Sport Fish Research Studies

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Annual Report

Colorado Parks & Wildlife

Aquatic Research Section

Fort Collins, Colorado

August 2023

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The results of the research investigations contained in this report represent work of the authors and may or may not have been implemented as Colorado Parks & Wildlife policy by the Director or the Wildlife Commission.

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Bellvue Fish Research Hatchery Production and Research Updates

The Hofer (GR or HOF; used interchangeably throughout) strain of Rainbow Trout *Oncorhynchus mykiss* is resistant to whirling disease (*Myxobolus cerebralis*), and has been incorporated into Colorado's hatchery program for both stocking into recreational fisheries and for crossing with other wild strains of Rainbow Trout to increase *M. cerebralis* resistance. A GR brood stock is maintained at the Colorado Parks and Wildlife (CPW) Bellvue Fish Research Hatchery (BFRH; Bellvue, Colorado) for both research and stocking purposes. The BFRH also rears and distributes other *M. cerebralis*-resistant Rainbow Trout strains and crosses, as well as other species of salmonids, for research purposes as the need arises. Additional sport fish research projects are conducted at the BFRH annually.

Fish and Brood Stock Production

The *M. cerebralis*-resistant Rainbow Trout brood stocks reared at the BFRH are unique, and each requires physical isolation to avoid unintentional mixing of stocks. Extreme caution is used during on-site spawning operations and throughout the rearing process to ensure complete separation of these different brood stocks. All lots of fish are uniquely fin-clipped and most stocks are individually marked with Passive Integrated Transponder (PIT) and/or Visible Implant Elastomer (VIE) tags before leaving the main hatchery. This allows for definitive identification before the fish are subsequently used for spawning.

Starting in mid-November 2022, BFRH personnel checked all of the two- and three-year-old GR brood fish weekly for ripeness. Eggs or milt flowing freely when slight pressure was applied to the abdomen of the fish indicated maturation. The first females usually maturated two to four weeks after the first group of males. As males were identified, they were moved into a separate section of the raceway to reduce handling and fighting injuries. On November 15, 2022, the first group of GR females were ripe and ready to spawn.

Before each fish was spawned, it was examined for the proper identification (fin clip, PIT, or VIE tag), a procedure that was repeated for each fish throughout the winter. Fish were spawned using the wet spawning method, where eggs from the female were stripped into a bowl along with the ovarian fluid. After collecting the eggs, milt from several males was added to the bowl. Water was poured into the bowl to activate the milt, and the bowl of eggs and milt was covered and left undisturbed for several minutes while the fertilization process took place. Next, the eggs were rinsed with fresh water to expel old sperm, feces, egg shells, and dead eggs. Eggs were poured into an insulated cooler with iodine to water harden for approximately one hour.

Water-hardened fertilized (green) eggs were moved to the BFRH main hatchery building. Upon reaching the hatchery, green eggs were tempered and disinfected (PVP Iodine, Western Chemical Inc., Ferndale, Washington; 100 ppm for 10 min at a pH of 7). Eggs were then put into vertical incubators (Heath Tray, Mari Source, Tacoma, Washington) with five gallons per minute (gpm) of 12.2ºC (54ºF) flow-through well water. The total number of eggs was calculated using number of eggs per ounce (Von Bayer trough count minus 10%) multiplied by the total ounces of eggs. Subsequent daily egg-takes were put into separate trays and recorded. To control fungus, eggs received a prophylactic flow-through treatment of formalin (1,667 ppm for 15 minutes) every other day until eye-up.

Eggs reached the eyed stage of development after 16 days in the incubator. The eyed eggs were removed from the trays and physically shocked to detect dead eggs, which turn white when disturbed. Dead eggs were removed both by hand and with a Van Gaalen fish egg sorter (VMG Industries, Longmont, Colorado) for two days following physical shock. The total number of good eyed eggs was calculated using the number of eggs per ounce multiplied by total ounces.

The on-site Rainbow Trout production spawn started on November 15, 2022, with the last group of GR females spawned December 6, 2022. The goal was to produce 1,000 GR eggs for brood stock replacement purposes, and this goal was met. No eggs were shipped from the BFRH in 2022/2023. All eggs were kept at the BFRH for brood stock purposes (Table 1.1).

Table 1.1. Bellvue Fish Research Hatchery on-site spawning information for the Hofer (GR) Rainbow Trout strain during the winter 2022-2023 spawning season.

Strain	Date Spawned	No. Spawned Females	No. Green Eggs	No. Eyed Eggs	Destination
GR	11/15/22-12/6/22	100	1,000	850	BFRH
Total	11/15/22-12/6/22	100	1,000	850	

As of 2022, the BFRH no longer maintains an HL brood stock or produces HL eggs. The transfer of all three year classes of HL brood fish to the CPW Poudre Rearing Unit occurred on June 21, 2022. The purpose of the transfer was two-fold: 1) create space at the BFRH for incoming YY Brook Trout *Salvelinus fontinalis*, and 2) increase the Poudre Rearing Unit production using *M. cerebralis*-resistant fish. The two primary brood stocks now maintained at the BFRH are the GR strain and YY Brook Trout (see below).

Annual Disease Testing

The BFRH annual disease inspection was conducted on March 6, 2023. All fish were negative for all diseases for which they were tested, including *Renibacterium salmoninarum*, the bacteria causing Bacterial Kidney Disease, which had been present on the unit in 2016 through 2020.

Brown Trout YY Production

Details regarding the involvement of the BFRH in the experimental development of YY Brown Trout *Salmo trutta* brood stock can be found in Fetherman et al. (2021, 2022). Brown Trout held at the BFRH were spawned in 2022 to make eggs for future YY brood stocks. All of the eggs (1,957) and adult Brown Trout (182; 1.45/lb) were transferred to the Los Ojos Hatchery in New Mexico to continue the brood stock development process.

Fetherman, E. R., B. Neuschwanger, T. B. Riepe, and B. W. Avila. 2021. Sport Fish Research Studies. Annual Report. Colorado Parks and Wildlife, Aquatic Research Section. Fort Collins, Colorado.

Fetherman, E. R., B. Neuschwanger, and T. B. Riepe. 2022. Sport Fish Research Studies. Annual Report. Colorado Parks and Wildlife, Aquatic Research Section. Fort Collins, Colorado.

REARING YY BROOK TROUT

YY Brook Trout were imported from the Hayspur Hatchery in Idaho on June 27, 2022. The Brook Trout were tested as part of the annual disease inspection on March 6, 2023 and were negative for all diseases for which they were tested, including Bacterial Kidney Disease. This is the first year class of YY Brook Trout that will be utilized for production in Colorado. A second year class of Brook Trout will be imported from the Hayspur Hatchery in August 2023. If males in the second year class received in August 2023 are ripe, the BFRH will produce its first replacement YY Brook Trout brood fish in fall 2023.

SPORT FISH RESEARCH PROJECT UPDATES

UPPER COLORADO RIVER SALMONID POPULATION MONITORING

Whirling disease (*Myxobolus cerebralis*) caused significant declines in Rainbow Trout populations throughout Colorado following its accidental introduction and establishment in the late 1980s. *M. cerebralis*-resistant Rainbow Trout have been developed by CPW and are currently stocked in a large number of locations across Colorado in an attempt to recover lost populations and create self-sustaining Rainbow Trout populations. The success of *M. cerebralis*-resistant Rainbow Trout introductions is highly variable, dependent on a number of factors including flow, temperature, stream type, habitat availability for different size classes, Brown Trout densities, prey availability, the size at which the Rainbow Trout are stocked, and strain type. Post-stocking evaluations conducted throughout Colorado allow comparisons of different management options to increase post-stocking survival, recruitment, and the potential to produce self-sustaining populations of *M. cerebralis*-resistant Rainbow Trout. Management actions, including stocking strategies, predator/competitor manipulations, habitat improvements, and increased river connectivity, continue to be evaluated in ongoing field experiments in the Colorado, Fraser, and Yampa rivers. Results from experiments conducted in the upper Colorado River within the last reporting cycle are presented below.

2022 Salmonid Fry Population Estimates

Rainbow Trout fry stocking evaluations began in the upper Colorado River in 2013. In 2013, 2014, and 2015, the 3.9-mile stretch of the upper Colorado River between Hitching Post Bridge on the Chimney Rock Ranch and the Sheriff Ranch (Figure 2.1) was stocked with 100,000 to 250,000 Hofer (HOF) by Colorado River Rainbow Trout (H×C) fry annually. Due to the detection of *Renibacterium salmoninarum* at the CPW Glenwood Springs Hatchery in late 2015, H×C fry were not available for stocking in 2016. Previous studies conducted in collaboration with Colorado State University showed that the HOF survived just as well as the H×C when stocked as fry into small streams (Avila et al. 2018), but the survival of the HOF had not been evaluated in a large river. As such, approximately 60,000-70,000 HOF fry were stocked into this stretch of the upper Colorado River in 2016, 2017, and 2018. Once *M. cerebralis*-resistance evaluations of the HOF by Gunnison River Rainbow Trout (H×G) were completed (Fetherman et al. 2018), survival evaluations of stocked H×G fry began in 2019 (Fetherman et al. 2020, 2021, 2022). Although the Rainbow Trout fry evaluation study begun in 2013 was completed following the fry estimates conducted in 2021, H×G fry continue to be stocked above and below Byers Canyon in an effort to increase the adult Rainbow Trout populations in these sections.

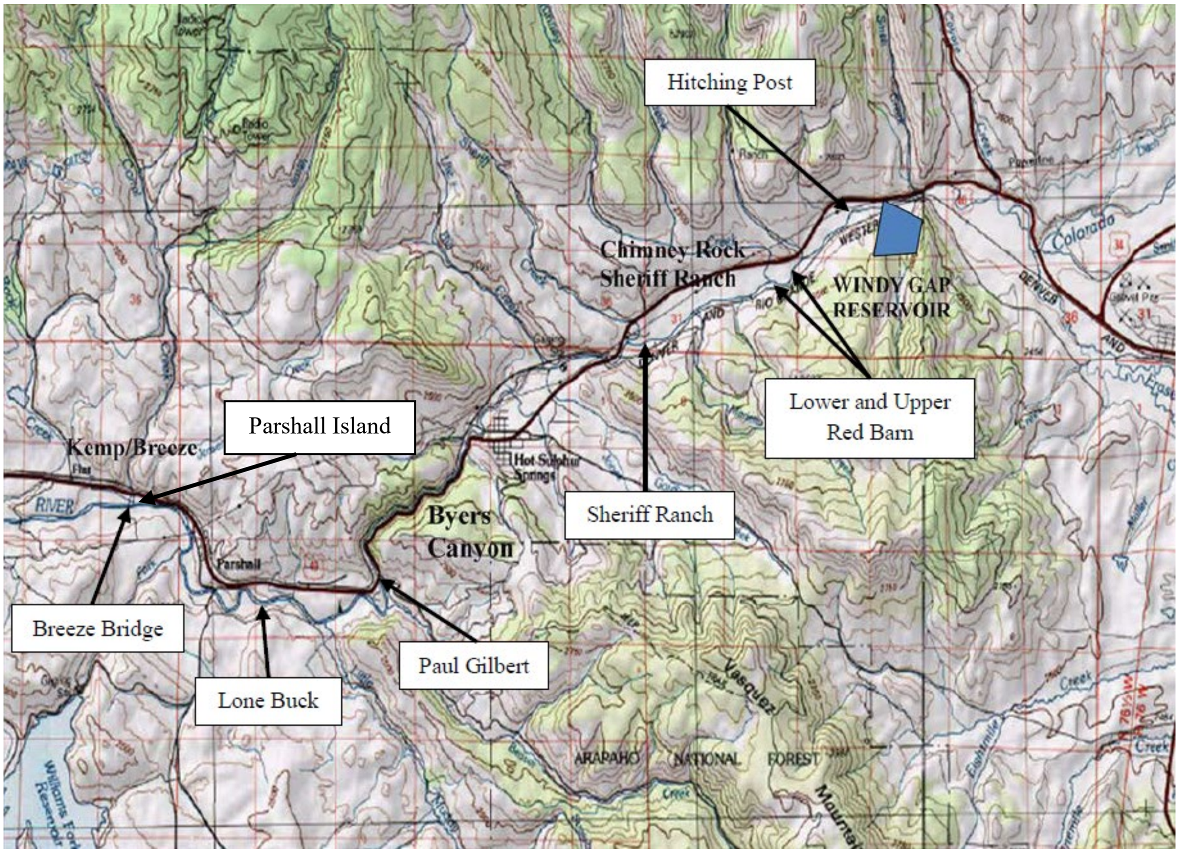

Figure 2.1. Upper Colorado River study area showing the eight sites at which salmonid fry population estimates were conducted in July, August, September, and October 2022.

On July 7, 2022, approximately 142,500 H×G fry were stocked into the upper Colorado River between Hitching Post Bridge on the Chimney Rock Ranch and the Sheriff Ranch (Figure 2.1). Approximately 25,000 Rainbow Trout fry were stocked by bucket at each of two access points below the Red Barn diversion structure, one at the Sheriff Ranch and one at Kinney Creek. Two-thirds of the remaining Rainbow Trout fry were loaded into large coolers supplied with a constant flow of oxygen on a stocking raft at the Hitching Post Bridge. Rainbow Trout were stocked in the margins on both sides of the river in the 0.8-mile stretch between Hitching Post Bridge and the upper extent of the Red Barn access road. The final third of the Rainbow Trout fry were loaded at the upper extent of the Red Barn access road, and fry were similarly stocked on both sides of the river from this point to Lower Red Barn (0.4 miles).

Pre-stocking fry population estimates were conducted at eight sites in the upper Colorado River in early July, and post-stocking fry population estimates were conducted at the end of July, August, September, and October 2022. Fry estimates completed prior to H×G stocking provided information on the number of Rainbow Trout and Brown Trout fry occurring from natural reproduction, whereas the estimates completed at the end of July, August, September, and October provided information regarding the post-stocking survival of the H×G fry and survival of wild Rainbow Trout and Brown Trout fry. Sampling sites (*n* = 4) in the Chimney Rock/Sheriff Ranch study section included the Sheriff Ranch, Lower and Upper Red Barn, and the Hitching Post Bridge (Figure 2.1), which are historical sites used to evaluate fry production and survival in this section of the Colorado River. Four reference sites below Byers Canyon were used to compare survival of stocked H×G fry above and below Byers Canyon. Sampling sites (*n* = 4) below Byers Canyon included sites in the Kemp-Breeze and Paul Gilbert State Wildlife Areas. The Parshall Island site was added in the Kemp-Breeze State Wildlife Area in 2019 to provide pre-construction fry estimates at multiple locations prior to habitat enhancement work that occurred on the State Wildlife Area in fall 2022 (Figure 2.1). The Colorado River below Byers Canyon had been stocked with H×C fry between 2010 and 2015, not stocked between 2016 and 2019 to allow evaluation of natural reproduction and determine if there was evidence for a self-sustaining Rainbow Trout population, and stocked with H×G fry in between 2020 and 2022 to increase Rainbow Trout recruitment in this section of the river.

Salmonid fry abundance estimates were accomplished using two Smith-Root LR-24 backpack electrofishing units running side-by-side to cover available fry habitat. Three passes were completed through each of the 50-foot study sites, and fry were removed on each pass. All salmonid fry encountered were measured (mm) and returned to the site. Fry density estimates were calculated using the three-pass removal equations of Seber and Whale (1970). In October 2022, up to ten Brown Trout and ten Rainbow Trout were collected from each of the eight fry sites to obtain myxospore counts. Myxospore enumeration was completed at the CPW Aquatic Animal Health Laboratory (Brush, Colorado).

Brown Trout fry were only encountered in and collected for myxospore enumeration from the Breeze Bridge and Parshall Island sites below Byers Canyon and the Hitching Post site above Byers Canyon in October 2022 (see fry estimate results below). Overall, Brown Trout averaged 30,148 (± 14,865) myxospores per fish, significantly higher than myxospore counts in 2020 or 2021 (Fetherman et al. 2021, 2022). Two Brown Trout of the ten collected exhibited signs of whirling disease, one fish with cranial deformities at the Parshall Island site, and one fish with opercular deformities at the Hitching Post site (myxospore counts of 153,600 and 46,900, respectively). Rainbow Trout fry, which were encountered in and collected from all eight fry sites in October 2022, averaged 989 (± 383) myxospores per fish, higher than the previous year (Fetherman et al. 2022), but similar to myxospore counts obtained in 2020 (Fetherman et al. 2021). Three Rainbow Trout fry of the 34 collected exhibited signs of whirling disease, one fish with opercular deformities at Breeze Bridge, one fish with cranial deformities at the Paul Gilbert site, and one fish with cranial, opercular, and lower jaw deformities at the Upper Red Barn site. However, myxospores were not found in any of the three fish exhibiting signs of disease suggesting that the deformities may have been genetic defects or a result of exposure to a different pathogen such as *Flavobacterium psychrophilum* in the hatchery prior to being stocked.

Brown Trout fry have historically been evenly distributed throughout the eight fry sites, with the patterns in abundance from upstream to downstream observed in 2019 (Figure 2.2) being representative of the distribution occurring over the course of the study from 2013 to 2019. In 2020, a different pattern in abundance was observed, with significant decreases in fry abundance in the sites located upstream of the Williams Fork confluence, and increases in abundance in the two sites located below the Williams Fork confluence. Although the cause of the change in Brown Trout fry abundances is unknown, draining of Windy Gap Reservoir for survey work in late 2019 may have played a role (Fetherman et al. 2021). Fine sediment from construction activities at Windy Gap Reservoir, as well as ash and sediment from major rain events occurring over the extent of the East Troublesome burn area, continued to be deposited in the Colorado River below Windy Gap Reservoir in 2021 and 2022. As a result, Brown Trout fry numbers were lower in almost all sites, including those below the Williams Fork confluence in 2022 (Figure 2.2). Overall, this has resulted in a nearly complete loss of the 2021 and 2022 age classes of Brown Trout within the study site, and potentially lower recruitment to the adult population below Byers Canyon. Flow conditions did not improve significantly in spring, summer, or fall 2022, and the fine sediment appeared to have remained in the system throughout the year, reducing cover in the form of interstitial spaces for both Brown Trout and Rainbow Trout fry throughout the system. However, runoff reached a higher peak in spring 2023 than in previous years, and during the spring adult salmonid population estimates conducted in the Chimney Rock/Sheriff Ranch section in June 2023, researchers observed less fine sediment and greater access to interstitial spaces than had been available in previous years. Although these flows may have come too late to be beneficial to the 2023 age class of Brown Trout, if fine sediment deposits do not increase significantly in the summer and fall of 2023, Brown Trout spawning may be more successful in fall 2023, with potential increase in fry numbers in 2024.

