

Sport Fish Research Studies

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Annual Report

Colorado Parks & Wildlife

Aquatic Research Section

Fort Collins, Colorado

August 2021

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BELLVUE FISH RESEARCH HATCHERY PRODUCTION AND RESEARCH UPDATES

The Hofer (GR or HOF; used interchangeably throughout report) strain of Rainbow Trout is resistant to whirling disease (*Myxobolus cerebralis*), and as such 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 report) 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, and their crosses, 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 2020, 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 matured 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 10, 2020, the first group of HL females were ripe and ready to spawn.

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

Water-hardened fertilized (green) eggs 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 12.2°C (54°F) 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 minute) 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 2020-2021 spawning season.

Strain	Date Spawned	No. Spawned Females	No. Green Eggs	No. Eyed Eggs	Destination
HL	11/10/20-12/29/20	100	12,160	7,904	BFRH
GR	12/15/20-12/29/20	102	11,869	9,851	BFRH
GR	12/15/20	36	44,590	37,610	BFRH and Seaman Reservoir
Total	11/10/20-12/29/20	238	68,619	55,365	

The on-site Rainbow Trout production spawn started on November 10, 2020, with the last group of HL and GR females spawned on December 29, 2020 (Table 1.1). The goal was to produce 1,000 eggs per strain for brood stock replacement purposes and 30,000 HL subcatchables for Seaman Reservoir. Both goals were met, and replacement brood and subcatchable fish are growing well. There were no additional GR or HL production requests for Colorado in 2021.

This year was unlike previous years in that the HL brood females were ripe early. On our first spawn date, 70% of HL females were ripe and ready to spawn. This resulted in only two spawn dates for the HL brood stock. Since the BFRH water temperature remains constant, daylight exposure was the other possible factor that could have caused the females to ripen early. This year we had a predominant smoky sky due to fires in the area, which could have changed perceived daylight exposure periods and possibly explained the shift in spawn timing observed.

ANNUAL DISEASE TESTING

Between 2016 and 2020, the BFRH conducted a brood stock cull program to reduce and attempt to eliminate the presence of *Renibacterium salmoninarum*, the pathogen causing Bacterial Kidney Disease (BKD), on the facility. During the program, only *R. salmoninarum*-negative progeny were retained for brood stock replacement purposes by discarding eggs and progeny whose parents tested positive for the bacteria. The first annual BFRH disease inspection following this program was conducted on April 6, 2020 to determine the status of *R.*

salmoninarum on the unit. This inspection detected no *R. salmoninarum* using the USFWS and AFS-FHS (2014) Blue Book standard for direct fluorescent antibody test (DFAT) kidney tissue sampling protocols. This initial inspection was followed up by two more sampling events conducted three months apart per state regulations. All samples from these additional inspections were also determined to be negative for *R. salmoninarum*. As such, the BFRH is now considered negative for the bacteria, and fish from the facility can once again be stocked into Colorado waters. The stocking of GR subcatchables into Seaman Reservoir in 2021 will be the first time that fish have been stocked from the BFRH since detection of *R. salmoninarum* on the unit in 2016.

USFWS and AFS-FHS (U.S. Fish and Wildlife Service and American Fisheries Society-Fish Health Section). 2014. Standard procedures for aquatic animal health inspections. *In* AFS-FHS. FHS blue book: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, 2016 edition.

BROWN TROUT YY PRODUCTION EXPERIMENT

The use of supermales, male fish that have two Y-chromosomes, shows promise as an alternative for eradication via mechanical removal or pesticides of nonnative or undesirable wild fish populations (Schill et al. 2017). Development of a YY Brook Trout brood stock has been successful at producing large numbers of fish for stocking (Schill et al. 2016). Simulations suggest that at a 50% annual stocking rate of the age-0 density, combined with a 50% annual selective suppression rate, wild Brook Trout could be extirpated in only 2-4 years, and in most populations, eradication could occur in less than 10 years regardless of the suppression rate (Schill et al. 2017). Since the successful production of the YY Brook Trout, the Idaho Department of Fish and Game has organized a program through which the development of YY brood stocks have been attempted with several other species. CPW and the BFRH are attempting to develop a YY Brown Trout brood stock as part of this program.

The first attempt to develop neo-females through the use of estradiol (E2)-coated feed, which will subsequently be used to develop and produce the supermale brood stock (Schill et al. 2016), occurred at the BFRH in 2019-2020. This experiment included three treatment groups to determine the minimum required E2 exposure for feminization (development of neo-females): a control group, a 30-day exposure group, and 60-day exposure group. The control fish were fed an ethanol-coated feed. The exposure groups were fed a diet of 20 mg/kg E2 top-coated feed for either 30 or 60 days. However, after several gonadal development evaluations of these fish, neither the 30- nor 60-day treatment resulted in complete neo-female development. As such, the parameters for the feed treatment and durations were reconfigured for a second experiment conducted at the BFRH in 2020-2021.

Brown Trout for the second experiment were spawned at North Delaney Lake on October 6, 2020 and eggs were transported to the CPW Glenwood Springs Hatchery to eye up. Due to COVID exposure concerns, BFRH staff could not be on-site to select specific parents to be spawned for this experiment, so the eggs received were from the sum total of fish spawned on that day. The eyed eggs were shipped to the BFRH from the Glenwood Springs Hatchery on November 18, 2020, and were distributed to egg cups in tanks assigned each treatment.

On November 29, 2020, 90% of the eggs had hatched. Initial feeding in both the control and treatment (E2-coated feed) tanks (100 fish per tank) began on December 24, 2020. In an attempt to find the correct combination of E2 concentration in the feed and exposure period, this experiment included more treatment combinations than the previous experiment. The experiment consisted of five E2 treatments and four exposure periods for a total of ten experimental groups with two tanks (replicates) each: 1) 10 mg/kg E2 fed for 120 days, 2) 20 mg/kg E2 fed for 90 days, 3) 20 mg/kg E2 fed for 120 days, 4) 30 mg/kg E2 fed for 60 days, 5) 30 mg/kg E2 fed for 75 days, 6) 30 mg/kg E2 fed for 90 days, 7) 30 mg/kg E2 fed for 120 days, 8) 60 mg/kg E2 fed for 60 days, 9) 60 mg/kg E2 fed for 90 days, and 10) controls. After each scheduled feeding period was completed, fish were switched over to a normal (no top coating) trout diet. A third control tank was included in the experiment and used for interval sampling to examine gonadal development in the young Brown Trout. The fish in this tank were sampled at 30, 60, 75, 90, 105, 120, and 135 days after initial feeding occurred, and ten fish were collected and preserved in formalin at each sampling period.

On April 27-28, 2020, 75 fish in each tank were PIT tagged using individual 12-mm pre-loaded 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. An additional five fish per tank were euthanized and a whole-fish health sample was taken to determine potential effects of E2 exposure on overall fish health metrics. Once fish outgrow their experimental tanks in FR1, they will be moved into troughs in the main hatchery building. Fish will remain in separate troughs by treatment (two tanks from the same treatment combined in each trough) within the main hatchery facility until further testing can occur after the development of ovaries in fall 2021.

Schill, D. J., J. A. Heindel, M. R. Campbell, K. A. Meyer, and E. R. Mamer. 2016. Production of a YY male Brook Trout broodstock for potential eradication of undesired Brook Trout populations. *North American Journal of Aquaculture* 78:72-83.

Schill, D. J., K. A. Meyer, and M. J. Hansen. 2017. Simulated effects of YY-male stocking and manual suppression for eradicating nonnative Brook Trout populations. *North American Journal of Fisheries Management* 37:1054-1066.

SPORT FISH RESEARCH PROJECT UPDATES

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 large 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 in many locations 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, that have the potential to impact Colorado's salmonid populations continue to be evaluated in ongoing field experiments in the Colorado, Fraser, and Yampa rivers.

UPPER COLORADO RIVER SALMONID POPULATION MONITORING

2020 Salmonid Fry Population Estimates

Upper Colorado River Rainbow Trout fry stocking evaluations began 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 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 Hofer (GR) survived just as well as the H×C when stocked as fry into small streams (Avila et al. 2018), but the survival of the GR had not been evaluated in a large river. As such, approximately 60,000-70,000 GR fry were stocked by raft into this stretch of the upper Colorado River in 2016, 2017, and 2018. Once *M. cerebralis*-resistance evaluations of the GR 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). On July 8, 2020, approximately 80,000 H×G fry were stocked into the upper Colorado River between Hitching Post Bridge and Lower Red Barn on the Chimney Rock Ranch (Figure 2.1). Two-thirds of the Rainbow Trout fry were loaded into large coolers on the stocking raft, supplied with a constant flow of oxygen, 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 onto the raft from 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). No fish were stocked below the diversion structure as they had been in previous years (Fetherman and Schisler 2016) due to the lower number of fry available.

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, and September 2020. October fry population estimates could not be conducted in 2020 due to access issues caused by the East Troublesome Fire. 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, and September 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), 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, Lone Buck, and Paul Gilbert State

Wildlife Areas. A second site, Parshall Island, 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 2021 (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 to increase Rainbow Trout recruitment in this section of the river.

