# Sport Fish Research Studies

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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. The Harrison Lake (HL or HAR; used interchangeably throughout) strain of Rainbow Trout is a wild lake strain from Harrison Lake, Montana that shows some natural resistance to *M. cerebralis* and survives well when stocked into lakes and reservoirs. Crosses of the GR and HL strains show increased resistance over the pure HL strain. Brood stocks of the GR and HL strains are 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 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 early November 2021, BFRH personnel checked all of the two- and three-year-old GR and HL 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 17, 2021, the first group of GR females were ripe and ready to spawn, followed by the first group of HL females on December 14, 2021.

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 from the GR and HL were moved to the BFRH main hatchery building. Extreme caution was used to keep each strain separate from the other. 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 and specific strains 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. Select groups of eggs were kept for brood stock purposes at the BFRH (Table 1.1).

**Table 1.1.** Bellvue Fish Research Hatchery on-site spawning information for the Hofer (GR) and Harrison Lake (HL) Rainbow Trout strains during the winter 2021-2022 spawning season.

| Strain | Date Spawned | No. Spawned Females | No. Green Eggs | No. Eyed Eggs | Destination |
|---|---|---|---|---|---|
| HL | 12/14/21-1/4/22 | 96 | 50,960 | 30,968 | BFRH and Seaman Reservoir |
| GR | 11/17/21-12/7/21 | 100 | 1,000 | 850 | BFRH |
| Total | 11/17/21-1/4/22 | 196 | 51,960 | 31,818 | |

The on-site Rainbow Trout production spawn started on November 17, 2021, with the last group of GR females spawned on December 7, 2021 and last group of HL females spawned on January 4, 2022 (Table 1.1). The goal was to produce 1,000 eggs per strain for brood stock replacement purposes, with an additional 25,000 HL eggs requested for Seaman Reservoir. Both goals were met, with 30,303 three-inch subcatchable HL fish produced for the Seaman Reservoir request.

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 BFRH for incoming YY Brook Trout *Salvelinus fontinalis*, and 2) increase the Poudre Rearing Unit production using *M. cerebralis*-resistant fish. The first year class of YY Brook Trout was imported from the Idaho Fish and Game (IDFG) Hayspur Fish Hatchery (Bellevue, Idaho) to the BFRH on June 27, 2022 to be utilized for production in Colorado. Subsequent year classes will be obtained from IDFG to build up the brood stock at the BFRH.

### Annual Disease Testing

The BFRH annual disease inspection was conducted on April 4, 2022. 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 EXPERIMENT

Details regarding the involvement of the BFRH in the experimental development of a YY Brown Trout *Salmo trutta* brood stock can be found in Fetherman et al. (2021). The following briefly describes the YY Brown Trout experiment conducted at the BFRH within the last reporting cycle.

**Table 1.2.** Proportion of fish determined to be female, intersexed, male, or undetermined by treatment (bold) and tank (T) in the YY Brown Trout experiment conducted at the BFRH in 2021. Treatment descriptions include amount of estradiol used to top coat feed (mg/kg; mg) and the duration (days; d) that the treated feed was fed to each experimental group.

| Treatment/Tank | Total | Prop Female | Prop Intersexed | Prop Male | Prop Undetermined |
|---|---|---|---|---|---|
| **Control** | 125 | 48.8% | 0% | 51.2% | 0% |
| T15 | 64 | 53.1% | 0% | 46.9% | 0% |
| T16 | 61 | 44.3% | 0% | 55.7% | 0% |
| **30 mg 60 d** | 120 | 80.8% | 4.2% | 13.3% | 1.7% |
| T8 | 60 | 85.0% | 3.3% | 11.7% | 0% |
| T9 | 60 | 76.7% | 5.0% | 15.0% | 3.3% |
| **60 mg 60 d** | 120 | 79.2% | 10.8% | 10.0% | 0% |
| T11 | 60 | 81.7% | 8.3% | 10.0% | 0% |
| T12 | 60 | 76.7% | 13.3% | 10.0% | 0% |
| **30 mg 75 d** | 120 | 85.0% | 6.7% | 8.3% | 0% |
| T2 | 60 | 90.0% | 6.7% | 3.3% | 0% |
| T3 | 60 | 80.0% | 6.7% | 13.3% | 0% |
| **10 mg 90 d** | 120 | 77.5% | 7.5% | 13.3% | 1.7% |
| T19 | 60 | 76.7% | 8.3% | 11.7% | 3.3% |
| T20 | 60 | 78.3% | 6.7% | 15.0% | 0% |
| **20 mg 90 d** | 120 | 86.7% | 3.3% | 7.5% | 2.5% |
| T13 | 60 | 83.3% | 3.3% | 13.3% | 0% |
| T14 | 60 | 90.0% | 3.3% | 1.7% | 5.0% |
| **30 mg 90 d** | 120 | 83.3% | 7.5% | 8.3% | 0.8% |
| T6 | 60 | 86.7% | 5.0% | 8.3% | 0% |
| T7 | 60 | 80.0% | 10.0% | 8.3% | 1.7% |
| **60 mg 90 d** | 60 | 85.0% | 10.0% | 3.3% | 1.7% |
| T10 | 60 | 85.0% | 10.0% | 3.3% | 1.7% |
| **20 mg 120 d** | 115 | 96.5% | 1.7% | 1.7% | 0% |
| T17 | 55 | 94.5% | 1.8% | 3.6% | 0% |
| T18 | 60 | 98.3% | 1.7% | 0% | 0% |
| **30 mg 120 d** | 123 | 93.5% | 0.8% | 4.1% | 1.6% |
| T4 | 61 | 95.1% | 1.6% | 3.3% | 0% |
| T5 | 62 | 91.9% | 0% | 4.8% | 3.2% |
| **Grand Total** | 1143 | 81.3% | 5.0% | 12.8% | 1.0% |

In the fall of 2020, the BFRH acquired Brown Trout eggs from the spawning operations conducted at North Delaney Buttes Lake. These fish were hatched and reared in the BFRH Isolation Building #1. The Brown Trout were divided into 19 tanks, each containing 75 fish. Ten treatments were randomly assigned to two tanks each, with the exception of the 60 mg/kg estradiol (E2)-coated feed fed for a duration of 90 days (Table 1.2). Fish were fed a diet of E2-coated feed (10, 20, 30, or 60 mg/kg) for a duration of 60, 75, 90, or 120 days. Control fish were fed an ethanol-coated feed for 120 days. Treatments were chosen based on the results of a previous experiment conducted at the BFRH (Fetherman et al. 2021). After each scheduled feeding period was completed, fish were switched over to a normal (no top coating) trout diet.

On April 27-28, 2021, 75 fish in each tank were tagged using individual 12-mm pre-loaded PIT tags, measured (mm), weighed (g), and a fin clip was taken for genetic analysis. Tagged fish were returned to their respective treatment tanks to continue normal feeding and rearing. Once fish outgrew their experimental tanks in FR1, they were moved into troughs in the main hatchery building to complete the experiment. A subset of the fish remaining in each treatment were euthanized and dissected to determine sex via visual inspection of the gonads between November 29 and December 2, 2021. Most of the treatments were successful in producing feminized males, especially relative to the control treatment (Table 1.2).

The BFRH continues to be involved in the evaluation of the treated fish from the experiments conducted at the hatchery in 2020 and 2021. In the fall of 2022, Brown Trout spawned in 2019 and experimentally treated with E2 in 2020 will be spawned with male Brown Trout spawned in 2020 and treated with E2 in 2021 to assess overall egg production, and hatch and swim-up rates. Results will be available in the next reporting cycle.

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.

## **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.

*2021 Salmonid Fry Population Estimates*

Rainbow Trout fry stocking evaluations began in the upper Colorado River Rainbow Trout 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).

On July 13, 2021, approximately 140,000 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 20,000 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 2021. 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. Although this current study is focused on the Chimney Rock/Sheriff Ranch study section, 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 starting on the State Wildlife Area in 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 2020 and 2021 to increase Rainbow Trout recruitment in this section of the river.

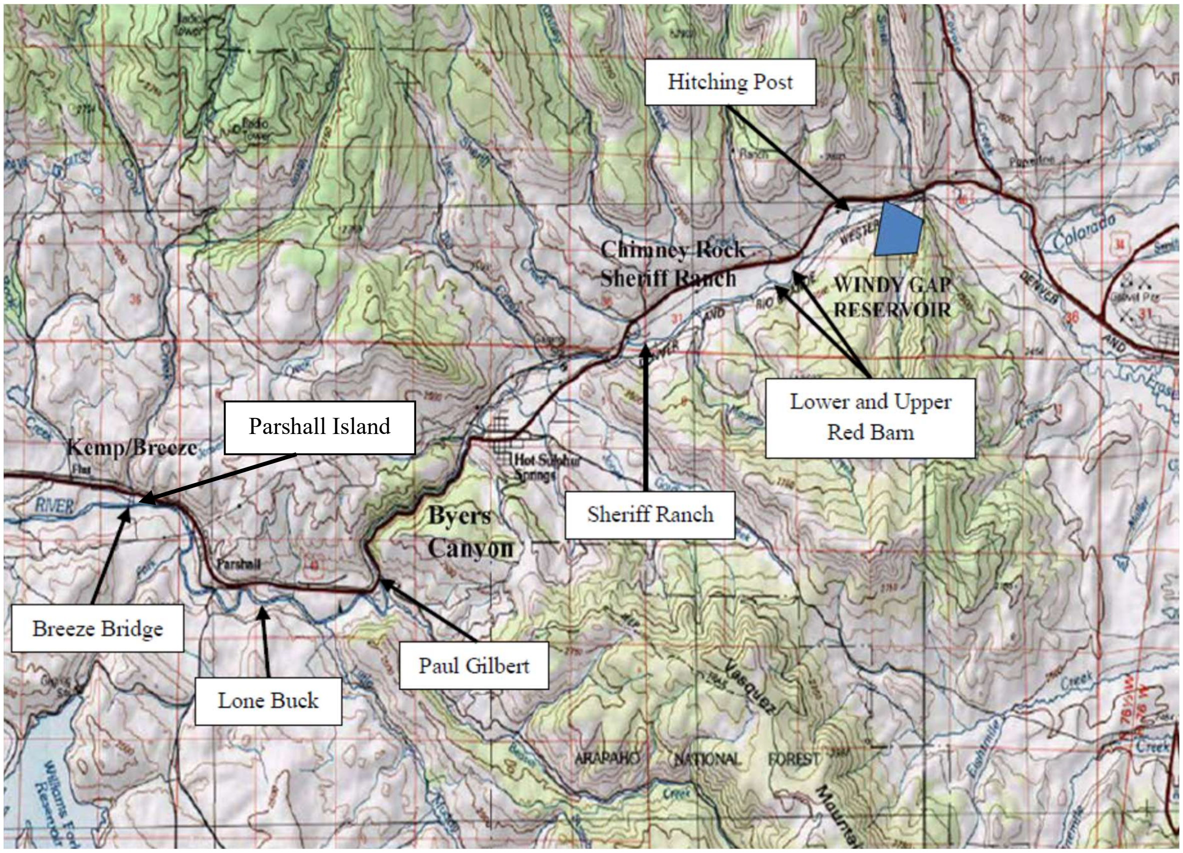


**Figure 2.1.** Upper Colorado River study area showing the eight sites at which salmonid fry population estimates were conducted in July, August, September, and October 2021.

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 2021, 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 from three sites below Byers Canyon in October 2021 for myxospore enumeration (see fry estimate results below). Below Byers Canyon, Brown Trout averaged 991 (± 637) myxospores per fish. None of the Brown Trout fry exhibited clinical signs of disease. Rainbow Trout fry, which were encountered in and collected from all eight fry sites in October 2021, averaged 301 (± 151) myxospores per fish, lower than the previous year (Fetherman et al. 2021). As with the Brown Trout fry, none of the Rainbow Trout fry collected for myxospore enumeration exhibited clinical signs of disease.

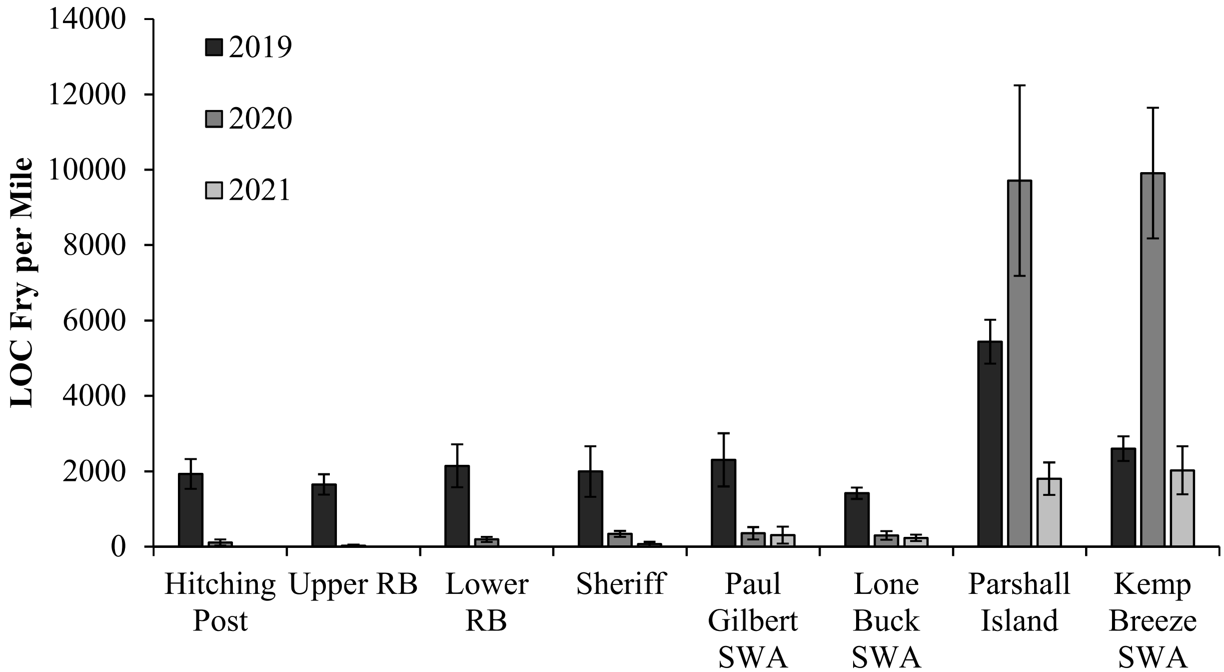


**Figure 2.2.** Average Brown Trout (LOC) fry abundance (fry per mile; SE bars) in 2019 (typical), 2020 (atypical), and 2021 (atypical) between Hitching Post Bridge, the furthest upstream site, and Kemp Breeze SWA, the furthest downstream site, in the Colorado River.

Brown Trout fry are typically 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). The issues persisted into 2021, further reducing Brown Trout fry abundances above Byers Canyon. Only four Brown Trout fry were captured across all sampling occasions in the Chimney Rock/Sheriff Ranch study section, all at the Sheriff Ranch fry site (one in early July and three in September), much lower than the number of Brown Trout fry captured below Byers Canyon (Figure 2.2). This suggests a nearly complete loss of the 2021 age class of Brown Trout in the study section. Again, the cause is unknown. However, researchers observed very fine sediment deposited in interstitial spaces and covering rocks in the study section in May 2021, prior to runoff. The sediment likely originated from a second draining of Windy Gap Reservoir in the fall of 2020, which may have covered gravels or suffocated Brown Trout eggs, leading to an unsuccessful spawn or hatch. Flow conditions did not improve significantly in spring, summer, or fall 2021, and the fine sediment appeared to have remained in the system throughout the year. Fry sampling in 2022 will provide insight as to whether this fine sediment continues to affect the success of the Brown Trout spawn below Windy Gap Reservoir.