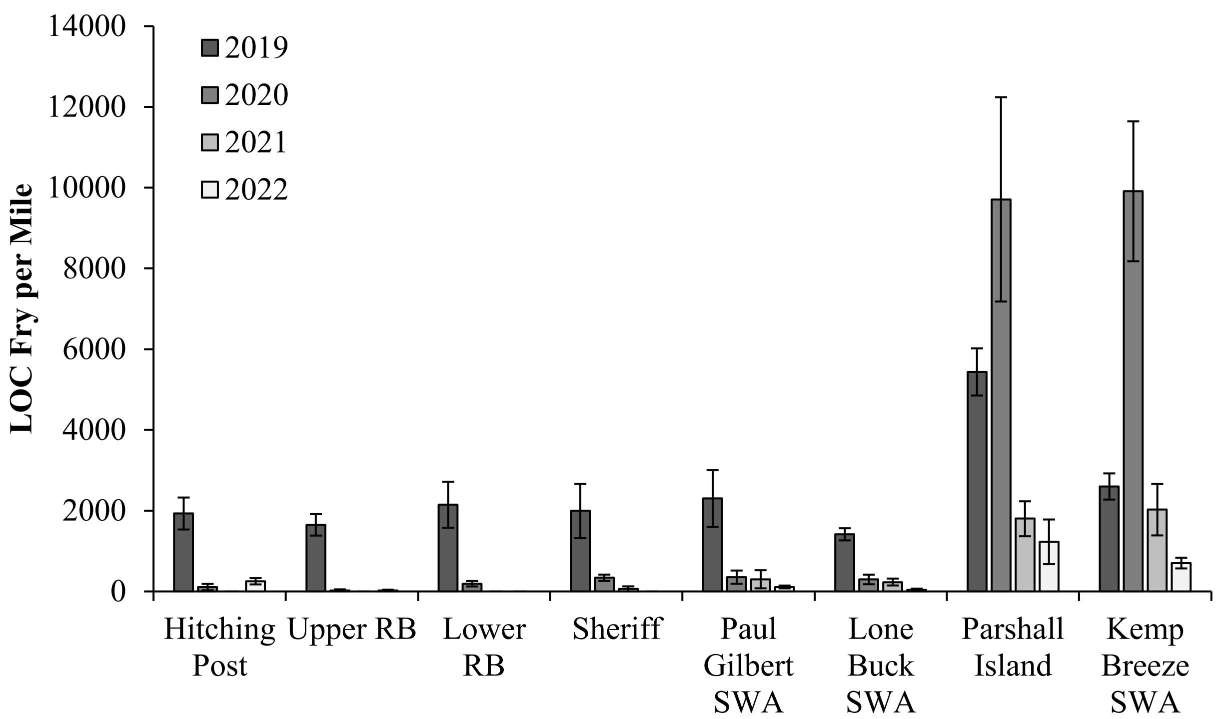

Figure 2.2. Average Brown Trout (LOC) fry abundance (fry per mile; SE bars) in 2019 (typical), 2020 (atypical), 2021 (atypical), and 2022 (atypical) between Hitching Post Bridge, the furthest upstream site, and Kemp Breeze SWA, the furthest downstream site, in the Colorado River.

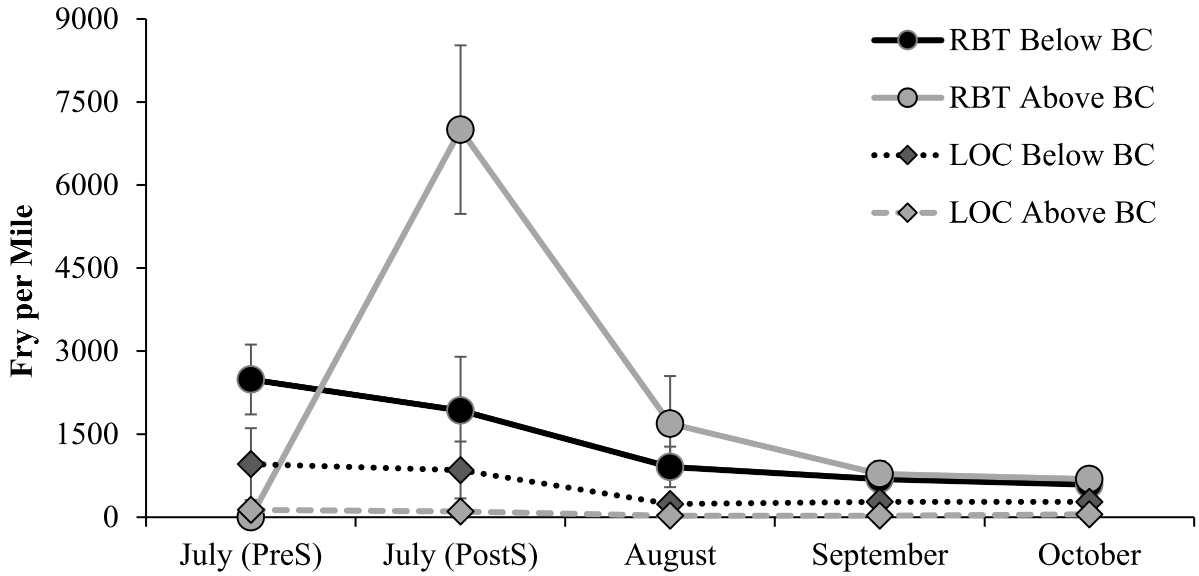

Figure 2.3. Average Rainbow Trout (RBT) and Brown Trout (LOC) fry abundance (fry per mile; SE bars) in the sites below Byers Canyon (BC; Breeze Bridge, Parshall Island, and Lower and Upper Paul Gilbert) and above Byers Canyon (Sheriff Ranch, Lower and Upper Red Barn, and Hitching Post Bridge) in 2022. H×G fry were stocked into all eight fry sites in July 2022.

A large number of wild Rainbow Trout fry were encountered prior to stocking H×G fry in the sites below Byers Canyon, but very few wild Rainbow Trout fry were encountered in the sites above Byers Canyon in 2022. It is suspected that the fine sediment observed in the river affected success of the Rainbow Trout spawn as well as the Brown Trout spawn in 2022. Rainbow Trout fry abundance was significantly increased by stocking H×G fry above Byers Canyon, but there was a significant reduction in H×G fry in the Chimney Rock/Sheriff Ranch study sites between July and August. By October, Rainbow Trout fry numbers were nearly identical above and below Byers Canyon (Figure 2.3). In addition to potentially disrupting the spawn, fry abundance estimates suggest that fine sediment accumulation in interstitial spaces throughout the Colorado River below Windy Gap Reservoir may have reduced the amount of available fry habitat and resulted in lower abundances than observed in the previous year (Fetherman et al. 2022). However, the reduced fine sediment and greater access to interstitial spaces that resulted from the higher peak runoff in spring 2023 will likely be beneficial to the H×G fry that will be stocked in the upper Colorado River later this year. The 2023 fry evaluation results will be available in the next reporting cycle.

Results obtained from the H×G fry stocking evaluations conducted between 2019 and 2021 suggest that the H×G fry appear to both survive and recruit well after being stocked in the upper Colorado River (Fetherman et al. 2020, 2021, 2022), and that H×Gs should be used to reestablish self-sustaining Rainbow Trout populations in the Colorado River and throughout the state. Although the reduced Brown Trout fry abundances continue to be a concern, the low number of Brown Trout fry and adults has likely reduced competition for the stocked H×G fry, and provided space for recruitment as these fish transition into the adult Rainbow Trout population. Given the current river conditions and performance of the H×G up to this point, H×G fry will continue to be stocked and evaluated as part of the monitoring plan associated with the Upper Colorado River Fish Movement Study through its completion in 2025.

Avila, B. W., D. L. Winkelman, and E. R. Fetherman. 2018. Survival of whirling-disease-resistant Rainbow Trout fry in the wild: A comparison of two strains. Journal of Aquatic Animal Health 30:280-290.

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2023 Adult Salmonid Population Estimates

An adult salmonid population estimate was conducted in the 3.9-mile Chimney Rock/Sheriff Ranch study section of the upper Colorado River in June 2023, with the mark run occurring on June 5, and the recapture run occurring on June 7. The adult population estimates are usually conducted in May prior to spring runoff, but earlier-than-normal high flows with low visibility due to the amount of fines in the water column caused the estimates to be moved back until after the runoff peak. Flows on the two sampling days ranged between 850 and 950 cfs. Two raft-mounted, fixed-boom electrofishing units were used to conduct the population estimates.

All fish captured on the mark run were given a caudal fin punch for identification during the recapture run, scanned for a passive integrated transponder (PIT) tag, measured (mm), and returned to the river. In addition, any Rainbow Trout encountered on the mark run that did not have a tag, were PIT tagged, adipose clipped, caudal fin punched, measured, and weighed. On the recapture run, fish were examined for the presence of a caudal fin punch and PIT tag, measured (mm), and weighed (g). On both runs, Brown Trout and Rainbow Trout that had been previously PIT tagged but had lost their tag, indicated by an adipose clip but no PIT tag upon scanning, were re-tagged, measured (mm), and weighed (g). Population estimates were calculated using the Lincoln-Peterson estimator with a Bailey (1951) modification, which accounted for fish being returned to the population following examination of marks on the recapture run, making them potentially available for subsequent recapture.

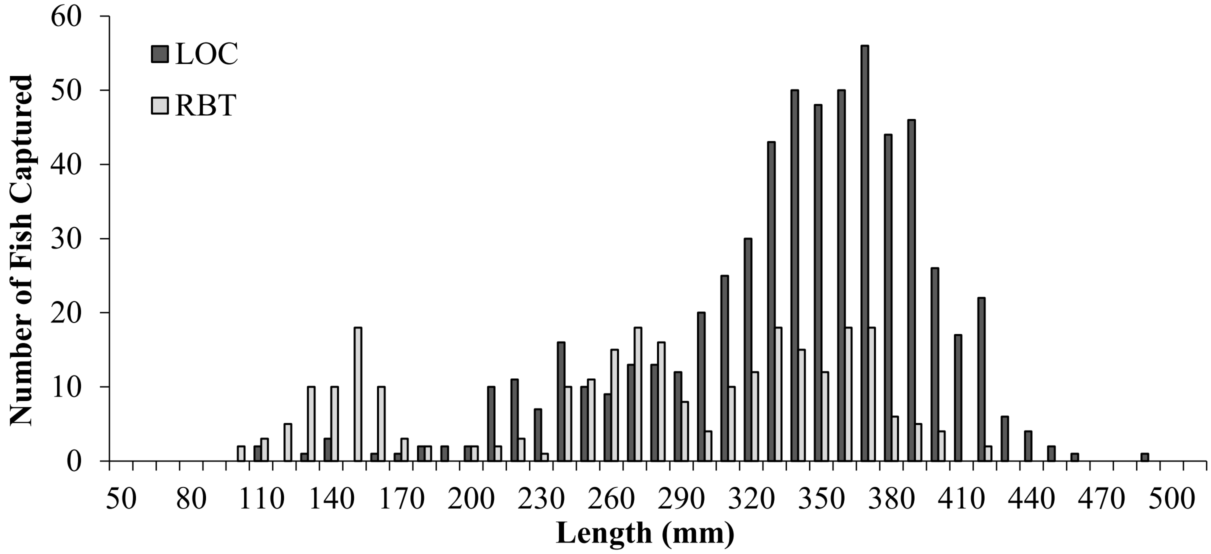

Figure 2.4. Number of Brown Trout (LOC) and Rainbow Trout (RBT) captured by total length (mm) during the 2023 adult salmonid population estimates in the Chimney Rock/Sheriff Ranch study section of the upper Colorado River.

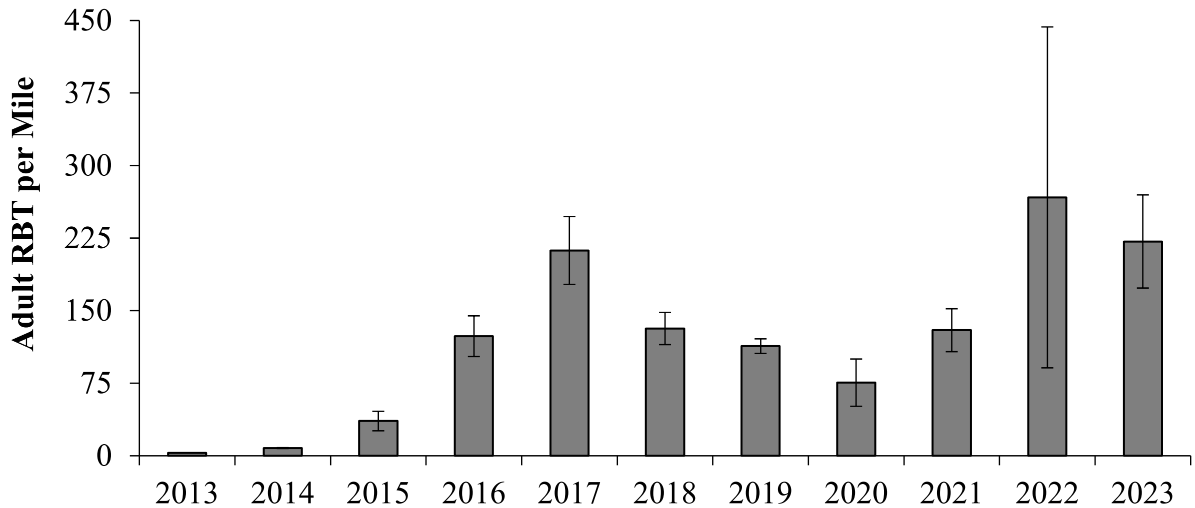

Figure 2.5. Estimated number of adult Rainbow Trout (RBT) per mile (SE bars) in the Chimney Rock/Sheriff Ranch study section of the upper Colorado River between 2013 and 2023.

An estimated 1,826 (± 212) adult Brown Trout were present in the Chimney Rock/Sheriff Ranch study section in 2023, approximately 4,160 less than in 2022 (Fetherman et al. 2022). Overall, only 468 (± 54) Brown Trout were present per mile in the study section. Adult Brown trout averaged 326 (± 55) mm TL and 364 (± 163) g. A small number of all age classes of Brown Trout ≥ 150 mm TL were represented in the sample, but the majority of the Brown Trout captured were age 3+ (Figure 2.4). Numbers of brown Trout fry and juveniles captured during the population estimates remained low, reflecting the trends observed in the fry estimates conducted in 2020 through 2022. This estimate represents one of the most significant declines in the Brown Trout population, as well as the lowest population estimates obtained from the study reach, since 1981. The cause of the reduction in the population is unknown, but is likely a combination of a lack of recruitment over the previous three years, and high spring flows with a lot fine sediment and ash from the East Troublesome Fire that may have resulted in mortality or migration out of the study reach.

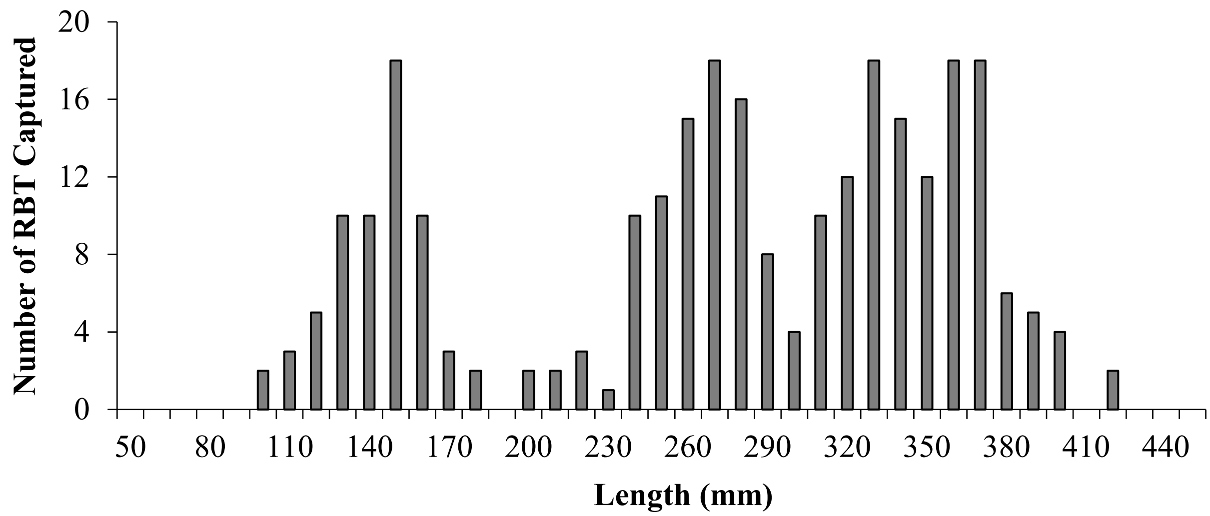

Figure 2.6. Number of Rainbow Trout (RBT) captured by total length (mm) during the 2023 adult salmonid population estimates in the Chimney Rock/Sheriff Ranch study section of the upper Colorado River.

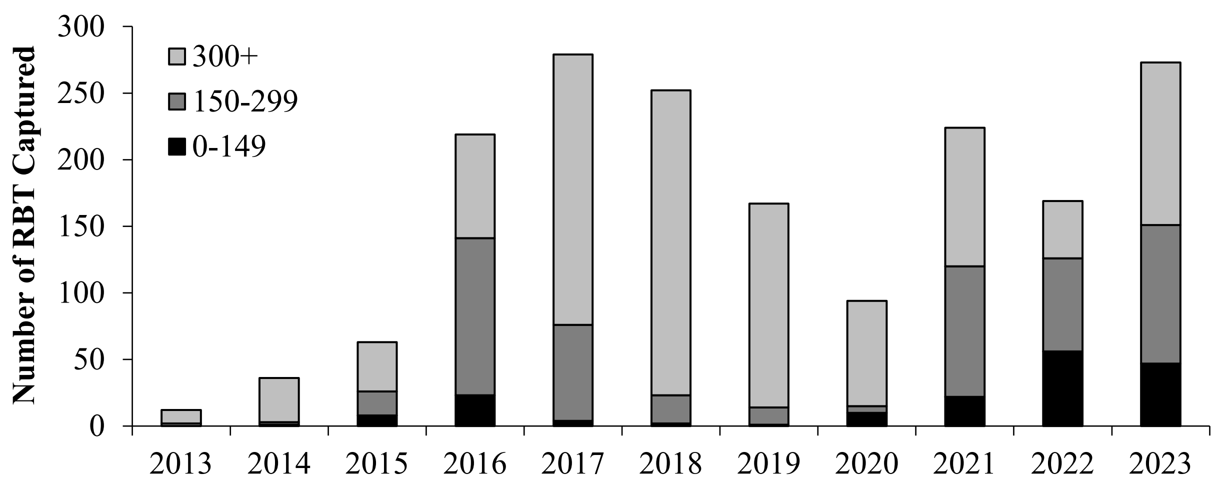

Figure 2.7. Number of age-1 (≤ 149 mm TL), age-2 (150-299 mm TL) and age-3+ (> 300 mm TL) Rainbow Trout (RBT) captured in the Chimney Rock/Sheriff Ranch study section of the upper Colorado River between 2013 and 2023.

Rainbow Trout abundance decreased, but within the range of error, between 2022 and 2023, with an estimated 1,041 (± 686) adult Rainbow Trout present in 2022 (Fetherman et al. 2022), and

864 (± 188) present in 2023. This resulted in an estimated 222 (± 48) adult Rainbow Trout per mile in the Chimney Rock/Sheriff Ranch study site (Figure 2.5). Adult Rainbow Trout averaged 297 (± 81) mm TL and 314 (± 154) g, and all age classes of Rainbow Trout were well represented in the sample (Figure 2.6). Although not recruited to the gear used, a number of age-1 Rainbow Trout were captured during the population estimates, suggesting that even though H×G fry numbers were lower in 2022 than in previous years, fry remaining in October 2022 had overwintered well. In addition, the age-2 and age-3+ age classes were also larger than the previous year, suggesting that survival of the H×Gs was high once they had recruited to the adult population (Figure 2.7), potentially a result of relaxed competition and predation due to the lower adult Brown Trout abundance.

In 2021, the adult Rainbow Trout population in the upper Colorado River exhibited an increase in abundance for the first time since 2017, which carried over into 2022 and 2023, with the 2023 estimates being significantly higher than the last population estimate conducted by raft in 2021. Abundance did not increase between 2022 and 2023, but it also did not exhibit a similar large decrease as the Brown Trout population. Survival of the H×Gs appears to be high once the fry have recruited beyond age one. With fewer Brown Trout present within the study reach, relaxed competition and predation should lead to higher survival in 2023. With the reduced Brown Trout abundances, Rainbow Trout now constitute one-third of the total salmonid population in the Colorado River, a ratio that has not been observed since whirling disease was established in the river in the mid-1990s. Adult population sampling will continue through completion of the Upper Colorado River Fish Movement Study in 2025, and 2024 spring population estimate results will be available in the next reporting cycle.

Bailey, N. T. J. 1951. On estimating the size of mobile populations from recapture data. Biometrika 38:293-306.

Fetherman, E. R., B. Neuschwanger, and T. B. Riepe. 2022. Sport Fish Research Studies. Annual Report. Colorado Parks and Wildlife, Aquatic Research Section. Fort Collins, Colorado.