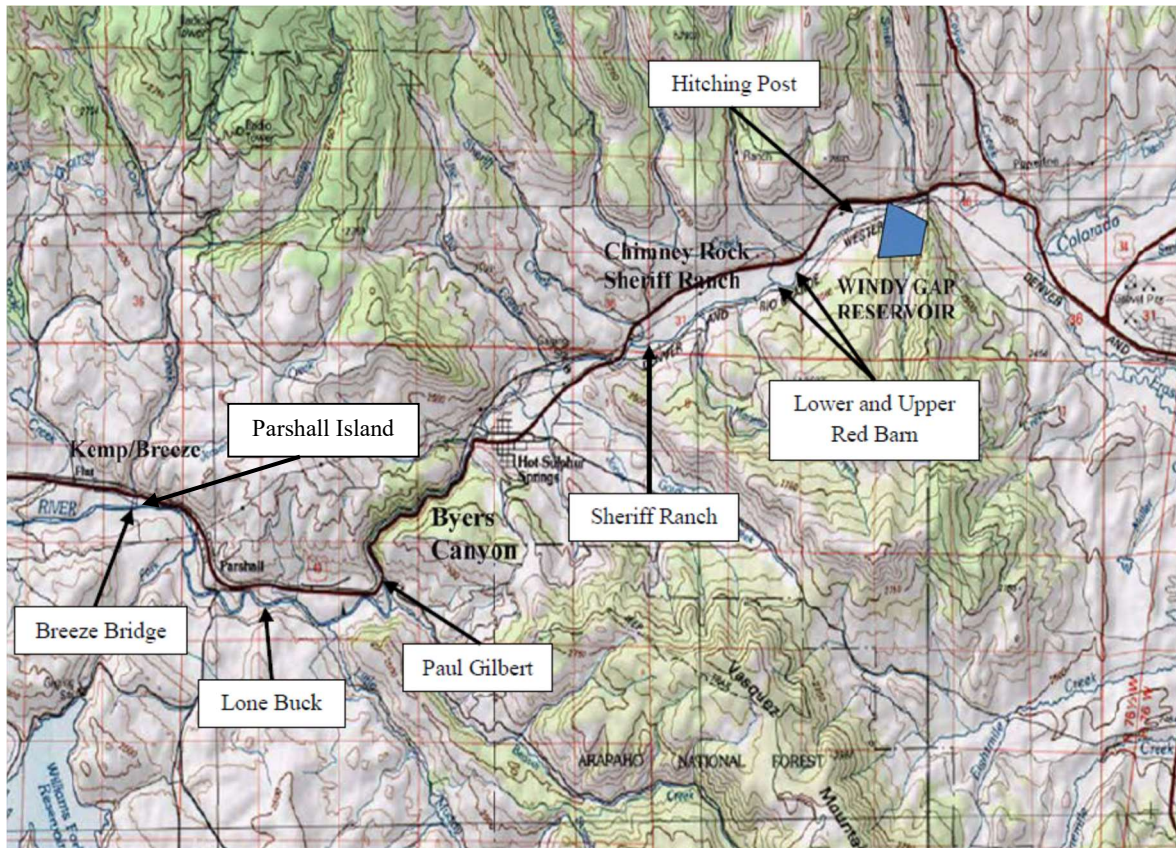


Figure 2.1. Upper Colorado River study area showing the eight sites at which salmonid fry population estimates were conducted in July, August, and September 2020.

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 November 2020, up to ten Brown Trout and ten Rainbow Trout were collected by Hot Sulphur Springs Aquatic Biologist Jon Ewert from the four sites within the Chimney Rock/Sheriff Ranch study section to obtain myxospore counts. Myxospore enumeration was completed at the CPW Aquatic Animal Health Laboratory (Brush, Colorado).

Brown Trout fry averaged 7,426 (\pm 2,383) myxospores per fish. One Brown Trout fry captured at the Sheriff Ranch fry site exhibited cranial deformities, but otherwise, no other signs of

disease were observed in Brown Trout collected in November 2020. Rainbow Trout fry averaged 1,411 (\pm 847) myxospores per fish, lower than the previous year (Fetherman et al. 2020). One Rainbow Trout fry captured at the Lower Red Barn fry site exhibited spinal deformities, but otherwise, no other signs of disease were observed in Rainbow Trout collected in November 2020. Overall, less than 1% of Brown Trout and Rainbow Trout fry exhibited clinical signs of disease in 2020.

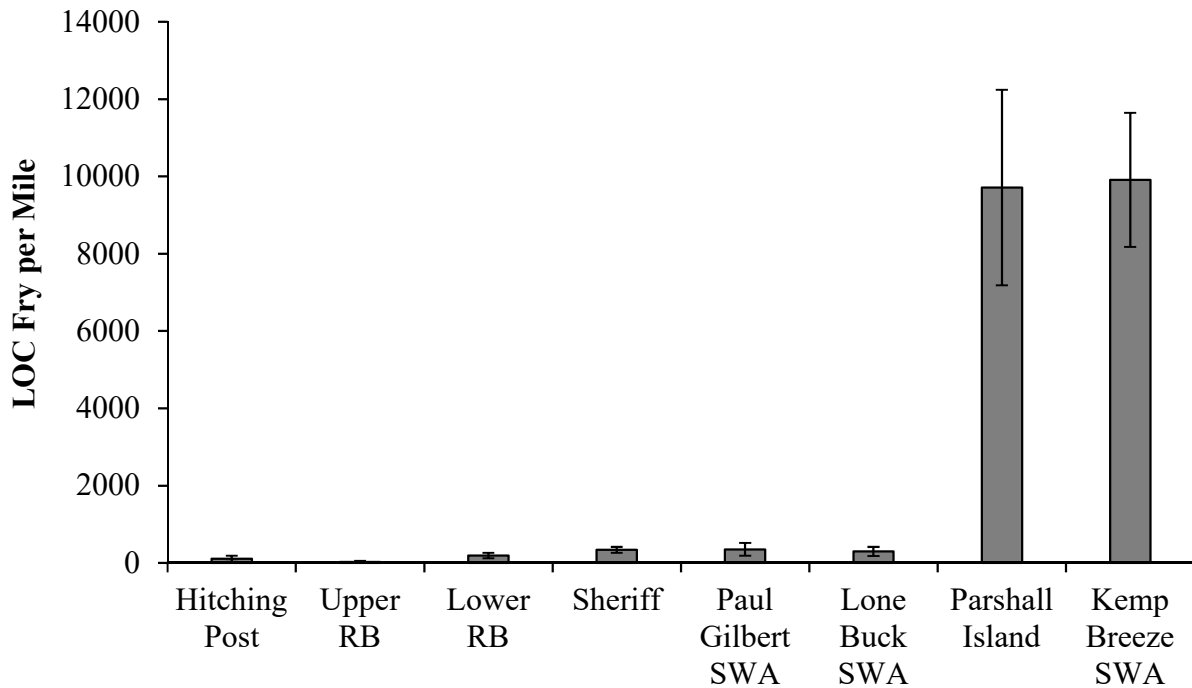


Figure 2.2. Average estimates of Brown Trout (LOC) fry abundance (fry per mile; SE bars) in 2020 between Hitching Post Bridge, the closest fry site to Windy Gap Reservoir on the upstream end of the study section, to the Parshall Island and Kemp Breeze SWA fry sites located below the Williams Fork confluence with the Colorado River.

Brown Trout fry, normally relatively evenly distributed throughout the eight fry sites, showed a significantly different pattern of abundance in 2020 than in previous years, with significantly higher abundances in the two sites located below the confluence with the Williams Fork compared to sites located above the confluence (Figure 2.2). On average, an estimated 219 (\pm 133) Brown Trout fry per mile were present above the Williams Fork confluence, whereas 9,811 (\pm 141) Brown Trout fry per mile were present below the Williams Fork confluence. In addition, Brown Trout fry were not observed in five sites above the Williams Fork confluence on at least one sampling occasion. It is not known what caused this pattern of Brown Trout fry abundance in 2020. However, in late 2019, Windy Gap Reservoir was drained to allow for survey work during the winter months. Draining occurred after the Brown Trout spawn, and changes in flow, temperature, or increased fine sediment settling into interstitial spaces and disrupting oxygen exchange in the spawning gravels may have killed Brown Trout eggs. Whatever the cause, the addition of flows from the Williams Fork likely diluted and reduced these effects in the Colorado River below the confluence, resulting in higher Brown Trout fry abundances in the Kemp-Breeze SWA.

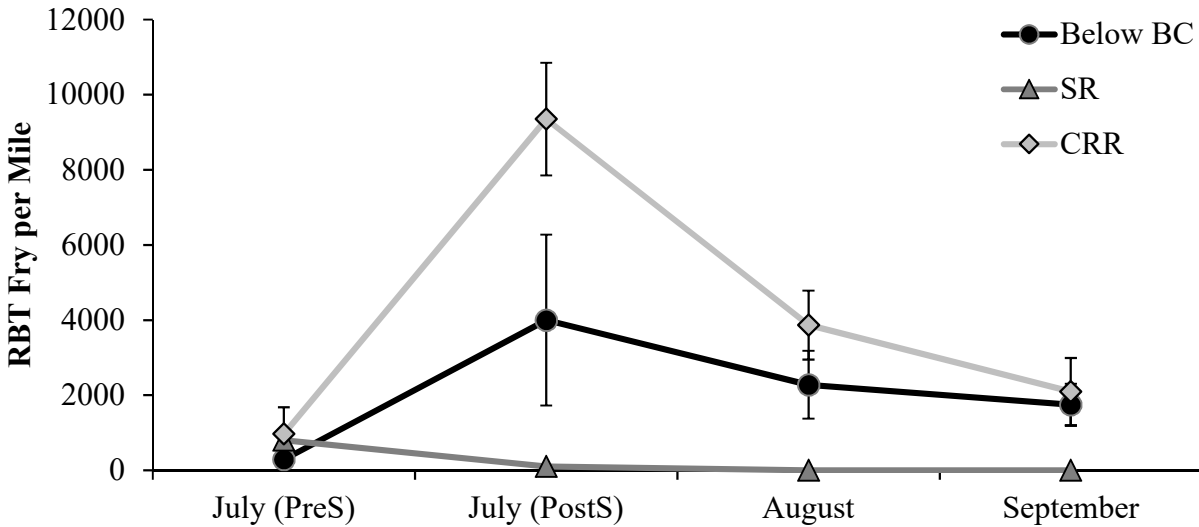


Figure 2.3. Average Rainbow Trout (RBT) fry abundance (fry per mile; SE bars) in the sites below Byers Canyon (BC; Breeze Bridge, Parshall Island, Lone Buck, and Paul Gilbert) stocked with H×G fry, at the Sheriff Ranch (SR) fry site (not stocked), and the sites on the Chimney Rock Ranch (CRR; Lower Red Barn, Upper Red Barn, and Hitching Post Bridge) stocked with H×G fry in 2020.