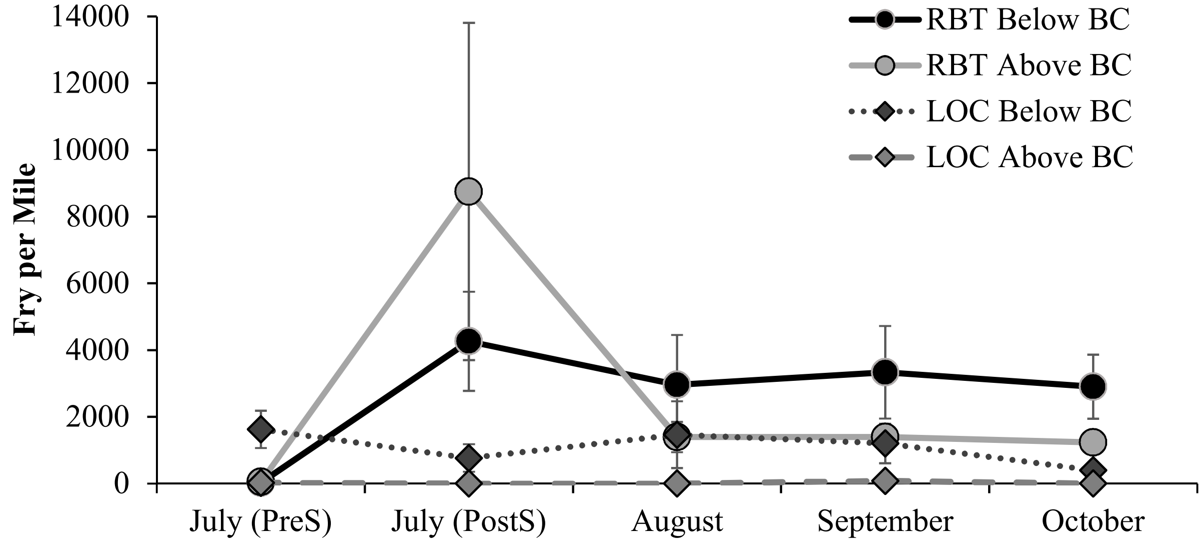


**Figure 2.3.** Average Rainbow Trout (RBT) and Brown Trout (LOC) fry abundance (fry per mile; SE bars) in the sites below Byers Canyon (BC; Breeze Bridge, Parshall Island, and Lower and Upper Paul Gilbert) and above Byers Canyon (Sheriff Ranch, Lower and Upper Red Barn, and Hitching Post Bridge) in 2021. H×G fry were stocked into all eight fry sites in July 2021.

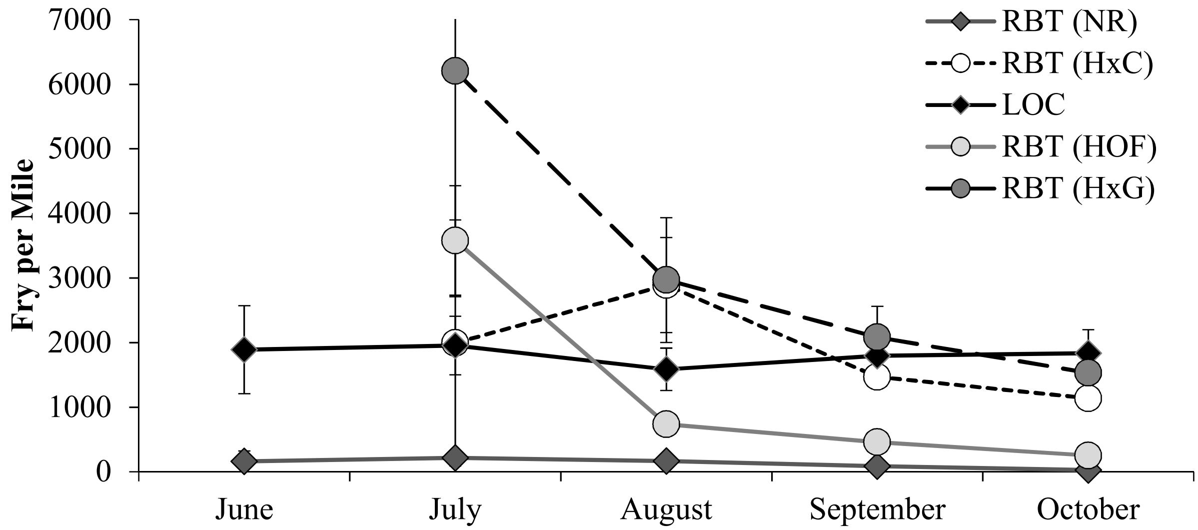


**Figure 2.4.** Upper Colorado River Brown Trout (LOC) fry abundance estimates averaged between 2013 and 2019, wild Rainbow Trout (RBT [NR]) abundance estimates averaged between 2008 and 2012, H×C fry (RBT [H×C]) abundance estimates averaged between 2013 and 2015, Hofer fry (RBT [HOF]) abundance estimates averaged between 2016 and 2018, and H×G fry (RBT [H×G]) abundance estimates averaged between 2019 and 2021 (fry per mile; SE bars). Stocking of H×C, HOF, and H×G fry occurred in July; no estimates are shown for June.

Very few wild Rainbow Trout fry were encountered in fry sites above or below Byers Canyon in early July 2021 (Figure 2.3). It is unknown whether the absence of wild Rainbow Trout fry is due to the relatively low adult Rainbow Trout abundances, or if the fine sediment observed in the river affected the success of the Rainbow Trout spawn as well as the Brown Trout spawn in 2021. Rainbow Trout fry abundance was significantly increased by stocking H×G fry in July. A few weeks after the fry were stocked, Rainbow Trout fry abundances were higher above Byers Canyon than below. However, there was a significant reduction in H×G fry in the Chimney Rock/Sheriff Ranch study site between July and August. Abundances in both locations remained relatively static between August and October, resulting in estimates of 1,235 (± 287) Rainbow Trout fry per mile above Byers Canyon and 2,904 (± 960) Rainbow Trout fry per mile below Byers Canyon in October 2021. In addition to potentially disrupting the spawn, fry abundance estimates suggest that fine sediment accumulation in interstitial spaces within the Chimney Rock/Sheriff Ranch study site may have reduced the amount of available fry habitat and resulted in lower abundances than both those below Byers Canyon and observed in the previous year (Fetherman et al. 2021).

H×G fry abundance was higher in 2019-2021 than had been observed for the H×C and HOF fry (Figure 2.4). H×G fry stocked in 2019 and 2020 were available for capture as age-2 and age-3 fish during the 2022 adult salmonid population estimates, and recruitment rates look promising. Following three years of decline in the adult Rainbow Trout population after stocking HOF fry, we observed similar increases in the adult population following H×G fry stocking as were seen following H×C fry stocking. Given the current results, the H×G appears promising for this use in the future. Although the reduced Brown Trout fry abundances in 2020 and 2021 were concerning, they may have reduced competition for the stocked H×G fry, and provided space for recruitment as these fish transition into the adult Rainbow Trout population.

The 2021 H×G stocking was the final stocking event for the fry evaluation study that began in 2013. Given the two year time lag between stocking and recruitment to the adult population, the adult Rainbow Trout population will continue to be monitored as part of this study through 2023. The H×G have performed well in the Colorado River, and will continue to be stocked above and below Byers Canyon to increase the adult Rainbow Trout population in these sections. Fry evaluations will continue as part of the monitoring plan associated with the Upper Colorado River Fish Movement Study through its completion in fall 2025.

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*2022 Adult Salmonid Population Estimates*

An adult salmonid population estimate was conducted in the Chimney Rock/Sheriff Ranch study section of the upper Colorado River in May 2022. Unlike previous years where raft electrofishing was used to sample the entire 3.9-mile study section, water levels were too low to run rafts. Therefore, CPW personnel chose to bank shock shorter reaches within the study section. Three reaches were chosen for sampling, Hitching Post, Red Barn, and Kinney Creek. The Hitching Post reach had historically been sampled via bank electorfishing prior to the initiation of the spring raft electrofishing in 2009. The Red Barn reach was chosen because it provided one of the longest continuous reaches in the middle of the section that had been stocked with Rainbow Trout fry between 2013 and 2021. The Kinney Creek reach was chosen because it was located downstream of where the majority of the fry stocking between 2013 and 2020 had occurred, although roughly 40,000 fry were stocked within the reach in 2021. The 760-foot Hitching Post reach and 555-foot Kinney Creek reach were sampled on May 2, 2022, and the 1,080-foot Red Barn Reach was sampled on May 5, 2022.

Population estimates in all three sites were accomplished using a two-pass removal with bank electrofishing gear. All fish captured were removed from the river and held in net pens separated by pass until they could be processed. Fish were measured (mm), weighed (g), and checked for a missing adipose fin, indicating that they had previously been tagged as part of the fish movement study. If fish had retained their tag, the tag number was recorded along with the total length (TL) and weight of the fish; if the tag was no longer present, the fish was retagged. Additionally, up to 90 Brown Trout and Rainbow Trout each were PIT tagged in each of the three sites, although the highest number of tagged Rainbow Trout released at a site was 56 at Hitching Post. Population estimates were calculated using the two-pass removal equations of Seber and Whale (1970), and the estimates and length-frequency data from the three sites were combined to allow comparison to previous years in which the entire study section had been sampled.

An estimated 5,986 (± 3,299) adult Brown Trout were present in the Chimney Rock/Sheriff Ranch study section in 2022, approximately 550 less than in 2021 (Fetherman et al. 2021). Overall, 1,535 (± 845) Brown Trout were present per mile in the study section, averaging 303 (± 63) mm TL and 317 (± 32) g. 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 2.5). The bank electrofishing gear allowed us to sample all age classes of fish, including fry and juveniles, effectively. Brown Trout fry and juvenile numbers remained low, reflecting the trends observed in the fry estimates conducted in 2020 and 2021. The adult population decreased in 2022 as a result, and will likely be reduced further as missing age classes fail to recruit to the adult population. These impacts will likely be more apparent in the 2023 adult population estimates.

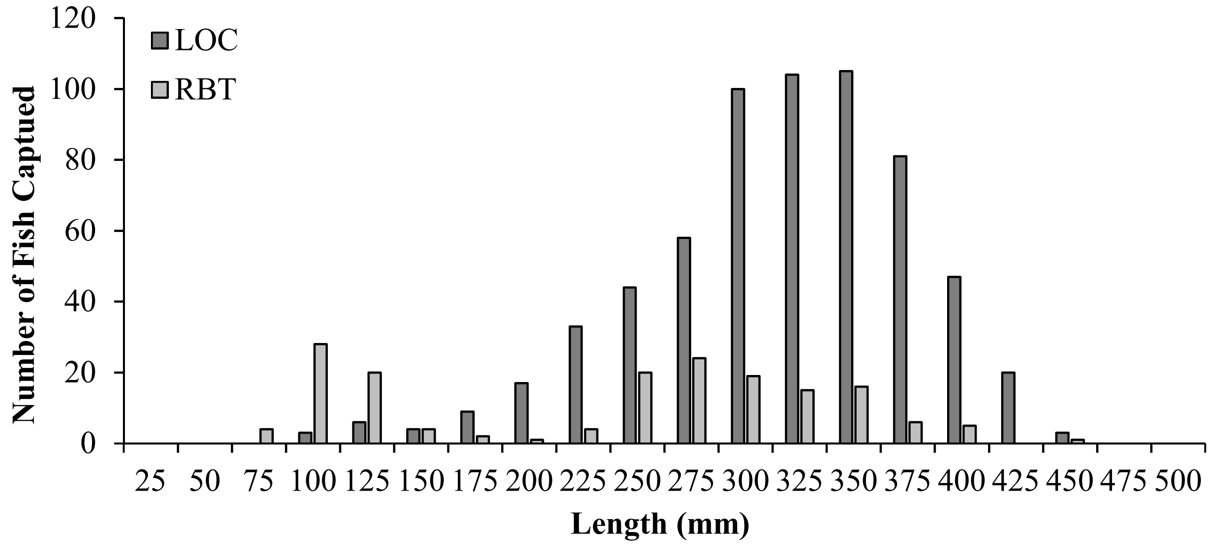


**Figure 2.5.** Number of Brown Trout (LOC) and Rainbow Trout (RBT) captured by total length (mm) during the 2022 adult salmonid population estimates in the Chimney Rock/Sheriff Ranch study section of the upper Colorado River.

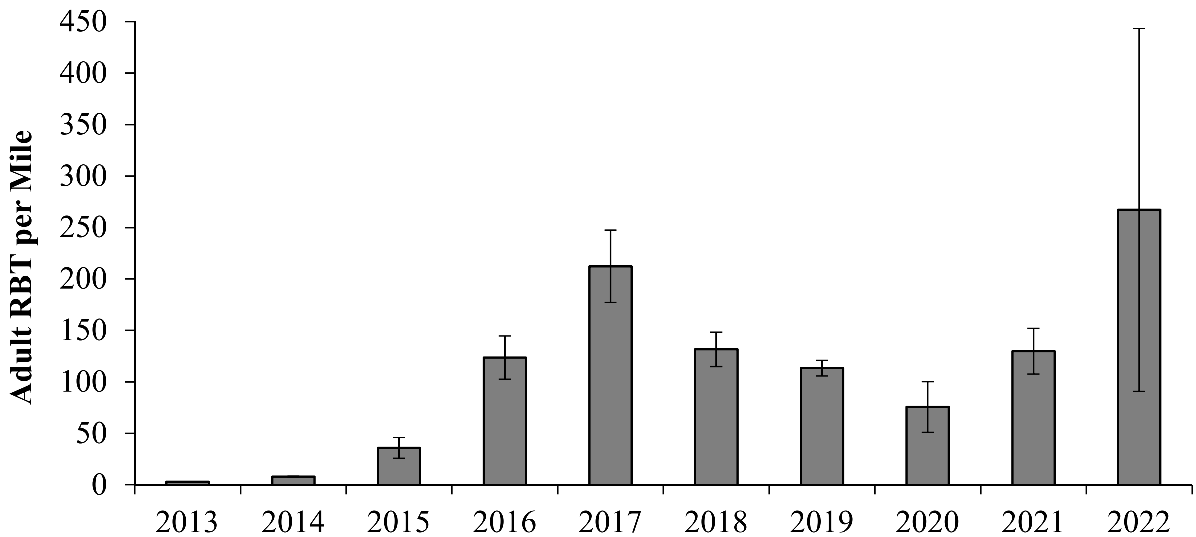


**Figure 2.6.** Estimated number of adult Rainbow Trout (RBT) per mile (SE bars) in the Chimney Rock/Sheriff Ranch study section of the upper Colorado River between 2013 and 2022.

Rainbow Trout densities increased between 2021 and 2022, with an estimated 506 (± 87) adult Rainbow Trout present in 2021 (Fetherman et al. 2021), and 1,041 (± 686) present in 2022. The adult Rainbow Trout abundance increased between 2013 and 2017 following H×C fry stocking, but the low survival of the GR fry resulted in fewer adult Rainbow Trout present in the study section between 2018 and 2020. The stocked H×G fry exhibited higher survival rates than the H×C or GR fry (Fetherman et al. 2021; Figure 2.4), resulting in an estimated 267 (± 176) adult Rainbow Trout per mile in 2022 (Figure 2.6), 138 fish per mile higher than in 2021 (Fetherman et al. 2021).

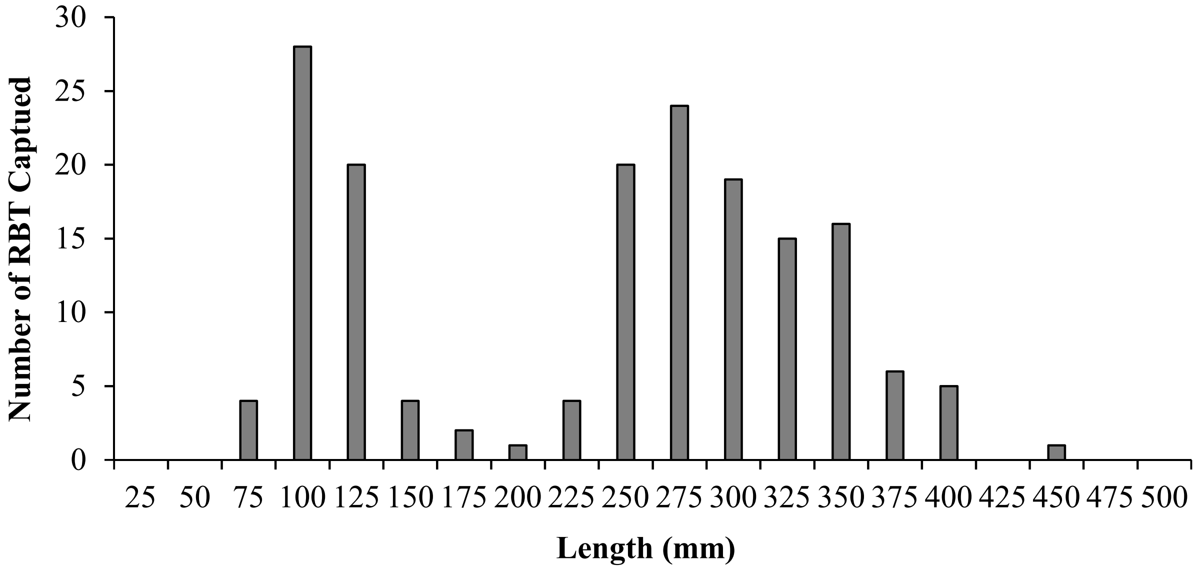


**Figure 2.7.** Number of Rainbow Trout (RBT) captured by total length (mm) during the 2022 adult salmonid population estimates in the Chimney Rock/Sheriff Ranch study section of the upper Colorado River.