UPPER COLORADO RIVER FISH MOVEMENT STUDY

The Upper Colorado River Fish Movement Study is being conducted in conjunction with and as a part of the Upper Colorado River Headwaters Projects Monitoring Plan. The fish movement study focuses specifically on fish use of the connectivity channel to be constructed around Windy Gap Reservoir, reconnecting the Colorado and Fraser rivers upstream of the reservoir with the Colorado River downstream of the reservoir for the first time in decades. Experimental design and timelines for the study were approved by all interested parties involved in the Upper Colorado River Headwaters Monitoring Plan in 2019, and the final draft of the study proposal can be found in Fetherman et al. (2020). The following describes the steps taken to implement the Upper Colorado River Fish Movement Study within the last year.

Fetherman, E. R., B. Neuschwanger, B. W. Avila, and T. B. Riepe. 2020. Sport Fish Research Studies. Annual Report. Colorado Parks and Wildlife, Aquatic Research Section. Fort Collins, Colorado.

Population Estimates and Tag Releases in the Fraser and Colorado Rivers

Two-pass removal population estimates were conducted in the Fraser River on the Fraser River Ranch and in Kaibab Park on August 30 and 31, and in two stations on the City of Granby property at River Run in the Colorado River on October 4, 2022. A bank electrofishing unit was used to complete the surveys in each location. Fish were held in separate net pens by pass, measured (mm), weighed (g), and a large portion of untagged fish were tagged with a 32 or 12 mm tag, dependent upon size. Fish were anesthetized prior to tagging using AQUI-S 20E, administered with permission and oversight from the US Fish and Wildlife Service Investigational New Animal Drug (INAD) program and CPW aquatic veterinarian Colby Wells. PIT tags were inserted posterior of the pectoral fin through the midventral body wall into the peritoneal cavity via a hypodermic needle (Prentice et al. 1990; Acolas et al. 2007). All fish were adipose clipped to indicate they had been tagged as part of the fish movement study, and to identify and quantify tag loss. Fin-clipped fish from previous tagging events were measured and weighed, and PIT tag numbers were recorded. If a recaptured fish lost their tag, they received a new tag prior to release. Fish were given time to recover in the net pens before being returned to the river. Any mortalities that occurred from the tagging procedure were scanned for a PIT tag number and removed from the released fish dataset. Population estimates were calculated using the two-pass removal equations of Seber and Whale (1970).

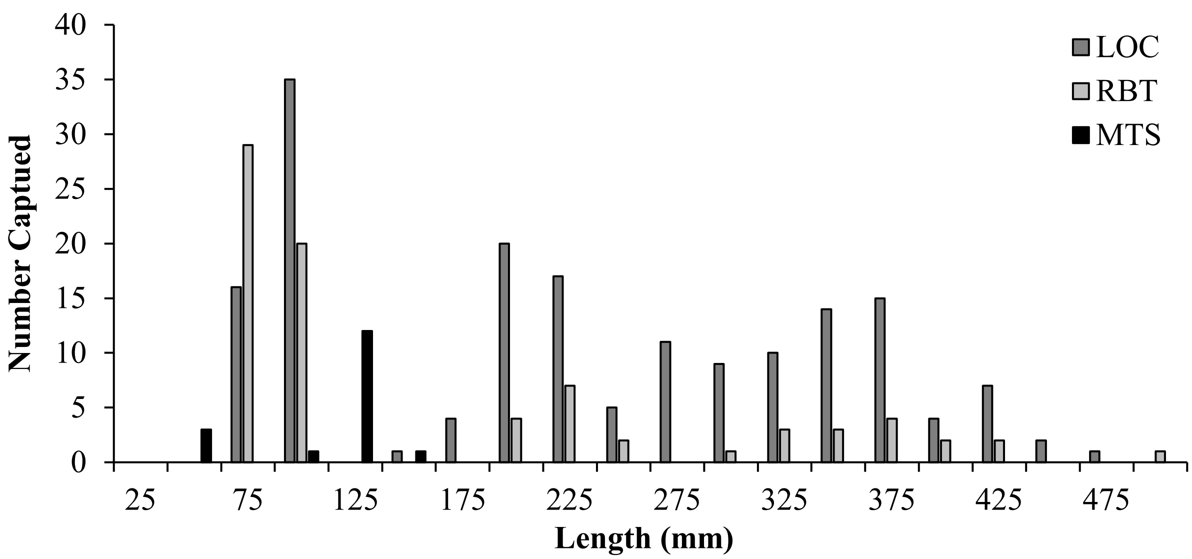

Figure 2.8. Number of Brown Trout (LOC), Rainbow Trout (RBT), and Mottled Sculpin (MTS) captured by total length (mm) during the Fraser River Ranch 2022 population estimate.

The 677-foot station located about 0.25 miles upstream of the railroad crossing at the lower end of the Fraser River Ranch contained 171 Brown Trout, 119 adults (≥ 150 mm total length [TL]) and 53 fry/juveniles (< 150 mm TL; Figure 2.8). An estimated 1,543 ± 94 Brown Trout per mile were present on the ranch, 1,047 ± 65 adult Brown Trout per mile and 505 ± 81 Brown Trout fry/juveniles per mile. More Brown Trout fry/juveniles and adults were present in 2022 compared to 2021 (Fetherman et al. 2022). Adult Brown Trout averaged 282 ± 7 mm TL and 280 ± 19 g, with the largest measuring 465 mm TL and weighing 912 g. Brown Trout outnumbered Rainbow Trout in the total catch, with 79 Rainbow Trout captured, 30 adults and 49 fry/juveniles (Figure 2.8). An estimated 666 ± 36 Rainbow Trout per mile were present on the ranch, 243 ± 13 adult Rainbow Trout per mile and 427 ± 39 Rainbow Trout fry/juveniles per mile. Fewer fry/juvenile and adult Rainbow Trout were present in 2022 compared to 2021, however, especially fry/juvenile estimates were more precise than they had been in the previous year (Fetherman et al. 2022). Adult Rainbow Trout averaged 305 ± 18 mm TL and 352 ± 57 g, with the largest measuring 575 mm TL and weighing 1,399 g. Seventeen Mottled Sculpin *Cottus bairdii* were captured in the site. An estimated 260 ± 250 Mottled Sculpin per mile were present on the ranch in 2022. Mottled Sculpin averaged 110 ± 2 mm TL and 16 ± 2 g. Longnose Sucker *Catostomus catostomus* (1,529 ± 2,362 per mile), Creek Chub *Semotilus atromaculatus* (490 ± 15 per mile), Speckled Dace *Rhinichthys osculus* (663 ± 94 per mile), Iowa Darter *Etheostoma exile* (35 ± 12), White Sucker *Catostomus commersonii* (8 ± 0 per mile), and Fathead Minnow *Pimephales promelas* were also captured on the Fraser River Ranch.

Twenty-four Brown Trout and nine Rainbow Trout were recaptured on the Fraser River Ranch in 2022. This represented a recapture rate of 13.8% and 10.8% of the Brown Trout and Rainbow Trout released on the ranch in 2020 and 2021, and 1.8% and 0.7% of the total 1,325 fish (all species) released above Windy Gap Reservoir in 2020 and 2021. Of those, three Brown Trout and three Rainbow Trout had lost their tags, a tag loss rate of 12.5% and 33.3% for the two species, respectively, and were retagged prior to release. Most of the fish recaptured had been released on the ranch the year prior. However, four Brown Trout and Two Rainbow Trout had been released on the ranch in 2020, and were recaptured in both 2021 and 2022. An additional Brown Trout and Rainbow Trout each had been released on the ranch in 2020, lost their tags and were retagged in 2021, and recaptured in 2022. Lastly, one recaptured Brown Trout had been released below the Red Barn antenna on the Chimney Rock Ranch in May 2021, and had passed through both Windy Gap dam and over the Fraser Gauge prior to being recaptured. This was the first physical recapture of a fish that had passed upstream through Windy Gap dam, although fish had been detected doing so with both the stationary Confluence antenna and mobile antennas. Brown Trout grew an average of 56 ± 4 mm TL and 197 ± 14 g since their last recapture, while Rainbow Trout grew an average of 42 ± 11 mm TL and 145 ± 20 g. One Mottled Sculpin was recaptured on the Fraser River Ranch in 2022 which had been previously tagged in September 2021, a recapture rate of 2.1% of the sculpin released on the ranch in 2020 and 2021, and 0.1% of all fish released above Windy Gap Reservoir in 2020 and 2021. The sculpin had grown 20 mm TL and 8 g. A total of 82 new fish were PIT tagged in the Fraser River Ranch, 50 Brown Trout, 19 Rainbow Trout, and 13 Mottled Sculpin. Mottled Sculpin (92 to 127 mm TL) were tagged with 12 mm PIT tags, whereas the Brown Trout (144 to 420 mm TL) and Rainbow Trout (186 to 412 mm TL) were tagged with 32 mm PIT tags.

A total of 149 Brown Trout, 101 adults and 48 fry/juveniles, were captured in the 643-foot electrofishing station in Kaibab Park. The majority of the Brown Trout captured were age-2 fish (Figure 2.9). Kaibab Park contained an estimated 1,253 ± 22 Brown Trout per mile, 865 ± 26 adult Brown Trout per mile and 395 ± 4 Brown Trout fry/juveniles per mile. The abundance of Brown Trout in Kaibab Park was higher in 2022 than in 2021 (Fetherman et al. 2022), exhibiting a recovery from the disturbances caused by construction activities conducted in 2021 immediately upstream of the site to make the Highway 40 diversion more fish passable. Adult Brown Trout averaged 210 ± 5 mm TL and 112 ± 9 g, with the largest measuring 347 mm TL and weighing 396 g. Many fewer Rainbow Trout were captured in Kaibab Park than on the Fraser River Ranch, with only six Rainbow Trout captured (Figure 2.9). An estimated 49 ± 0 Rainbow Trout per mile were present in Kaibab Park, 33 ± 0 adult Rainbow Trout per mile and 16 ± 0 Rainbow Trout fry/juveniles per mile. Adult Rainbow Trout averaged 240 ± 32 mm TL and 143 ± 47 g, with the largest measuring 313 mm TL and weighing 257 g. One hundred four Mottled Sculpin were captured in Kaibab Park, providing an estimate of 899 ± 31 Mottled Sculpin per mile. Mottled Sculpin averaged 90 ± 1 mm TL and 12 ± 1 g. Longnose Sucker (1,039 ± 28 per mile), Creek Chub, Speckled Dace (300 ± 8 per mile), Fathead Minnow (59 ± 5 per mile), and Iowa Darter (66 ± 0 per mile) were also captured in Kaibab Park.

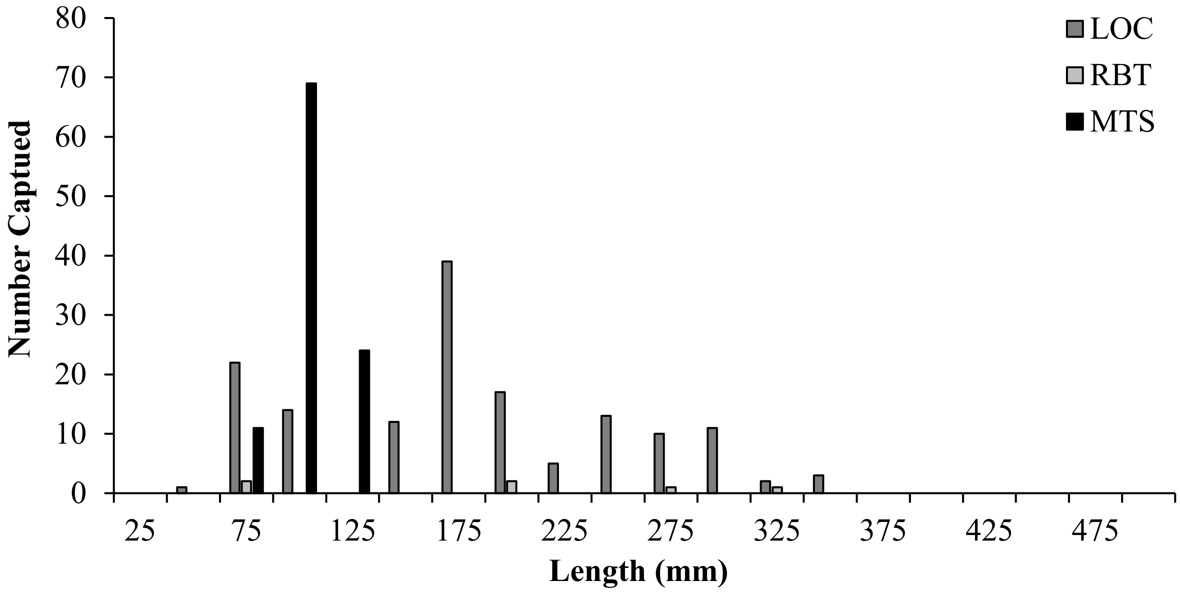

Figure 2.9. Number of Brown Trout (LOC), Rainbow Trout (RBT), and Mottled Sculpin (MTS) captured by total length (mm) during the Kaibab Park 2022 population estimate.

One hundred twenty-six new fish were PIT tagged in Kaibab Park, 67 Brown Trout, three Rainbow Trout, and 56 Mottled Sculpin. Mottled Sculpin (78 to 118 mm TL) were tagged with 12 mm PIT tags, whereas the Brown Trout (132 to 323 mm TL) and Rainbow Trout (181 to 313 mm TL) were tagged with 32 mm PIT tags. Thirty-three Brown Trout, one Rainbow Trout, and six Mottled Sculpin were recaptured in Kaibab Park in 2022, a recapture rate of 14.8%, 9.1%, and 3% of the Brown Trout, Rainbow Trout, and Mottled Sculpin released in Kaibab Park in 2020 and 2021, and 2.5%, 0.1%, and 0.5% of the 1,325 fish released above Windy Gap Reservoir in 2020 and 2021. Of those, two Brown Trout and the one Rainbow Trout had lost their tags, a tag loss rate of 6.1% and 100%, respectively, and were retagged prior to release. Nine Brown Trout had been released in Kaibab Park in 2020, and were recaptured in both 2021 and 2022. An additional four Brown Trout and two Mottled Sculpin had been released in 2020, but not recaptured until 2022, whereas the remainder of the recaptured fish had been released in the previous year. Brown Trout grew an average of 61 ± 5 mm TL and 98 ± 8 g since their last recapture, while Mottled Sculpin grew an average of 14 ± 2 mm TL and 6 ± 1 g. No growth information was available for the recaptured Rainbow Trout due to tag loss.

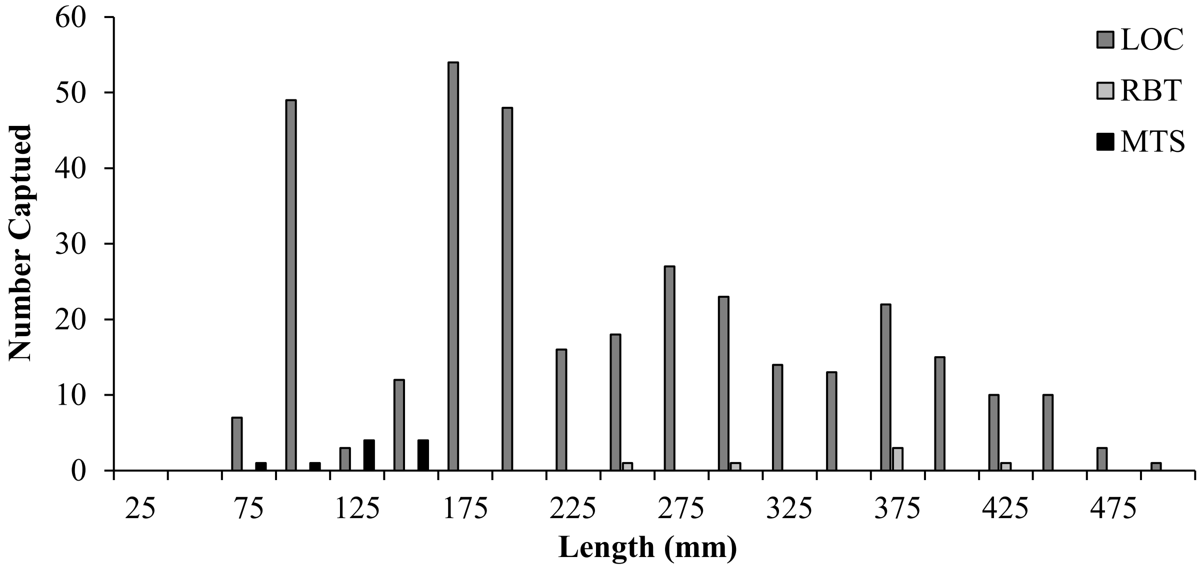

Figure 2.10. Number of Brown Trout (LOC), Rainbow Trout (RBT), Mottled Sculpin (MTS) and cutbows (RXN) captured by total length (mm) during the 2022 River Run population estimates.

Two sites were sampled in the River Run section of the Colorado River, a 630 foot site located downstream of the River Run bridge, and a 493 foot site located upstream of the bridge. The data from both sites were combined for the purposes of this summary. The River Run sites contained 346 Brown Trout, 275 adults and 71 fry/juveniles (Figure 2.10). An estimated 1,809 ± 86 Brown Trout per mile were present in River Run, 1,391 ± 53 adult Brown Trout per mile and 592 ± 463 Brown Trout fry/juveniles per mile. The average number of Brown Trout fry/juveniles and adults per mile decreased between 2021 and 2022 (Fetherman et al. 2022). Adult Brown Trout averaged 264 ± 6 mm TL and 253 ± 16 g, with the largest measuring 560 mm TL and weighing 1,894 g. Only seven adult Rainbow Trout were captured in 2022 (Figure 2.10), for an estimated 33 ± 0 adult Rainbow Trout per mile. Adult Rainbow Trout averaged 390 ± 51 mm TL and 778 ± 359 g, with the largest measuring 663 mm TL and weighing 2,884 g. Only ten Mottled Sculpin were captured within the two sites, providing an estimate of 206 ± 152 Mottled Sculpin per mile. Mottled Sculpin averaged 115 ± 6 mm TL and 23 ± 3 g. Longnose Sucker, White Sucker (56 ± 25 per mile), Speckled Dace (443 ± 61 per mile), Creek Chub, Fathead Minnow (20 ± 6 per mile), and Iowa Darter (4 ± 0 per mile) were also captured in River Run.

Twenty-five Brown Trout and four Mottled Sculpin were recaptured in the River Run reach of the Colorado River in 2022, a recapture rate of 6.2% of the Brown Trout and 3.4% of Mottled Sculpin released in River Run in 2020 and 2021. This equated to 1.9% and 0.4%, respectively, of the 1,325 fish released above Windy Gap Reservoir in 2020 and 2021. No Rainbow Trout were recaptured in the River Run reach in 2022. Recapture rates were likely lower for all species due to cloudy water conditions resulting from fine sediment and ash being released from Willow Creek Reservoir during the sampling. Of the fish recaptured, seven Brown Trout had lost their tags, a tag loss rate of 28%, and were retagged prior to release. Nine Brown Trout had been released in River Run in 2020, and recaptured in both 2021 and 2022, of which, two had lost their tag and had been retagged in 2021. Three Brown trout had been tagged in River Run in 2020 and not recaptured until 2022, while the remainder had been released the previous year. One Brown Trout had been released in Kaibab Park in 2020, representing one of the few fish tagged in Kaibab Park that had moved to another study site, as well as changed rivers above Windy Gap. Two Mottled Sculpin had been released in the River Run reach in 2020 and not recaptured until 2022, whereas the other two had been released the previous year. Brown Trout grew an average of 53 ± 9 mm TL and 150 ± 27 g since their last recapture, while Mottled Sculpin grew and average of 9 ± 2 mm TL and 6 ± 3 g. A total of 183 new fish were PIT tagged in River Run, 172 Brown Trout, seven Rainbow Trout, and four Mottled Sculpin. Mottled Sculpin (91 to 124 mm TL) were tagged with 12 mm PIT tags, whereas the Brown Trout (147 to 560 mm TL), and Rainbow Trout (242 to 663 mm TL) were tagged with 32 mm PIT tags.