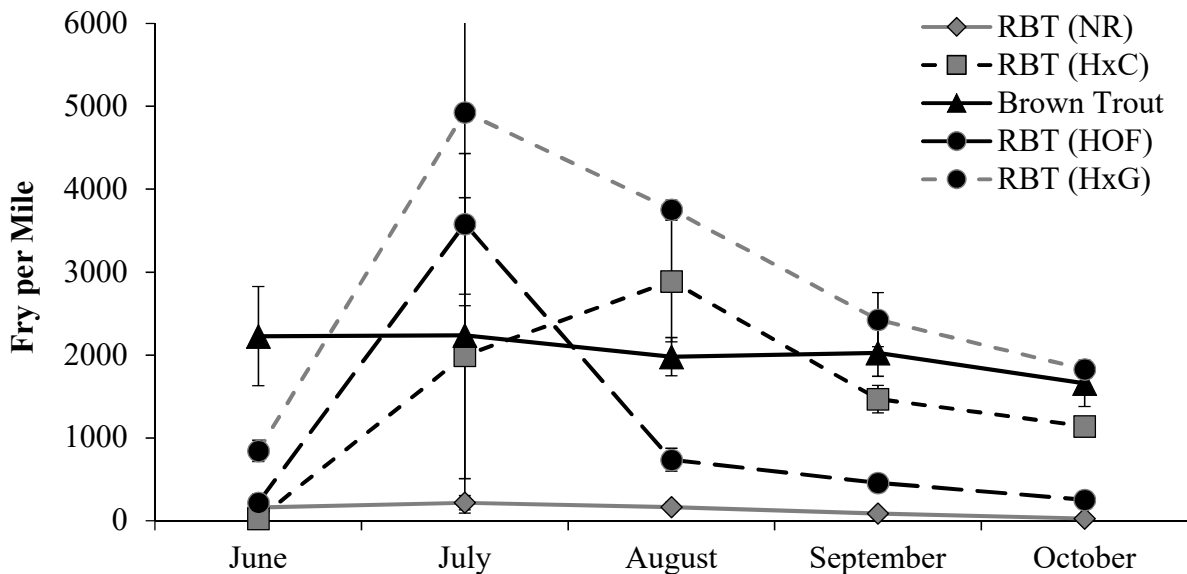


Figure 2.4. Upper Colorado River Brown Trout and wild Rainbow Trout (RBT [NR]) fry abundance estimates averaged between 2013 and 2019, H×C fry (RBT [H×C]) abundance estimates averaged between 2013 and 2015, Hofer fry (RBT [HOF]) abundance estimates averaged between 2016 and 2018, and H×G fry (RBT [H×G]) abundance estimates averaged between 2019 and 2020 (fry per mile; SE bars).

Wild Rainbow Trout fry densities were similar among sites below Byers Canyon, at the Sheriff Ranch, and the sites on the Chimney Rock Ranch in early July (Figure 2.3). Rainbow Trout fry abundance estimates dropped to zero by September in the Sheriff Ranch fry site, the only one of

the eight fry sites not stocked with H×G fry in 2020. Stocked H×G fry were less abundant below Byers Canyon, potentially because of the high Brown Trout fry abundances on the Kemp-Breeze SWA, which likely increased competition in the Parshall Island and Breeze Bridge fry sites. The low Brown Trout fry abundances on the Chimney Rock Ranch may have reduced competition with the stocked H×G fry, resulting in higher Rainbow Trout fry abundance estimates in July and August. This pattern may have continued into September as well, however, Windy Gap Reservoir was again drained in mid-September to allow for survey work, and fine sediment was observed in interstitial spaces at all three sites on the Chimney Rock Ranch during the September sampling occasion. Although Rainbow Trout fry abundances did not change in the Upper Red Barn fry site, abundances were reduced from 5,297 (\pm 143) fry per mile to 2,982 (\pm 172) fry per mile in the Lower Red Barn fry site and 3,989 (\pm 210) fry per mile to 316 (\pm 0) fry per mile in the Hitching Post fry site between the August and September sampling occasions. As a result, Rainbow Trout fry abundance estimates were similar below Byers Canyon and on the Chimney Rock Ranch in September 2020 (Figure 2.3).

H×G fry abundance was higher in 2019-2020 than had been observed for the H×C and GR fry (Figure 2.4). H×G fry stocked in 2019 were available for capture as age-2 fish during the 2021 adult salmonid population estimates, and recruitment rates look promising. Following three years of decline in the adult Rainbow Trout population after stocking GR fry, we observed similar increases in the adult population following H×G fry stocking as was seen following the H×C fry stocking. Although the reduced Brown Trout fry abundances in 2020 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. H×G fry will be stocked again in July 2021, with adult population estimates used to estimate fry recruitment rates continuing into 2022. The ultimate objective of this study is to determine which strain to stock as fry to increase the adult Rainbow Trout abundance in the Upper Colorado River. Given the current results, the H×G appears promising for this use in the future.

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2021 Adult Salmonid Population Estimates

An adult salmonid population estimate was conducted in the 3.9-mile Chimney Rock/Sheriff Ranch study section of the upper Colorado River in May 2021, with the mark run occurring on May 4, and the recapture run occurring on May 6. Two raft-mounted, fixed-boom electrofishing units were used to conduct the population estimates. All adult Brown Trout and Rainbow Trout captured on the mark run were adipose clipped and passive integrated transponder (PIT) tagged as part of the upper Colorado River fish movement study and a mark-recapture assumption study. PIT-tagged fish were measured (mm), weighed (g), and placed in a recovery net pen to check for mortalities from the tagging procedure. Fish in the net pen were released after shocking crews left a work-up station to prevent tagged fish from being recaptured on the mark run. On the recapture run, fish were examined for an adipose fin clip and scanned for a PIT tag if the adipose fin was missing. Additionally, to meet tagged-fish release goals of the fish movement study, all untagged Rainbow Trout were adipose clipped, PIT tagged, measured, and weighed. Otherwise, recaptured fish or new captures were measured only. Population estimates were calculated using the Lincoln-Peterson estimator with a Bailey (1951) modification, which accounted for fish being returned to the population following examination of marks on the recapture run, making them potentially available for subsequent recapture.

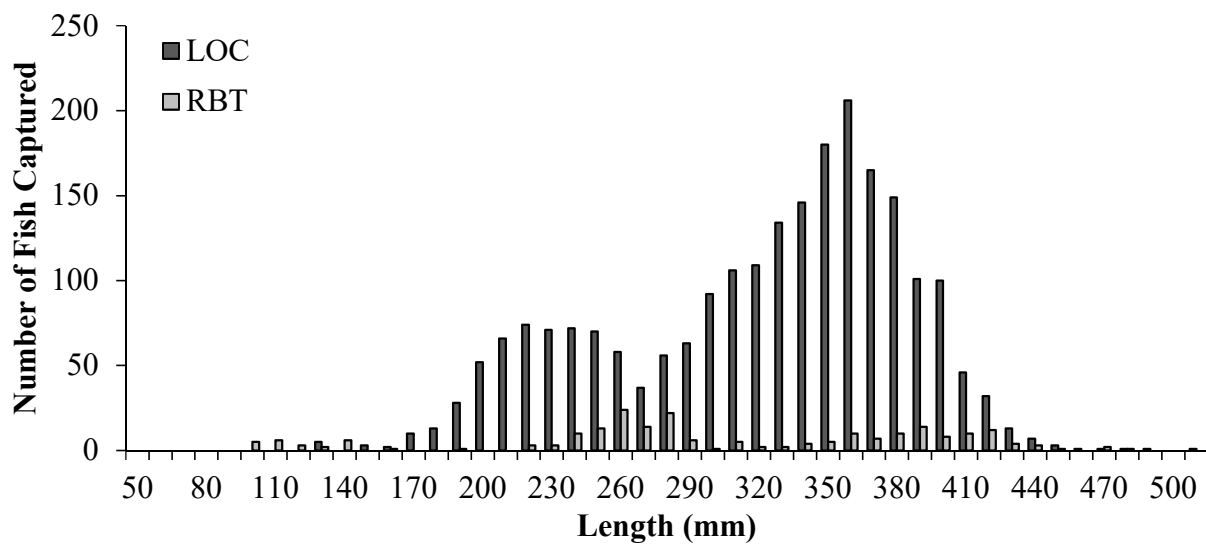


Figure 2.5. Number of Brown Trout (LOC) and Rainbow Trout (RBT) captured by total length (mm) during the 2021 adult salmonid population estimates in the Chimney Rock/Sheriff Ranch study section of the upper Colorado River.

PIT-tagged Brown Trout ($N = 1,132$) ranged in size from 150 to 470 mm total length (TL) and 28 to 1,004 g. An estimated $6,527 (\pm 383)$ adult Brown Trout were present in the Chimney Rock/Sheriff Ranch study section in 2021, approximately 500 less than in 2020 (Fetherman et al. 2020). Overall, $1,673 (\pm 98)$ Brown Trout were present per mile in the study section, averaging $306 (\pm 63)$ mm TL and $325 (\pm 162)$ 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 potential impacts of the reduced Brown Trout fry abundances in 2020 will not be apparent in the adult Brown Trout population until 2022.

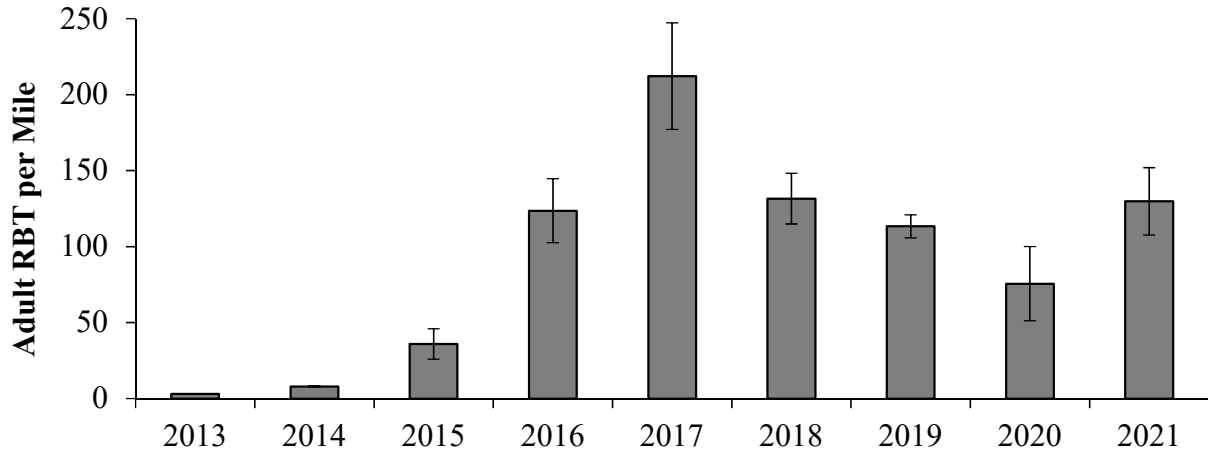


Figure 2.6. Estimated number of adult Rainbow Trout (RBT) per mile (SE bars) in the Chimney Rock/Sheriff Ranch study section of the upper Colorado River between 2013 and 2021.