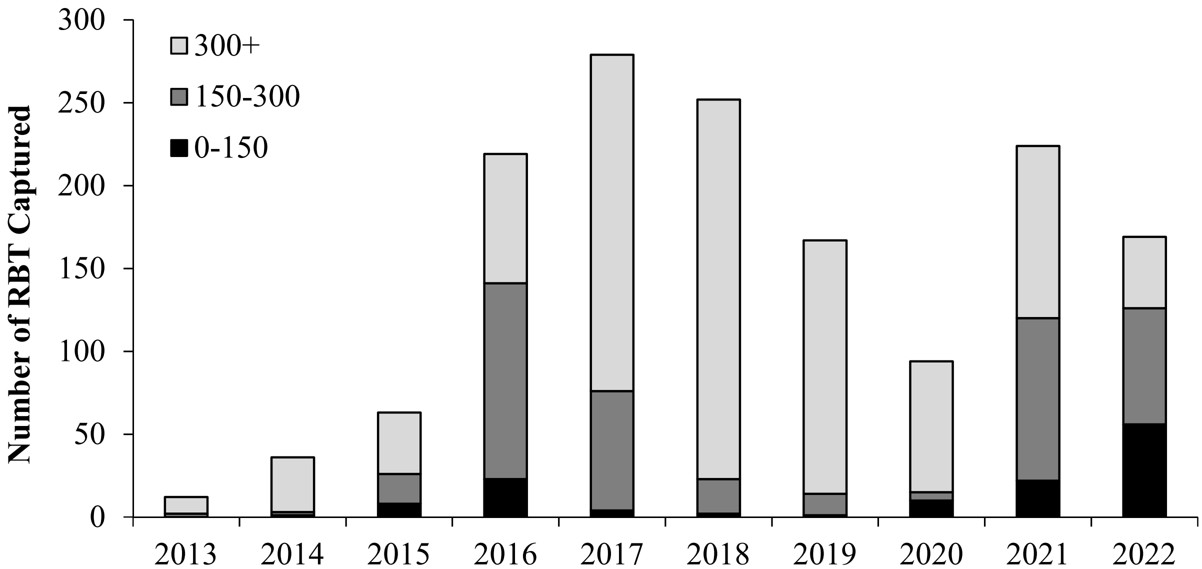


**Figure 2.8.** Number of age-1 (≤ 150 mm TL), age-2 (150-300 mm TL) and age-3+ (> 300 mm TL) Rainbow Trout (RBT) captured in the Chimney Rock/Sheriff Ranch study section of the upper Colorado River between 2013 and 2022.

Adult Rainbow Trout averaged 289 (± 50) mm TL and 253 (± 48) g, smaller than in 2021, potentially due to the large numbers of age-1 and age-2 fish (Figure 2.7). The bank electrofishing gear allowed us to sample the age-1 fish more effectively, the number of which was higher than in any year since the fry stocking evaluations began in 2013 (Figure 2.8). This suggests that H×G fry stocked in 2021 had survived well. Fewer age-2 and age-3 Rainbow Trout were captured than in previous years (Figure 2.8). However, this was likely due to the short sampling reaches, which did not cover as much of the available or occupied Rainbow Trout habitats as the raft electrofishing. Therefore, it is likely that Rainbow Trout estimates were conservatively low in 2022 due to the type of sampling conducted.

The adult Rainbow Trout population in the upper Colorado River exhibited an increase in abundance for the first time since 2017 in 2021, and an even larger increase in 2022. The H×G fry have exhibited high survival rates during their first year in the river (Fetherman et al. 2021), and appear to continue to do so as they recruit to the adult population. The reduced Brown Trout fry abundance may also have contributed to increased survival of H×G fry in 2020 and 2021. As fry recruit to the juvenile and adult population, these reduced age classes of Brown Trout may result in less competition for resources and higher survival for the H×G in future years. As a result, the adult Rainbow Trout population is expected to continue to increase into 2023.

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.

Seber, G. A. F., and J. F. Whale. 1970. The removal method for two and three samples. Biometrics 26(3):393-400.

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 31 and September 1, and in two stations on the City of Granby property at River Run in the Colorado River on October 5, 2021. 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 identify and quantify tag loss. Fin-clipped fish from previous tagging events were measured and weighed, and PIT tag number was 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).

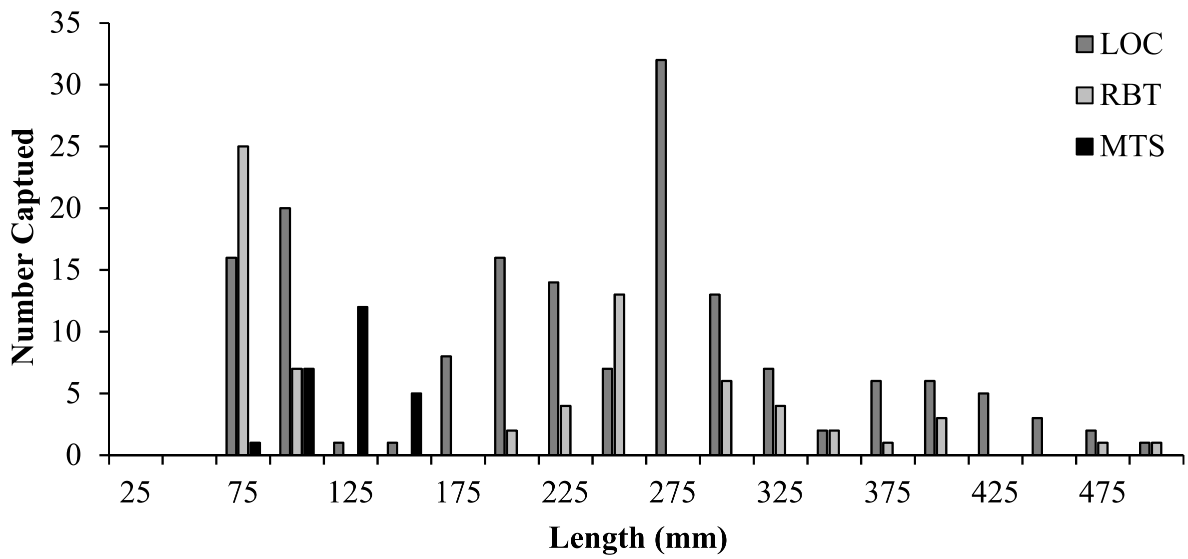


**Figure 2.9.** Number of Brown Trout (LOC), Rainbow Trout (RBT), and Mottled Sculpin (MTS) captured by total length (mm) during the Fraser River Ranch 2021 population estimate.

The 677-foot station located about 0.25 miles upstream of the railroad crossing at the lower end of the Fraser River Ranch contained 160 Brown Trout, 122 adults (≥ 150 mm total length [TL]) and 38 fry/juveniles (< 150 mm TL; Figure 2.9). An estimated 1,306 ± 34 Brown Trout per mile were present on the ranch, 971 ± 16 adult Brown Trout per mile and 406 ± 109 Brown Trout fry/juveniles per mile. Fewer adult Brown Trout, but similar numbers of fry/juvenile Brown Trout were present in 2021 compared to 2020 (Fetherman et al. 2021). Brown Trout averaged 226 ± 8 mm TL and 241 ± 19 g, with the largest measuring 476 mm TL and weighing 1,008 g. Brown Trout outnumbered Rainbow Trout in the total catch, with 70 Rainbow Trout captured, 38 adults and 32 fry/juveniles (Figure 2.9). An estimated 598 ± 39 Rainbow Trout per mile were present on the ranch, 298 ± 5 adult Rainbow Trout per mile and 938 ± 1,558 Rainbow Trout fry/juveniles per mile. Similar numbers of adult Rainbow Trout, but fewer fry/juvenile Rainbow Trout were present in 2021 compared to 2020 (Fetherman et al. 2021). Rainbow Trout averaged 190 ± 15 mm TL and 300 ± 41 g, with the largest measuring 502 mm TL and weighing 1,118 g. Twenty-five Mottled Sculpin *Cottus bairdii* were captured in the site. Twelve Mottled Sculpin were captured on the first pass, while 13 were captured on the second pass, and as such it was not possible to calculate a population estimate. Mottled Sculpin averaged 108 ± 4 mm TL and 17 ± 2 g. Longnose Sucker *Catostomus catostomus* (2,246 ± 6,982 per mile), Creek Chub

*Semotilus atromaculatus* (841 ± 85 per mile), Speckled Dace *Rhinichthys osculus*, and Iowa Darter *Etheostoma exile* were also captured on the Fraser River Ranch.

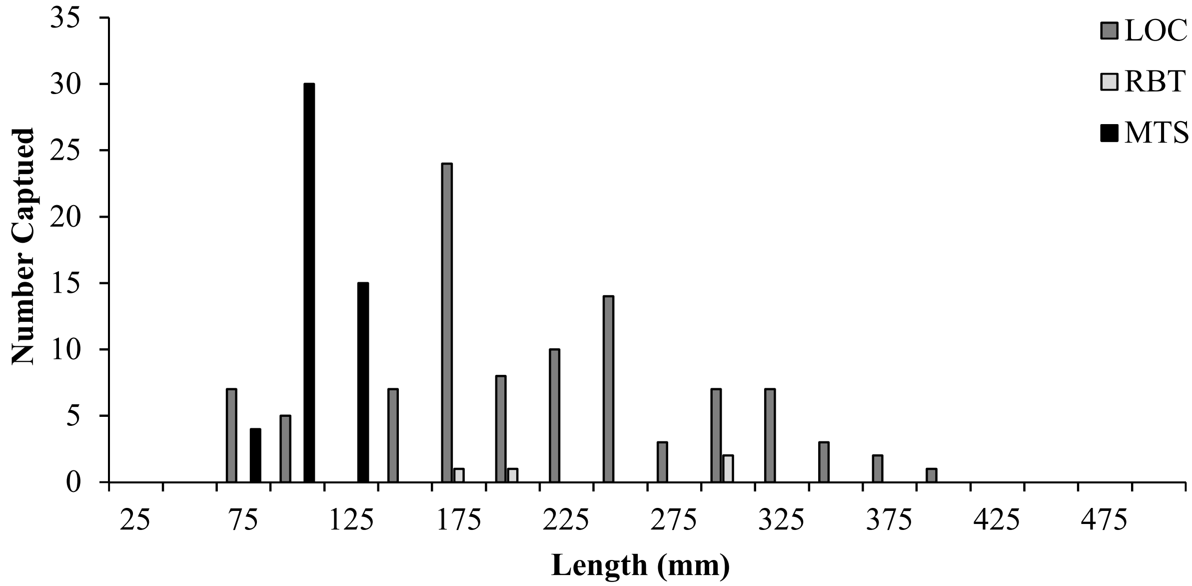


**Figure 2.10.** Number of Brown Trout (LOC), Rainbow Trout (RBT), and Mottled Sculpin (MTS) captured by total length (mm) during the Kaibab Park 2021 population estimate.

A total of 98 Brown Trout, 79 adults and 19 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 2.10). Kaibab Park contained an estimated 977 ± 102 Brown Trout per mile, 801 ± 100 adult Brown Trout per mile and 179 ± 30 Brown Trout fry/juveniles per mile. The abundance of Brown Trout in Kaibab Park was much lower in 2021 than in 2020 (Fetherman et al. 2021), likely a result of construction activities conducted in 2021 immediately upstream of the site to allow the Highway 40 diversion to be more fish passable. Brown Trout averaged 202 ± 8 mm TL and 129 ± 12 g, with the largest measuring 394 mm TL and weighing 624 g. Many fewer Rainbow Trout were captured in Kaibab Park than on the Fraser River Ranch, with only four age-2 Rainbow Trout captured (Figure 2.10), for an estimated 37 ± 12 adult Rainbow Trout per mile. Rainbow Trout averaged 229 ± 29 mm TL and 143 ± 47 g, with the largest measuring 281 mm TL and weighing 230 g. Forty-nine Mottled Sculpin were captured in Kaibab Park, but with 20 captured on the first pass and 29 captured on the second pass, a population estimate could not be calculated. Mottled Sculpin averaged 93 ± 2 mm TL and 11 ± 1 g. Longnose Sucker (1,324 ± 2,442 per mile), Creek Chub (103 ± 87 per mile), and Speckled Dace were also captured in Kaibab Park.

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 697 Brown Trout, 461 adults and 236 fry/juveniles (Figure 2.11). An estimated 3,605 ± 108 Brown Trout per mile were present in River Run, 2,262 ± 63 adult Brown Trout per mile and 1,452 ± 194 Brown Trout fry/juveniles per mile. The average number of Brown Trout adults per mile increased and fry/juveniles per mile decreased between 2020 and 2021 (Fetherman et al.

2021). Brown Trout averaged 253 ± 4 mm TL and 224 ± 11 g, with the largest measuring 530 mm TL and weighing 1,250 g. Only 13 adult Rainbow Trout were captured in 2021 (Figure 2.11), for an estimated 60 ± 0 adult Rainbow Trout per mile. Rainbow Trout averaged 357 ± 24 mm TL and 515 ± 78 g, with the largest measuring 467 mm TL and weighing 1,054 g. Additionally, two cutbows were captured in the lower site, for an estimated eight cutbows per mile, likely reservoir escapees. The cutbows averaged 434 ± 79 mm TL and 903 ± 472 g, with the largest measuring 512 mm TL and weighing 1,374 g. Forty-eight Mottled Sculpin were captured in the two sites, providing an estimate of 263 ± 105 Mottled Sculpin per mile. Mottled Sculpin averaged 120 ± 2 mm TL and 26 ± 1 g. Longnose Sucker, White Sucker *Catostomus commersonii* (437 ± 605 per mile), Speckled Dace (403 ± 617 per mile), Creek Chub (8 ± 0 per mile), Longnose Dace *Rhinichthys cataractae*, Fathead Minnow *Pimephales promelas* (57 ± 170 per mile), and Iowa Darter were also captured in River Run.

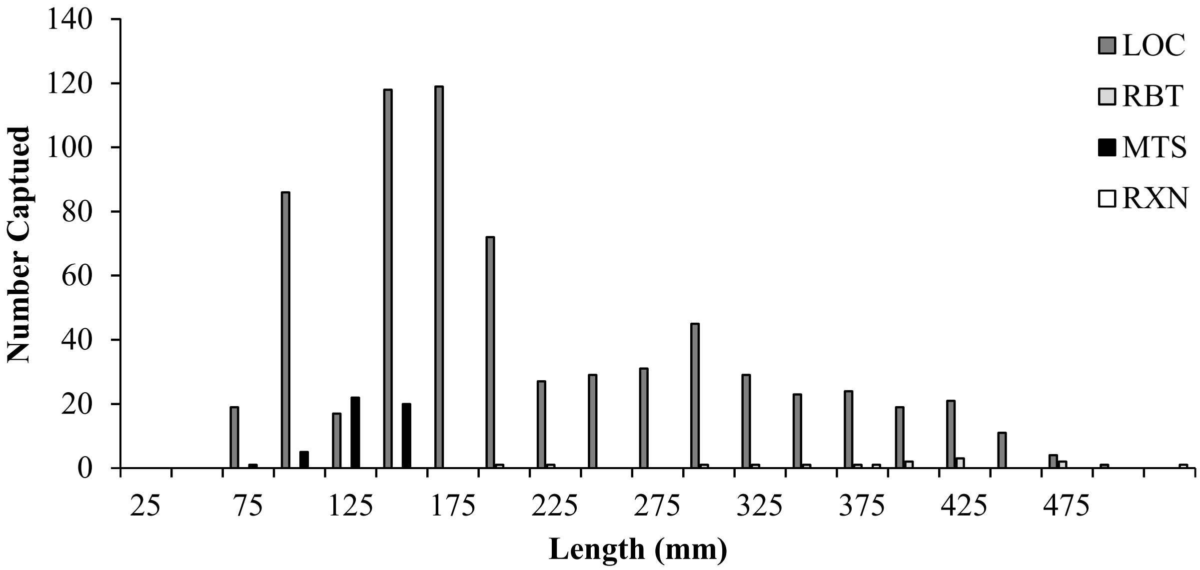


**Figure 2.11.** Number of Brown Trout (LOC), Rainbow Trout (RBT), Mottled Sculpin (MTS) and cutbows (RXN) captured by total length (mm) during the 2021 River Run population estimates.