In summary, 391 fish were PIT tagged in the Fraser and Colorado rivers above Windy Gap Reservoir in 2022. The goal had been to release a minimum of 250 tagged fish of each species. We met this goal for Brown Trout with 289 tagged, but did not meet this goal with Mottled Sculpin or Rainbow Trout, with only 29 and 73 tagged of each, respectively. All Rainbow Trout and Mottled Sculpin captured in the four sampling sites were tagged; the inability to meet this goal was subject to the availability of these species in the sites in 2022. Since the beginning of the study, 1,716 fish have been tagged and released in the Fraser and Colorado rivers above Windy Gap Reservoir.

One hundred nineteen new fish were PIT tagged with 32 mm tags immediately downstream of Windy Gap Reservoir in October 2022, 93 Brown Trout (177 to 575 mm TL) and 26 Rainbow Trout (139 to 402 mm TL). Twelve Brown Trout were recaptured downstream of Windy Gap Dam, a recapture rate of 6.6% of the fish released in the site in 2020 and 2021, and 0.5% of the 2,422 fish (all species) released below Windy Gap Reservoir. Of those, three had lost their tag, a tag loss rate of 25%, and were retagged prior to release. One of the recaptured Brown Trout had been released in the site in 2020 but not recaptured until 2022, one had been released below the Red Barn diversion structure in 2021, and the remainder had been released within the site in 2021. Recaptured Brown Trout grew an average of 40 ± 11 mm TL and 85 ± 51 g since their last recapture.

Fifty-two Brown Trout and 17 Rainbow Trout were recaptured during the adult salmonid population estimates conducted in the Chimney Rock/Sheriff Ranch study section of the Colorado River below Windy Gap Reservoir in June 2023. This represents a recapture rate of 2.5% of Brown Trout and 3.6% of Rainbow Trout previously released below Windy Gap Dam. Of the fish captured, 27 Brown Trout and nine Rainbow Trout had lost their tags, a tag loss rate of 52% and 53%, respectively, and were retagged prior to release. Two Brown Trout that had lost their tags and were retagged in 2022 were recaptured in 2023. One Rainbow Trout had been tagged at 108 mm TL and 16 g with a 12 mm PIT tag in 2020, and was recaptured both in 2022 and 2023, having grown to 307 mm TL and 359 g. Only one other Brown Trout and one other Rainbow Trout that had been tagged in 2020 were recaptured in 2023, otherwise, all other Brown Trout and Rainbow Trout recaptures had been released in the site in either 2021 or 2022 and were recaptured for the first time in 2023. Brown Trout grew an average of 44 ± 6 mm TL and 168 ± 20 g since their last recapture, while Rainbow Trout grew and average of 83 ± 27 mm TL and 208 ± 30 g. Only Rainbow Trout were targeted for new PIT tags on this sampling occasion to increase their numbers within the study section. Seventy-six new Rainbow Trout (152 to 411 mm TL) were tagged with 32 mm PIT tags during the population estimates conducted in 2023.

In summary, 195 fish were PIT tagged in the Colorado River below Windy Gap Reservoir in fall 2022 and spring 2023, bringing the total number of tagged fish released below the reservoir since the beginning of the study to 2,617. By design, fewer fish were tagged below Windy Gap Reservoir in spring 2023. The pre-construction phase of the fish movement study was completed following the release of PIT tagged fish below Windy Gap Dam in October 2022. The Colorado River Connectivity Channel (CRCC) is scheduled to be completed in fall 2023, with the majority of 2023 representing the construction phase of the study. The post-construction phase of the study will begin with the release of PIT tagged fish in the Fraser River in September 2023, which will occur only a week or two prior to the installation of stationary antennas in the CRCC. From there, PIT tag releases will follow a similar pattern to those made during the pre-construction phase of the study. More information regarding these PIT tag releases, with an emphasis on recaptured of fish that moved through the CRCC, will be available in the next reporting cycle.

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Stationary Antenna Installations, Performance Evaluations, and Data Collection

Stationary antennas used to detect the movements of PIT-tagged fish were installed at three sites in the Colorado River in 2020: 1) immediately downstream of the confluence of the Fraser and Colorado rivers above Windy Gap Reservoir on Northern Water property (Confluence; CF), 2) just downstream of the Hitching Post (CR 57) bridge on the Chimney Rock Ranch (Hitching Post; HP), and 3) in the Red Barn area of the Chimney Rock Ranch upstream of the Red Barn diversion structure (Red Barn; RB). Two portable Biomark wagon wheel antennas were additionally deployed downstream of Windy Gap Reservoir and upstream of the Fraser River diversion structure below Highway 40 in September-November 2022. The wagon wheel antenna below Windy Gap Reservoir was deployed in the Schmuck channel as the majority of the flow out of the reservoir was coming through this channel. Additionally, this helped avoid potential damage to the antenna from construction activities occurring below the dam, including the construction of a temporary road that essentially blocked fish access to the dam outlet in fall 2022. The wagon wheel antenna in the Fraser River was deployed to detect fish that successfully passed the new diversion structure installed below Highway 40 in 2021, and was put in the same location in fall 2022. Due to the need to change batteries and download data from these portable antennas every two weeks, they were only deployed only during the fall Brown Trout spawning period when fish activity was expected to be high. Wagon wheel antennas were not deployed in spring 2023 to reduce data collection and field personnel requirements during the construction phase of the fish movement study, and allow researchers to focus on other parts of the project (e.g., bird predation and ghost tags).

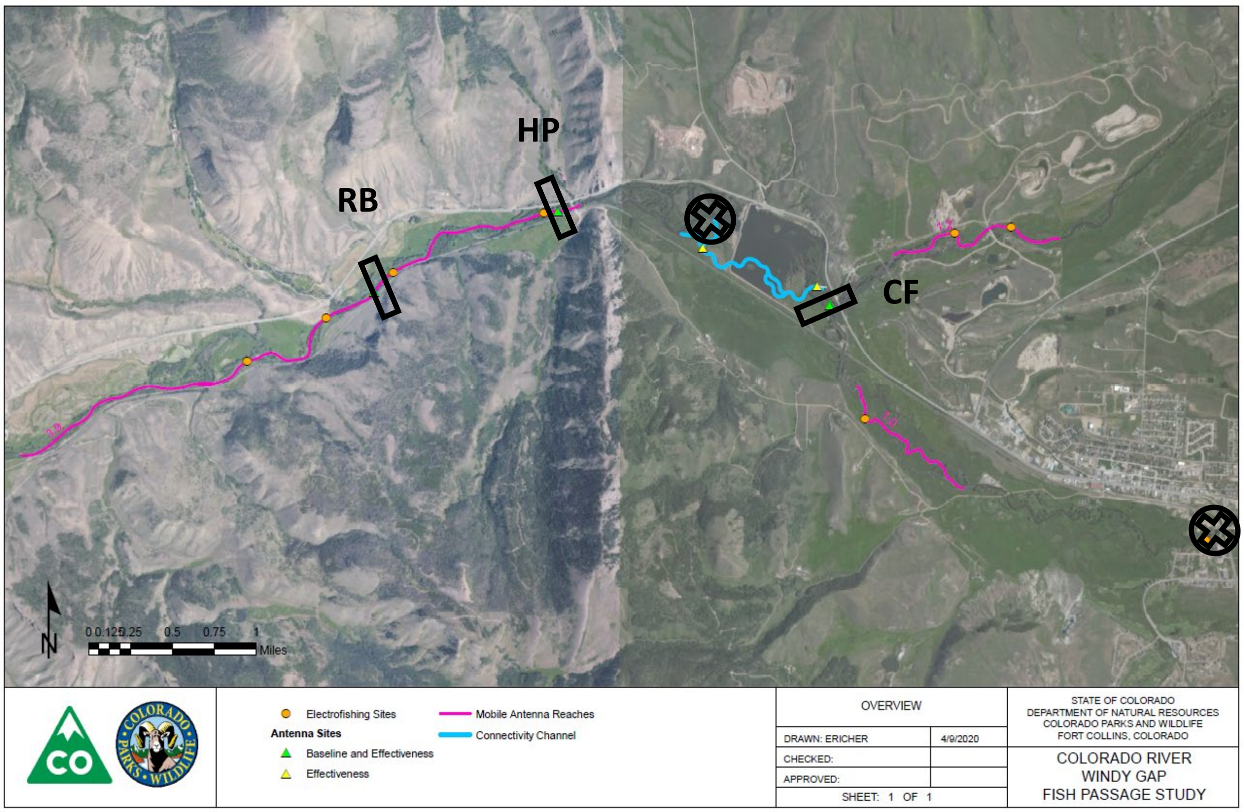

Figure 2.11. Locations of the Red Barn (RB), Hitching Post (HP), and Confluence (CF) stationary antenna stations (rectangles), and the portable Biomark wagon wheel antennas (circle with X) temporarily deployed downstream of Windy Gap Reservoir and upstream of the Fraser River diversion structure below Highway 40.

Releases of a large volume of water from Willow Creek Reservoir due to a pump failure caused trees and other large woody debris to be dislodged from a beaver dam complex located in the Colorado River just upstream of the Confluence antenna in spring 2022. Several of these trees got hung up on the downstream antenna (CF5) at the Confluence site, fracturing the protective PVC housing around the antenna wire and resulting in the wire loop being broken. Due to water levels, as well as scheduling, researchers were not able to get out and repair the antenna until August 2022. Repair of the antenna required disconnection from the antenna tuner boxes and complete removal of the river spanning PVC housing around the antenna wire. Similar to the installation of antennas at the beginning of the study in 2020 (Fetherman et al. 2021), the PVC housing was repaired on-shore, reusing as much of the original unbroken structure as possible. Antenna wire was run through the PVC prior to gluing all of the new pieces together. Following completion of the repairs, a crew of about twelve people was utilized to lift and support the antenna as it was taken back into the river and reinstalled in its original location. The crew utilized as many of the duckbill anchors holding down the original antenna as possible, and new anchors were driven in where they had broken or additional support was needed to hold the antenna closer to the substrate. The antenna was secured to the substrate and duckbill anchors using both river and ratchet straps. After reconnecting the wire to the tuning boxes and readers, the antenna was tuned to optimize read range. Although no data were collected between May and August on the downstream antenna of the pair, the upstream antenna continued to collect fish movement data during this time. Both antennas have been operating continuously since the downstream antenna was repaired and reinstalled in August 2022.

Antenna detection distances were measured at stationary antenna sites in summer 2022 following reinstallation of the damaged Confluence antenna to determine if vertical detection distances exceeded average water depth. Detection distances were measured using a 32 or 12 mm PIT tag on a PVC stick run perpendicular to the antenna wire (optimal tag orientation and most likely orientation of a fish crossing the antenna). The tag was raised from the antenna until an audible beep from the reader, indicating detection, was no longer heard. The tag was then lowered back down towards the antenna until beeping resumed. The distance from the antenna to the tag was measured (tenths of feet), and a measurement of 0.2 feet was added to account for the distance from the top of the PVC to where the wire sat on the bottom of the pipe. Previous work had revealed that antenna detection distances did not differ between upstream and downstream sides of the antenna (Fetherman et al. 2020), so detection distances, along with water depth and velocity measurements, were taken every ten feet along the upstream side of each antenna only.

Velocities in summer 2022 averaged 0.50 ± 0.15, 0.46 ± 0.18, and 0.58 ± 0.12 m/s at the Red Barn, Hitching Post, and Confluence sites, respectively. On average, velocities did not significantly exceed 0.50 m/s, the maximum velocity measured by Fetherman (2013) at which detection probability remained 1.0. Detection distances for a 32 mm PIT tag in summer 2022 ranged between an average of 1.42 and 1.90 feet, and for a 12 mm PIT tag between 0.36 and 0.58 feet. Detection distances for both 32 and 12 mm PIT tags were similar between the two antennas at the Hitching Post and Red Barn sites, and between the two sites. Confluence showed the largest discrepancy in detection distances within a site, with CF5, the antenna that was recently replaced, having greater detection distances for both tag sizes relative to CF6. Detection distance for the 32 mm PIT tags was similar to or exceeded the average water depth at each antenna, suggesting full coverage of the water column. Read ranges for the 12 mm PIT tags were significantly lower than the water depth, with the exceptions of the HP3 and CF5 antennas (Figure 2.12). However, 12 mm PIT tags were primarily used to tag Mottled Sculpin. Given their sedentary nature and the likelihood that movement occurs near the substrate, 12 mm PIT tag detection distances should be sufficient for detecting Mottled Sculpin movements. Overall, preliminary detection distance results continue to suggest that detection probability should be high for both salmonids and Mottled Sculpin at all antennas. A more formal analysis of detection probability will be completed using the long-term tag detection data set towards the end of the fish movement study.

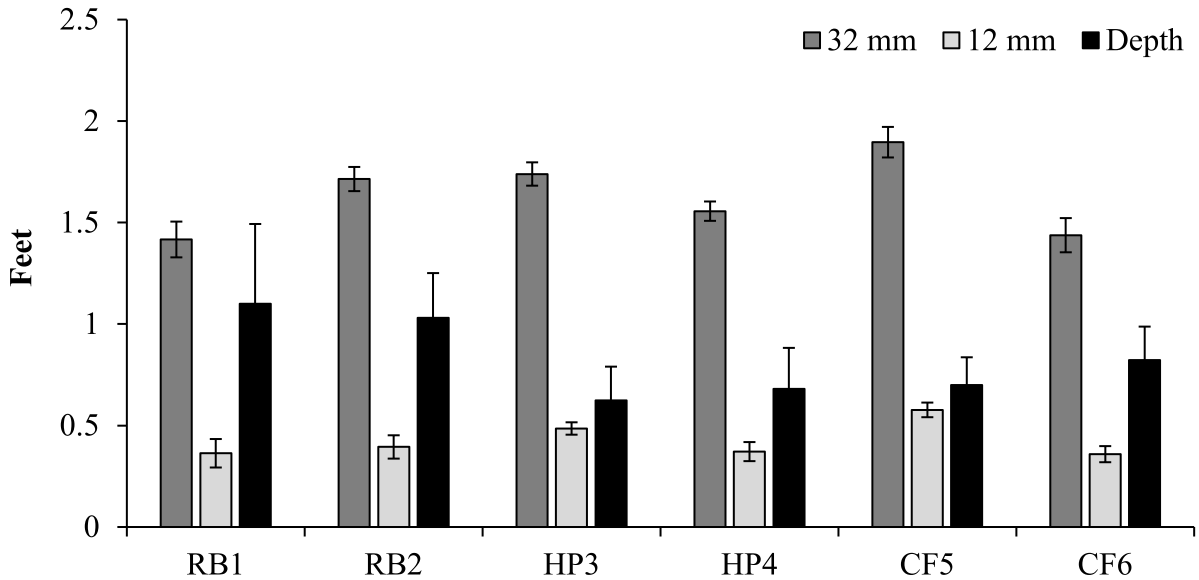

Figure 2.12. Detection distances for 32 mm and 12 mm PIT tags and water depths (feet; 2 SE bars) for the two antennas located at Red Barn (RB), Hitching Post (HP), and Confluence (CF) in summer 2022.

Flows in spring and summer 2023 were higher than those experienced since the fish movement study began in 2020. High flows have caused additional damage and issues with continuous operation of the antenna stations. At the Hitching Post site, a large cottonwood tree got hung up on the lower antenna (HP3). Although flows have precluded an opportunity to fully assess the damage, it appears that the tree broke the PVC housing and is pulling on the antenna wire, but the wire has not broken. Strain on the wire pulled it out from the tuning box, but only by a few feet. Despite not having a complete loop of wire, the reader was still recording a small number of tag detections, but significantly fewer than the intact loop of the upstream antenna (HP4). On June 6, an additional length of 1/0 wire was soldered onto the existing wire to complete the loop. After completing this repair, the antenna was retuned, and began reading tags within the detection field as normal. Unfortunately, after the repair, flows increased again in the Colorado River, and it is currently unknown whether more damage to the antenna has occurred. High flows also threatened to flood the battery and reader boxes at the Red Barn antenna site, however assessment of the site after flows receded showed that no damage to the site due to high flows had occurred.

With the exceptions mentioned above, antennas have been operating continuously since late August 2020, and have collected tens of thousands of data points from moving fish. Additional data points have been obtained from marker tags located at each antenna, which reveal a tag with a known number every 15 minutes. These marker tag detections allow researchers to determine if there are gaps in operation and tag detection (e.g., due to power failure) in the time between visits to the stations. Data are downloaded from the readers once a month, at which time antennas are visually inspected and cleared of debris, ratchet straps are tightened as needed, and read ranges are checked to ensure the antennas continue to function as designed. Antennas are also retuned on each visit to optimize read range for the current flows, temperatures, and environmental conditions. Data are being stored in a large PIT tag database developed for the fish movement study, and an R script has been written to provide visual summaries of the data.

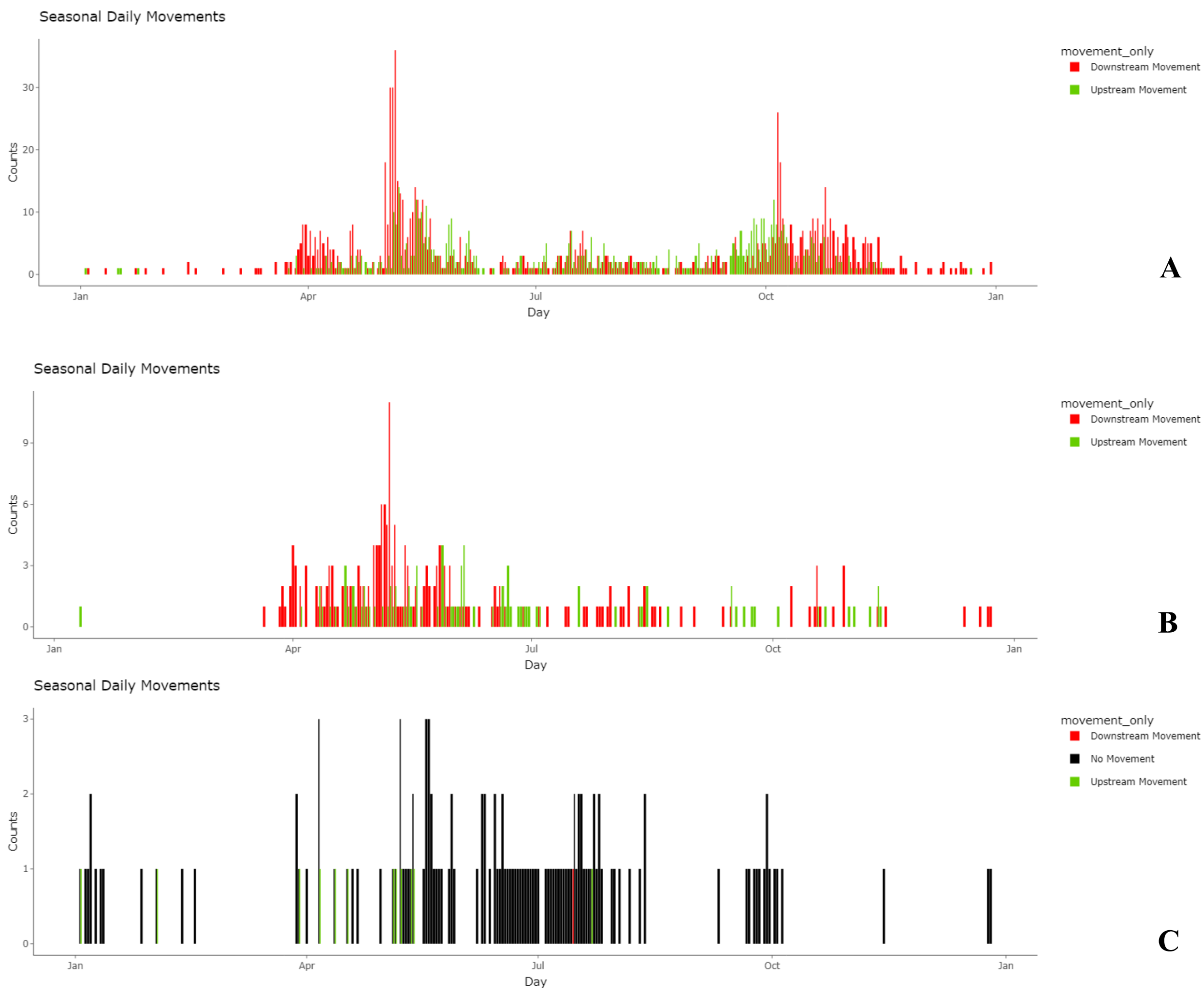

Figure 2.13. Number of Brown Trout (A) and Rainbow Trout (B) detected per day within a calendar year at the Red Barn, Hitching Post, and Confluence stationary antenna sites (combined across sites and years), and number of Mottled Sculpin detected per day within a calendar year at the Confluence site (C). Upstream and downstream movements are defined by detection at both antennas at a site such that the direction of movement was known and the fish remained above or below the site for the remainder of the day once that movement was made. No movement (Mottled Sculpin only) is defined as detection at a single antenna at a site with no additional detections to inform directionality of movement, or a fish that passed both antennas at a site going each direction such that it began and ended the day in the same location relative to the antenna site.