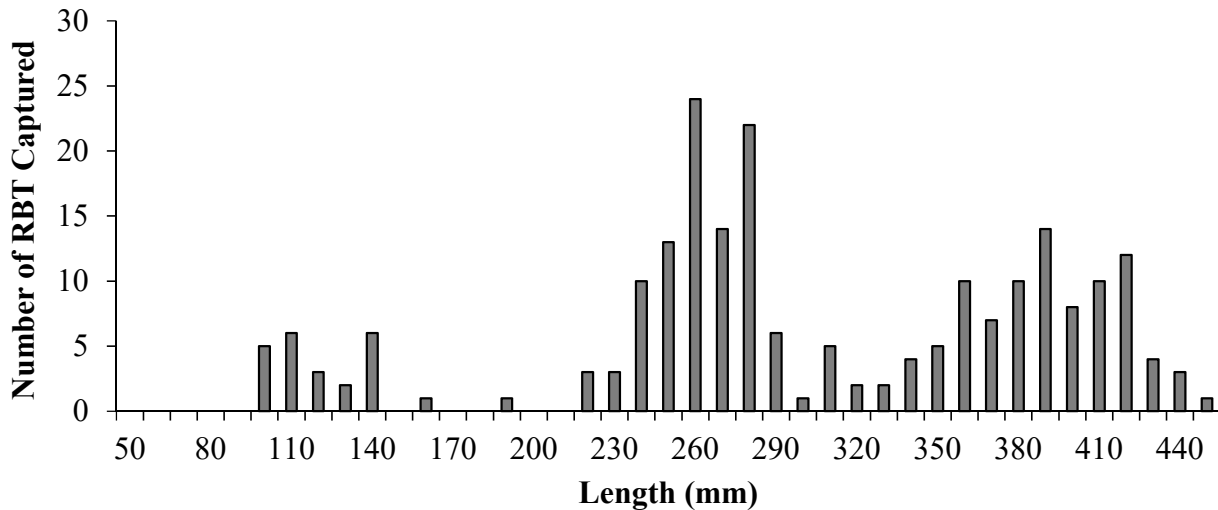


Figure 2.7. Number of Rainbow Trout (RBT) captured by total length (mm) during the 2021 adult salmonid population estimates in the Chimney Rock/Sheriff Ranch study section of the upper Colorado River.

PIT-tagged Rainbow Trout (N = 196) ranged in size from 126 to 591 mm TL and 22 to 1,925 g. Rainbow Trout densities increased between 2020 and 2021, with an estimated 295 (\pm 95) adult Rainbow Trout present in 2020 (Fetherman et al. 2020), and 506 (\pm 87) present in 2021. The Rainbow Trout population had exhibited an increase in abundance between 2013 and 2017, 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. 2020; Figure 2.4), resulting in an estimated 130 (\pm 22) adult Rainbow Trout per mile in 2021 (Figure 2.6), nearly 50 fish per mile higher than in 2020 (Fetherman et al. 2020). Adult Rainbow Trout averaged 303 (\pm 80) mm TL and 350 (\pm 273) g, smaller than in 2020, potentially due to the large numbers of age-1 and age-2 fish (Figure 2.7). Although not recruited to the gear used, a number of age-1 Rainbow Trout were captured during the population

estimates (Figure 2.8), suggesting that H×G fry stocked in 2020 had survived well. Similarly, a large number of age-2 fish were captured, higher than the age-2 population in any year following GR fry stocking, and similar to that observed following H×C fry stocking. The number of age-3+ Rainbow Trout was similar to that observed in 2020 (Figure 2.8), and is suspected to be made up largely of surviving GR fish as H×Gs were not old enough to recruit and it has been five years since H×C fry have been stocked, although a few older H×Cs may remain in the population. Given the apparent survival rates of the H×G, the adult Rainbow Trout population is expected to continue to increase into 2022.

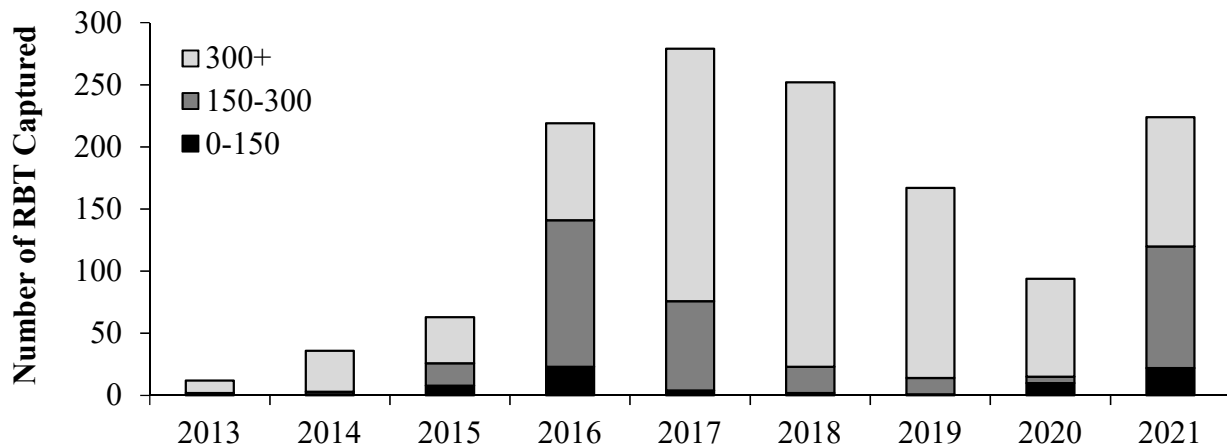


Figure 2.8. Number of age-1 (≤ 150 mm TL), age-2 (150-300 mm TL) and age-3+ (> 300 mm TL) Rainbow Trout (RBT) captured in the Chimney Rock/Sheriff Ranch study section of the upper Colorado River between 2013 and 2021.

The adult Rainbow Trout population in the upper Colorado River exhibited an increase in abundance for the first time since 2017. This is largely a result of the higher numbers of age-2 H×G Rainbow Trout captured in 2021 compared to previous years. The H×G fry exhibited high survival rates in their first year in the river (Fetherman et al. 2020). Although small fish are usually not targeted by raft electrofishing gear, several age-1 H×G were collected during the 2021 population estimates, suggesting H×G fry stocked in 2020 were surviving and recruiting. Additionally, the large age-2 size class indicates H×G survival remains high beyond the fry life stage. If the majority of the fish in the age-2 and age-3+ size classes persist into 2022, and a similar or larger age-2 size class is observed, the Rainbow Trout abundance in this section of the Colorado River could be higher in 2022 than in any year since resistant Rainbow Trout stocking began in 2008. The reduced Brown Trout fry abundance may have contributed to increased survival of H×G fry in 2020. Additionally, as these fish recruit to the juvenile and adult population, this reduced 2020 class of Brown Trout may result in less competition for resources and higher survival for the H×G in future years.

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

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.

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.

Stationary Antenna Installations

Stationary antennas used to detect the movements of passive integrated transponder (PIT)-tagged fish were installed at three sites in the Colorado River: 1) immediately downstream of the confluence of the Fraser and Colorado rivers above Windy Gap Reservoir on Northern Water property (hereafter, Confluence), 2) just downstream of the Hitching Post (CR 57) bridge on the Chimney Rock Ranch (hereafter, Hitching Post), and 3) in the Red Barn area of the Chimney Rock Ranch upstream of the Red Barn diversion structure (hereafter, Red Barn). Antennas and reader stations were constructed onshore the week prior to installation (Figure 2.9). Antennas consist of 6 AWG (Confluence and Red Barn) or 1/0 (Hitching Post) welding cable run through 2-inch schedule 80 PVC pipe to prevent water contact from affecting antenna read range or wire corrosion and keep debris from catching on and breaking the wire. Cross members were installed every ten feet along the 90-130 foot long by 2-foot wide antennas to reinforce the frame and maintain shape. Onshore, antenna wire was run through conduit to the reader station for weatherproofing and protection from animals. Reader stations consist of a constructed platform housing three solar panels for charging the batteries used to power the readers and antennas, a solar panel charge controller, and a job box containing the battery bank, Oregon RFID ORSR single readers (one per antenna), and electronic equipment needed to download tag detection data from the readers. A GNSS GPS connection is used to update and maintain reader time, date, and location. A standalone unit houses the antenna tuner boxes that are used to adjust antenna tuning for optimal read range given antenna inductance, flow, temperature, reader power, and environmental conditions (Figure 2.9). A pressure transducer was installed in the river just upstream of each station, which allows researchers to correspond fish movements to water flows and temperature. A cattle fence was also constructed around Red Barn to prevent cattle from breaking the equipment (Figure 2.10).

Two antennas, used to determine directionality of movement, were installed at each of the three stations the week of August 3-7, 2020 (Figure 2.10). A crew of 25 people was used to complete the antenna installations. Antennas were placed in shallow runs to maximize read range relative to water depth. Prior to installation, crews cut trenches in the substrate in which the antennas would sit to hold them in line with versus above the substrate. After preparing the substrate, crewmembers positioned themselves at the antenna cross members along the pre-constructed antennas onshore. Working together, crews picked up the antennas, navigated onshore obstacles, and walked the antennas into the river, laying them into the trenches. Once the antennas were

settled into the substrate, crewmembers stood on the antennas to keep them from floating since the PVC was watertight and filled with air. Smaller crews of three to four people each used gas-powered t-post pounders to sink DB-40 duckbill earth anchors into the substrate adjacent to the antenna cross members upstream and downstream of the antenna. Nine-foot river straps were threaded through the wire loops of the anchors sticking up just above the substrate, and tightened down over the antenna cross members to hold the antenna tight to the substrate. In areas of higher velocity and sheer stress, ratchet straps were also used to ratchet the antenna down to the substrate (Figure 2.10).