Seventeen Brown Trout and five Rainbow Trout were recaptured on the Fraser River Ranch in 2021, a recapture rate of 15.2% and 10% of the Brown Trout and Rainbow Trout released on the ranch in 2020, and 1.9% and 0.5% of the total 917 fish (all species) released above Windy Gap Reservoir in 2020. Of those, six Brown Trout and two Rainbow Trout had lost their tags, a tag loss rate of 35% and 40% for the two species, respectively, and were retagged prior to release. Of the fish that retained their tags, all but one Brown Trout that had been released in the lower River Run site in October 2020 had been released in on the Fraser River Ranch the year prior. Brown Trout grew an average of 49 ± 7 mm TL and 135 ± 25 g between 2020 and 2021, while Rainbow Trout grew an average of 178 ± 49 mm TL and 103 ± 61 g. No Mottled Sculpin were recaptured on the Fraser River Ranch in 2021. A total of 127 new fish were PIT tagged in the Fraser River Ranch, 69 Brown Trout, 33 Rainbow Trout, one cutbow, and 24 Mottled Sculpin. Mottled Sculpin (79 to 137 mm TL) were tagged with a 12 mm PIT tag, whereas the Brown

Trout (161 to 476 mm TL), Rainbow Trout (193 to 502 mm TL), and cutbow (389 mm TL) were tagged with 32 mm PIT tags.

Ninety-one new fish were PIT tagged in Kaibab Park, 46 Brown Trout, four Rainbow Trout, and 41 Mottled Sculpin. Mottled Sculpin (71 to 122 mm TL) were tagged with a 12 mm PIT tag, whereas the Brown Trout (128 to 321 mm TL) and Rainbow Trout (174 to 281 mm TL) were tagged with 32 mm PIT tags. Thirty-two Brown Trout and five Mottled Sculpin were recaptured in Kaibab Park in 2021, a recapture rate of 18.1% and 3.1% of the Brown Trout and Mottled Sculpin released in Kaibab Park in 2020, and 3.5% and 0.5% of the 917 fish released above Windy Gap Reservoir in 2020. Of those, six Brown Trout had lost their tags, a tag loss rate of 18%, and were retagged prior to release. All fish that retained their tags had been released in Kaibab Park in 2020. Brown Trout grew an average of 41 ± 3 mm TL and 59 ± 4 g between 2020 and 2021, while Mottled Sculpin grew an average of 12 ± 2 mm TL and 3 ± 2 g. No Rainbow Trout were recaptured in Kaibab Park in 2021.

Forty-five Brown Trout, two Rainbow Trout, and seven Mottled Sculpin were recaptured in the River Run reach of the Colorado River in 2021, a recapture rate of 16.9% of the Brown Trout, 16.6% of the Rainbow Trout, and 8.9% of Mottled Sculpin released in River Run in 2020. This equated to 4.9%, 0.2%, and 0.8%, respectively, of the 917 fish released above Windy Gap Reservoir in 2020. Of those, 16 Brown Trout, one Rainbow Trout, and one Mottled Sculpin had lost their tags, a tag loss rate of 36%, 50%, and 14% for the three species, respectively, and were retagged prior to release. All fish that retained their tags had been released in River Run in 2020. Brown Trout grew an average of 63 ± 6 mm TL and 124 ± 13 g between 2020 and 2021, while Mottled Sculpin grew and average of 5 ± 2 mm TL and 3 ± 2 g, and the Rainbow Trout grew 26 mm TL and 96 g. A total of 190 new fish were PIT tagged in River Run, 136 Brown Trout, 12 Rainbow Trout, two cutbows, and 40 Mottled Sculpin. Mottled Sculpin (79 to 138 mm TL) were tagged with a 12 mm PIT tag, whereas the Brown Trout (129 to 530 mm TL), Rainbow Trout (183 to 467 mm TL), and cutbows (355 to 512 mm TL) were tagged with 32 mm PIT tags.

In summary, 408 fish were PIT tagged in the Fraser and Colorado rivers above Windy Gap Reservoir in 2021. The goal had been to release a minimum of 250 tagged fish of each species. We met this goal for Brown Trout with 251 tagged, but did not meet this goal with Mottled Sculpin or Rainbow Trout, with only 105 and 49 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 2021. Since the beginning of the study, 1,325 fish have been tagged and released in the Fraser and Colorado rivers above Windy Gap Reservoir.

Brown Trout and Rainbow Trout were also tagged immediately downstream of Windy Gap Reservoir in October 2021, and at three population estimation sites, Hitching Post, Red Barn, and Kinney Creek, in May 2022. The Windy Gap site was used to capture and tag fish that had moved upstream to spawn, but whose upstream movements were impeded by the dam. The other three sites were sampled in place of the typical raft electrofishing population estimates conducted on the Chimney Rock Ranch due to low water levels (see 2022 Adult Salmonid Population Estimates). The same tagging protocols used above Windy Gap were followed below Windy Gap. Fish were anesthetized in an AQUI-S bath, measured, weighed, tagged, and adipose clipped. Recaptured fish that had lost their tags were retagged, while the tag number and new length and weight measurements were recorded for those that retained their tags. Fish were held in net pens to allow them to recover prior to being released, and mortalities were scanned and removed from the release dataset.

One hundred sixteen new fish were PIT tagged with 32 mm tags immediately downstream of Windy Gap Reservoir in October 2021, 100 Brown Trout (132 to 508 mm TL), 15 Rainbow Trout (126 to 577 mm TL), and one cutbow (383 mm TL). Three Brown Trout were recaptured downstream of Windy Gap Dam, a recapture rate of 3.7% of the fish released in the site in 2020, and 0.2% of the 1,940 fish (all species) released below Windy Gap Reservoir. Of those three, two had lost their tag, a tag loss rate of 67%, and were retagged prior to release. The one Brown Trout that retained its tag had been released immediately downstream of Windy Gap Reservoir in October 2020, and had grown 18 mm TL and 45 g.

Twenty-nine Brown Trout and seven Rainbow Trout were recaptured in the Hitching Post site in 2022. The majority of the Brown Trout had been tagged and released at the Hitching Post in October 2020 or May 2021, although many came from other locations in the river including the next release site downstream, a release site near the Red Barn antenna, and two release sites below the Red Barn diversion. Rainbow Trout recaptures had been released at Hitching Post or the next release site downstream in 2020 or 2021, but one fish had been released immediately downstream of Windy Gap Reservoir in October 2020. The recapture rate for Brown Trout was 1.5% and for Rainbow Trout was 0.4% of the 1,940 fish released below Windy Gap Reservoir. Of those, 12 Brown Trout and two Rainbow Trout had lost their tags, a tag loss rate of 41% and 29%, respectively, and were retagged prior to release. Brown Trout grew an average of 41 ± 8 mm TL and 60 ± 18 g, and Rainbow Trout grew an average of 56 ± 16 mm TL and 101 ± 25 g. A total of 141 new fish were PIT tagged with 32 mm tags at Hitching Post, 85 Brown Trout (185 to 393 mm TL) and 56 Rainbow Trout (155 to 448 mm TL).

One hundred eighteen new fish were PIT tagged with 32 mm tags in the Red Barn site in 2022, 90 Brown Trout (221 to 447 mm TL) and 28 Rainbow Trout (214 to 347 mm TL). Eleven Brown Trout and one Rainbow Trout were recaptured at Red Barn, a recapture rate of 0.5% and 0.05%, respectively, of the 1,940 fish released below Windy Gap Reservoir. Of those, eight Brown Trout had lost their tag, a tag loss rate of 73%, and were retagged prior to release. Both the Brown Trout and Rainbow Trout that retained their tags originated from release sites in the Red Barn area. Brown Trout grew and average of 46 ± 20 mm TL and 97 ± 51 g. The Rainbow Trout had been tagged with a 12 mm tag in October 2020, and had grown 144 mm TL and 172 g. Unfortunately, the adipose clip on this fish had partially regenerated, and it was tagged as a new fish with a 32 mm PIT tag 2022, so both tag numbers are now associated with future detections of this fish.

Thirteen Brown Trout and two Rainbow Trout were recaptured in the Kinney Creek site in 2022, all of which were released in nearby sites in spring 2021. The recapture rate for Brown Trout was 0.6% and for Rainbow Trout was 0.1% of the 1,940 fish released below Windy Gap Reservoir. Of those, five Brown Trout and both Rainbow Trout had lost their tags, a tag loss rate of 38% and 100%, respectively, and were retagged prior to release. Brown Trout grew an average of 44 ± 10 mm TL and 97 ± 29 g. Rainbow Trout growth could not be assessed due to tag loss. A total of 107 new fish were PIT tagged with 32 mm tags at Kinney Creek, 90 Brown Trout (162 to 422 mm TL), 16 Rainbow Trout (248 to 392 mm TL), and one cutbow (425 mm TL).

In summary, 482 fish were PIT tagged in the Colorado River below Windy Gap Reservoir in 2021 and 2022, meeting our goal of 250 tagged fish per species for Brown Trout (365 tagged), but not Rainbow Trout (115). It may have been possible to tag more Rainbow Trout had the raft surveys been conducted, but we were limited by the number of fish available in the three population estimate sites. Hopefully, more tagged Rainbow Trout can be added to the system in future years as the adult population continues to increase from recent H×G fry stocking efforts (see 2021 Salmonid Fry Population Estimates). Since the beginning of the study, 2,422 fish have been tagged and released in the Colorado River below Windy Gap Reservoir.

Acolas, M. L., J.–M. Roussel, J. M. Lebel, and J. L. Baglinière. 2007. Laboratory experiment on survival, growth and tag retention following PIT injection into the body cavity of juvenile Brown Trout (*Salmo trutta*). Fisheries Research 86:280-284.

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Seber, G. A. F., and J. F. Whale. 1970. The removal method for two and three samples. Biometrics 26(3):393-400.

*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 2021 and April-June 2022. The wagon wheel antenna below Windy Gap Reservoir was deployed immediately downstream of the auxiliary outlet of the dam in 2021 to increase detections of fish, particularly Brown Trout, that approached the dam to spawn. The deployment location for this antenna was changed to the Schmuck channel in 2022 as the majority of the flow out of the reservoir was coming through this channel. 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. Due to the need to change batteries and download data from these portable antennas every two weeks, they were only deployed during the fall Brown Trout and spring Rainbow Trout spawning periods when fish activity was expected to be high.

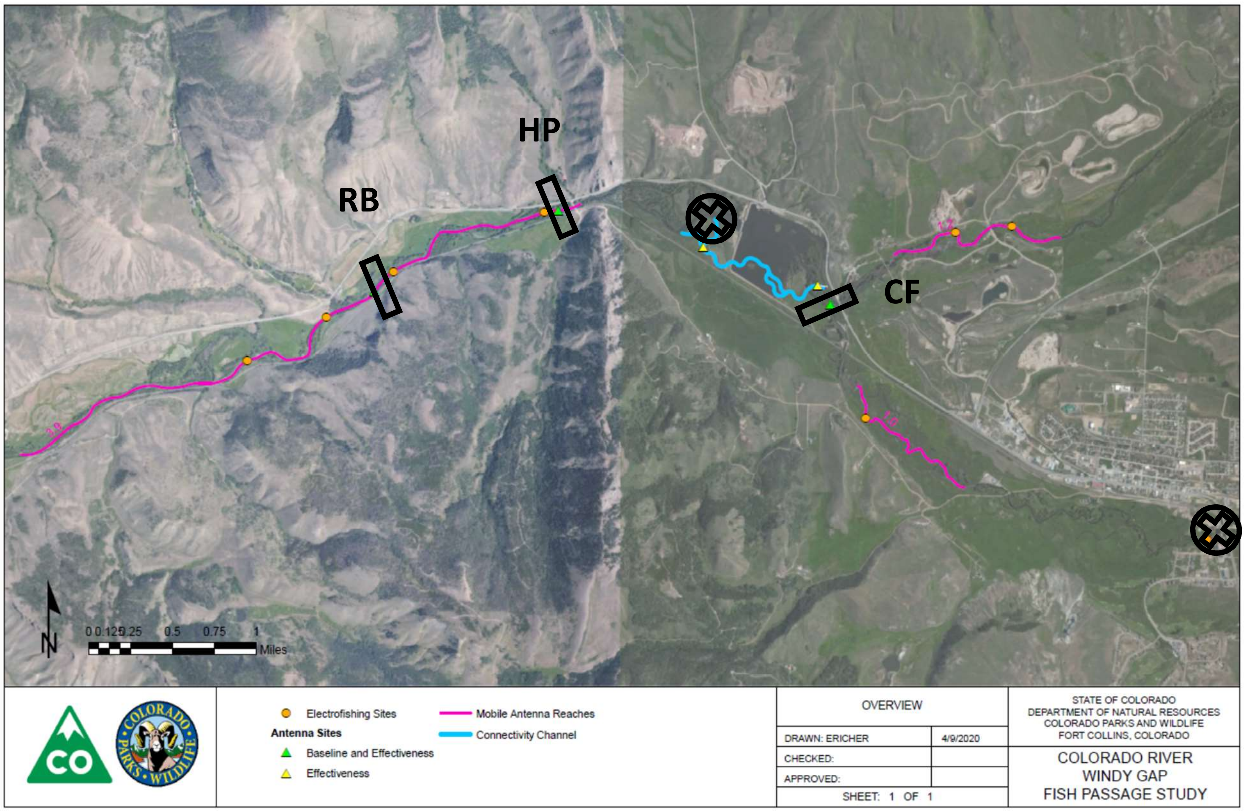


**Figure 2.12.** Locations of the Red Barn (RB), Hitching Post (HP), and Confluence (CF) stationary antenna stations (rectangles), and the portable Biomark wagon wheel antennas (circle with X) temporarily deployed downstream of Windy Gap Reservoir and upstream of the Fraser River diversion structure below Highway 40.

Antenna detection distances were measured at stationary antenna sites in fall 2021 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 shown 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 fall 2021 averaged 0.28 ± 0.11, 0.26 ± 0.17, and 0.31 ± 0.11 m/s at the Red Barn, Hitching Post, and Confluence sites, respectively. On average, velocities remained below 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 fall 2021 ranged between an average of 1.22 and 2.09 feet, and for a 12 mm PIT tag between 0.26 and 0.52 feet. Detection distances for both 32 and 12 mm PIT tags were similar between the two antennas at the Hitching Post and Confluence sites, and between the two sites. Red Barn showed the largest discrepancy in detection distances within a site. RB1 had a shorter detection distance for both tag sizes than all other sites, whereas RB2 detection distances were greater than at all other sites. 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 (Figure 2.13). 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 suggest 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.

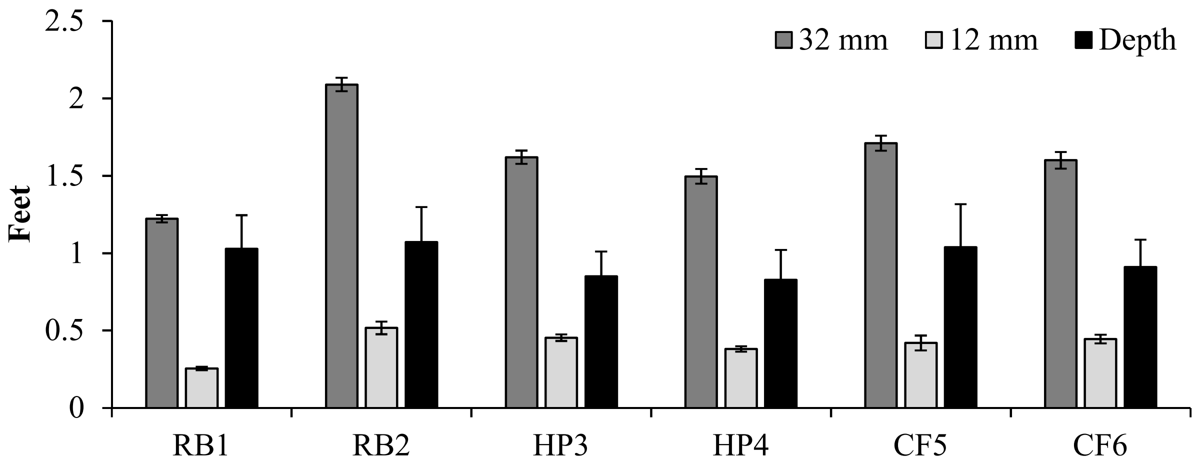


**Figure 2.13.** Detection distances for 32 mm and 12 mm PIT tags and water depths (feet; 2 SE bars) for the two antennas located at Red Barn (RB), Hitching Post (HP), and Confluence (CF) in fall 2021.

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.

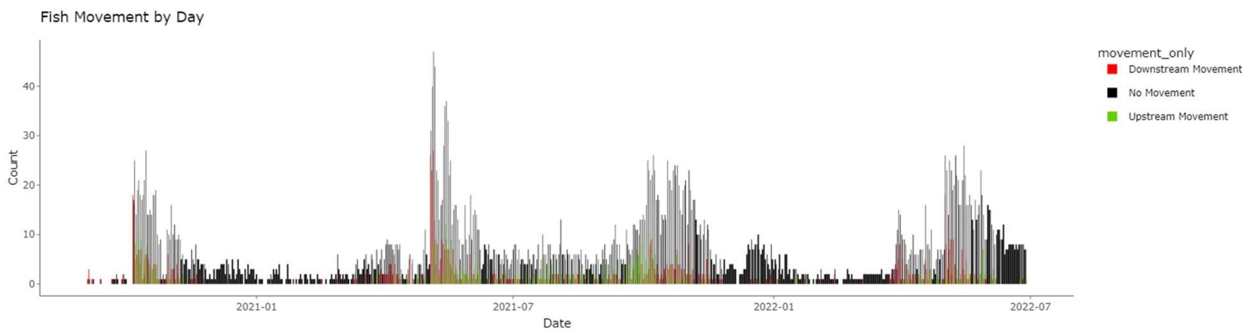


**Figure 2.14.** Number of Brown Trout detected per day at the Red Barn, Hitching Post, and Confluence sites (combined). No movement is defined as detection at a single antenna at a site with no additional detections to inform directionality of movement, or a fish that passed both antennas at a site going each direction such that it began and ended the day in the same location relative to the antenna site. 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.