Evaluating fish detections at the population level has revealed interesting patterns in movement. This is especially the case between January and December averaged over both the years data was collected, and across antenna stations. Brown Trout appear to show an increase in movement during the fall spawning period, with activity peaking in early to mid-October. Unexpectedly, it also appears that handling the fish during the spring adult salmonid population estimates induces movement. A small spike in movement in early winter suggests that Brown Trout may be moving to more favorable over-winter habitats just before ice forms on the river, and movement is also induced by ice-off in early April. Rainbow Trout show a spike in activity following ice-off and during the spring spawning period, with activity peaking in mid- to late-May. Mottled Sculpin movements appear to be more erratic (Figure 2.13). However, the majority of the Mottled Sculpin data has come from a small subset of fish released below the Confluence antenna site in September 2020 (Fetherman et al. 2021). Mobile antenna detections of Mottled Sculpin have shown that they are generally sedentary and do not move far from their release location in either the Fraser or Colorado rivers.

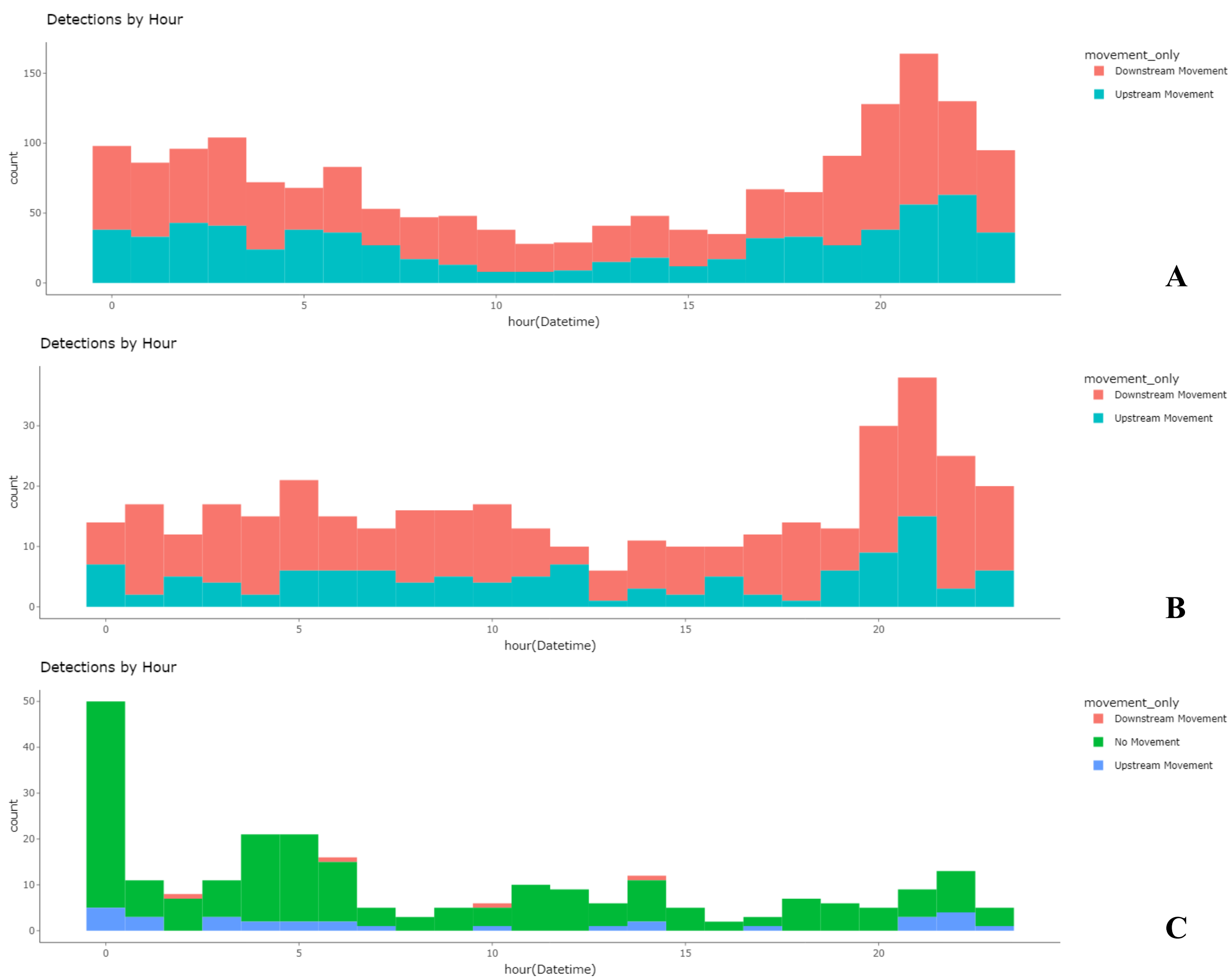

Figure 2.14. Counts of number of Brown Trout (A), Rainbow Trout (B), and Mottled Sculpin (C) making upstream and downstream movements, or no movements in the case of Mottled Sculpin, by time of day (24 hour scale from midnight at 0 hours to 11 pm at 23 hours).

Brown Trout and Rainbow Trout appear to be more active and move more often in late evening, with both upstream and downstream movements spiking between 9 and 10 pm, and the lowest levels of activity and movement during the middle of the day. Mottled Sculpin similarly show a large spike in movement around midnight, with additional spikes occurring in the early morning hours, midday, and late evening (Figure 2.14). Diurnal movement patterns of all fish species seem to suggest that movement occurs primarily at night when fish are less likely to encounter predators.

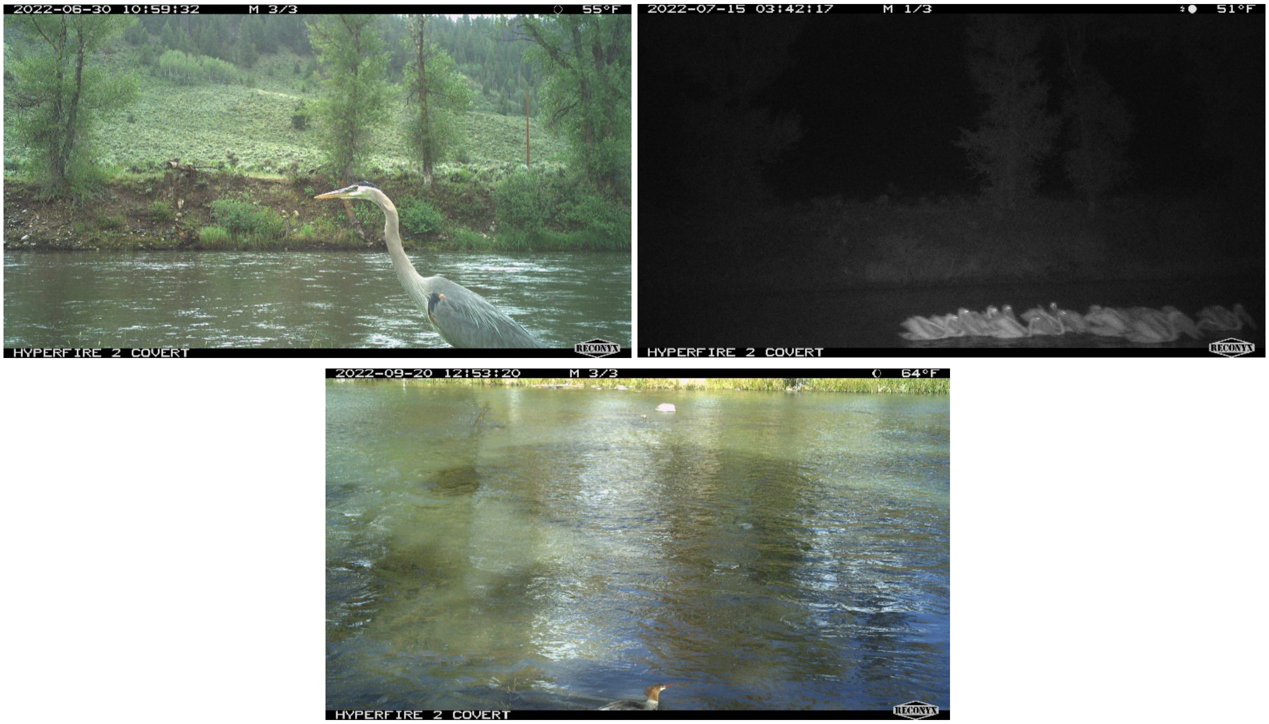

Figure 2.15. Avian predators (great blue heron, American white pelicans, common merganser) captured crossing stationary antennas by the Reconyx game cameras in 2022.

Although the fish movement study is focused on the movement rates of the tagged fish population as a whole, examination of individual fish movements has also revealed some interesting and unexpected results. Most notably, 59 tags have been observed passing through (or over) Windy Gap Reservoir. Of these, 35 were confirmed as typical spawn or post-spawn migration behaviors, or exploratory behaviors of smaller fish to find favorable feeding or refuge habitats, and generally occurred in the fall when the reservoir was drained and fish could pass through the auxiliary outlet of Windy Gap dam. However, after examining the movement patterns, timing of the reservoir draining and potential passage through the auxiliary outlet, and fish size, it was determined that not all of the remaining 24 fish may have moved through the reservoir of their own accord. This was based on the observation that tags were not being detected in a logical sequence at the antenna sites or missed at one of the sites, were moving too quickly through the reservoir between detections at the sites when moving upstream, or were moving when a path through the reservoir would have made those movements improbable.

Upon further examination of these tags, avian predation is the suspected cause of the detections above and below the reservoir for 14 of these fish, three Rainbow Trout (89 to 262 mm TL), one Mottled Sculpin (114 mm TL), and ten Brown Trout (71 to 198 mm TL). These movements were typically observed in the spring, concurrent with the appearance of fish-eating birds, specifically common mergansers *Mergus merganser*, but also potentially American white pelicans *Pelicanus erythrorhynchos* and great blue herons *Ardea herodias*. Additionally, tags were frequently observed at the Red Barn and Confluence sites while being missed at Hitching Post, and movements in the upstream direction occurred in a matter of hours during a time of year when the reservoir was full and the water was coming through the Schmuck channel. Because the tag of a consumed fish will remain intact in the stomach or intestine of a fish-eating bird, and read ranges generally surpass the water surface, these tags could be detected when a bird floats over the antennas. Researchers deployed Reconyx game cameras at all three sites in June 2022 to confirm that fish-eating birds are active around the sites. Since then, the cameras have captured several pictures of picivorous bird species crossing the stationary antennas (Figure 2.15). In the case of the group of white pelicans, three brown trout tags were detected passing the Red Barn antenna at the same time, although it is difficult to tell whether these tags were in fish that were fleeing the pelicans or in the pelicans themselves.

Researchers in the aquatic research section have designed a merganser PIT tagging experiment with researchers in the avian research section to determine if PIT tag movement patterns align with the movement patterns of piscivorous bird species. This information is important for correctly interpreting movement patterns of the PIT tagged fish in the Fraser and Colorado rivers before and after the completion of the CRCC. The avian predation experiment will be implemented within the study section in summer 2023. See the CPW Stream Habitat Investigations 2023 Annual Report for more details and the study proposal.

Stationary antenna stations will continue to remain operational as conditions allow through the remainder of the fish movement study in fall 2025. Movement data collected in 2023 will allow comparisons of baseline movement data collected in 2020 to 2022 with movements that may be affected by construction activities associated with completion of the CRCC. In addition, two stationary antenna stations will be installed in the CRCC in September 2023, or as soon as the construction of the CRCC has been completed, prior to water being run through the CRCC. It is important to get these antennas installed while the channel is still dry, as movement through the CRCC may occur as soon as water is flowing through the channel. Information regarding installation of these antennas, as well as any data collected by these stations once operational, will be available in the next reporting cycle.

Fetherman, E. R. 2013. Introduction and management of *Myxobolus cerebralis*-resistant Rainbow Trout in Colorado. Ph.D. dissertation. Department of Fish, Wildlife and Conservation Biology, Colorado State University, Fort Collins, CO.

Fetherman, E. R., B. Neuschwanger, B. W. Avila, and T. B. Riepe. 2020. Sport Fish Research Studies. Annual Report. Colorado Parks and Wildlife, Aquatic Research Section. Fort Collins, Colorado.

Fetherman, E. R., B. Neuschwanger, T. B. Riepe, and B. W. Avila. 2021. Sport Fish Research Studies. Annual Report. Colorado Parks and Wildlife, Aquatic Research Section. Fort Collins, Colorado.

Mobile Antenna Deployments and Data Collection

Mobile antennas (Figure 2.16) are being deployed as part of the fish movement study to supplement movement and detection data obtained from the stationary antennas. Data obtained from mobile antenna deployments will be used to adjust estimates of movement and survival probabilities when conducting the full analysis of the fish movement data. GPS locations of detected fish from the mobile antennas can be used to inform distance moved by tagged fish, especially those never detected at a stationary antenna station. Repeat detections in the same location by the mobile antennas can help identify ghost tags, PIT tags that are no longer inside the fish due to tag expulsion or mortality (Richer et al. 2017). Failure to account for ghost tags can lead to incorrect interpretations regarding fish location and fate (Fetherman et al. 2014).

Figure 2.16. (A) Antenna wire encased in PVC to maintain rigidity, shape, and tuning during deployment. (B) Pelican box, containing batteries, reader, tuner box, and Campbell Scientific datalogger, and external GPS unit for marking the location of detected PIT tags. (C) Portable antennas set to deploy on the Fraser River Ranch.

Mobile antennas were deployed in three reaches in October 2022: 1) the Fraser River Ranch reach in the Fraser River upstream of Windy Gap Reservoir (1.0 miles), 2) the River Run reach in the Colorado River upstream of Windy Gap Reservoir (1.2 miles), and 3) the Chimney Rock reach in the Colorado River downstream of Windy Gap Reservoir (4.5 miles; Figure 2.17). Two rafts were used to complete a single pass, one running the left side of the river and one running the right, to provide the greatest detection coverage. Rafts remained about 100 yards apart during deployment to prevent reader interference. The starting location for deployment through River Run was located on the River Run property just downstream of the Miller Ranch, and the reach included portions of the Colorado River through River Run, the Horn Ranch, and Northern Water property. Upon reaching the confluence of the Colorado and Fraser rivers, the rafts were walked upstream in the Fraser River to the pullout located downstream of the Fraser River gauge. The starting location for the Fraser River Ranch was at the upstream-most end of the property, just downstream of the Granby water treatment plant. The Fraser River splits just downstream of the starting location. The north channel was run to avoid beaver dams and a water diversion structure located on the south channel, and the rafts were pulled out upstream of the Fraser River gauge. Rafts were deployed in the Colorado River immediately below Windy Gap Reservoir, covering the river section from immediately below the dam downstream through the Chimney Rock Ranch, and rafts were pulled out at the Sheriff Ranch. Each reach was run on a separate day, and two passes were completed in the same day in all three reaches. The antennas recorded tags along with a GPS location and temperature for each detection. After completing both passes, the data were downloaded and stored in a Toughbook until transfer to the database could be completed. Data were used to map the locations of detected tags relative to their release location to examine movement patterns within and among the reaches.

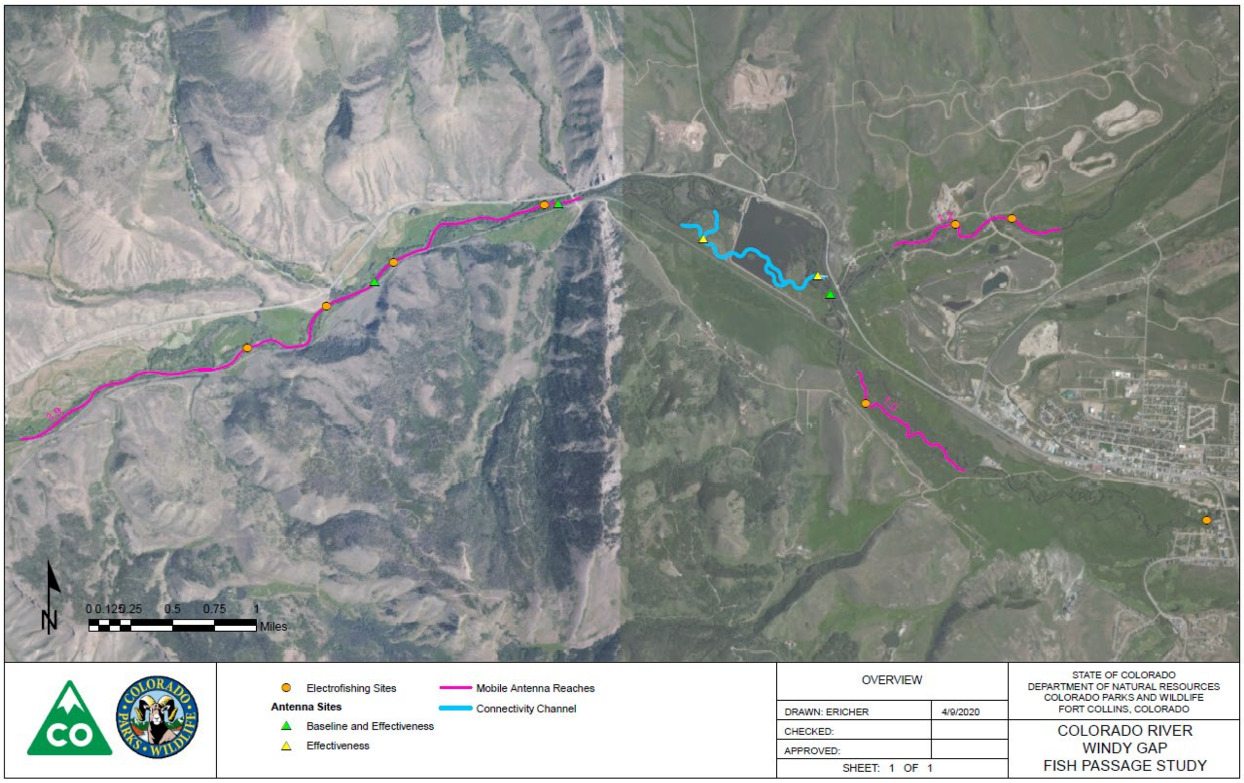

Figure 2.17. Mobile antenna reaches in the Colorado and Fraser rivers above Windy Gap Reservoir and the Colorado River below Windy Gap Reservoir (red lines). The blue line represents a future mobile antenna reach following completion of the Colorado River Connectivity Channel.