Figure 2.9. Antennas were constructed onshore prior to installation (A), as were the reader stations containing solar panels, (B) the battery bank powering the Oregon RFID ORSR readers (C), a charge controller (D), and a standalone housing unit for the antenna tuner boxes (B and E).

Onshore, crews dug trenches for the conduit containing the antenna wire to the tuner box housing and containing the twin axial cable from the tuner box housing to the job boxes housing the readers. The conduit was buried to prevent damage to the wires. An inductance reading was taken from each antenna to ensure it was within the range to allow tuning ($< 120 \mu\text{H}$). Inductances ranged between 60 and $80 \mu\text{H}$. After connecting all wires, antennas were tuned using Oregon RFID autotuners (Red Barn, Hitching Post, and one antenna at Confluence) or a manual tuner (one antenna at Confluence). Read range for a 32 mm PIT tag was greater than one foot at all sites. After ensuring that all components were operational, antenna stations were shut down until one week prior to releasing tagged fish in the Fraser River on September 1, 2020, at which time the readers were turned back on and checked to make sure tags were being detected and recorded correctly.



Figure 2.10. Antenna crews navigated the long, pre-made antennas through onshore obstacles (A), walked the antennas into the river (B), and placed them in the trenches dug in the substrate (C). Duckbill anchors were installed, and river and ratchet straps were used to hold the antennas in place (D). A cattle fence was also constructed around Red Barn to prevent damage (E). Two antennas were used to determine directionality of movement at each site (F).

Antenna detection distances were measured in fall 2020 and spring 2021 to determine if vertical detection distances exceeded average water depth and if detection distance fluctuated with flow and environmental conditions. Ice precluded the measurement of detection distances during the winter. 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 PVC 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.

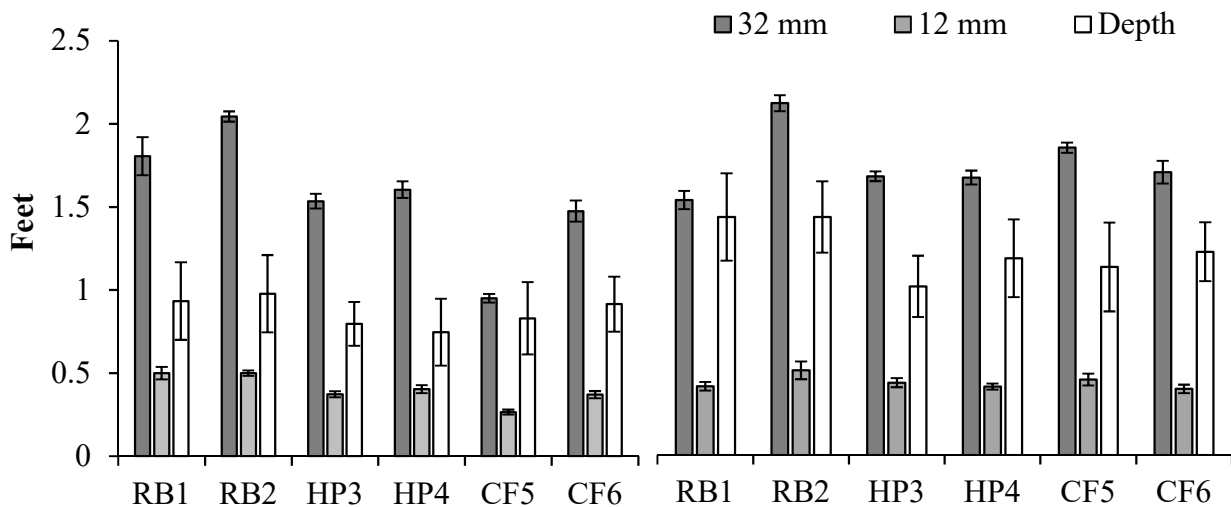


Figure 2.11. Detection distances for 32 mm and 12 mm PIT tags and water depths (feet; 2 SE bars) for the two antennas located at Red Barn (RB), Hitching Post (HP), and Confluence (CF) in fall 2020 (left panel) and spring 2021 (right panel).

Velocities in fall 2020 averaged 0.21 ± 0.15 , 0.19 ± 0.17 , and 0.34 ± 0.15 m/s at the Red Barn, Hitching Post, and Confluence sites, respectively. In spring 2021, velocities averaged 0.48 ± 0.15 , 0.42 ± 0.19 , and 0.49 ± 0.12 m/s at the three sites. 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 2020 ranged between an average of 0.95 and 2.04 feet, and for a 12 mm PIT tag between 0.27 and 0.5 feet. In spring 2021, detection distances for a 32 mm PIT tag ranged between 1.51 and 2.08 feet, and for a 12 mm PIT tag between 0.39 and 0.5 feet. Detection distances were similar among seasons at all antennas with the exception of CF5, at which detection distance for a 32 mm PIT tag increased from 0.95 to 1.81 feet between fall 2020 and spring 2021, likely due to retuning of the antenna between seasons. Within both seasons, detection distance for the 32 mm PIT tags was similar to or significantly exceeded the average water depth at each antenna (Figure 2.11), suggesting full coverage of the water column. Read ranges for the 12 mm PIT tags were always significantly

lower than the water depth (Figure 2.11). However, with the exception of a few smaller salmonids, 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.

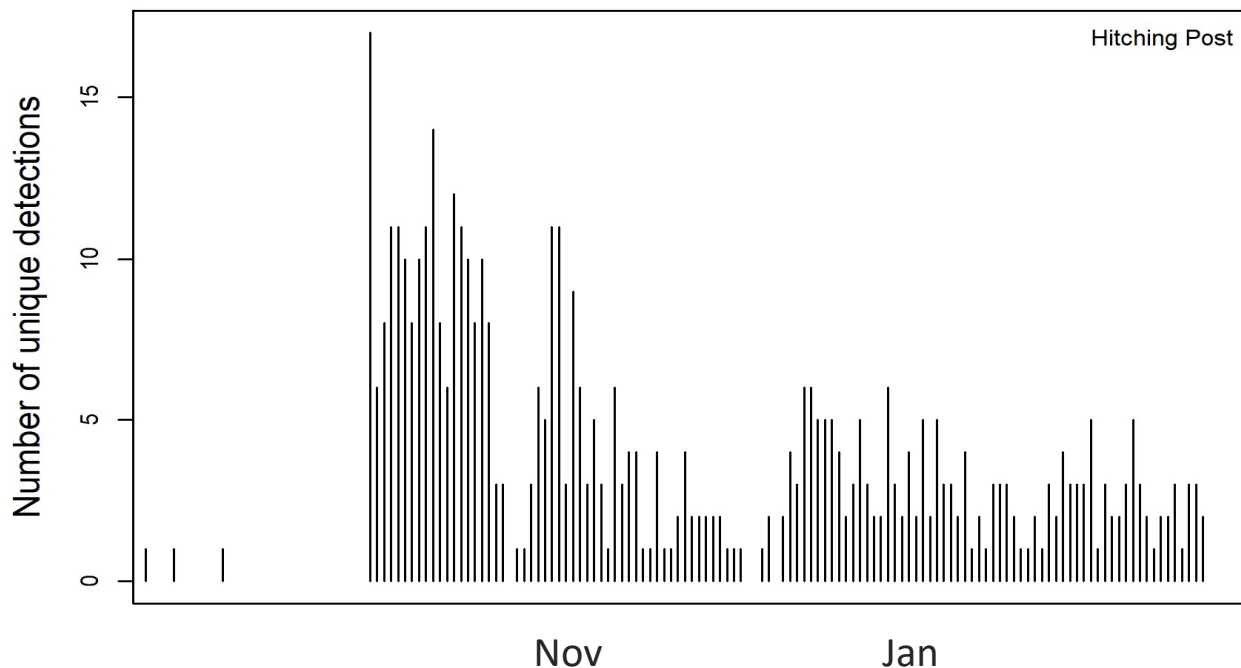


Figure 2.12. Number of unique PIT-tagged Brown Trout detected per day at Hitching Post between September 2020 and February 2021.

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 2.12). Data organization and analysis will continue throughout the study as time allows. Preliminary data regarding distance moved are presented later in this report.

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.

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 September 1 and 2, and in two stations on the City of Granby property at River Run in the Colorado River on October 5, 2020. A bank electrofishing unit was used to complete the surveys in each location. Fish were held in separate net pens by pass until being measured (mm) and weighed (g), and a large portion were tagged with a 32 or 12 mm tag, dependent upon size. Fish were anesthetized prior to tagging using AQUI-S 20E, a clove oil derivative, administered with permission and oversight from the US Fish and Wildlife Service Investigational New Animal Drug (INAD) program and CPW aquatic veterinarian Colby Wells (study numbers 11-741-20-303F and 11-741-20-333F). 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). Additionally, the right pelvic fin was removed from all fish tagged in the Fraser River and left pelvic fin from all fish tagged in the Colorado River to determine river of origin in the event of tag loss. 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.

