg/L generally lead to oxygen distress for salmonids (Davis 1975, as cited in Carter et al 2005a).

Brewitt and Danner (2014) observed a diel behavioral shift in young *O. mykiss* suggesting they may be resource-limited in refuges due to density-dependent competition, and may move into mainstem habitat during the day to forage, but seek thermal refuge at night when the metabolic cost of remaining in warmer water rises due to limited foraging success. In this study, the lack of observations during periods when *O. mykiss* would be expected to feed, especially during periods of increased metabolic benefit, coupled with poor water quality that results in greater predation vulnerability and the observation of several potential predators and competitors to *O. mykiss* (e.g., Sacramento Pikeminnow, Striped Bass, Largemouth Bass) during video surveys, suggest a severely limited number, if not absence of *O. mykiss* from the LAR during the survey period.

Two important observations must be taken into account when reviewing these data: The relatively short duration of survey (2.5 hours during daylight hours) and what appeared to be a single *O. mykiss* hooking mortality observed just above the Sunrise boat launch on 23 September 2021.

Adult Chinook Salmon Migration and Holding

Adult fall run Chinook Salmon generally migrate at 10.6 – 19.6°C (Bell 1990 as cited in Carter 2005b) and 21°C is considered a thermal barrier to immigrating adult Chinook Salmon (Reiser and Bjornn 1979, as cited by Armour et al. 1991). Overall, average temperatures recorded during this short survey were 21.6 °C. DO levels near 4.5 – 5 mg/L are considered a barrier to immigrating adult Chinook Salmon (Hallock et al. 1970), and while DO levels did not reach this threshold they were quite low, particularly in the upper river where temperature management is actively occurring.

We observed a single adult Chinook Salmon (male hatchery return, as indicated by clipped adipose fin) moving up through a shaded channel lined by Mehrten just upstream of Gristmill, and at least 8 individual adult Chinook Salmon were observed in Nimbus Basin along with several adult Striped Bass. These are early returning adult salmon holding prior to spawning, possibly searching for upstream temperature refuge. These adults were observed for at least 24 hours suggesting they were surviving diel temperature and DO fluctuations for at least this short duration.

While we were not specifically surveying for Chinook Salmon carcasses, three were observed. This suggests that mortality has occurred even during the early stages of adult immigration and reflects the poor water quality for spawning salmon we observed. Hooking mortality cannot be ruled out.

Environmental DNA Survey

The point source sampling experiment confirmed that the 1000m sampling interval was sufficient to detect eDNA from the high biomass fish (Ballyhoo, 3172 grams) and was therefore determined to be a suitable approach for surveying the 22-km section of the LAR, as proposed.

The evaluation of exogenous eDNA inputs from the hatchery was inconclusive. *O. mykiss* eDNA was detected at all sites, including at the Nimbus Basin above the hatchery outflow. If the hatchery outflow was inputting large quantities of *O. mykiss* eDNA into the river, we would expect to see a high concentration of *O. mykiss* eDNA at the outflow (0m), and a gradual decrease in eDNA concentration with distance. Instead, *O. mykiss* eDNA was present, at low concentrations, at Nimbus Basin and throughout the LAR, with no decrease over distance from outflow. Possible explanations for these results include (1) *O. mykiss* are present throughout the system (living or dead), (2) eDNA signal from hatchery outflow is present but indistinguishable from naturally occurring eDNA, (3) eDNA is entering LAR from above Lake Natoma. Further analysis of hatchery introduced eDNA using a cultured species that is not present in the LAR, such as Brown Trout (*Salmo trutta*) or Lahontan Cutthroat Trout (*Oncorhynchus clarkii henshawi*) (both raised at the American River Hatchery), is recommended to address this question.