Using different colors for the various release sites, researchers have been able to examine unique patterns in movement in the Fraser and Colorado rivers (Figure 2.18). Dispersion of tagged fish appears to be greater below Windy Gap Reservoir compared to above. Overall, electrofishing recaptures and mobile detections of fish released in the Fraser River at Kaibab Park have been relatively low. However, a few fish tagged in Kaibab Park have moved downstream into the Fraser River Ranch reach, and at least one moved downstream to the confluence and a short distance upstream in the Colorado River. Several fish tagged in the Fraser River Ranch made movements downstream to the confluence, and were detected in sections of the River Run reach in the Colorado River. Most of the fish tagged within the River Run reach remained in the

Colorado River, although at least one fish moved downstream to the confluence and over the Fraser River gauge into the Fraser River Ranch. Several of the sculpin tagged below the Confluence antenna site in September 2020 made short upstream movements past the antenna station, being detected in both the Fraser and Colorado rivers. At least one fish tagged below Windy Gap Reservoir was detected in the Colorado River above Windy Gap Reservoir in 2022.

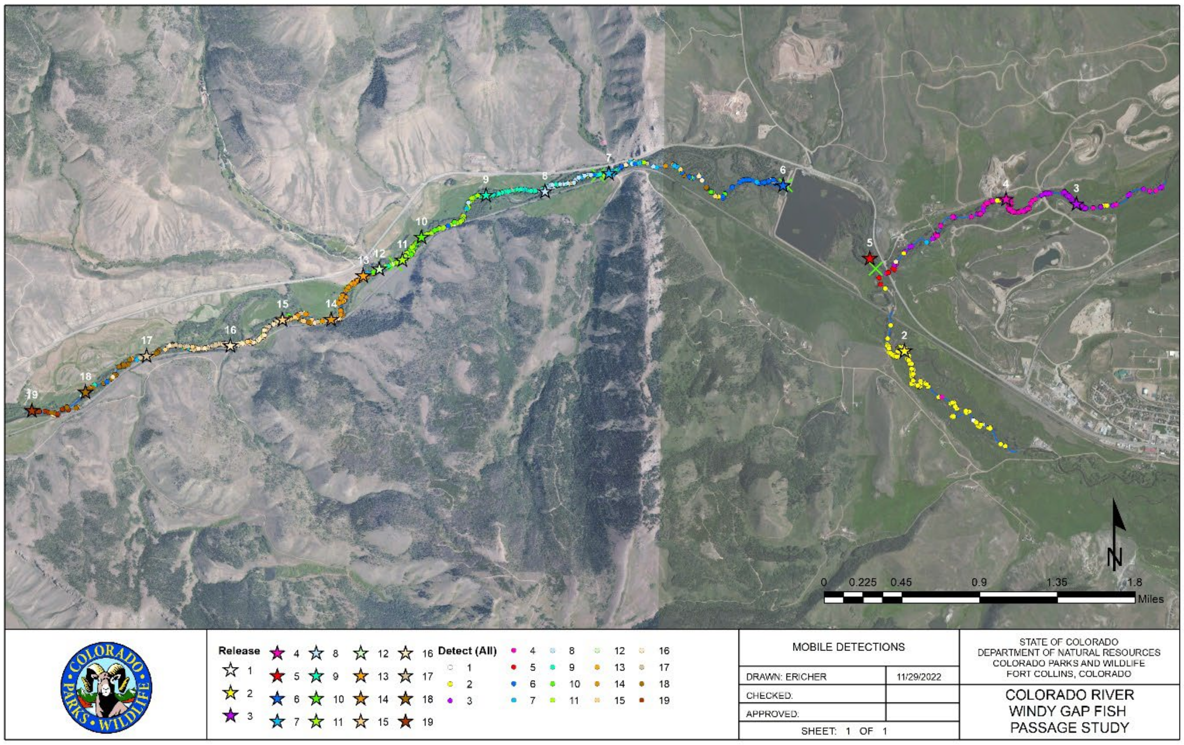

Figure 2.18. Location of detected PIT tags in the Fraser and Colorado rivers upstream of Windy Gap Reservoir and the Colorado River downstream of Windy Gap Reservoir in fall 2022. Colored stars correspond to release locations to show distance moved.

In the Colorado River below Windy Gap Reservoir, fish appear to redistribute upstream after being tagged (Figure 2.18). This is likely because fish were collected by the raft electrofishing moving downstream; upstream movement from the release sites is thought to represent fish returning to the locations at which they were captured during the electrofishing efforts. One exception is the release site immediately downstream of Windy Gap Reservoir, where the only option for movement is downstream due to the dam restricting upstream movements. Tagged individuals from all of the release sites were found throughout the Colorado River below Windy Gap Reservoir. However, the largest mixture from release sites occurred between Hitching Post and Windy Gap dam, suggesting that fish throughout the river are moving upstream, likely to spawn. Many of these fish were tagged several miles downstream from where they were detected. Similarly, fish that had been tagged immediately downstream of Windy Gap Reservoir and at Hitching Post were found in the downstream sections of the study reach near the Sheriff Ranch. Detections at both locations represent some of the longest distance movements in either direction that could be observed during the first phase of this study.

Many detections appear to be grouped around release sites (Figure 2.18), which could either indicate a lack of movement by fish tagged in those sites, or alternatively, a grouping of ghost tags due to mortality or tag loss near the release sites. With six mobile antenna runs completed since the study began in 2020, researchers were able to use the mobile antenna data to determine if tags that had been detected a minimum of three times were still in a fish or if the tags had not moved and could be classified as a ghost tags. First, a list was generated of potential ghost tags that had been detected at least three times during mobile runs. The list contained 315 unique tags, 175 that had been detected three times, 84 that had been detected four times, 40 that had been detected five times, and 16 that had been detected on all six mobile antenna runs. Every tag on the list was then examined for release and movement locations using the GIS function within the R shiny app. A tag was confirmed as a ghost tag if it had not moved, or moved very little in the downstream direction but stayed within the same pool-riffle complex, when detected on three mobile antenna runs. A tag was determined to not be a ghost tag if it made longer range movements, especially in the upstream direction, if the tag was detected moving past a stationary antenna between mobile antenna runs, or if the fish was physically recaptured during population estimates.

Table 2.1. Number of tags detected on three, four, five, or all six mobile antenna runs that were confirmed to be ghost tags, confirmed to be tags that were still in fish, or for which the tag status is currently unknown.

Number of times detected	Number of tags	Confirmed ghost tags	Confirmed tags in fish	Status unknown	Percent ghost tags
3	175	111	35	29	63%
4	84	70	5	8	83%
5	40	36	4	0	90%
6	16	15	0	1	94%

The percent of tags confirmed to be ghost tags increased, and the number of tags confirmed to still be in a fish or for which the status is currently unknown decreased, with the number of mobile antenna runs on which a tag was detected (Table 2.1). Although 63% of tags were confirmed to be ghost tags if detected on just three mobile antenna runs, the percent of ghost tags increased to over 80% with four or more mobile runs. These results suggest that a minimum of four mobile antenna run detections may be more useful in determining tag status in future investigations. One tag confirmed to be a ghost tag showed patterns of movement similar to that identified as avian predation using the criteria defined above by the stationary antenna detection analyses, and is suspected to have been evacuated in the river in its current location by a bird. For tags confirmed to still be in fish, patterns of movement or physical recaptures of the tagged fish made it relatively easy to confirm tag status. The majority of the tags for which the status was unknown were classified as such because movement had been detected in the first couple mobile antenna runs, and the tag had been detected in the same location for only the last two mobile antenna runs conducted. Additionally, some tags had moved downstream at a greater distance than would have been expected given flow conditions if the tag was no longer in a fish.

None of the tags confirmed to be ghost tags originated from Mottled Sculpin. Three tags for which the status was unknown were Mottled Sculpin tags, but due to the relative lack of movement observed from sculpin over the course of the study, field confirmation is needed to determine the status of these tags. Two hundred twelve ghost tags originated from Brown Trout, while the remaining 25 ghost tags originated from Rainbow Trout. Brown Trout that lost their tags ranged from 129 to 506 mm TL, and averaged 316 ± 5 mm TL, and Rainbow Trout that lost their tags ranged 72 to 435 mm TL, and averaged 304 ± 19 mm TL. Fish of both species on the smaller end of the range were likely fish that died from the tagging procedure shortly after release. However, the average length data suggests that the majority of tags were likely lost during spawning, which is especially likely for the Brown Trout that are often tagged only a few weeks prior to the fall spawning period. Fish on the upper end of the size range could also have been tagged near the end of their lives, dying of old age during the study.

Field surveys conducted in August 2023 will determine if tags identified using the above protocols are indeed ghost tags. If possible, ghost tags will be removed from the Fraser and Colorado Rivers prior to the start of the post-construction monitoring phase beginning in fall 2023. Summary data regarding the number and location of ghost tags confirmed during the field surveys will be available in the next reporting cycle.

Fetherman, E. R., B. W. Avila, and D. L. Winkelman. 2014. Raft and floating radio frequency identification (RFID) antenna systems for detecting and estimating abundance of PIT-tagged fish in rivers. North American Journal of Fisheries Management 34:1065-1077.

Richer, E. E., E. R. Fetherman, M. C. Kondratieff, and T. A. Barnes. 2017. Incorporating GPS and mobile radio frequency identification to detect PIT-tagged fish and evaluate habitat utilization in streams. North American Journal of Fisheries Management 37(6):1249-1264.

Water Filtrations for Triactinomyxon Quantification

Whirling disease is established in the upper Colorado River, and Windy Gap Reservoir is one of the primary sources of triactinomyxon (TAM) production in the system. With the construction of the CRCC, water will bypass Windy Gap Reservoir, potentially reducing TAM production and overall infection prevalence in the system. To monitor the potential change in TAM production, we began taking water samples in 2020 to quantify the amount of TAMs in the water column at multiple times of year.

Water samples were taken at four locations in the Chimney Rock/Sheriff Ranch study section during the adult population estimates in May 2022: 1) Hitching Post, 2) Red Barn, 3) below the Red Barn diversion, and 4) Sheriff Ranch. Samples were also collected from each of four fry sites at Hitching Post, upper Red Barn, lower Red Barn, and Sheriff Ranch during each of four fry sampling occasions in July, August, September, and October 2022 to determine if TAM counts are correlated with myxospore counts in salmonid fry. At each location and on each sampling occasion, four consecutive 1-L samples were collected by placing the sample bottle at 0.67 the depth of the water column and removing the lid to quickly fill the bottle. Samples were kept on ice until filtering could occur. Water was vacuum filtered through 5 μm filters, one to three per 1-L sample depending on turbidity. The entire filters were folded, placed in a 2-ml tube with several drops of 100% ethanol to stabilize the sample, and frozen.

Samples were sent to Sascha Hallett and Steven Atkinson at Oregon State University (OSU) for TAM quantification. At the time of writing, samples were still being processed. Results from 2022 will be available in the next reporting cycle. Samples will be collected from the same sites in 2023 and will continue to be collected through two years post-construction of the CRCC. These data will be used not only to determine patterns across the sites and sampling dates as above, but also annual fluctuations in TAM production in the upper Colorado River.

YAMPA RIVER RAINBOW TROUT BROOD STOCK

Renibacterium salmoninarum in Yampa River Rainbow Trout

In April 2019, the wild Rainbow Trout brood stock in Harrison Creek and Lake Catamount (Steamboat Springs, Colorado) tested positive for *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease (BKD), during a routine annual health inspection. It is unknown how *R. salmoninarum* was introduced to the system. The Yampa River was stocked with thousands of fish that tested negative for *R. salmoninarum* from CPW hatcheries in 2017-2018 for the Yampa River Rainbow Trout post-stocking survival experiment (Fetherman et al. 2018). In addition, Rainbow Trout having an unconfirmed infection status were stocked within the system from a private hatchery in 2018. The brood stock in Harrison Creek and Lake Catamount was developed using Hofer x Harrison Lake Rainbow Trout (H×H) and was being maintained to conduct wild egg takes for hatchery supplementation if the need were to arise. State regulations prevent moving fish or collecting eggs from *R. salmoninarum* positive locations, preventing utilization of the brood stock after it tested positive for *R. salmoninarum*.

In April 2020, a wild spawn was conducted in Harrison Creek to collect several genetically unique male-female pair families for comparison of wild H×H genetics to those from fish reared in CPW hatcheries (Fetherman et al. 2021). Fish utilized in the wild spawn were kept in net pens, along with fish from Lake Catamount, and tested for infectious pancreatic necrosis virus (IPNV), viral hemorrhagic septicemia virus (VHSV), infectious hematopoietic necrosis virus (IHNV), *Oncorhynchus masou* virus (OMV), and *R. salmoninarum* during the annual disease inspection conducted on May 5, 2020. Six of the samples tested positive by DFAT (50%) and were confirmed positive for *R. salmoninarum* DNA by single-round PCR. Eggs from the April 2020 spawn were taken to the salmonid Disease and Sport Fish Research Lab in Fort Collins, Colorado, and offspring were reared for five months post-hatch under relatively high density, high stress conditions for additional testing of vertical transmission from the Harrison Creek brood stock (Fetherman et al. 2021). Fifteen fish were found to be positive for *R. salmoninarum* by DFAT. However, all 15 samples were found negative for *R. salmoninarum* DNA by single-round PCR. A positive with DFAT followed by a negative confirmatory PCR test is considered a negative result. Therefore, the risk of vertical transmission from the *R. salmoninarum*-positive brood stock, even when the parents were known to be positive, was presumed to be low.

In May 2021, 57 fish were again collected from Lake Catamount for disease testing. Interestingly, all 57 fish tested negative for *R. salmoninarum* by DFAT. However, it is important to note that no fish were collected from Harrison Creek in 2021 as they had been in previous years because discharge in the creek was lower than usual and very few fish had entered the creek to spawn. Because previous collections had grouped fish from Harrison Creek and Lake Catamount, it was unknown which individuals from each location previously tested positive. Therefore, it was possible that fish that had remained in and were collected from the lake were not spawning, and therefore had lower-than-detectable concentrations or no bacteria compared to the fish that typically spawn in Harrison Creek. To determine if *R. salmonimiarum* infection status differed by the location from which the fish were collected, 30 fish were collected from Lake Catamount and 30 from Harrison Creek in May 2022, and maintained separately throughout the entirety of the testing process. All 60 fish again tested negative for *R. salmoninarum* by DFAT.

Per state regulations, a previously positive site must test negative three times within a year, with the tests occurring at least three months apart, and with 12 months between the initial and final negative test, in order for a site to return to a negative status. The negative results obtained from the fish in Harrison Creek and Lake Catamount in May 2022 began this process. A sixty fish sample was collected from Lake Catamount in September 2022, and all fish tested negative by DFAT, providing the second required negative for the disease certification process. The final sample of 60 fish was collected from Lake Catamount in May 2023. No fish were collected from Harrison Creek due to a late ice off delaying the spawn. Unfortunately, one five-fish pooled sample tested positive by DFAT, and was confirmed positive for *R. salmoninarum* DNA using single-round PCR. Therefore, the Lake Catamount and Harrison creek system maintained its status as positive in 2023. The future of this brood stock is in question, as eggs will not be able to be taken from this location until a negative status is returned via the three-negative testing regulation described above. The next test for *R. salmoninarum* in these fish will occur in spring 2024. If they test negative, the regulation testing process will begin again, but as such, Harrison Creek and Lake Catamount will not be able to return to a negative status until at least spring 2025.

Fetherman, E. R., B. Neuschwanger, T. B. Riepe, and B. W. Avila. 2021. Sport Fish Research Studies. Annual Report. Colorado Parks and Wildlife, Aquatic Research Section. Fort Collins, Colorado.

Fetherman, E. R., G. J. Schisler, and B. W. Avila. 2018. Sport Fish Research Studies. Federal Aid Project F-394-R18. Federal Aid in Fish and Wildlife Restoration, Job Progress Report. Colorado Parks and Wildlife, Aquatic Wildlife Research Section. Fort Collins, Colorado.

Yampa River H×H Spawning Plans

The CPW Crystal River Hatchery is interested in receiving wild eggs to supplement the H×H brood stock maintained by the hatchery. There is some evidence that the genetic resistance of the H×H brood stock at the Crystal River Hatchery has changed over time due to being reared in a *Myxobolus cerebralis* negative environment (Avila et al. 2022). Taking eggs from an *M. cerebralis* positive wild brood stock would incorporate more wild-type genetics and potentially increase the whirling disease resistance of the brood stock. Plans were initially made to collect eggs from Harrison Creek and Lake Catamount in spring 2023 for this purpose. However, prior to the spring spawn, the decision was made to do a dry run of the wild spawning procedure and collect genetic samples from spawning fish in both Harrison Creek and Lake Catamount, as well as the Stagecoach Tailwater, which may be a good alternative location for egg takes if the Harrison creek and Catamount Lake portion of the system continues to remain *R. salmoninarum* positive. Therefore, no eggs were collected for hatchery supplementation purposes in 2023.

With other pathogens such as *Myxobolus cerebralis*, once established in a portion of a connected system, the entire system is considered positive for that pathogen. However, recent field experiments and risk mapping suggest that *R. salmoninarum* may not have a continuous distribution within a system depending on species distributions, spawning locations, habitat, and water flows and temperatures. One of the goals of the Yampa River post-stocking survival experiment was to establish H×H and increase Hofer *M. cerebralis*-resistance characteristics throughout the entire Yampa River between Stagecoach Reservoir and Lake Catamount (Fetherman et al. 2018), which would allow egg takes to occur anywhere in this section of the river. Therefore, during the annual disease inspection conducted on May 3, 2021, in addition to testing 57 Rainbow Trout from Lake Catamount to maintain the disease history, 60 Rainbow Trout were collected from the Yampa River and tested for *R. salmoninarum* only. Although Harrison Creek, Lake Catamount, and the Yampa River are connected, it is possible that fish positive for *R. salmoninarum* remain in the lake or spawn in Harrison Creek only, but do not move up the Yampa River to spawn. If fish do move up into the Yampa River from Lake Catamount, there are 6.5 miles of river between Lake Catamount and the Stagecoach Reservoir tailwater, which could prevent fish that are stressed and infected with *R. salmoninarum* from moving this far to spawn. Additionally, three tributaries, Green Creek, Sarvis Creek, and Morrison Creek, are encountered along the way and are known spawning locations for salmonids, potentially diverting fish before reaching the tailwater. Therefore, 30 fish were collected on the Weller Ranch above Morrison Creek and 30 from the upper Stagecoach Tailwater above the habitat project (about 0.75 miles between locations).

All of the Rainbow Trout collected from the Yampa River tested negative for *R. salmoninarum* by DFAT. The majority of the fish collected were of spawning age and *R. salmoninarum* should have been detectable, if present. These results suggest that *R. salmoninarum* may not be distributed throughout the entire system and egg takes from the Weller Ranch or Stagecoach Tailwater may be possible in the future. The Yampa River between Lake Catamount and Stagecoach Reservoir is a different water code than Lake Catamount and Harrison Creek. Therefore, a full disease inspection and three-year disease history is needed for this site before taking eggs. Sixty fish, 30 each from the Wellar Ranch above Morrison Creek and the Stagecoach Tailwater, were collected and tested in May 2022, and 60 fish were collected and tested from the Stagecoach Tailwater in May 2023. In both 2022 and 2023, the fish tested negative for all pathogens of concern, including *R. salmoninarum*. One more year of testing in 2024 is needed to ensure fish remain negative and finalize the disease history for this location. It is possible that this sampling could occur prior to the spawn in spring 2024 such that eggs could be taken from this location immediately after the disease testing results were obtained, but it is more likely, given the *R. salmoninarum* concerns in other parts of the system, that wild egg takes will not occur until 2025.