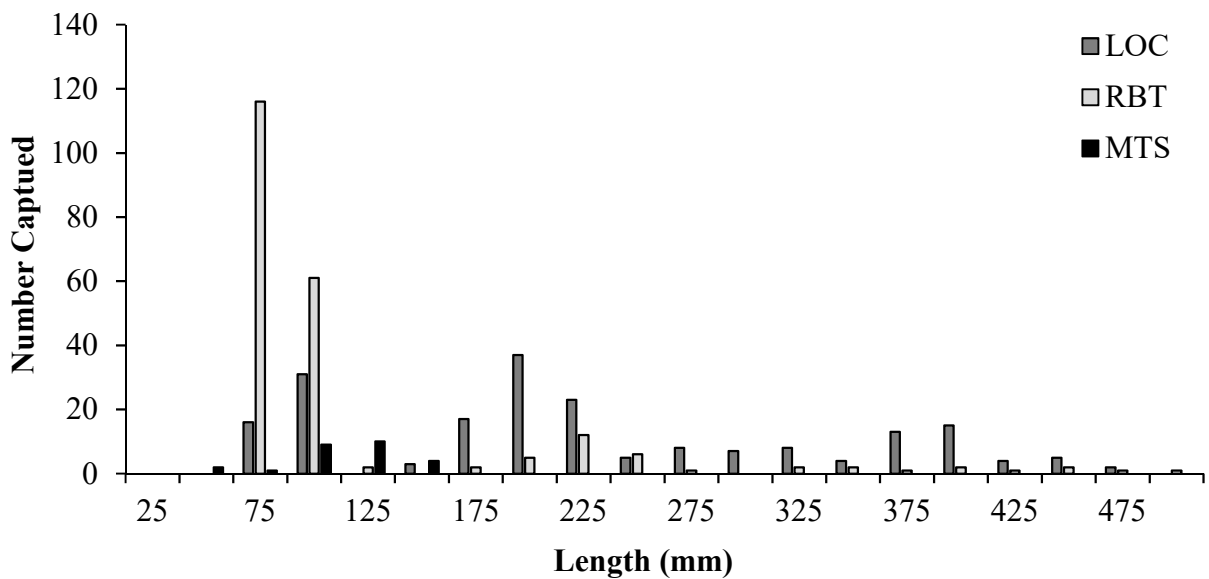


Figure 2.13. Number of Brown Trout (LOC), Rainbow Trout (RBT), and Mottled Sculpin (MTS) captured by total length (mm) during the Fraser River Ranch 2020 population estimate.

The 677-foot station located about 0.25 miles upstream of the railroad crossing at the lower end of the Fraser River Ranch contained 198 Brown Trout, 148 adults (≥ 150 mm total length [TL]) and 50 fry/juveniles (< 150 mm TL; Figure 2.13). An estimated $1,566 \pm 16$ Brown Trout per mile were present on the ranch, $1,163 \pm 10$ adult Brown Trout per mile and 409 ± 20 Brown Trout fry/juveniles per mile. Brown Trout averaged 261 ± 7 mm TL and 251 ± 20 g, with the

largest measuring 457 mm TL and weighing 1,016 g. Rainbow Trout outnumbered Brown Trout in the total catch, with 217 Rainbow Trout captured, although only 38 of these were adults whereas the other 179 were fry/juveniles (Figure 2.13). An estimated $1,868 \pm 76$ Rainbow Trout per mile were present on the ranch, 296 ± 2 adult Rainbow Trout per mile and $1,642 \pm 108$ Rainbow Trout fry/juveniles per mile. Rainbow Trout averaged 270 ± 15 mm TL and 282 ± 47 g, with the largest measuring 485 mm TL and weighing 1,072 g. Twenty-six Mottled Sculpin were captured in the site, and although the number was relatively low, there were only four fewer Mottled Sculpin captured on the second pass relative to the first, providing an estimate of 438 ± 10 Mottled Sculpin per mile. Mottled Sculpin averaged 103 ± 5 mm TL and 18 ± 2 g. Longnose Sucker (estimated 412 ± 139 per mile), Creek Chub (16 ± 0 per mile), White Sucker (55 ± 0 per mile), Speckled Dace (210 ± 29 per mile), and Iowa Darter (16 ± 0 per mile) were also captured on the Fraser River Ranch.

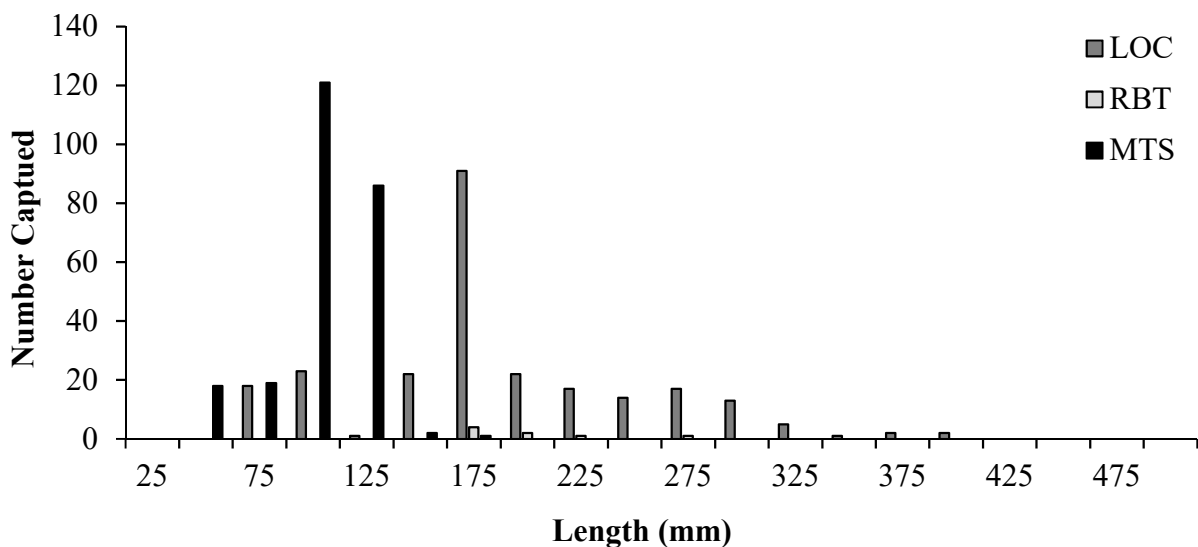


Figure 2.14. Number of Brown Trout (LOC), Rainbow Trout (RBT), and Mottled Sculpin (MTS) captured by total length (mm) during the Kaibab Park 2020 population estimate.

A total of 248 Brown Trout, 186 adults and 62 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 2.14). Kaibab Park contained an estimated $2,093 \pm 30$ Brown Trout per mile, $1,565 \pm 24$ adult Brown Trout per mile and 529 ± 19 Brown Trout fry/juveniles per mile. Brown Trout averaged 196 ± 4 mm TL and 97 ± 7 g, with the largest measuring 384 mm TL and weighing 576 g. Many fewer Rainbow Trout were captured in Kaibab Park than on the Fraser River Ranch, with only eight age-2 Rainbow Trout captured (Figure 2.14), for an estimated 102 ± 87 adult Rainbow Trout per mile. Rainbow Trout averaged 187 ± 12 mm TL and 72 ± 14 g, with the largest measuring 257 mm TL and weighing 156 g. In contrast to the Fraser River Ranch, 247 Mottled Sculpin were captured in Kaibab Park, providing an estimate of $2,261 \pm 90$ Mottled Sculpin per mile. Mottled Sculpin averaged 90 ± 1 mm TL and 13 ± 1 g. Longnose Sucker (estimated $1,504 \pm 89$ per mile), Creek Chub (131 ± 260 per mile), White Sucker (154 ± 22 per mile), Speckled Dace (210 ± 37 per mile), and Fathead Minnow (33 ± 0 per mile) 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 669 Brown Trout, 346 adults and 323 fry/juveniles (Figure 2.15). An estimated $4,135 \pm 284$ Brown Trout per mile were present in River Run, $1,870 \pm 101$ adult Brown Trout per mile and $2,831 \pm 875$ Brown Trout fry/juveniles per mile. Brown Trout averaged 244 ± 5 mm TL and 220 ± 15 g, with the largest measuring 506 mm TL and weighing 1,475 g. Only 18 Rainbow Trout were captured, nine adults and nine fry/juveniles (Figure 2.15). An estimated 101 ± 33 adult Rainbow Trout per mile were present in River Run, 44 ± 4 adult Rainbow Trout per mile and 78 ± 133 fry/juvenile Rainbow Trout per mile. Rainbow Trout averaged 398 ± 43 mm TL and 858 ± 299 g, with the largest measuring 656 mm TL and weighing 3,383 g. Eighty-one Mottled Sculpin were captured in the two sites, providing an estimate of $555 \pm 1,053$ Mottled Sculpin per mile in River Run. Mottled Sculpin averaged 117 ± 1 mm TL and 25 ± 1 g. Longnose Sucker (estimated 41 ± 13 per mile), White Sucker (148 ± 278 per mile), Speckled Dace (4 ± 7 per mile), Fathead Minnow (86 ± 321 per mile), and Iowa Darter (11 ± 0 per mile) were also captured in River Run.

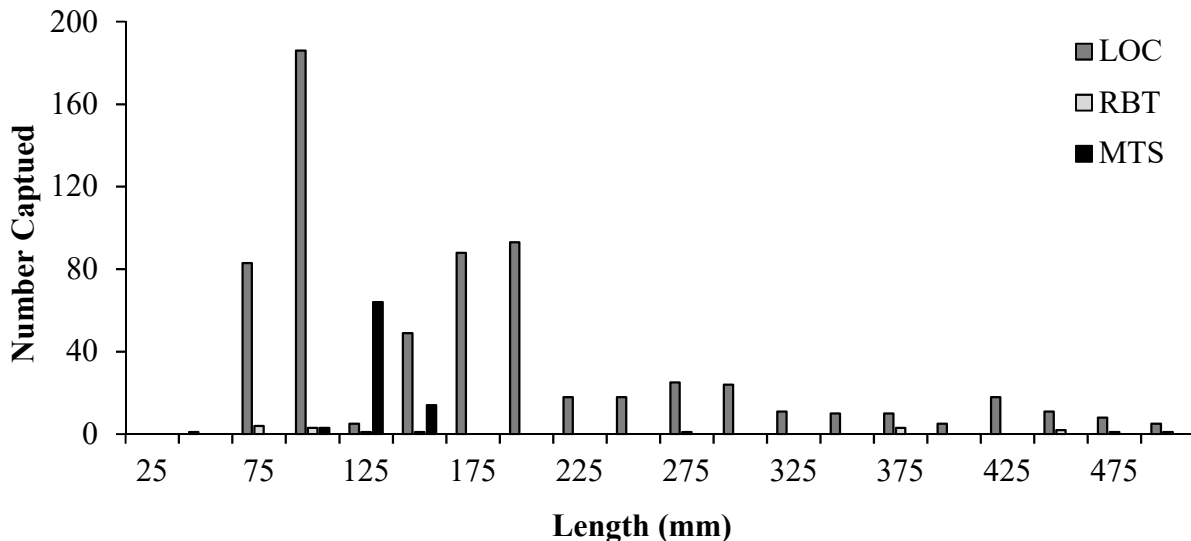


Figure 2.15. Number of Brown Trout (LOC), Rainbow Trout (RBT), and Mottled Sculpin (MTS) captured by total length (mm) during the 2020 population estimates in River Run.