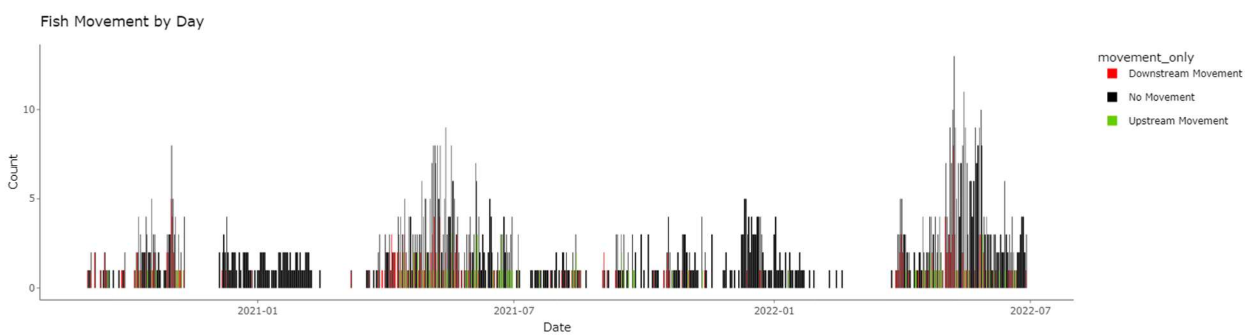


**Figure 2.15.** Number of Rainbow Trout detected per day at the Red Barn, Hitching Post, and Confluence stationary antenna sites (combined).

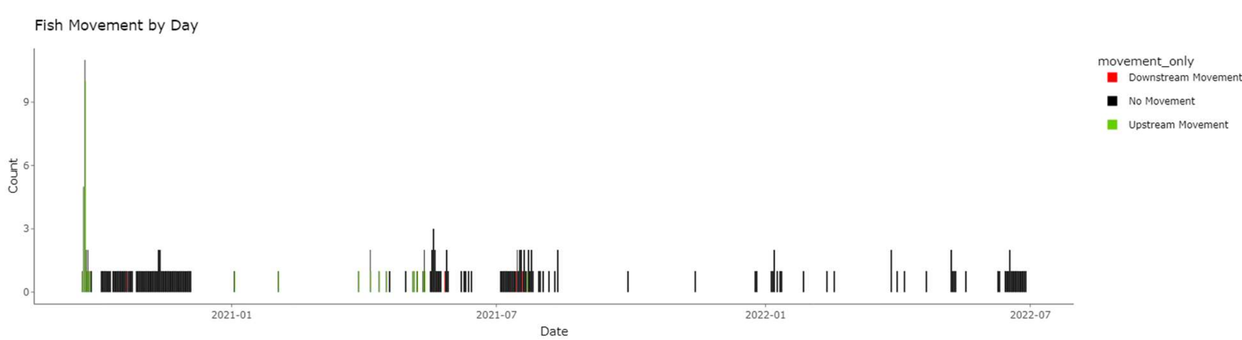


**Figure 2.16.** Number of Mottled Sculpin detected per day at the Confluence site.

Looking at fish detections at the population level has revealed some interesting patterns in movement. As was expected, Brown Trout appear to show an increase in movement during the fall spawning period, with activity peaking in 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 (Figure 2.14). Rainbow Trout show a similar spike in activity during the spring spawning period, with activity peaking in mid- to late-May. Additionally, a spike in movement in early winter suggests that the Rainbow Trout also may be moving to more favorable over-winter habitats as ice forms (Figure 2.15). Mottled Sculpin movements appear to be more erratic (Figure 2.16). 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.

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*. 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 game cameras at all three sites in June 2022 to confirm that fish-eating birds are active around the sites. The cameras will capture a bird passing a site, allowing researchers to match the time stamps from the camera and the reader to confirm the tag was in a bird. Additionally, researchers in the aquatic research section have designed a potential 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 Colorado River Connectivity Channel. The avian predation experiment will be implemented within the study section in spring 2023.

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 2.17) 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 2.17.** (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.

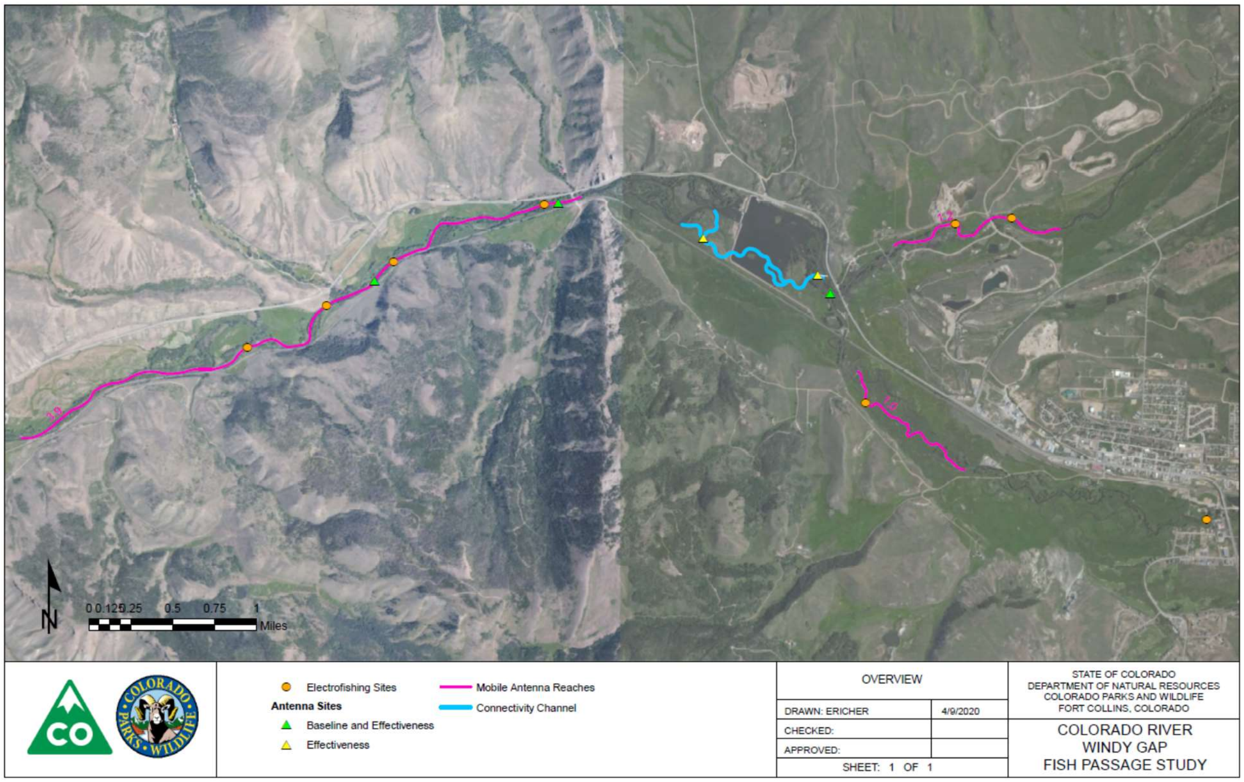


**Figure 2.18.** Mobile antenna reaches in the Colorado and Fraser rivers above Windy Gap Reservoir and the Colorado River below Windy Gap Reservoir (red lines). The blue line represents a future mobile antenna reach following completion of the Colorado River Connectivity Channel.

Mobile antennas were deployed in three reaches in July 2021, October 2021, and April 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 2.18). 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 everything from 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.

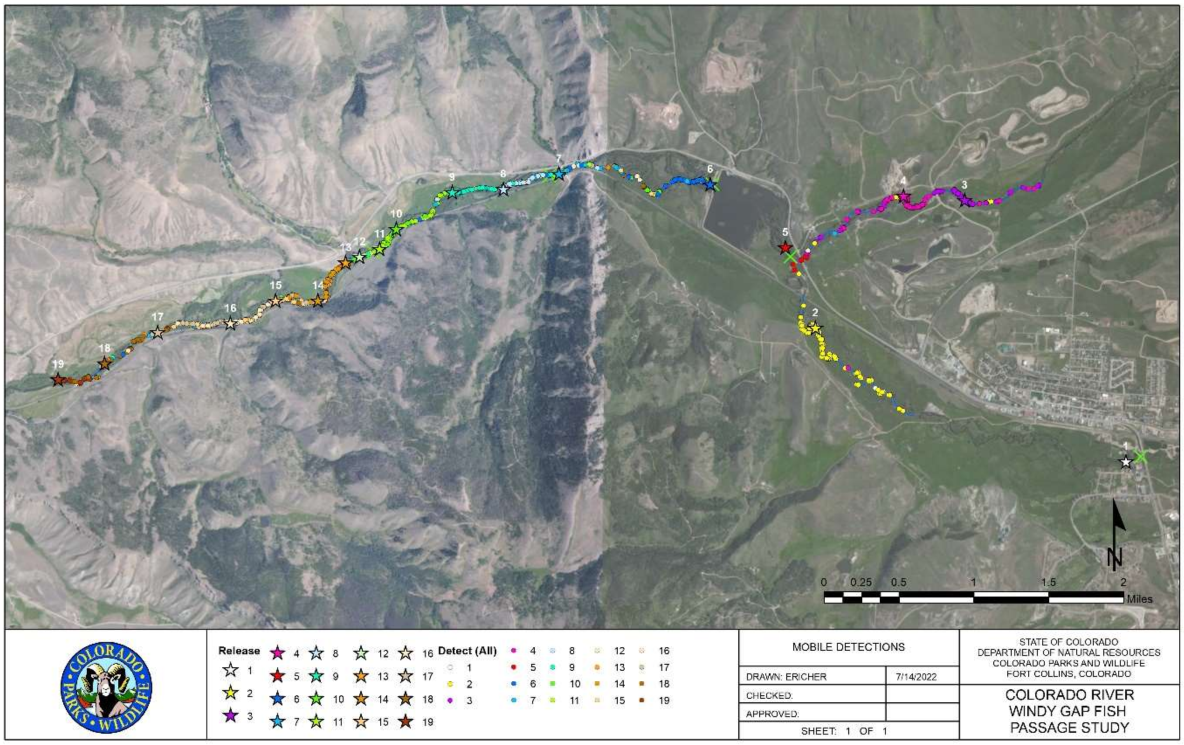


**Figure 2.19.** Location of detected PIT tags in the Fraser and Colorado rivers upstream of Windy Gap Reservoir and the Colorado River downstream of Windy Gap Reservoir in spring 2022. Colored stars correspond to release locations to show distance moved.

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 2.19). 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 gage 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.

In the Colorado River below Windy Gap Reservoir, fish appear to redistribute upstream after being tagged (Figure 2.19). 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 site 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 2.19), 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. Future GPS mapping will be used to determine candidate ghost tags based on a lack of movement between mobile antenna deployments, and protocols will be implemented to locate and confirm the identity of ghost tags and remove them from the dataset. Surveys to find and remove ghost tags will occur in 2023.

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 Colorado River Connectivity Channel, 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 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 2021: 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, and September 2021 to determine of 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 per 1-L sample. The entire filter was 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.

**Table 2.1.** Number of triactinomyxons per liter (TAMs/L; T) and number of wells that fluoresced (F) for each sample collected on seven dates and from seven locations (Confluence antenna, CF; Hitching Post, HP; upper Red Barn, URB; Red Barn antenna, RB; lower Red Barn, LRB; below the Red Barn diversion, BRBD; Sheriff Ranch, SR) in the upper Colorado River in 2021. NA indicates that samples were not collected from a given location/date.

| Site | 5/6/21 | | 7/13/21 | | 7/29/21 | | 9/2/21 | | 9/16/21 | | 9/27/21 | | 10/21/21 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | T | F | T | F | T | F | T | F | T | F | T | F | T | F |
| CF1 | NA | NA | NA | NA | NA | NA | NA | NA | 0 | 0 | NA | NA | NA | NA |
| CF2 | NA | NA | NA | NA | NA | NA | NA | NA | 0.6 | 3 | NA | NA | NA | NA |
| CF3 | NA | NA | NA | NA | NA | NA | NA | NA | 0.2 | 2 | NA | NA | NA | NA |
| HP1 | 0.2 | 3 | 6 | 3 | 0 | 2 | 0 | 0 | 0 | 0 | 0.1 | 2 | 0 | 3 |
| HP2 | 0.8 | 3 | 2 | 3 | 0.2 | 3 | 2 | 3 | 0.1 | 3 | 3 | 3 | 2 | 3 |
| HP3 | 0 | 0 | 3 | 3 | 5 | 3 | 0 | 0 | 1 | 3 | 1 | 3 | 1 | 3 |
| URB1 | NA | NA | 0 | 0 | 0 | 0 | 0 | 0 | NA | NA | 0.2 | 3 | 1 | 3 |
| URB2 | NA | NA | 0.6 | 3 | 0.1 | 3 | 0.1 | 2 | NA | NA | 0 | 0 | 2 | 3 |
| URB3 | NA | NA | 0.1 | 2 | 0 | 0 | 0 | 0 | NA | NA | 2 | 3 | 5 | 3 |
| RB1 | NA | NA | NA | NA | NA | NA | NA | NA | 0.1 | 3 | NA | NA | NA | NA |
| RB2 | NA | NA | NA | NA | NA | NA | NA | NA | 0 | 0 | NA | NA | NA | NA |
| RB3 | NA | NA | NA | NA | NA | NA | NA | NA | 2 | 3 | NA | NA | NA | NA |
| LRB1 | 0 | 0 | 1 | 3 | 0 | 1 | 0.7 | 3 | NA | NA | 0 | 0 | 3 | 3 |
| LRB2 | 0.4 | 3 | 0 | 0 | 0 | 1 | 0 | 0 | NA | NA | 3.2 | 3 | 2 | 3 |
| LRB3 | 0.7 | 3 | 0.2 | 2 | 0 | 1 | 1 | 3 | NA | NA | 4.9 | 3 | 0.5 | 3 |
| BRBD1 | 0.8 | 3 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| BRBD2 | 2 | 3 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| BRBD3 | 2 | 3 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| SR1 | 1.5 | 3 | 0 | 0 | 0 | 0 | 0.1 | 1 | NA | NA | 2 | 3 | 1 | 3 |
| SR2 | 3.9 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | NA | NA | 0 | 1 | 1 | 3 |
| SR3 | 0.7 | 3 | 0 | 2 | 0 | 0 | 0 | 0 | NA | NA | 1 | 3 | 2 | 3 |

Three of the four filters from each site and date were processed by OSU, with the fourth retained for processing if needed. Filters were extracted and total *M. cerebralis* DNA purified according to the method of processing environmental samples presented in Hallett et al. (2012). The amount of *M. cerebralis* DNA was assayed by qPCR (Kelley et al. 2004), with modifications in chemistry and machine programing consistent with current technology. For calibration purposes, reference control samples were prepared from in-house cultures of *M. cerebralis*. Replicates of five TAMs were counted and added to filter papers, and non-target carrier DNA was added. Control samples were then extracted using the same protocol as used with the environmental water samples. A second positive control reference of diluted *M. cerebralis*-infected Rainbow Trout was also used on some plates. All samples were diluted 1:10 in Qiagen buffer AE prior to running qPCR to reduce the effect of environmental PCR inhibitors that may have co-purified with the *M. cerebralis* DNA. Each sample was run in triplicate through qPCR (technical replicates). Samples were considered positive when two or three wells fluoresced, and considered "not detected" when zero or one well fluoresced.

*M. cerebralis* TAMs were consistently detected at Hitching Post, but not always other sites downstream Table 2.1). Assuming Windy Gap Reservoir was the primary source of TAMs, higher detection at this upstream-most site was expected, as was lower detection at Sheriff Ranch, the downstream-most site and furthest from Windy Gap Reservoir. In late July, flows may not have been sufficient for transporting TAMs further downstream to the other sites. Samples were collected at the three antenna sites in mid-September 2021 on the last day that Windy Gap Reservoir was being drained for fall survey and construction activities. TAMs were detected at the Confluence antenna site, being produced in either the Fraser or Colorado rivers upstream of Windy Gap Reservoir. Interestingly, TAMs/L were lower than expected on September 16, 2021 downstream of Windy Gap Reservoir following reservoir draining. However, TAMs/L increased at the end of September, suggesting that there may have been a pulse of TAMs released that took some time to make their way downstream. TAMs/L remained high in October 2021, while the Colorado River was freely flowing through Windy Gap Reservoir, suggesting that draining the reservoir may have disturbed the worms, causing them to release more TAMs than they typically do in an undisturbed state. This disturbance effect has been observed in experimental worm cultures maintained for TAM production purposes.