Researchers, biologists, and hatchery managers have developed a plan for taking eggs and bringing them into the hatchery when that occurs in the future. Single male-female pairs will be spawned together to obtain the eggs, and eggs originating from these pairs will be maintained separately until the genetic results from the samples collected from each spawning fish are received. This will require up to 30 egg cups and tanks to maintain the separate groups through eye-up, hatch, and swim-up. The genetic samples will be tested for the presence of the WDRES-9 quantitative trait loci (QTL; Fetherman et al. 2020, 2022). Eggs will initially be retained from families of which both parents have two copies of the WDRES-9 QTL (classified as resistant-resistant; RR), but may also be kept from families of which one or both parents have only one copy of the WDRES-9 QTL (classified as resistant-susceptible; RS). No families in which one or both parents do not have a copy of the WDRES-9 QTL (classified as susceptible-susceptible; SS) will be retained to maintain higher genetic resistance to *M. cerebralis* in the hatchery brood stock. Once genetic samples have been obtained and eggs from SS parents have been culled, the fish will be combined and sent to the Crystal River Hatchery for incorporation into their brood stock program.

Although no eggs were collected from the Yampa River in 2023, genetic samples were collected from all fish taken for disease testing in both Lake Catamount and the Stagecoach Tailwater, a total of 117 samples, on May 2, 2023. Of these, 96 samples were sent to the Genomics Variation Laboratory at UC Davis on May 3, 2023. The number sent was reduced from the total collected to allow a single 96-well plate run and reduce costs. The first round of genetic results was received on June 2, 2023. However, 28 of the samples did not amplify correctly and were rerun. The updated genetic results were received on June 8, 2023, five weeks after the samples were collected. This provided a good estimate for the length of time, plus or minus one to two weeks, for which eggs and fish will need to be maintained as separate families during future egg takes. Fifteen of the samples still did not amplify correctly after the second run. Had these samples been associated with families from which eggs had been collected, those eggs would have been discarded due to the lack of genetic information for one or both parents.

Table 2.2. Total number of arbitrarily paired spawns for which genetic results were obtained from Lake Catamount and the Stagecoach Tailwater, and number of families for which the parents would be genetically RR-RR, RR-RS, RR-SS, RS-RS, RS-SS, or SS-SS.

Location	Total	RR-RR	RR-RS	RR-SS	RS-RS	RS-SS	SS-SS
Lake Catamount	20	9	7	1	3	0	0
Stagecoach Tailwater	20	4	5	2	3	5	1

More fish from Lake Catamount contained two copies of the WDRES-9 QTL compared to the Stagecoach Tailwater, and there were more fish from the Stagecoach Tailwater that did not have a copy of the WDRES-9 QTL. Overall, 65% of the fish from Lake Catamount were classified as RR, 32.5% were classified as RS, and 2.5% were classified as SS, whereas 41% of fish from the Stagecoach Tailwater were classified as RR, 37% were classified RS, and 22% were classified as SS. Sex was not determined during genetic sample collection, so fish were arbitrarily paired by sample collection number within a location to create families for a preliminary examination of how many families would have originated from parents with the various possible combinations of resistant and susceptible genetic markers (Table 2.2). The eggs from only one family (5%) in

Lake Catamount would have been discarded due to one of the parents being classified as SS, with an additional three families (15%) that may or may not have been retained, depending on the number eggs requested, due to both parents being classified as RS. In contrast, eggs from eight families (40%) would not have been retained from the Stagecoach Tailwater due to one or both parents being classified as SS. Given this, eggs from the three families (15%) where both parents were classified as RS would likely have been retained to attempt to meet egg requests.

Taken together, the genetic results suggest that eggs should be taken from Lake Catamount versus the Stagecoach Tailwater to maintain a higher percentage of *M. cerebralis*-resistant fish for brood stock supplementation. However, given the current *R. salmoninarum*-positive status of Lake Catamount, the Stagecoach Tailwater will be the only location from which eggs can be taken for the foreseeable future. Forty-five percent of the families collected from the Stagecoach Tailwater had the correct combination of parental genetic characteristics to be retained. The goal in future years will be to collect and retain roughly 30,000 eggs for brood stock supplementation purposes from either or both locations. Assuming a conservative estimate of 1,000 eggs per female, had eggs been collected this year, only 9,000 eggs would have been retained from the Stagecoach Tailwater spawns, with the potential to retain an additional 3,000 eggs from families in which both parents were classified as RS. Therefore, if eggs are collected only from the Stagecoach Tailwater in the future, and genetic proportions of the fish in the tailwater do not move towards more resistant fish, approximately 66 families will need to be spawned and maintained separately to meet the egg request. Genetic samples will continue to be collected from the Stagecoach Tailwater to monitor changes in resistance and adjust spawning family projections as needed.

Avila, B. W., D. L. Winkelman, and E. R. Fetherman. 2022. Dual resistance to *Flavobacterium psychrophilum* and *Myxobolus cerebralis* in rainbow trout (*Oncorhynchus mykiss*, Walbaum). Journal of Fish Diseases 45:801-813.

Fetherman, E. R., B. Neuschwanger, B. W. Avila, and T. B. Riepe. 2020. Sport Fish Research Studies. Annual Report. Colorado Parks and Wildlife, Aquatic Research Section. Fort Collins, Colorado.

Fetherman, E. R., B. Neuschwanger, and T. B. Riepe. 2022. Sport Fish Research Studies. Annual Report. Colorado Parks and Wildlife, Aquatic Research Section. Fort Collins, Colorado.

Fetherman, E. R., G. J. Schisler, and B. W. Avila. 2018. Sport Fish Research Studies. Federal Aid Project F-394-R17. Federal Aid in Fish and Wildlife Restoration, Job Progress Report. Colorado Parks and Wildlife, Aquatic Wildlife Research Section. Fort Collins, Colorado.

COLLABORATIVE RESEARCH PROJECTS WITH COLORADO STATE UNIVERSITY

Collaborations with graduate students at Colorado State University (CSU) provide an opportunity to expand on management and research questions of interest to the State of Colorado. One such project focused on bacterial kidney disease and conducted in conjunction with sport fish research was initiated in 2022. Bacterial kidney disease, caused by *Renibacterium salmoninarum*, is a major disease of concern for Colorado hatcheries. As a regulated pathogen in Colorado, current regulations prevent the transfer or stocking of infected eggs or fish. Additionally, *R. salmoninarum* can be transmitted in two ways, presenting challenges for prevention and management. Understanding the rate of vertical and horizontal transmission in Colorado hatcheries, the role these transmission routes play in maintaining infection prevalence, and determining the optimal tissues for detecting *R. salmoninarum* infections can help with management and regulatory decisions for this pathogen. Additionally, bacterial strain and virulence differences may affect mortality rates of fish infected with *R. salmoninarum* in both wild and hatchery environments, and this is the focus of the current study.

BACTERIAL KIDNEY DISEASE RESEARCH

Project Collaborators: Rebecca E. McDevitt, M.S. student, and Dana L. Winkelman, Ph.D.

Renibacterium salmoninarum, the causative agent of bacterial kidney disease (BKD), is a gram-positive, intracellular bacteria that primarily infects members of the family Salmonidae, with varying levels of susceptibility among species. Infection with *R. salmoninarum* is known to cause high mortality in both wild and cultured salmonids, especially Coho Salmon *Oncorhynchus kisutch* and Chinook Salmon *O. tshawytscha*. *R. salmoninarum* is found in salmonid populations throughout the U.S., and has been detected within several of Colorado's wild trout fisheries and hatcheries (Fetherman et al. 2020; Kowalski et al. 2022; Riepe et al. 2023). Previous research has shown that Sockeye Salmon *O. nerka*, Chinook Salmon, Coho Salmon, Atlantic Salmon *Salmo salar*, and Chum Salmon *O. keta* are more susceptible to an *R. salmoninarum* infection compared to some inland trout species such as Lake Trout *Salvelinus namaycush*, Brown Trout *S. trutta*, Bull Trout *S. confluentus*, Brook Trout *S. fontinalis*, and Rainbow Trout and steelhead *O. mykiss* (Rhodes and Mimeault 2019; Starliper et al. 1997). Susceptibility to infection may depend on whether the pathogen and host originate from the same location. If the host and pathogen are not of the same origin, the pathogen may have a greater effect on the survival of the host (Johnson et al. 2020).

Chinook Salmon are one of the most susceptible species to *R. salmoninarum* infection, and are typically infected with a virulent strain found in the Pacific Northwest (ATCC 33209) that causes high mortality rates. However, the susceptibility of this species to other strains, such as the one found in Colorado, is unknown. Therefore, we evaluated the response of Chinook Salmon to two strains of *R. salmoninarum*, the ATCC 33209 strain isolated from the Pacific Northwest (hereafter the ATCC strain) and a suspected attenuated virulent strain (Riepe 2022) that was isolated from a Colorado hatchery (hereafter the Unknown strain). Our goal was to infect Chinook Salmon with the two strains and evaluate the cumulative percent survival over a 10 week period following exposure. We predicted that the Chinook Salmon would exhibit lower survival following exposure to the Unknown strain compared to the ATCC strain given that Unknown strain originates from outside of the native range of the Chinook Salmon. However, if the Unknown strain is an attenuated virulent strain, the Chinook Salmon exposed to the Unknown strain would be expected to show similar or higher survival rates compared to those exposed to the potentially more virulent ATCC strain.

In November 2022, we collected and transported 300 age-1 Chinook Salmon from the Pahsimeroi Fish Hatchery (May, Idaho). The fish were transported in tanks with a steady flow of oxygen and temperature controlled to maintain a temperature range of 8-14°C. Upon arrival, ten fish each were placed into 24 tanks located in a wet lab in the basement of the Anatomy/Zoology building on the CSU main campus. Fish in each tank were treated twice with 10% formalin after they arrived to clear any external parasites that could affect mortality prior to or during the exposure experiment.

Our exposure experiment consisted of ten tanks (replicates) per bacterial strain, with ten fish in each tank (200 fish total: 100 fish for the ATCC strain, 100 fish for the Unknown strain). All ten fish in each tank were injected with the bacteria strain assigned to that tank. The experiment additionally contained two tanks in which fish received a mock saline injection, and two tanks serving as naïve controls (no injection). Using ten fish per tank allowed for an assessment of mortality in each tank and comparison to other studies that had used similar exposure evaluation methods.

The dosage necessary for injection (1×0^6) was determined from a previous study (Mckibben et al. 1999). We used the American Type Culture Collection (ATCC) 33209D-5 isolate of live *R. salmoninarum* for ATCC injection exposures to 100 fish, while injection exposures to the other 100 fish used the Unknown strain that was previously isolated in our lab. Using the well-known ATCC strain provided for consistency in comparisons of mortality rates with previous laboratory studies using injections for inoculation. The bacteria were cultured and prepared in diluted phosphate buffered solution at CSU prior to injection. Fish were anesthetized with tricane methanesulfonate (MS-222) and injected in the intraperitoneal cavity anterior to the pelvic fin with 20 uL of one of the two strains of *R. salmoninarum*. Fish in the mock injection tanks were injected with saline. After injections, fish were monitored in 5-gallon buckets containing fresh water and aeration until they recovered. Fish were then placed back into their designated, and randomly assigned 20-gallon flow-through tanks with aeration for the duration of the experiment.

Fish were fed once a day with size 3 BioOregon feed at the manufactures recommendation of 3% body weight per day. Every two weeks, fish were batch weighed to determine the amount of feed to be distributed to each tank. Fish were monitored twice a day after injections. Moribund and dead fish were removed from each tank twice daily, lengths and weights were recorded, and internal liver, kidney, and spleen tissues were collected from all fish removed to determine the timing of mortality and infection severity after injection. All fish remaining at ten weeks post-injection were euthanized, and lengths, weights, and tissues were collected to test for *R. salmoninarum* using DFAT and qPCR to determine if clearance of the infection occurred among the surviving fish.

Following Riepe (2022), tissues were thawed, homogenized together, and prepared for DFAT and qPCR analyses. Specifically, DFAT samples were smeared on a 12-well slide in duplicate, stained with an FITC-conjugated *R. salmoninarum* antisera, and counter-stained using Eriochrome Black (USFWS and AFS-FHS 2014). All slides were analyzed using a Nikon compound microscope fitted with a 420 nm fluorescent emission high-pressure mercury lamp and examined at 1000X magnification (Fetherman et al. 2020). Any detection of *R. salmoninarum* was considered infected tissue. DNA extractions were completed with Qiagen DNeasy Blood and Tissue Kits with an additional elution step (Elliot et al. 2013). We used previously determined primer and probe sets (RS 1238 F, RS 1307 R, and RS 1262 MGB probe; Chase et al. 2006; Elliott et al. 2012) to complete the qPCR analyses. Samples were determined positive when the Cq values were less than 37.75 (Riepe 2022). A previously developed standard curve was used to determine the number of bacteria in each sample.

We calculated the survival of infected fish over time of exposure (days) with the *survival* package in Rstudio version 4.2.1. The package allowed us to use the Kaplan-Meier Survival Curve to estimate cumulative survival of the ATCC and Unknown infected fish. Specifically, we modeled and plotted Kaplan-Meier estimates of probability of survival over time for each bacterial strain.

All fish used in the four treatments (control, saline, ATCC, Unknown) were approximately the same weight at the start of the experiment (Figure 3.1). Therefore, fish size-at-exposure was not considered to be a factor affecting mortality during the exposure experiment.

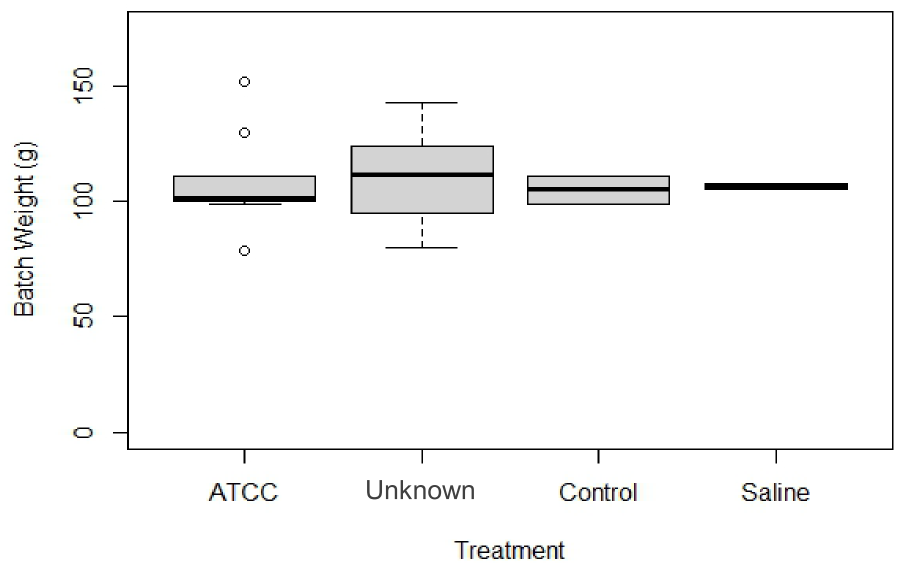

Figure 3.1. Chinook Salmon batch weights (g) by treatment (ATCC, Unknown, Control, Saline) at time of injection with *R. salmoninarum.*

There were no mortalities in our control or saline injected tanks, indicating that all mortality in the injection tanks occurred as a result of *R. salmoninarum* infection. However, we detected *R. salmoninarum* in both the control and saline injected fish with qPCR and DFAT, but bacterial loads were low compared to fish injected with the ATCC and Unknown *R. salmoninarum* strains (Table 3.1). We had anticipated that control fish would have a mild infection because they came from an *R. salmoninarum* positive hatchery at which they had been previously treated for the bacteria, but the infection loads were so low that they didn't affect the experiment. Many fewer control and saline injected fish were determined to be infected with *R. salmoninarum* when using the DFAT compared to the qPCR assay (Table 3.1).

Table 3.1. Summary of qPCR and DFAT results from each of the four treatments. Data include the percent of fish that died during the experiment (Mortality) or were euthanized at ten weeks post-injection (Survived) that were infected with *R. salmoninarum* (% Infect), and the average *R. salmoninarum* cells g^{-1} in the homogenized tissue samples of the two groups, estimated from the qPCR standard curve.

	qPCR				DFAT	
Treatment	Mortality (% Infect)	Mortality (cells g^{-1})	Survived (% Infect)	Survived (cells g^{-1})	Mortality (% Infect)	Survived (% Infect)
Control	NA	NA	100	22	NA	25
Saline	NA	NA	100	8	NA	10
ATCC	100	765,108	100	351,187	100	100
Unknown	100	683,463	100	716,880	100	100

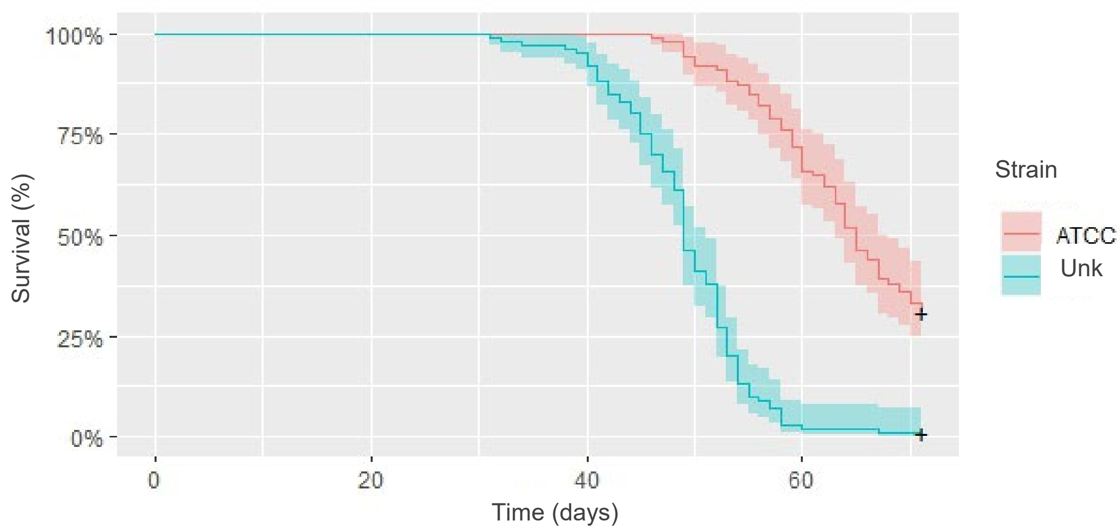

Figure 2. Kaplan-Meier cumulative percent survival of Chinook Salmon injected with the ATCC strain (red) and the Unknown strain (blue), plotted daily over the 71-day exposure experiment.

The exposure experiment lasted 71 days. Fish injected with the Unknown strain started dying from an *R. salmoninarum* infection at 31 days post-injection, whereas fish injected with the ATCC strain started dying from an *R. salmoninarum* infection at 46 days post-injection. Overall, only 1% of the fish injected with the Unknown strain compared to 31% of the fish injected with the ATCC strain survived to the end of the experiment (Figure 3.2). Fish injected with either the ATCC or Unknown strain that died during the experiment had a similar number of bacterial cells g^{-1} as determined by the qPCR standard curve. However, fish injected with the ATCC strain that survived to the end of the experiment had fewer bacterial cells g^{-1} than did those injected with the Unknown strain, suggesting that Chinook Salmon were able to clear the ATCC strain infection (Table 3.2). Taken together, the results of this experiment suggest that 1) location-of-origin of the bacterial strain and exposed fish has an effect on both mortality and the ability of exposed fish to clear an infection, and 2) that the Unknown strain isolated from Colorado may not be less virulent than the ATCC strain isolated from the Pacific Northwest when fish from outside the strains range are exposed to the bacteria.

The Chinook Salmon injection experiment is the first of two experiments that will occur as part of this M.S. project. The next step is to start another exposure experiment in which three Cutthroat Trout *O. clarkii* strains (Lake Nanita, Yellowstone Cutthroat Trout originating from LeHardy Rapids, and Trappers Lake) will be injected with the two bacterial strains to understand cumulative survival. We predict that injection with the ATCC strain may cause lower survival than injection with the Unknown strain because the fish have not been previously exposed to the ATCC strain. Results from the second injection experiment will be available in the next reporting cycle.

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Elliott, D. G., L. J. Applegate, A. L. Murray, M. K. Purcell, and C. L. McKibben. 2013. Bench-top validation testing of selected immunological and molecular *Renibacterium salmoninarum* diagnostic assays by comparison with quantitative bacteriological culture. Journal of Fish Diseases 36(9):779-809.