There were more adult Brown Trout per mile on average in the Colorado River than in the Fraser River (Figure 2.16). Brown Trout adults were larger in the Fraser River Ranch and River Run than in Kaibab Park, which contained mostly age-2 Brown Trout. Adult Rainbow Trout estimates were highest in the Fraser River Ranch, although they did not differ from Kaibab Park due to a poor depletion during that estimate. Kaibab Park supported large numbers of Mottled Sculpin compared to the other two sections, though the error for the estimates in both Fraser River Ranch and River Run was large due to poor depletion (Figure 2.16). Brown Trout spawn in River Run, which, in addition to adults, produced the highest estimate of Brown Trout fry/juveniles per mile compared to the other two sites. The Fraser River Ranch proved to be good nursery habitat for fry/juvenile Rainbow Trout (Figure 2.16) and is likely the primary source of recruitment for Rainbow Trout in the Fraser River, and possibly the Colorado River.

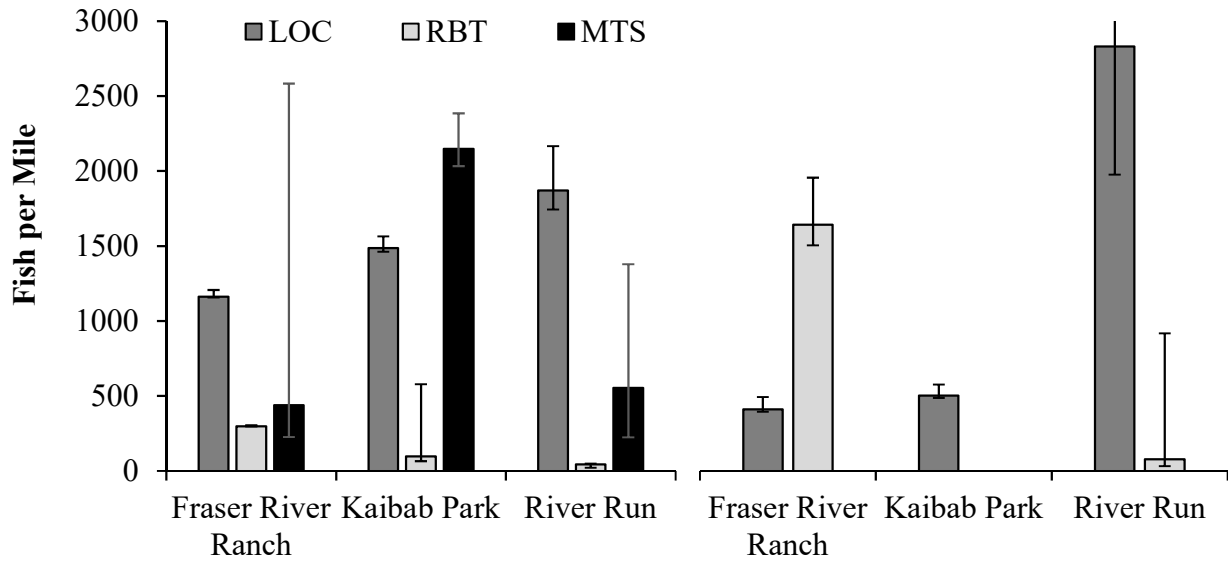


Figure 2.16. Comparison of estimates of adult Brown Trout (LOC), Rainbow Trout (RBT), and Mottled Sculpin (MTS) per mile (left panel), and Brown Trout and Rainbow Trout fry per mile (right panel), in the Fraser River Ranch, Kaibab Park, and River Run sections of the Fraser and Colorado rivers in fall 2020.

A total of 178 fish were PIT tagged in the Fraser River Ranch, 105 Brown Trout, 50 Rainbow Trout, and 23 Mottled Sculpin. Mottled Sculpin (85 to 133 mm TL) were tagged with a 12 mm PIT tag, as were 11 Brown Trout (77 to 98 mm TL) and 12 Rainbow Trout (72 to 102 mm TL). The remaining 94 Brown Trout (138 to 457 mm TL) and 38 Rainbow Trout (165 to 485 mm TL) were tagged with 32 mm PIT tags. Given the estimated number of fish per mile and length of the Fraser River Ranch, an estimated 5.4%, 8.5%, and 3.5% of the Brown Trout, Rainbow Trout, and Mottled Sculpin populations were tagged on the ranch, respectively. Only three mortalities were removed from the nets prior to release, a short-term survival rate from the tagging procedure of 98.3%.

Three hundred forty-five fish were PIT tagged in Kaibab Park, 177 Brown Trout, 7 Rainbow Trout, and 161 Mottled Sculpin. Mottled Sculpin (71 to 131 mm TL) were tagged with a 12 mm PIT tag, as were two Brown Trout (85 to 86 mm TL). The remaining 175 Brown Trout (135 to 384 mm TL) and all seven Rainbow Trout (151 to 257 mm TL) were tagged with 32 mm PIT tags. Given the estimated number of fish per mile and length of Kaibab Park, an estimated 56%, 35%, and 36% of the Brown Trout, Rainbow Trout, and Mottled Sculpin populations were tagged, respectively. Only four mortalities were removed from the nets prior to release, a short-term survival rate of 98.8%.

A total of 357 fish were PIT tagged in River Run, 266 Brown Trout, 12 Rainbow Trout, and 79 Mottled Sculpin. Mottled Sculpin (82 to 133 mm TL) were tagged with a 12 mm PIT tag, as were 10 Brown Trout (79 to 104 mm TL) and three Rainbow Trout (72 to 103 mm TL). The remaining 256 Brown Trout (153 to 506 mm TL) and nine Rainbow Trout (257 to 656 mm TL) were tagged with 32 mm PIT tags. Given the estimated number of fish per mile and length of River Run, an estimated 11.4%, 17.1%, and 11.9% of the Brown Trout, Rainbow Trout, and

Mottled Sculpin populations were tagged in the Colorado River, respectively. The short-term survival rate from the tagging procedure was 99.7%.

In addition to the Fraser River Ranch, Kaibab Park, and River Run population estimates, a Mottled Sculpin population estimate was conducted by CPW researcher Dan Kowalski between the Confluence antenna station and Windy Gap Reservoir as part of the Upper Colorado River Monitoring Program on September 21, 2020. Thirty-seven Mottled Sculpin (78 to 142 mm TL) were tagged following the estimate and released in a riffle ~100 feet downstream of Confluence. The majority of the Mottled Sculpin detected at Confluence between September 21 and October 21, 2021 were from this release event.

In summary, 917 fish were PIT tagged in the Fraser and Colorado rivers above Windy Gap Reservoir in 2020. The goal had been to release a minimum of 250 tagged fish of each species. We exceeded this goal for Brown Trout and Mottled Sculpin, releasing 548 tagged Brown Trout and 300 tagged Mottled Sculpin. However, only 69 Rainbow Trout were tagged above the reservoir in 2020. If we are to meet our goal in future years, additional electrofishing efforts may be needed to find Rainbow Trout outside of our population estimate sites in both rivers.

Tagged fish were also released at six sites in the Colorado River below Windy Gap Reservoir in 2020. The six sites included immediately below Windy Gap Reservoir, Hitching Post Bridge above the Hitching Post antenna station, above the Red Barn antenna station, below the Red Barn diversion, Kinney Creek, and Sheriff Ranch. 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 remainder of the five sites were used to distribute fish throughout the study section above, between, and below the antenna stations. A barge-mounted electrofishing unit was used to capture a minimum of 80 fish at each site for tagging. The same tagging protocols were followed. Fish were anesthetized in an AQUI-S bath, measured, weighed, and tagged. The adipose fin was removed from all tagged fish to differentiate fish tagged below the reservoir from those tagged above when the connectivity channel is complete and movement around the reservoir occurs. 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.

Table 2.1. Number of Brown Trout (LOC), Rainbow Trout (RBT), and Mottled Sculpin (MTS) tagged with 12 or 32 mm PIT tags at each of six sites in the Colorado River below Windy Gap Reservoir in October 2020. NA indicates that a species was not captured or tag size not used.