Water samples were again collected in May 2022 during the adult population estimates in the Chimney Rock/Sheriff Ranch study section. Samples will be collected from the four fry sites on each of five sampling occasions in 2022, and water sampling will continue through two years post-construction of the Colorado River Connectivity Channel. 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.

Hallett, S. L., R. A. Ray, C. N. Hurst, R. A. Holt, G. R. Buckles, S. D. Atkinson, and J. L. Bartholomew. 2012. Density of the waterborne parasite *Ceratomyxa shasta* and its biological effects on salmon. Applied and Environmental Microbiology 78(10):3724-3731.

Kelley, G. O., F. J. Zagmutt-Vergara, C. M. Leutenegger, K. A. Myklebust, M. A. Adkison, T. S. McDowell, G. D. Marty, A. L. Kahler, A. L. Bush, I. A. Gardner, and R. P. Hedrick. 2004. Evaluation of five diagnostic methods for the detection and quantification of *Myxobolus cerebralis*. Journal of Veterinary Diagnostic Investigation 16(3):202-211.

### Genetics of Rainbow Trout in the Yampa River

Genetic samples (upper caudal fin clip) were collected from coded-wire-tagged (CWT) and PIT-tagged Rainbow Trout stocked in the Yampa River between 2017 and 2019, and wild fry/juvenile, age-2, and age-3+ Rainbow Trout in 2019 and 2020. Stocked Rainbow Trout were used as a baseline to determine if hatchery fish maintained similar genetics to those expected from 87.5:12.5 Hofer:Harrison fish. Wild Rainbow Trout samples were collected to determine if stocking H×H in the Yampa River has increased Hofer genetics in wild fish. Initially, all of these samples were analyzed for the percent Hofer genetics. Fetherman et al. (2021) found that the stocked Rainbow Trout, both PIT-tagged catchable fish and CWT fingerlings, maintained a high percent Hofer, and did not deviate from genetic expectations for the current hatchery brood stocks. Additionally, percent Hofer was lower (25% on average) and similar across all age classes in the Yampa River in 2019 and 2020. It was suspected that the lower percent Hofer in the wild fish did not necessarily indicate a lack of reproduction and recruitment from the stocked fish, but rather that more wild-type fish that retained the Hofer genetic resistance to *M. cerebralis* were recruiting in the Yampa River. To test whether or not this was the case, the same samples were rerun for the WDRES-9 quantitative trait locus (QTL). Fish were classified as homozygous resistant-resistant (RR) if they had two copies of the resistance allele, heterozygous resistant-susceptible (RS) if they had only one copy of the resistance allele, or homozygous susceptible-susceptible (SS) if they did not have the resistance allele.

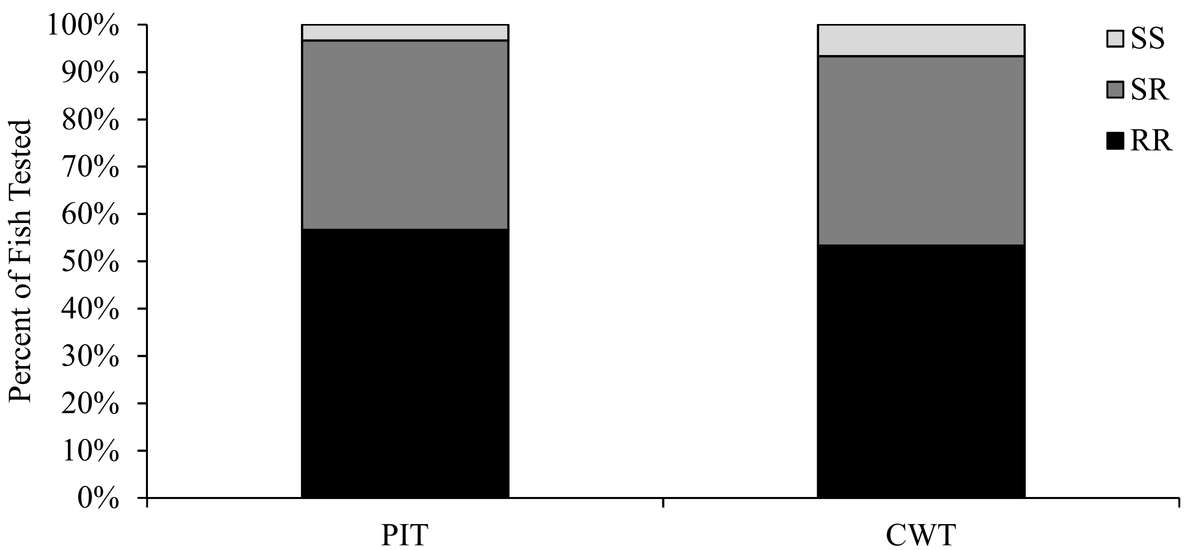


**Figure 2.20.** Percent of PIT-tagged (PIT) and coded-wire-tagged (CWT) H×H Rainbow Trout tested for the WDRES-9 QTL that had two copies of the resistance allele (RR), one copy of the resistance and one copy of a susceptible allele (SR), or two copies of a susceptible allele (SS).

The results of the genetic testing revealed that 53-56% of the stocked H×H originating from the CPW Crystal River Hatchery were RR, while 40% were SR and 3-7% were SS (Figure 2.20). Similar to what Fetherman et al. (2021) found after testing Crystal River progeny used in a lab exposure experiment, the percentage of RS and SS individuals was higher than would be expected in a population that is 87.5% Hofer and 12.5% Harrison. Genetic predictions suggest that if these fish were maintaining expected resistance characteristics, some individuals would present as RS, but there should be no individuals presenting as SS. Similarly, the percentage of the resistance and susceptible alleles in the population would be expected to be similar to the genetic expectations, i.e., 87.5% R and 12.5% S, but the actual percentages were 75% R and 25% S. Spawning procedures and/or a lack of selection pressure for maintaining the resistance allele may have resulted in the deviation from genetic expectations over time. The high proportion of RS and the presence of SS individuals may cause the brood stock to continue to lose resistance over time without additional backcrossing with pure Hofer individuals or introductions from wild brood stocks. Additionally, 9 ± 5 genes are estimated to be responsible for conferring resistance, the effects of which are likely additive (Fetherman et al. 2012). These results therefore also suggest that resistance may be lost over time due to disassociation of multiple alleles, in addition to the WDRES-9 QTL, if the same proportions are present at other resistance loci.

**Table 2.2.** Percent of wild age-1, age-2, and age-3 Rainbow Trout tested by sampling location for the WDRES-9 QTL that had two copies of the resistance allele (RR), one copy of the resistance and one copy of a susceptible allele (SR), or two copies of a susceptible allele (SS).

| Status | Age 1 | Age 2 | Age 3 | Average |
|---|---|---|---|---|
| | | **Green Creek/Kuntz** | | |
| RR | 50% | 50% | 25% | 42% |
| SR | 40% | 40% | 65% | 48% |
| SS | 10% | 10% | 10% | 10% |
| | | **BLM** | | |
| RR | 55% | 40% | 48% | 48% |
| SR | 30% | 55% | 37% | 41% |
| SS | 15% | 5% | 11% | 11% |
| | | **Sarvis Creek SWA** | | |
| RR | 50% | 45% | 68% | 54% |
| SR | 35% | 45% | 26% | 35% |
| SS | 15% | 10% | 6% | 11% |
| | | **Wellar Ranch** | | |
| RR | 35% | 70% | 50% | 52% |
| SR | 60% | 25% | 50% | 45% |
| SS | 5% | 5% | 0% | 3% |
| | | **Stagecoach Tailwater** | | |
| RR | 25% | 20% | 30% | 25% |
| SR | 60% | 40% | 35% | 45% |
| SS | 15% | 40% | 35% | 30% |
| | | **Average** | | |
| RR | 43% | 45% | 44% | 44% |
| SR | 45% | 41% | 43% | 43% |
| SS | 12% | 14% | 13% | 13% |

The percent of wild fish that were RR, SR, or SS differed among age classes (Table 2.2). Locations further downstream, e.g., Green Creek/Kuntz and BLM showed an increase in the percentage of RR fish between the three-year-old and fry/juvenile (age 1) age classes. This was expected given that stocked fish exhibited similar proportions of RR, SR, and SS fish and should have started spawning and contributing offspring to the fry/juvenile age class. Additionally, myxospore counts from fish collected in 2020 increased from upstream to downstream sampling locations (Fetherman et al. 2021), suggesting that selective pressure is likely higher for resistant individuals in the BLM and Green Creek/Kuntz sites. Similar increases in resistance alleles were not observed in sites further upstream where selective pressures are lower. Lower selective pressure not only allows more susceptible fish to be retained in the spawning population, but susceptible fry/juveniles are less likely to die due to a reduced exposure to *M. cerebralis*.

Differences in percent RR, SR, and SS were also observed across sampling locations (Table 2.2). Notably, higher percentages of RR fish were observed in the Wellar Ranch than in the Stagecoach Tailwater immediately upstream. Previous genetic testing showed that the percent Hofer was lower in fish collected from the tailwater than from fish collected in Harrison Creek and Catamount Lake lower down in the system (Fetherman et al. 2018). Not only is infection severity lower in the tailwater (Fetherman et al. 2021), but wild Rainbow Trout are numerous in this section and relatively little spawning habitat exists here relative to other sections (e.g., Wellar Ranch and BLM) further downstream. Therefore, it is probable that more susceptible fish were retained in this location, and stocking has not yet increased resistance alleles. The Wellar Ranch provides excellent spawning and nursery habitat. Therefore, it is likely that the stocked fish spawn on the ranch, resulting in an increase in resistance alleles in this section.

In 2021 and 2022, disease samples were collected from both the Wellar Ranch and the Stagecoach Tailwater during the Rainbow Trout spawning season. Samples collected in 2021 were only tested for *Renibacterium salmoninarum*, and were found to be negative for the bacteria (Fetherman et al. 2021). Samples collected in 2022 were tested for all pathogens of concern to develop a disease history such that eggs could be collected from these locations in the future if wild supplementation of hatchery brood stocks is needed. Both locations tested negative for all pathogens of concern. The WDRES-9 QTL results suggest that eggs should be collected from the Wellar Ranch rather than the Stagecoach Tailwater since maintaining higher resistance alleles would be a priority in hatchery brood stocks supplemented by this population.

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-R17. Federal Aid in Fish and Wildlife Restoration, Job Progress Report. Colorado Parks and Wildlife, Aquatic Wildlife Research Section. Fort Collins, Colorado.

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## 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 recently completed. 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.

## BACTERIAL KIDNEY DISEASE RESEARCH

Project Collaborators: Tawni B. Riepe, Ph.D., and Dana L. Winkelman, Ph.D.

*Renibacterium salmoninarum*, the causative bacterial agent of bacterial kidney disease (BKD), is difficult to prevent and manage in salmonid populations due to the slow fastidious progression of infection throughout the fish, the lack of a gold standard diagnostic method, and its multiple modes of transmission. Bacterial kidney disease is associated with high mortalities among salmonid species at all life stages, and the bacteria can exist subclinically, presenting no signs of disease. Management of *R. salmoninarum* infections in hatchery facilities often relies on the testing of fish through routine health inspections to prevent outbreaks. However, while decades of advances in molecular and serological diagnostics have helped to establish methods to test for *R. salmoninarum*, which include culture, the enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), quantitative PCR (qPCR), and the direct fluorescent antibody test (DFAT), they are often problematic due to variability in the specificity and reliability of each test (Pascho et al. 2002; Elliot et al. 2013; Elliott et al. 2015). Prevention of infections also relies on the ability to control transmission of the bacteria, but given the bacteria utilizes two routes (vertical and horizontal transmission), it is difficult to develop management protocols since the rate at which transmission occurs via each route is unknown. The experiments described below provide new insights for further refinement of management protocols for *R. salmoninarum* in hatchery-reared inland salmonids.


Elliot, 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 36:779-809.

Elliott, D. G., C. L. McKibben, C. M. Conway, M. K. Purcell, D. M. Chase, and L. J. Applegate. 2015. Testing of candidate non-lethal sampling methods for detection of *Renibacterium salmoninarum* in juvenile Chinook Salmon *Oncorhynchus tshawytscha*. Diseases of Aquatic Organisms 114:21-43.

Pascho, R. J., D. G. Elliott, and D. M. Chase. 2002. Comparison of traditional and molecular methods for detection of *Renibacterium salmoninarum*. Pages 157-209 *In* C. Cunningham, editor. Molecular diagnosis of salmonid diseases. Kluwer Academic Publishers, Dordrecht, The Netherlands.

*Detection and Transmission of Renibacterium salmoninarum in Colorado Inland Trout*

Tawni Riepe completed her dissertation entitled "Detection and Transmission of *Renibacterium salmoninarum* in Colorado Inland Trout", which was submitted to the CSU Graduate School on June 9, 2022. An electronic version of the dissertation will be available from the CSU Library in fall 2022. The following is the extended abstract from the dissertation describing the bacterial kidney disease research conducted by Dr. Riepe in conjunction with Dr. Dana Winkelman and CPW Aquatic Research and Aquatic Animal Health Lab staff.

<u>Detection and Transmission of *Renibacterium salmoninarum* in Colorado Inland Trout</u> (Tawni B. Riepe, Ph.D., Extended Abstract)

*Renibacterium salmoninarum*, the causative agent of bacterial kidney disease (BKD), is known to cause high mortality in both wild and cultured salmonids, causing concern for many salmonid populations. Bacterial kidney disease caused up to 80% mortality in cultured Pacific salmonids and 40% in Atlantic salmonids. Due to high mortality among salmonid species, the American Fisheries Society has defined *R. salmoninarum* as a regulated pathogen. Due to its regulated status, research efforts have focused on advancing fish health diagnostics and understanding the transmission of the bacteria. However, many of these studies focus on Pacific northwest salmonids and the infection dynamics of *R. salmoninarum* are not well known among inland salmonids.

Aquaculture propagation of Greenback Cutthroat Trout (*Oncorhynchus clarkii*) is a necessary component of their management. Since their protection under the Endangered Species Act, broodstock of Greenback Cutthroat Trout have been established at Colorado Parks and Wildlife (CPW) hatcheries to allow more rapid reintroduction through stocking. In 2017, hatcheries rearing isolated strains of the Greenback Cutthroat Trout contributed 1.5 million eggs during the spawning season. However, one major constraint to maintaining spawn production of the Greenback is the spread of disease within a facility. Increased contact rates between fish in raceways may influence the transmission of a pathogen. To ensure fish health and promote best practices in fish culture, fish health inspections have served as a critical step in identifying prohibitive and regulated pathogens entering or exiting hatchery systems.

Various diagnostic methods have been established to detect *R. salmoninarum* in salmonids. Culturing the bacteria is the most accurate and reliable assay for detection; however, it is a slow process and not suited for rapid assessment. Other methods used to detect *R. salmoninarum* include Direct Fluorescent Antibody Tests (DFAT), Polymerase Chain Reaction (PCR), and Enzyme-Linked Immunosorbent Assays (ELISA) and are typically performed using lethally collected kidney tissue. Currently, kidney tissues are used to screen for the presence of the bacteria using DFAT as the initial test and PCR as a confirmatory test, following the American Fisheries Fish (AFS) Health Blue Book protocol. The protocol was developed using highly susceptible Pacific northwest salmonids and it is unknown if the protocol is appropriate for testing inland salmonids which may be less susceptible. In addition, the current protocol requires sacrificing fish, which is undesirable in valuable and sometimes irreplaceable broodstocks. Therefore, I examined the efficacy of the current AFS detection protocol and compared it to other potential approaches (Chapter 2). I also assessed several non-lethal approaches to detecting the bacteria (Chapter 1 and 2).

In chapter 1, I compared non-lethal sampling methods with standard lethal kidney tissue sampling that is used to detect *R. salmoninarum* infections in salmonids. I collected anal, buccal, and mucus swabs (non-lethal qPCR) and kidney tissue samples (lethal DFAT) from 72 adult Brook Trout (*Salvelinus fontinalis*) reared at the Colorado Parks and Wildlife Pitkin Brood Unit and tested each sample to assess *R. salmoninarum* infections. Brook Trout were used as a model species for Cutthroat Trout because they are highly susceptible to infection. Standard kidney tissue detected *R. salmoninarum* 1.59 times more often than mucus swabs, compared to 10.43 and 13.16 times more often than buccal or anal swabs, respectively, indicating mucus swabs were the most effective and a useful non-lethal method. My study highlights the potential of non-lethal mucus swabs to sample for *R. salmoninarum* and suggests future studies are needed to refine this technique for use in aquaculture facilities and wild populations of inland salmonids.