Chinook Salmon eDNA was detected at all 22 of 22 sites along the reach of LAR from Nimbus Basin to the Watt Avenue bridge, with highest eDNA concentrations observed between Upper Sunrise Park and El Manto. A hook and line capture of an adult Chinook Salmon was observed near the El Manto site during the survey, confirming live Chinook were present in the area during sampling. Although Nimbus Basin is a known pre-spawning holding location and the site where multiple Chinook were observed on video several weeks prior to the eDNA survey, the eDNA concentrations were lower in Nimbus Basin than in the Upper Sunrise to El Manto reach. This may reflect the true concentration of eDNA in the environment. The Nimbus Basin site required eDNA collection from shore due to low water conditions and lack of launch access above the hatchery weir, while all other samples were collected from a drift boat, in the middle of the river. Lack of lateral flow and mixing towards the sampling site at the Nimbus Basin may have contributed to a weak eDNA signal.

Within the entire study area, the relative quantity of Chinook Salmon eDNA was higher than that observed for *O. mykiss*. This was expected given the documentation of Chinook Salmon spawners and mortalities (and absence of *O. mykiss*) during the video survey and the typical timing of Chinook Salmon immigration and spawning behavior in the LAR.

O. mykiss eDNA was similarly detected at 22 of 22 sampling sites, with hotspots at Upper Sunrise Park, near El Manto/the San Juan Rapids and at Gristmill Bar. A single *O. mykiss* mortality (presumed to be a hook and line death) was observed near the Upper Sunrise Park boat ramp in the eddy of a pool, providing visual confirmation of *O. mykiss* presence. Although the two Sunrise Park eDNA samples were collected upstream from the carcass and several hundred meters downstream, and in flowing water in both cases, it is possible that the relatively high eDNA density was influenced by the presence of this carcass. While no *O. mykiss* were observed during the video survey to corroborate the eDNA density and concentration distribution, the two survey types were completed on different dates (eDNA September 23; video September 2 and 7), leaving 2-3 weeks between events for river conditions and fish distributions to change. Additionally, eDNA from target organisms can be detected at low biomass (612 grams) up to 100m away from source and high biomasses (3172 grams) reliably detected up to 1km away from source, as documented during the fixed-point source experiment. Environmental DNA provides a broader spatial and temporal assessment of fish distributions, including refuge habitat in difficult-to-access areas, that may not be captured with targeted conventional monitoring.

CONCLUSIONS

Water quality conditions (temperature, DO) were generally poor during this short survey period. Although Nimbus Basin generally had the lowest water temperatures, DO was also relatively low, indicating stressful conditions for salmonids. Temperature refugia were observed at the mouths of Cordova and Buffalo creeks (LAR tributaries), where mixing area water temperatures were 1-2°C cooler than ambient river conditions immediately upstream. Dissolved oxygen was also relatively high in these locations. These creek confluences should be studied further, as during drought periods they may provide unique thermal refugia within the LAR.

No *O. mykiss* and only a few adult Chinook Salmon were observed by video in two locations within the LAR. Three Chinook salmon carcasses were anecdotally observed, suggesting a possibility of high mortality rates for early immigrating adult Chinook Salmon. Populations will continue to be monitored by CDFW, including quantifying Chinook Salmon pre-spawn mortality and relating mortality rates to water quality (including both temperature and DO), particularly in Nimbus Basin where water temperature is being actively managed.

The eDNA survey provided a single day snapshot (23 September 2021) of relative Chinook Salmon and *O. mykiss* distribution between the Nimbus Basin and the Watt Avenue Bridge. Highest eDNA concentrations for both species were observed between Upper Sunrise Park and downstream to San Juan Rapids, with detections of both species at all sites above and below the hatchery. Relative eDNA concentrations suggest Chinook Salmon were present in greater abundance than *O. mykiss* throughout the LAR. These observations were corroborated by the video survey. Repeated eDNA surveys at regular intervals along this same stretch of the LAR over longer periods of time could show patterns of habitat usage, variation and relative abundance of species, and could indicate sites of refugia during years affected by drought, supporting ongoing monitoring efforts. Effects of hatchery-introduced eDNA from *O. mykiss* were not quantifiable within the scope of this project, and further investigation of hatchery-introduced eDNA is needed to fully understand the effect, if any, hatchery eDNA has on the observation of naturally occurring eDNA.