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Riepe, T. B. 2022. Detection and transmission of *Renibacterium salmoninarum* in Colorado inland trout. Dissertation. Colorado State University, Fort Collins, Colorado.

Riepe, T. B., E. R. Fetherman, B. Neuschwanger, T. Davis, A. Perkins, and D. L. Winkelman. 2023. Vertical transmission of *Renibacterium salmoninarum* in Cutthroat Trout (*Oncorhynchus clarkii*). Journal of Fish Diseases 46(4):309-319.

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TECHNICAL ASSISTANCE

Effective communication between researchers, fishery managers and hatchery supervisors is essential to the management of fish populations in Colorado and across the globe. The objective of technical assistance is to provide information on impacts of fish disease on wild trout populations to the Management and Hatchery Sections of CPW and other resource agencies through publications, presentations, and research collaborations, as well as contribute editorial assistance to professional journals and other organizations upon request.

Internal presentations to CPW staff were used to update managers on current research and inform management decisions in Colorado. One presentation was given at the CPW Aquatic Research Section Meeting, and one at the CPW Aquatic Section Meeting:

- Fetherman, E. R. 2022. Salmonid disease and sport fish research updates. Colorado Parks and Wildlife 2022 Aquatic Research Section Meeting. Fort Collins, Colorado. November 30, 2022.
- Richer, E., E. Fetherman, J. Ewert, D. Kowalski, and M. Kondratieff. 2023. Windy Gap fish passage project Colorado River. Colorado Parks and Wildlife Aquatic Section Meeting. Florence, Colorado. January 19, 2023.

External presentations and posters provided an opportunity to give research updates to managers both within and outside Colorado. One talk was given at the First National Stream Restoration Conference, six talks were given at the American Fisheries Society Annual Meeting, two talks at the annual meeting of the Colorado/Wyoming Chapter of the American Fisheries Society, a presentation was given to the Fraser River Ranch Fishing Club, and one talk was given at the annual meeting of the Western Division of the American Fisheries Society:

- Kondratieff, M. C., T. Swarr, E. R. Fetherman, K. Kiel, and E. E. Richer. 2022. Response of trout populations and pool depths to large wood in streams. First National Stream Restoration Conference. Nashville, Tennessee. August 1, 2022.
- Winkelman, D. L., T. B. Riepe, E. R. Fetherman, and B. W. Avila. 2022. Introduction to enhancing salmonid disease management by understanding pathogen transmission. 152nd Annual Meeting of the American Fisheries Society. Spokane, Washington. August 25, 2022.
- Riepe, T. B., D. L. Winkelman, E. R. Fetherman, and R. McDevitt. 2022. Horizontal and vertical transmission of *Renibacterium salmoninarum* in Cutthroat Trout. 152nd Annual Meeting of the American Fisheries Society. Spokane, Washington. August 25, 2022.
- McDevitt, R., T. B. Riepe, D. L. Winkelman, and E. R. Fetherman. 2022. The effects of *Renibacterium salmoninarum* on Brook Trout population characteristics. 152nd Annual Meeting of the American Fisheries Society. Spokane, Washington. August 25, 2022.
- Fetherman, E. R., and B. W. Avila. 2022. Whirling disease-resistant Rainbow Trout development and post-stocking survival evaluations. 152nd Annual Meeting of the American Fisheries Society. Spokane, Washington. August 25, 2022.
- Avila, B. W., D. L. Winkelman, and E. R. Fetherman. 2022. Assessing resistance to *Flavobacterium psychrophilum* and *Myxobolus cerebralis* in Rainbow Trout. 152nd Annual Meeting of the American Fisheries Society. Spokane, Washington. August 25, 2022.
- Nehring, J., T. Swarr, E. R. Fetherman, J. Spohn, and R. B. Nehring. 2022. Challenges of eradicating whirling disease from high elevation lakes. 152nd Annual Meeting of the American Fisheries Society. Spokane, Washington. August 25, 2022.
- Wright III, F. B., E. R. Fetherman, and E. A. Krone. 2023. Rapid surveillance of *Myxobolus cerebralis* in small headwater streams using environmental DNA. 2023 Annual Meeting of the Colorado/Wyoming Chapter of the American Fisheries Society. Fort Collins, Colorado. March 2, 2023.
- Riepe, T. B., R. McDevitt, E. R. Fetherman, and D. L. Winkelman. 2023. The effects of *Renibacterium salmoninarum* infection on Brook Trout population characteristics. 2023 Annual Meeting of the Colorado/Wyoming Chapter of the American Fisheries Society. Fort Collins, Colorado. March 2, 2023.
- Fetherman, E. R., E. E. Richer, M. C. Kondratieff, J. Ewert, and D. A Kowalski. 2023. Update on Fraser River Ranch salmonid population and the Windy Gap fish movement study. Fraser River Ranch Fishing Club Party. Lakewood, Colorado. March 30, 2023.
- Fetherman, E. R., B. Neuschwanger, T. Davis, and D. Karr. 2023. Balancing feed costs and growth, health, and angler preference for catchable Rainbow Trout reared on the standard feeds from four commercial feed manufacturers. 2023 Annual Meeting of the Western Division of the American Fisheries Society. Boise, Idaho. May 11, 2023.

In addition to public and professional meeting presentations, two presentations were given to the fisheries management class at Front Range Community College in Fort Collins, Colorado. The first, an informal presentation/laboratory, was presented at the BFRH. During this lab, students learned about the various tagging methods used in research and management across Colorado, and were given a chance to try the tagging methods on live fish. The second, a formal presentation, was given to the class in March 2023.

- Fetherman, E. R. 2023. Salmonid disease research in Colorado. Guest lecture, Introduction to Fisheries. Front Range Community College. Fort Collins, Colorado. March 23, 2023.

Manuscripts published in peer-reviewed scientific journals help to inform fisheries management decisions locally, nationally, and internationally. One manuscript was published in Pathogens, one in the Journal of Fish Diseases, one in the North American Journal of Aquaculture, two in the North American Journal of Fisheries Management, one in Conservation Science and Practice, and one in Animals:

- Avila, B. W., K. P. Huyvert, D. L. Winkelman, and E. R. Fetherman. 2022. Factors affecting post-challenge survival of *Flavobacterium psychrophilum* in susceptible rainbow trout from the literature. Pathogens 11:1318. https://doi.org/10.3390/pathogens11111318.
- Riepe, T. B., E. R. Fetherman, B. Neuschwanger, T. Davis, A. Perkins, and D. L. Winkelman. 2023. Vertical transmission of *Renibacterium salmoninarum* in cutthroat trout (*Oncorhynchus clarkii*). Journal of Fish Diseases 46:309-319. DOI: https://doi.org/10.1111/jfd.13745.
- Kopack, C. J., E. D. Broder, E. R. Fetherman, R. Fitzpatrick, L. M. Angeloni. 2023. Assessing antipredator behavior and the potential to enhance it in a species of conservation concern. North American Journal of Aquaculture 85:136-145. DOI: https://doi.org/10.1002/naaq.10281
- Erickson, T. A., G. J. Schisler, and E. R. Fetherman. 2023. Post-stocking survival and myxospore evaluation of whirling disease resistant Rainbow Trout strains. North American Journal of Fisheries Management 43:586-599. DOI: https://doi.org/10.1002/nafm.10878
- Avila, B. W., D. L. Winkelman, and E. R. Fetherman. 2023. Biotic and abiotic factors affecting short-term survival of two age-0 Rainbow Trout strains in Colorado streams. North American Journal of Fisheries Management 43:786-793.
- Kopack, C. J., E. R. Fetherman, E. D. Broder, R. Fitzpatrick, and L. M. Angeloni. 2023. The effects of environmental enrichment on the behavior, morphology, and survival of a species of conservation concern. Conservation Science and Practice 2023:e12999. https://doi.org/10.1111/csp2.12999
- Fetherman, E. R., B. Neuschwanger, C. Praamsma, and T. Davis. 2023. Concentration, life stage, feeding, density, flow, and strain effects on formalin sensitivity in rainbow trout (*Oncorhynchus mykiss*). Animals 2023(13):2425. https://doi.org/10.3390/ani13152425

Three manuscripts were additionally submitted for publication in peer-reviewed scientific journals:

- Kopack, C. J., E. D. Broder, C. McDonald, E. R. Fetherman, K. L. Hoke, and L. M. Angeloni. *In review*. The effect of environmental enrichment on whole-brain gene expression in an imperiled fish. Submitted to Molecular Ecology.
- Avila, B. W., D. L. Winkelman, E. R. Fetherman. *In review*. Hatchery rearing density affects post-stocking survival. Submitted to North American Journal of Aquaculture.
- Reipe, T. B., E. R. Fetherman, and D. L. Winkelman. *In review*. The potential for horizontal transmission of *Renibacterium salmoninarum* in a flow-through hatchery system. Submitted to Journal of Aquatic Animal Health.

Additionally, Eric Fetherman is coauthor on Nutrition and Feeding chapter of Piper Redux, the third edition of Fish Hatchery Management being published by the American Fisheries Society.

Technical assistance milestones included consultation and assistance with experimental design, data collection and analysis on projects being conducted by CPW researchers and biologists, as well as Colorado State University:

- Discussed results of whirling disease retrospective study from 2022 and future directions for the study.
- Provided input regarding analysis of half heads for measuring *Myxobolus cerebralis* infection rates using PTD and qPCR techniques.
- Discussed best practices for filtering water samples for triactinomyxons.
- Advised on tagging techniques, tag sizes, and equipment needs for tagging tiger muskie.
- Provided direction on Rock Creek sentinel cage project to determine whirling disease persistence in reclaimed creek.
- Guided post-doc and CPW researcher through the Program Mark interface and how to create models using the design matrix.
- Developed ideas for population genetics projects to be completed by CPW and CSU in 2023-2024.

Sport Fish Research staff supported research projects using AQUI-S 20E under the Investigational New Animal Drug Program as program administrator:

- Fetherman et al. 2022. PIT tagging fish in Fraser River. August 2022-October 2022.
- Fetherman et al. 2022. PIT tagging fish in Colorado River. August 2022-November 2022.
- Britain et al. 2022. PIT tagging fish in St. Vrain River. September 2022-November 2022.

Technical assistance included peer review of manuscripts submitted to scientific journals:

- Rzhechitskiy, Y., A. Gurkov, N. Bolbat, E. Shchapova, A. Nazarova, M. Timofeyev, and E. Borvinskaya. *In review*. Adipose fin as a natural "optical window" for implantation of fluorescent sensors into salmonid fish. Submitted to *Animals*.
- Anonymous. *In review*. Effects of elevated water temperatures on trout angler catch rates and catch-and-release mortality. Submitted to *Fisheries Management and Ecology*.
- Rosendal, K., A. Kettunen, and I. Olesen. *In review*. Policies to promote breeding for lice-resistant salmon – incentives designed for resilient and sustainable growth in aquaculture? Submitted to *Journal of Fish Biology*.

An internal CPW review was also conducted prior to manuscript submission for publication:

- Riepe, T. B., Z. Hooley-Underwood, R. E. McDevitt, A. Sralik, and P. Cadmus. *In preparation*. Effects of temperature on egg development, hatch success, larval survival, and thermal tolerance of Bluehead Sucker (*Catostomus discobolus*). Intended for submission to Journal of Thermal Biology.

Service outside of CPW:

- Member of the North American Journal of Aquaculture subcommittee of the AFS Publication Awards Committee. 2022-2023.
- President-Elect of the Western Division of the American Fisheries Society. 2022-2023.
    - Officer Liaison to the Western Division Early Career Professionals Committee.
    - Member of the Western Division Diversity and Inclusion Committee.
    - Member of the AFS Management Committee.

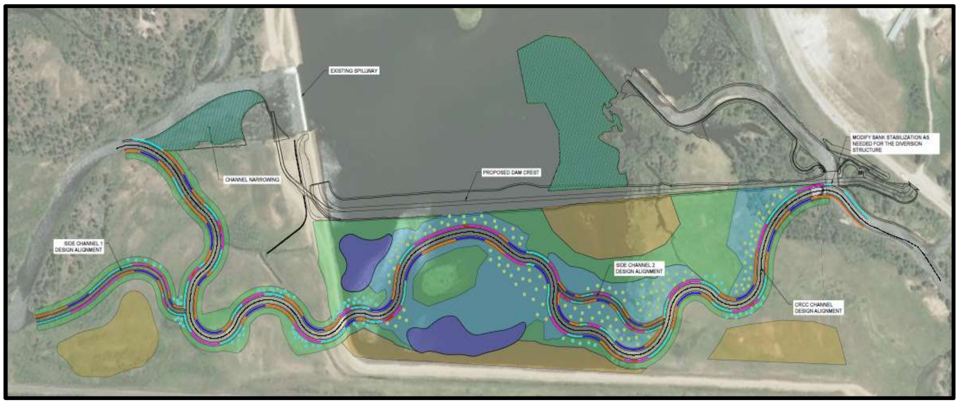
- Member of the AFS Governing Board.
- Member of the Grand Rapids Program Committee.
- Member of the Boise Meeting Core Planning Team.
- Co-chair of the Boise Meeting Program Committee.
- Organizer of the Best of the Western Division symposium.
- Member of the 2024 Honolulu Meeting Core Planning Team.
- Local Team Lead for the 2024 Honolulu Meeting Planning Team.
- Member of the 2024 Honolulu Meeting Program Committee.

C O L O R A D O   P A R K S   &   W I L D L I F E

Windy Gap Fish Movement Study

EVALUATING MOVEMENT IN THE COLORADO AND FRASER RIVERS PRIOR TO CONSTRUCTION OF THE CRCC

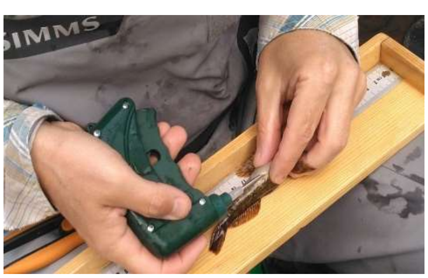
Windy Gap Fish Movement Study Objectives



The Windy Gap fish movement study is being conducted in Grand County in conjunction with the construction of the Colorado River Connectivity Channel (CRCC), which will reconnect the Colorado and Fraser rivers above Windy Gap Reservoir to the Colorado River below Windy Gap Reservoir for the first time since the early 1980s. Construction of the CRCC around Windy Gap Reservoir will allow fish movement and provide access to favorable habitats, such as optimal spawning locations, that have been previously unavailable to populations upstream and downstream of the reservoir. The primary objectives of the study are to evaluate fish movement patterns prior to the construction of the CRCC, and that the CRCC is being used for fish passage after construction. Evaluations are taking place on Northern Water, City of Granby, and private properties above and below Windy Gap Reservoir. To accomplish this, the fish movement study involves collaboration between these entities, as well as CPW aquatic researchers and biologists, Trout Unlimited, and Grand County Learning by Doing.

Population Estimates and PIT Tagging

Population estimates conducted in 2020-2022 provide baseline data on fish populations, and allow researchers to monitor and compare changes in these populations before and after construction of the CRCC. These estimates also allow opportunities to tag fish with Passive Integrated Transponder (PIT) tags used to evaluate fish movements. PIT tags use the same technology as those commonly used to identify pets, providing a unique ID for every fish that is detected by an antenna. PIT-tagged fish were released annually in the fall at two sites in the Colorado River and two sites in the Fraser River above Windy Gap Reservoir. Additionally, PIT-tagged fish were released in the spring throughout a four-mile stretch of the Colorado River below Windy Gap Reservoir. Since the study began in 2020, 4,234 fish have been PIT tagged in the Colorado and Fraser rivers, including 3,138 Brown Trout, 611 Rainbow Trout, 6 Cutbows (hybrids of Cutthroat Trout and Rainbow Trout), and 479 Mottled Sculpin. Mottled Sculpin are currently absent downstream of Windy Gap Reservoir. The CRCC is intended to facilitate sculpin distribution, increasing the diversity of the Colorado River downstream of the reservoir.

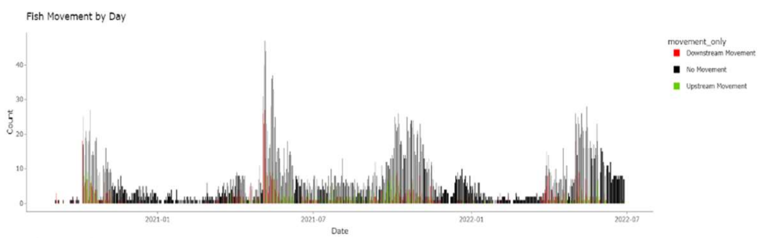



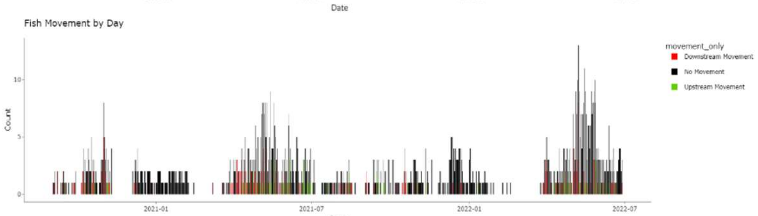

Left to right: 32-mm PIT tag for evaluating Brown Trout and Rainbow Trout movements in the Colorado and Fraser rivers; 12-mm PIT tag being inserted into a Mottled Sculpin; paired stationary antennas installed on the bottom of the river used to evaluate directionality of movement; antenna station containing readers for recording tags as they pass stationary antennas; mobile antennas deployed in the Fraser River.

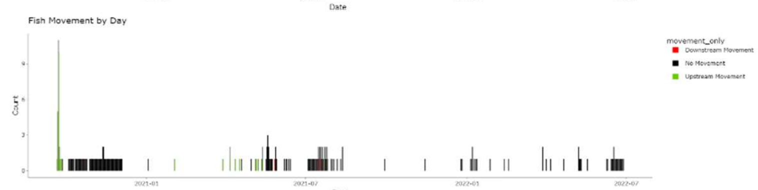
Monitoring Movement with Stationary and Mobile Antennas

Movements of PIT-tagged fish are passively monitored using two stationary antenna sites in the Colorado River below Windy Gap Reservoir and one stationary antenna site at the confluence of the Colorado and Fraser rivers above Windy Gap Reservoir. Paired antennas were installed on the bottom of the river to allow researchers to determine direction of movement, depending upon which of the two antennas a fish passes first. The antennas are connected to readers that store the date and time for every tag detected and are operated continuously 24 hours a day, 365 days a year. Antennas constructed in the bottom of rafts use GPS sensors to actively locate PIT-tagged fish in the Colorado and Fraser rivers in April, July, and October. These mobile antennas allow researchers to determine the location of sedentary fish such as Mottled Sculpin or certain age classes of Brown Trout and Rainbow Trout that move shorter distances, making it less likely that they would be detected as a stationary antenna site.

Data collected by the stationary antennas have provided some valuable insights into fish movement within the study area. Generally, fish move more often at night than during the day, especially Brown Trout, likely to avoid predators. Brown Trout and Rainbow Trout exhibit seasonal movements concurrent with their spawning seasons, Brown Trout in the fall and Rainbow Trout in the spring. Movement distance and timing is also dependent on fish size. Mottled sculpin do not exhibit seasonal movements, but rather move sporadically throughout the year.







Visualizing the Data

Maps such as this one depicting tagged fish release sites in colored stars and the location of fish from those release sites in the same colored circles allow researchers to draw conclusions about the distances fish have moved and overall movement patterns of the populations as a whole. Using data obtained from the mobile antenna surveys, we have seen that, upstream of Windy Gap Reservoir, some fish released in the Fraser River moved into the Colorado River, and vice versa, and that long distance movements are made in the Colorado River below Windy Gap Reservoir during certain times of year. Data collected from the stationary antenna sites, mobile antenna surveys, and recaptures of tagged fish during the population estimates will be used to obtain estimates of detection, survival, and movement probabilities, by species and fish size, before and after construction of the CRCC.