In chapter 2, I assessed the probability of detecting the bacteria in several tissues using standard diagnostic tests. I collected three lethal tissue (kidney, liver, and spleen) and three nonlethal serum (blood, ovarian fluid, and mucus swabs) samples from 781 adult Greenback Cutthroat Trout at the Colorado Parks and Wildlife Poudre Rearing Unit. All tissues were tested for *R. salmoninarum* via DFAT and qPCR. The overall prevalence of *R. salmoninarum* among the fish was 22.7% with DFAT and 81.8% with qPCR. Kidney and liver tissues resulted in the greatest number of detections using either assay. To calculate the probability of infection among kidney and liver tissues and probability of detection between assays, I developed a hierarchical occupancy model. The liver had the highest probability of infection among all fish (0.69) and the probability of detection within the liver was highest with qPCR (0.79). DFAT produced a high probability of false negative detections (0.58). Thus, I suggest that testing a combination of both kidney and liver tissues with qPCR may yield a higher detection rate that better predicts the probability of infection when performing fish health inspections.

Management of *R. salmoninarum* is particularly difficult because the bacterium utilizes both vertical and horizontal transmission. Vertical transmission occurs when infected brood fish transmit the bacterium to their eggs and progeny. Previous studies suggest the bacterium cannot be paternally transmitted due to limited success of bacterial entry into the egg from the spermatozoa. Thus, vertical transmission is suggested to be maternal. Horizontal transmission occurs among individuals through the ingestion of contaminated fecal matter or through direct contact with infected fish or water. In previous studies, horizontal transmission has been suggested to contribute more toward infection persistence than vertical transmission in wild and hatchery fish populations. However, the relative importance of horizontal transmission in hatcheries, where flow-through systems may expose multiple fish lots, has received little attention. I conducted experiments to determine rates of vertical and horizontal transmission.

In chapter 3, I examined the potential for horizontal transmission among hatchery-reared brood fish at an *R. salmoninarum*-positive hatchery facility. Juvenile Cutthroat Trout were placed in sentinel cages near positive adult Rainbow Trout and Cutthroat Trout for three, 30-day periods during optimal temperatures for infection. After exposure, the caged Cutthroat Trout were euthanized, and kidney tissue was tested for *R. salmoninarum* with qPCR. Only one out of 360 potentially exposed fish tested positive. My data suggest that horizontal transmission may play a small role in maintaining infection in hatchery-reared inland trout. However, I also show that horizontal transmission can occur in a short time, an important consideration when moving fish both within a hatchery or from one unit to another.

In chapter 4, I assessed whether the bacterium is vertically transmitted in Cutthroat Trout from the Poudre Rearing Unit in Colorado and the rate of transmission from paternal and maternal brood fish. Adult brood fish were lethally tested for *R. salmoninarum* and stripped of gametes to create 32 families among four *R. salmoninarum* infection treatments (MNFN, MNFP, MPFN, MPFP; M: male, F: female, P: positive, N: negative). Progeny from each spawning treatment were sampled at 6- and 12-months post swim-up to test for the presence of *R. salmoninarum* with an enzyme-linked immunosorbent assay (ELISA) and quantitative polymerase chain reaction (qPCR). My study indicates that vertical transmission occurs in inland Cutthroat trout and transmission is high when examined at the family level but is low within a family. These results suggest that hatcheries should limit vertical transmission through practices such as lethal culling, but also that adopting other methods such as testing eggs for *R. salmoninarum* should be considered in the future.

*Determination of an Agglutination Property Expressed by Renibacterium salmoninarum Isolated from Rainbow Trout in Colorado*

The following was included as an appendix in Dr. Riepe's dissertation and describes an agglutination property of *Renibacterium salmoninarum* isolated from Rainbow Trout in a Colorado hatchery. Evaluation of this property suggests that the strain of bacteria found in Colorado may be less virulent than a strain of the bacteria found in the Pacific Northwest. The appendix is included in this report because the findings described within have spurred additional research questions that will be investigated in a CSU M.S. project that will begin in fall 2022.

Determination of an Agglutination Property Expressed by *Renibacterium salmoninarum* Isolated from Rainbow Trout in Colorado (Tawni B. Riepe, Ph.D., Appendix I)

**Introduction**

*Renibacterium salmoninarum* is a non-motile and slow-replicating, Gram-positive diplobacillus that exhibits intracellular replication and survival capabilities within host macrophages (Young and Chapman 1978; Bruno 1987; Gutenberger et al. 1997). Mechanisms allowing *R. salmoninarum* to attach and colonize host macrophages vary between bacterial strains (Lindahl et al. 1981; Bruno 1988), but can play a critical role in subclinical or chronic infections. Strains of *R. salmoninarum* with auto-agglutination properties and increased hydrophobicity of the cell surface are considered most virulent and may invade, multiply, and increase infection intensity within the host compared to strains that are non-agglutinating and do not possess a hydrophobic cell surface (Bruno 1987; Bruno and Munro 1986; Evelyn et al. 1984). These properties allow virulent strains to resist host defense mechanisms, whereas other strains may be more readily phagocytized. Visual evidence for host immune and inflammatory responses in fish with chronic infections include external lesions or blisters, exophthalmos, swollen abdomen, hemorrhaging on the abdominal wall and viscera, and nodular lesions in the kidney or other internal organs (AFS-FHS 2016).

Previous studies have suggested that a commonly used assay, direct fluorescent antibody test (DFAT), can perform differently depending on the amount of surface associated p57, which can be strain dependent (Bruno 1988; O'Farrell et al. 2000). The strain found in Colorado hatcheries is currently unknown (hereby referred to as the CO strain). The ATCC 33209 strain (referred to as ATCC strain) has similar properties as the GL-64 strain optimized for the AFS Fish Health Blue Book (2016) DFAT standard operating procedures. Therefore, I examined the potential differences in detection probability of *R. salmoninarum* with DFAT found in another study (Chapter 2) due to the agglutination properties of the CO strain versus the virulent ATCC strain.

## Methods

The CO strain of *R. salmoninarum* was obtained from a natural infection in Rainbow Trout at the Colorado Parks and Wildlife (CPW) Bellvue Fish Research Hatchery and the ATCC strain came from the American Tissue Culture Collection (ATCC 33209; Sanders and Fryer 1980). Bacterial cultures were stored at -80°C in 10% glycerol prior to the auto-agglutination test. Both strains were cultured in KDM broth in two replicate flasks at continual agitation and maintained at 15°C. Subculturing of the two bacteria strains occurred every seven days, over two passes into new media. We verified purity of the culture with a Gram stain by identifying only one bacterial morphology that is described for *R. salmoninarum* under 50 times magnification (AFS-FHS 2016). Following Bruno (1988), the bacteria from each strain were resuspended in PBS and adjusted to an absorbance level of 1.0 at 420 nm. Cell suspensions of 1.5 mL of *R. salmoninarum* in PBS obtained from the two cultures was added to four glass cuvettes for both strains and examined for auto-agglutination. Two cuvettes contained 1.5 mL of PBS for blank controls. Measurements of absorbance were collected at 30-minute intervals up to six hours with an UV-Vis Spectrophotometer set at a wavelength of 420 nm. The temperature of the samples was maintained at 20°C between each absorbance measurement. The absorbance levels were plotted as a function of time to determine if the CO strain had similar agglutination properties as the ATCC strain.

## Results

The ATCC strain of *R. salmoninarum* resulted in clumping of the bacteria in PBS during the auto-agglutination test, with a decline of the absorbance measurements over time (Figure 3.1). The clumping of the ATCC strain suggests the presence of the auto-agglutination factor (Bruno 1988; O'Farrell et al. 2000) which contrasts to the CO strain isolated from naturally infected Rainbow Trout at the CPW Bellvue Fish Research Hatchery. Absorbance levels of the isolated strain in Rainbow Trout did not vary significantly over time showing little evidence for cell clumping. The absorbance measurements observed are similar to attenuated virulent strains with non-agglutinating factors from other studies (MT-239, originally isolated from Atlantic Salmon in Scotland; Bruno 1988).

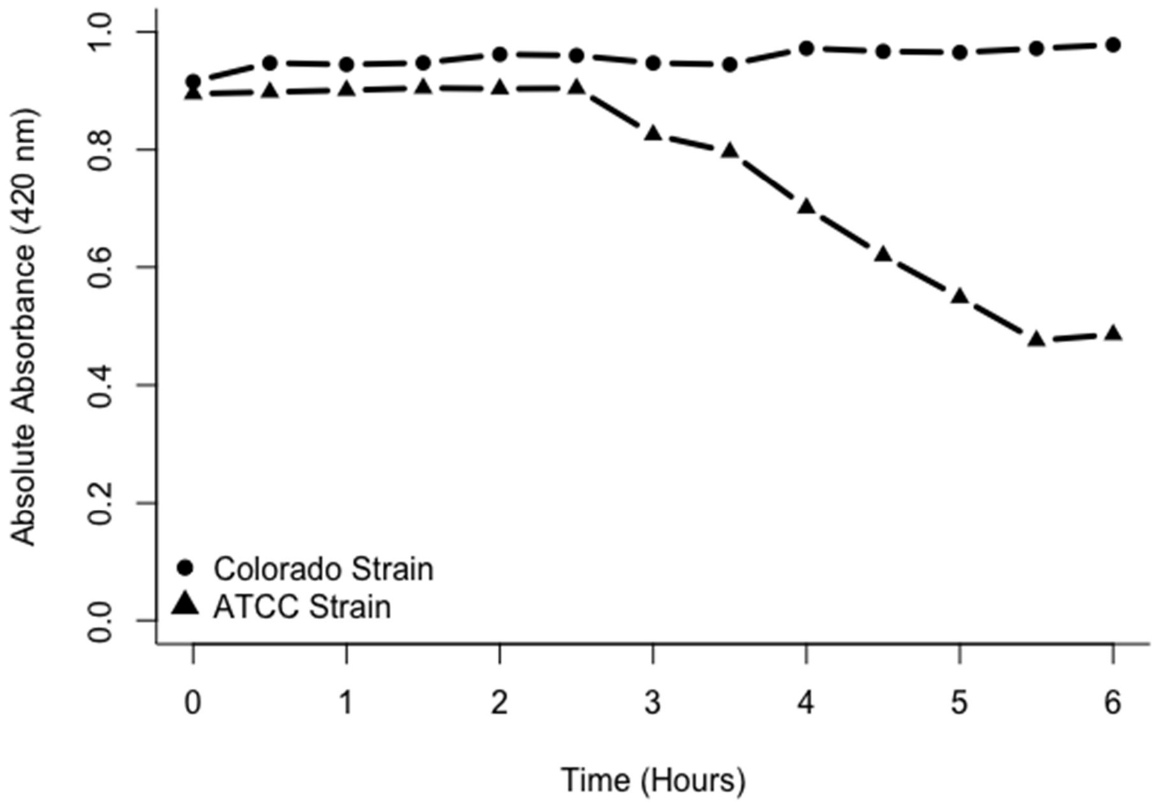


**Figure 3.1.** Change in absorbance measurements over six hours for the ATCC 33209 strain and Colorado strain found in Rainbow Trout from the CPW Bellvue Fish Research Hatchery.

**Discussion**

Typical fish health diagnostic methods are optimized from strains of pathogens that are highly transmissible between hosts and thus are typically highly virulent. Various virulent factors contribute to the capabilities of pathogens to infect and invade the hosts cells. One of the abilities of *R. salmoninarum* to colonize and infect the host tissues and cells depends on adherence of the bacteria (Kroniger et al. 2022). Adherence involves the protein p57, which is located on the cell surface providing the ability for auto-agglutination of the cells (Bruno 1988; Daly and Stevenson 1990). In contrast, attenuated virulent strains have been described in previous studies (Bruno 1988; O'Farrell et al. 2000; Elliott et al. 2013) and often show minimal detectable p57 on the cell surface (Senson and Stevenson 1999). The presence of p57 is presumed to lead to the auto-agglutination property of the bacteria and is the detectable protein for which the DFAT assay has been optimized. Thus, without the presence of the p57 protein, the *Fluorescein*-labeled antibody to *R. salmoninarum* used in the assay cannot bind to the bacteria, resulting in a lack of or poor fluorescence. O'Farrell et al. (2000) noted tissues extracted from fish injected with an attenuated virulent strain had poor fluorescence compared to fish infected with a virulent strain, and detecting the bacteria with DFAT did not occur with every injected fish. This could indicate a low specificity of DFAT to an attenuated strain of *R. salmoninarum*, and I sought to investigate the potential causes for the poor diagnostic performance I found in Chapter 2.

I tested the ability for auto-agglutination of the CO strain of *R. salmoninarum* compared to the highly virulent ATCC 33209 strain. The ATCC strain showed auto-agglutination properties, with decreased absorbance measurements over time as seen in other virulent strains of *R. salmoninarum* (Bruno 1988). By contrast, the CO strain was non-agglutinating as there was little evidence for cell clumping. The low detection abilities with DFAT in this study and others described in Colorado (Kowalski et al. 2022), may be a result of the non-agglutinating properties of this strain. In addition, my study indicates the strain of *R. salmoninarum* I have isolated is not only non-agglutinating, but may also be an attenuated virulent strain. The lack of noticeable clinical disease during fish health inspections in Colorado hatcheries and little evidence for disease through immune-histopathological analysis in a previous study of adult Cutthroat Trout at the CPW Poudre Rearing Unit may also suggest low virulence in the strain. High detection probabilities with qPCR found in my study and others, however, do seem reasonable. The major soluble antigen gene (*msa*) sequence detected with PCR assays are nearly equivalent as indicated by genetic analysis of an attenuated virulent and virulent strains of *R. salmoninarum* (O'Farrell et al. 2000). Thus, I have reason to believe that Colorado may be dealing with an attenuated strain with non-agglutination properties, which would explain why my detection probabilities with DFAT are much lower than with qPCR and could explain why I observed lower vertical and horizontal transmission rates than other studies.

It is possible that low within-family vertical transmission (Chapter 4) could be due to the differences in bacterial strains. Attenuated virulent strains of *R. salmoninarum* may have the limited ability to colonize the eggs from the lack of a surface protein allowing for it to occur. Several studies have suggested that the p57 has an attachment ability or inability to cells between strains (Bruno 1988; Daly and Stevenson 1989; Piganelli et al. 1999). It is therefore possible that although the adult brood fish have high bacterial loads, the bacteria will have a lower probability of successful vertical transmission during oogenesis or post-ovulation development if the strain cannot successfully attach to cells.

It is well known that *R. salmoninarum* is capable of surviving without a host for up to 21 days when water conditions are favorable (Austin and Rayment 1985; Evelyn 1988; Balfry et al. 1996). The bacteria are likely to bind to feces that are extruded from the fish and settle in the water. *Renibacterium salmoninarum* has been known to have a hydrophobic surface that allows it to have an affinity for organic matter which can be found within fecal matter (Austin & Rayment 1985; Gurijala and Alexander 1990; Balfry et al. 1996). However, this hydrophobic surface has also been known to be variable in different strains of the bacteria. Virulent strains of *R. salmoninarum* that typically express the p57 surface have a hydrophobic cell surface (Piganelli et al. 1999). Previous studies have tested the hydrophobic nature of various strains by salt aggregation assays, adherence to hydrocarbons and nitrocellulose filters, and have shown that the strains without a hydrophobic surface tend to be less virulent (Bruno 1988; Daly and Stevenson 1989; Bandin et al. 1989; Piganelli et al. 1999). Thus, if the strain found at the PRU during our study is less virulent, it may not be binding well to the fecal matter coming from infected fish, thus limiting the success of horizontal transmission through fecal ingestion.

Although I did successfully detect *R. salmoninarum* in one fish (Chapter 3), if the strain detected at the PRU is an attenuated strain, transmission may have been limited because the bacteria did not have an affinity to the feces, the suspected route of horizontal transmission on the unit.

My use of an auto-agglutination test to determine the potential virulence of the strain of *R. salmoninarum* found in inland trout of Colorado is interesting, but needs to be followed up with an end-point mortality experiment comparing the CO strain to a virulent strain, as well as a test of the hydrophobicity of the CO strain as this also contributes to the overall virulence.

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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 biologist meeting, and one at the CPW Northeast Region Biology Days:

- Fetherman, E. R., and B. W. Avila. 2022. Habitat associations of Rainbow Trout and Brown Trout fry. 2022 Colorado Parks and Wildlife Aquatic Biologist Meeting. Virtual. January 20, 2022.
- 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. Colorado Parks and Wildlife Northeast Region Biology Days. Brighton, Colorado. April 28, 2022.