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APPENDIX A: EXAMPLE IMAGES COLLECTED DURING VIDEO SURVEYS



Figure A1. Chinook Salmon in Nimbus Basin.



Figure A2. Striped bass in Nimbus Basin.

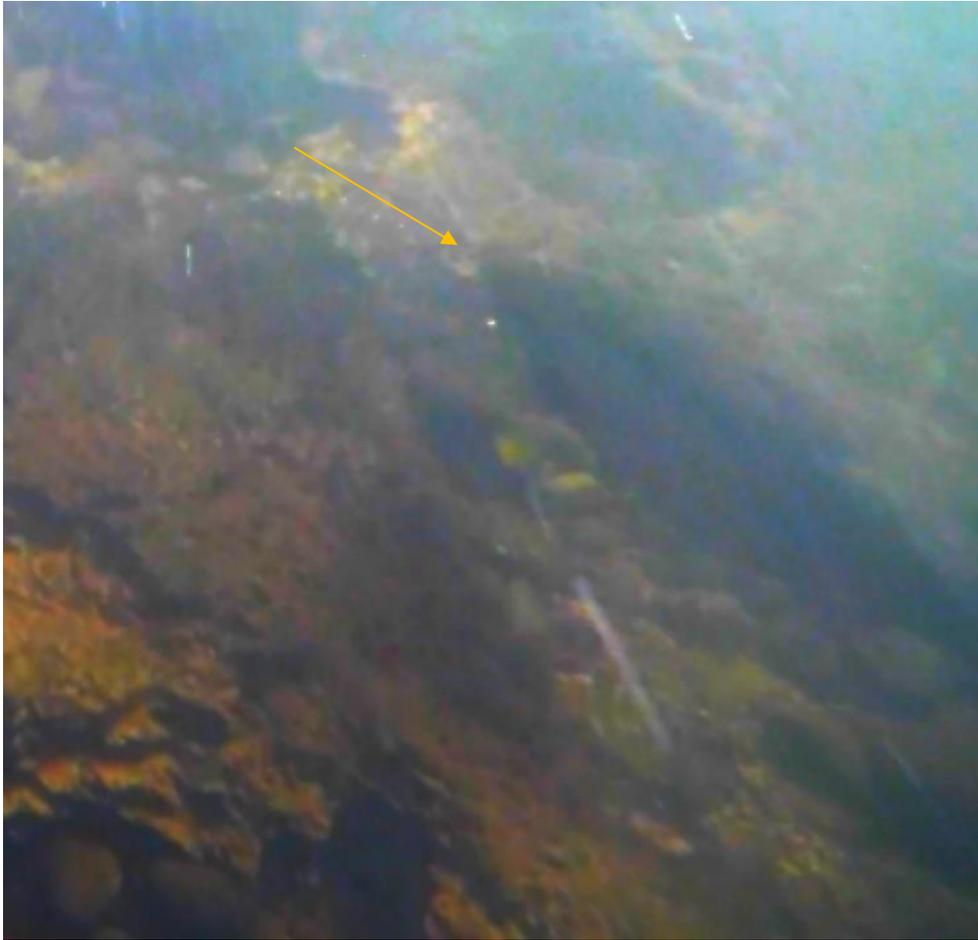


Figure A3. Chinook salmon (yellow arrow is pointing at the nose of fish) above Gristmill Bar.



Figure A4. Relatively high levels of algae in water column was observed at several locations suggesting potential dissolved oxygen issues. This photo is from pool just above Hazel Avenue.



Figure A5. Blooms of filamentous algae, such as *Didymosphenia geminata* have been associated with reduced benthic invertebrate production and poor water quality (Anderson et al. 2014).