External presentations and posters provided an opportunity to give research updates to managers both within and outside Colorado. One talk was given at the American Fisheries Society Annual Meeting, a seminar was presented at Colorado State University, a presentation was given to the Fraser River Ranch Fishing Club, and a talk was given to the Wyoming Game and Fish Department:

- Riepe, T. B., E. R. Fetherman, and D. L. Winkelman. 2021. Comparison of tissues for the detection of *Renibacterium salmoninarum* in Cutthroat Trout. 151$^{st}$ Annual Meeting of the American Fisheries Society. Baltimore, Maryland. November 9, 2021.
- Fetherman, E. R. 2022. Whirling disease-resistant Rainbow Trout development and post-stocking survival and recruitment evaluations in the upper Colorado River. Department of Fish, Wildlife and Conservation Biology seminar series, Colorado State University. Fort Collins, Colorado. February 11, 2022.
- Fetherman, E. R., E. E. Richer, M. C. Kondratieff, J. Ewert, and D. A Kowalski. 2022. Update on Fraser River Ranch salmonid population and the Windy Gap fish movement study. Fraser River Ranch Fishing Club Party. Lakewood, Colorado. March 30, 2022.
- 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. Wyoming Game and Fish Department. Laramie, Wyoming. April 29, 2022.

In addition to public and professional meeting presentations, one presentation was given to the Wildlife Disease Ecology class at Colorado State University, and 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 2022.

- Fetherman, E. R. 2021. Salmonid disease research and management in Colorado. Guest lecture, Wildlife Disease Ecology. Colorado State University. Fort Collins, Colorado. November 16, 2021.
- Fetherman, E. R. 2022. Salmonid disease research in Colorado. Guest lecture, Introduction to Fisheries. Front Range Community College. Fort Collins, Colorado. March 24, 2022.

Manuscripts published in peer-reviewed scientific journals help to inform fisheries management decisions locally, nationally, and internationally. One manuscript was published in the Journal of Fish Biology, and one in the Journal of Fish Diseases:

- Fetherman, E. R., and B. W. Avila. 2022. Habitat associations of Rainbow Trout *Oncorhynchus mykiss* and Brown Trout *Salmo trutta* fry. Journal of Fish Biology 100:51-61.
- 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. DOI: 10.111/jfd.13605.

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

- Kopack, C. J., E. R. Fetherman, E. D. Broder, R. M. Fitzpatrick, and L. M. Angeloni. *In review*. Assessing antipredator behavior and the potential to enhance it in a species of conservation concern. Submitted to *Aquatic Conservation*.
- Avila, B. W., K. P. Huyvert, D. L. Winkelman, and E. R. Fetherman. *In review*. A meta-analysis of factors affecting *Flavobacterium psychrophilum* experimental infections. Submitted to *PLoS Pathogens*.
- Reipe, T. B., E. R. Fetherman, and D. L. Winkelman. *In review*. Horizontal transmission of *Renibacterium salmoninarum* in a flow-through hatchery system. Submitted to *North American Journal of Aquaculture*.
- Erickson, T. A., G. J. Schisler, and E. R. Fetherman. *In review*. Post-stocking survival and myxospore evaluation of whirling disease resistant Rainbow Trout strains. Submitted to *North American Journal of Fisheries Management*.
- Avila, B. W., D. L. Winkelman, and E. R. Fetherman. *In review*. Biotic and abiotic factors affecting survival of two Rainbow Trout strains in streams in Colorado. Submitted to *North American Journal of Fisheries Management*.

Sport Fish Research staff participated in an interview for an article regarding the history of whirling disease in Colorado and the development of whirling disease resistant Rainbow Trout, specifically the Gunnison River Rainbow, for an article published in the Durango Herald entitled "In Colorado's rivers, genetic research helps Rainbow Trout rebound" on November 18, 2021 (Author: Aedan Hannon).

Technical assistance milestones included drafting a response to an angler interested in providing an injectable mix of CBD and essential nutrients to fish prior to release after angling; providing Brown Trout genetic, diet, and scale data from the Middle Fork of the South Platte for inclusion of concepts in the Brown Trout chapter of the Fishes of Colorado Book; discussing current WD exemption process with senior hatchery staff; reviewing captive kokanee brood stock plan with a focus on whirling disease and gill lice infection concerns; discussing materials and methods for installation of stationary antennas that will be used to track stocked tiger muskie in Shadow

Mountain Reservoir; discussing results of whirling disease exposures in Squaretop Lake to determine if the lake was negative and ready to be stocked with Cutthroat Trout; and, discussing implications of fry shocking and *M. cerebralis* infection results on management of Rainbow Trout in the Arkansas River. Additional external technical assistance milestones included consultation on Program MARK analyses and review of thesis chapters with CSU graduate students not associated with Sport Fish Research Studies; assistance with design of a project to evaluate the presence and distribution of *M. cerebralis* in LaBarge Creek, Wyoming; discussing Colorado eDNA results and potential sampling designs for detecting *M. cerebralis* in Banff National Park, Canada; and, discussing potential whirling disease research projects to be conducted by CSU graduate students on the Trinchera Ranch, Colorado.

Technical assistance milestones included assistance with experimental design, data collection and analysis on projects being conducted by CPW researchers and biologists:

- Submitted 622 sampling surveys for inclusion in ADAMAS.
- Completed data analysis and wrote the methods section for a manuscript examining the efficacy of eDNA sampling for *M. cerebralis* in relation to population reclamation and barrier construction in George and Cornelius creeks.
- Reared and collected samples for myxospore enumeration from fish held in sentinel cages in Lower Squaretop Lake and maintained for a minimum of 2,000 degree-days post-exposure in the CPW Salmonid Disease and Sport Fish Research lab.
- Assisted with fry sampling, collection of heads for myxospore enumeration, and water samples for triactinomyxon enumeration in the Arkansas River.
- Assisted with aquatic invertebrate collections in the upper Colorado River.
- Analyzed data examining the effects of toe wood on fish abundance and biomass.
- Collected samples for developing a disease history on Rainbow Trout in new locations within the Yampa River.
- Reviewed study design and provided input for a whirling disease retrospective study.

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

- Fetherman et al. 2021. PIT tagging fish in Fraser River. September 2021-October 2021.
- Fetherman et al. 2021. PIT tagging fish in Colorado River. October 2021-December 2021.
- Brittain et al. 2022. PIT tagging fish in St. Vrain River. March 2022-July 2022.

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

- Ying, C., X. Fang, H. Wang, Y. Yang, P. Xu, K. Liu, and G. Yin. *In review*. Anisakidae parasitism activated immune response and induced liver fibrosis in wild anadromous *Coilia nasus*. Submitted to *Journal of Fish Biology*.
- Sarais, F., R. Montero, S. Ostermann, A. Rebl, B. Köllner, and T. Goldammer. *In review*. The early immune response of lymphoid and myeloid head-kidney cells of Rainbow Trout (*Oncorhynchus mykiss*) stimulated with *Aeromonas salmonicida*. Submitted to *Fishes*.
- Reviewed final report of the project "The role of SOCS proteins in salmonid whirling disease" for the Austrian Science Fund (FWF).
- Anonymous. *In review*. Digital hydrography underestimates stream length and leads to underestimates of trout population size. Submitted to *North American Journal of Fisheries Management*.

- Sliger, R., and G. D. Grossman. *In review*. Foraging dynamics of hatchery Brook Charr (*Salvelinus fontinalis*). Submitted to *North American Journal of Fisheries Management.*
- Anonymous. *In review*. Zero altered modeling of an aquatic parasite host with application to invasive species risk assessment. Submitted to *Management of Biological Invasions*.
- Shivam, S., M. El-Matbouli, and G. Kumar. *In review*. Kinetics of parasite-specific antibody and B cell associated gene expression in Brown Trout, *Salmo trutta* during Proliferative Kidney Disease. Submitted to *Biology*.
- Born-Torrijos, A., A. Kosakyan, S. Patra, J. Pimentel-Santos, B. Panicucci, J. T. H. Chan, T. Korytář, and A. S. Holzer. *In review*. Method for isolation of myxozoan proliferative stages from fish at high yield and purity: an essential prerequisite for *in vitro*, *in vivo* and *in silico* research developments. Submitted to *Cells*.

Service outside of CPW:

- Member of the Colorado/Wyoming Chapter of the American Fisheries Society (AFS) Budget Review Committee. 2021, 2022.
- Member of the Colorado/Wyoming Chapter of AFS Paper/Poster Judging Committee. 2022.
- Member of the Western Division of AFS Resource Policy and Environmental Concerns Committee. 2021.
- Member of the North American Journal of Aquaculture subcommittee of the AFS Publication Awards Committee. 2021-2022.
- Vice President of the Western Division of the American Fisheries Society. 2021-2022.
  - Chair of the Division Membership Committee.
  - Member of the AFS Membership Committee.
  - Officer Liaison to the Western Division Diversity and Inclusion Committee.
  - Member of the Spokane Local Planning Team.
  - Member of the Society Planning Team.
  - Member of the Pacific Islands Chapter Formation Committee.
  - Organizer of 2022 Aquatic Film and Photo Festival.

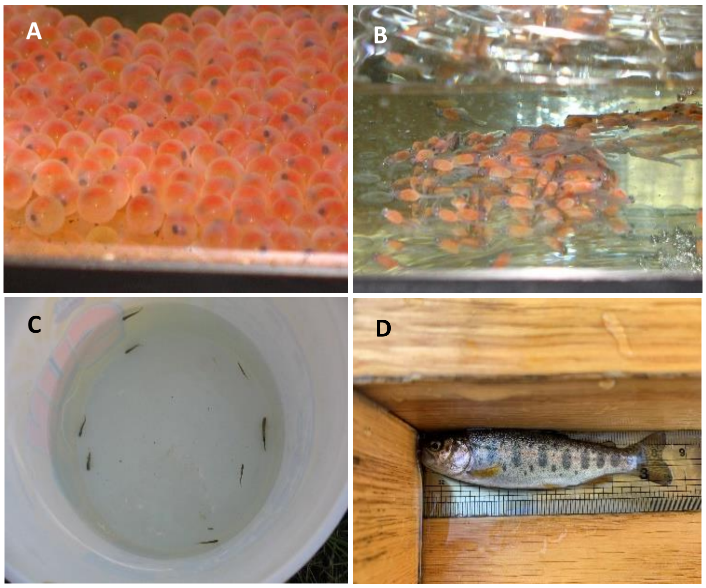
Highlights:

- Eric Fetherman became an American Fisheries Society Certified Fisheries Professional in July 2022.

# Rainbow Trout Fry Investigations

**FRY SAMPLING INFORMS SURVIVAL, DISEASE ISSUES, AND HABITAT RESTORATION IN LARGE RIVER SYSTEMS**

## Early Life Stages of Rainbow Trout in Colorado's Rivers



Rainbow Trout eggs (A) and sac fry (B). After swimming up from the gravel, fry are available for sampling (C and D).

Rainbow Trout are spring spawners, and deposit their eggs in gravel at sites known as redds. The eggs hatch in 4-7 weeks depending on water temperature. After hatching, fish spend up to two weeks as sac fry, remaining in the gravel and absorbing their yolk sac. Once the yolk sac is absorbed, the fry emerge from the gravel to begin feeding. Fry spend the first several months of their life in shallow areas found along the river margins, using vegetation and interstitial spaces between rocks as cover from predators. During this time, they are also easily sampled to estimate population size. Changes in the fry population over time can indicate whether factors such as water quality, flow, temperature, or presence of parasites are affecting survival. Additionally, fry estimates conducted later in the year can be indicative of the number of fish that will recruit to the juvenile and adult populations. Finding ways to increase fry survival in rivers has been the focus of many CPW aquatic research projects.

## Conducting Fry Population Estimates

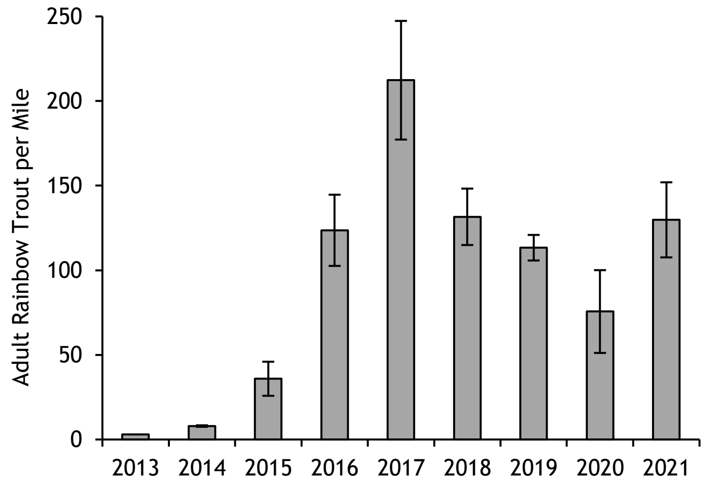

Electrofishing for Rainbow Trout fry

Fry population estimates are conducted using one to three backpack electrofishing units and making three passes through a 50-foot site along the river margins. Fish are removed on each pass, and kept separate until they can be counted and measured after all three passes have been completed. Flows, habitat complexity, and differences in how the electricity affects each individual causes some fish to be missed on each pass. Equations used to calculate a population estimate account for this imperfect detection between passes to generate a population estimate. This estimate per 50 feet of riverbank is then standardized to number of fry per mile to allow comparison between sites or sections of the river. The example below produced an estimate of 70 (± 1) salmonid fry per 50 feet or 7,376 (± 1,978) fry per mile.

Forty-five salmonid fry were removed from this 50-foot site on pass 1. An additional 13 were removed on pass 2 and eight removed on pass 3. A total of 66 fry were collected across the three passes.

## Fry as Disease and Management Indicators

The disappearance of Rainbow Trout fry from many of Colorado's large rivers was one of the first indicators of a problem after whirling disease (caused by *Myxobolus cerebralis*) first arrived in these systems in the early 1990s. Fry are most susceptible to infection, and susceptible Rainbow Trout fry exposed to the parasite rarely survive past their first year of life. With no fry available to recruit to the adult population, these losses resulted in the collapse of Rainbow Trout populations across Colorado. Whirling disease resistant Rainbow Trout developed and produced by CPW have been stocked throughout the state to reestablish these lost Rainbow Trout populations.




CPW started stocking whirling disease resistant Rainbow Trout fry in the upper Colorado River to increase post-stocking survival in 2013. At that time, there were only three adult Rainbow Trout per mile in the river. The first strain CPW stocked was the Hofer strain crossed with Colorado River Rainbow Trout (H×C), which was stocked in 2013-2015. H×C fry survived well, with over 1,100 H×C fry remaining in October of each year. In response, the adult population showed an exponential increase, with up to 212 Rainbow Trout per mile in 2017. Unfortunately, a separate hatchery disease outbreak in the brood stock (bacterial kidney disease) caused that strain to be discontinued in 2015. Pure Hofer strain fry were stocked in the upper Colorado River in 2016-2018, but they did not survive as well in this river as the H×Cs. Only 250 Hofer fry per mile remained in October of each year, not enough to sustain the adult population, which declined between 2018 and 2020. By that time, CPW had created a replacement strain for the H×C, a cross between the Hofer and Gunnison River Rainbow (H×G). H×G fry were stocked in the upper Colorado River in 2019-2021 and showed excellent survival, with over 1,500 H×G fry per mile remaining in October of each year. The H×G fry also showed good recruitment, with an increase of 50 adult Rainbow Trout per mile between 2020 and 2021, and a continued increase in the adult population expected in 2022 and 2023.

## Fry Habitat Associations

Data obtained from fry evaluations show how important fry survival is for recruitment to and management of adult populations. Although habitat restoration projects typically focus on adult fish habitat, creating ideal fry habitat will help to establish self-sustaining whirling disease resistant Rainbow Trout populations. A recent study in the upper Colorado River showed that Brown Trout and Rainbow Trout fry overlap in their habitat associations, but there are opportunities for both single- and multi-species management. Brown Trout and Rainbow Trout fry were most often found together in sites with pebble sizes of 151 mm, velocities between 0.20 and 0.23 m/s, and depths of 0.18 m. However, more Rainbow Trout fry were found in sites with velocities ≥ 0.23 m/s, and depths ≤ 0.17 m. Larger particle sizes and higher velocities in near-shore habitats can reduce fine sediment habitats for the intermediate host of whirling disease, the *Tubifex* worm, and fry infection severity. Overall, interspecific competition, fry stocking and strain type stocked, and the presence of pathogens such as *M. cerebralis*, cause deviations from published habitat suitability indices typically used to design restored habitat reaches. Evaluating system-specific differences in these factors on fry habitat use may allow future restoration activities to be more effective.

### Associated Literature

Fetherman, E. R., and B. W. Avila. 2022. Habitat associations of Rainbow Trout *Oncorhynchus mykiss* and Brown Trout *Salmo trutta* fry. Journal of Fish Biology 100:51-61.