Site	LOC		RBT		MTS	Total
	32 mm	12 mm	32 mm	12 mm	12 mm	
Below Windy Gap	81	NA	13	2	NA	96
Hitching Post	62	NA	31	1	NA	94
Above Red Barn Antenna	83	NA	20	22	NA	125
Below Red Barn Diversion	88	NA	6	18	NA	112
Kinney Creek	82	NA	5	3	1	91
Sheriff Ranch	85	1	7	4	NA	97
<i>Total</i>	<i>481</i>	<i>1</i>	<i>82</i>	<i>50</i>	<i>1</i>	<i>615</i>

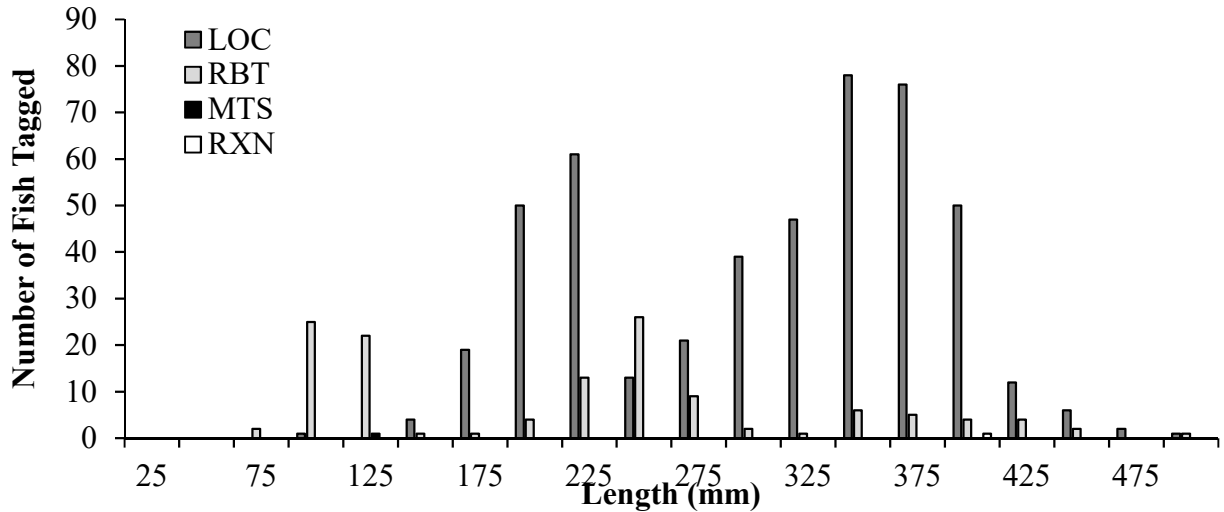


Figure 2.17. Number of Brown Trout (LOC), Rainbow Trout (RBT), Mottled Sculpin (MTS), and cutbows (RXN) PIT tagged by total length (mm) in the Colorado River below Windy Gap Reservoir in October 2020.

The number of fish PIT tagged within a site ranged between 90 and 112, with 615 fish tagged in the Colorado River below Windy Gap Reservoir in October 2020 (Table 2.1). Short-term survival from the tagging procedure was 99.8%. Brown Trout ranged from 91 to 630 mm TL, with the majority being age-2 or age-3+, although one age-0 fish was tagged with a 12 mm PIT tag at the Sheriff Ranch site (Figure 2.17). PIT tagged Rainbow Trout ranged in size from 68 to 547 mm TL, with the majority of fish tagged between 75 and 275 mm TL, although several larger fish were tagged as well (Figure 2.17). All of the Rainbow Trout tagged with 12 mm PIT tags were suspected to be H×Gs stocked as fry in July 2020. In addition to Brown Trout and Rainbow Trout, one cutbow (409 mm TL, 620 g) was tagged below Windy Gap. Surprisingly, a Mottled Sculpin (140 mm TL, 44 g) was captured and tagged at Kinney Creek. It is not known whether this fish resides in the Colorado River or moved down into the river from Kinney Creek. Regardless, this fish will provide additional movement data for a rarely observed species downstream of the reservoir if detected by stationary or portable antennas during future surveys. The goal was to tag 250 Brown Trout and Rainbow Trout below the reservoir. We met this goal with the Brown Trout, but not the Rainbow Trout despite tagging a large number of smaller fish. It is unlikely that this goal will be met in future years until the Rainbow Trout population has recovered and become reestablished in this section of the Colorado River.

Brown Trout and Rainbow Trout were also PIT tagged during the spring 2021 adult population estimates conducted in the Chimney Rock/Sheriff Ranch study section of the Colorado River below Windy Gap Reservoir. All adult fish captured during the mark run were tagged with a 32 mm PIT tag. Untagged Rainbow Trout captured on the recapture run were also tagged with a 32 mm PIT tag. All fish captured on the mark run that had been previously tagged in the section in October 2020 but had lost their tags, as indicated by an adipose clip but no PIT tag detected when scanned, were given a new tag. However, on the recapture run, fish that had lost their tags were returned to the river without being retagged. All fish were measured and weighed to maintain similar release records for tagged fish as those obtained during previous tagging efforts.

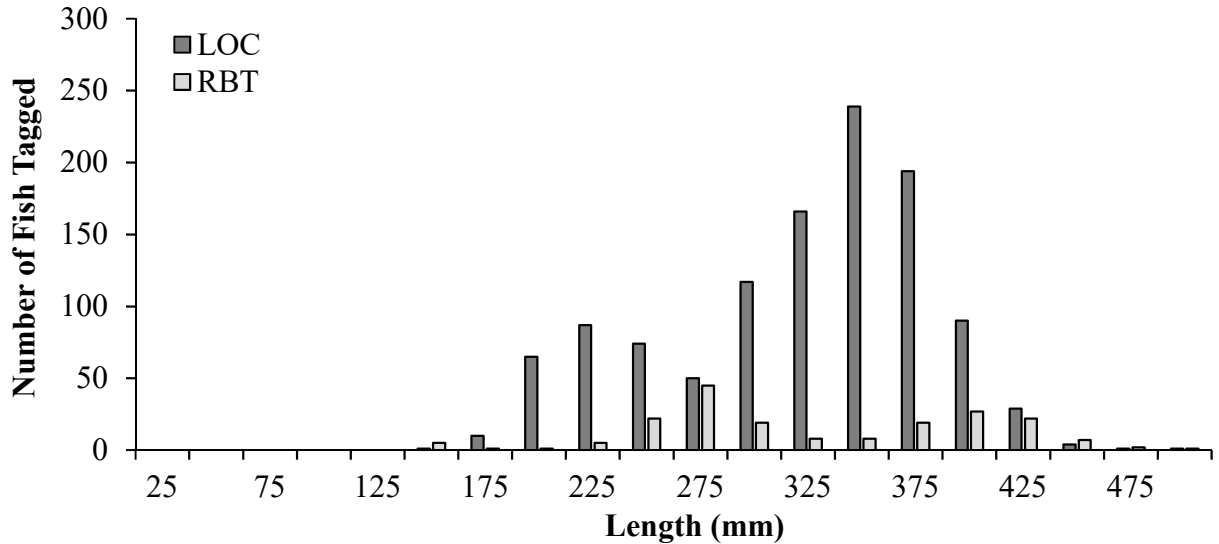


Figure 2.18. Number of Brown Trout (LOC) and Rainbow Trout (RBT) PIT tagged by total length (mm) in the Colorado River below Windy Gap Reservoir in May 2021.

A total of 1,325 fish were PIT tagged in the Chimney Rock/Sheriff Ranch study section of the Colorado River in May 2021, 1,129 Brown Trout (150 to 497 mm TL) and 196 Rainbow Trout (126 to 591 mm TL; Figure 2.18). Five Brown Trout and three Rainbow Trout lost their tags between the mark and recapture runs, providing a short-term tag retention rate of 97.9% for Brown Trout and 87% for Rainbow Trout. Fourteen of the tagged fish had been previously tagged in this section in October 2020 and were retagged during the mark run, 13 Brown Trout and one Rainbow Trout. An additional 20 Brown Trout and one Rainbow Trout with lost tags were caught on the recapture run. Given the estimated abundance for each species and the length of the study section, 17.3% of the Brown Trout population and 38.7% of the Rainbow Trout population was tagged in the Chimney Rock/Sheriff Ranch study section during this effort.

Recaptures of previously tagged fish provided our first evaluation of growth and long-term tag retention of Brown Trout and Rainbow Trout for the fish movement study. On average, Brown Trout grew 6 ± 4 mm and gained 21 ± 41 g since their release in October 2020. Two fish lost weight, with one losing 98 g between October and May 2021, which may represent a loss of egg weight if the fish was a female tagged prior to spawning. Rainbow Trout grew an average of 8 ± 8 mm and gained 49 ± 53 g between October 2020 and May 2021. In total, 62 Brown Trout and 10 Rainbow Trout with missing adipose fins were captured during the estimates, representing a 10% and 5% recapture rate of the fish released in October 2020. Of those, 33 Brown Trout and two Rainbow Trout had lost their tags, providing a long-term tag retention rate of 46.8% for Brown Trout and 80% for Rainbow Trout. Tag loss appears to be an issue with Brown Trout in the Colorado River. We suspect that because these fish were tagged immediately prior to spawning, with several tagged fish being mature, ripe females, that the tags were spawned out within a few weeks of the fish being tagged. In contrast, Brown Trout in the Fraser River Ranch and Kaibab Park were tagged 1.5 months prior to spawning, and Brown Trout tagged in the Chimney Rock/Sheriff Ranch study section in May 2021 were tagged five months prior to spawning. Increasing the time period between tagging and spawning could help with retention

by allowing time for the tag to be encapsulated and no longer loose within the body cavity by the time spawning occurs. This hypothesis will be examined further during future population estimates and tagging efforts.

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.

Prentice, E. F., T. A. Flagg, C. S. McCutcheon, D. F. Brastow, and D. C. Cross. 1990. Equipment, methods, and an automated data-entry station for PIT tagging. Pages 335-340 in N. C. Parker, A. E. Giorgi, R. C. Heidinger, D. B. Jester, Jr., E. D. Prince, and G. A. Winans, editors. *Fish marking techniques*. American Fisheries Society Symposium 7, Bethesda, Maryland.

Portable Antenna Deployments

Portable antennas are being deployed as part of the fish movement study to supplement movement and detection data obtained from the stationary antennas. Data obtained from portable 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 using the portable 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 with the portable 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 2.19. (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.

Portable antenna design improved upon previous portable antennas developed and evaluated by CPW researchers (Fetherman et al. 2014; Richer et al. 2017). Antenna wire is encased in PVC and placed in the self-bailing channel of a 14-foot NRS raft to maintain rigidity, shape, and tuning for optimal read range during deployment (Figure 2.19). Using rafts, compared to previous designs requiring walking deployment (Richer et al. 2017), allows the antennas to cover greater distances in a shorter period of time. The antenna wire is connected to a tuner box, tuned for optimal read range given antenna inductance and shape, which in turn is connected to an Oregon RFID ORSR single reader. The reader and antenna are powered by two batteries, which, along with the reader, tuner box, and a Campbell Scientific datalogger, are housed in waterproof Pelican case. The Campbell Scientific datalogger merges the tag data from the reader and location data from an external GPS unit mounted on a pole (Figure 2.19). Two crewmembers deploy each antenna, one rower, and one crewmember that helps maneuver the raft through shallow riffles and over obstacles when deployed during low flows.

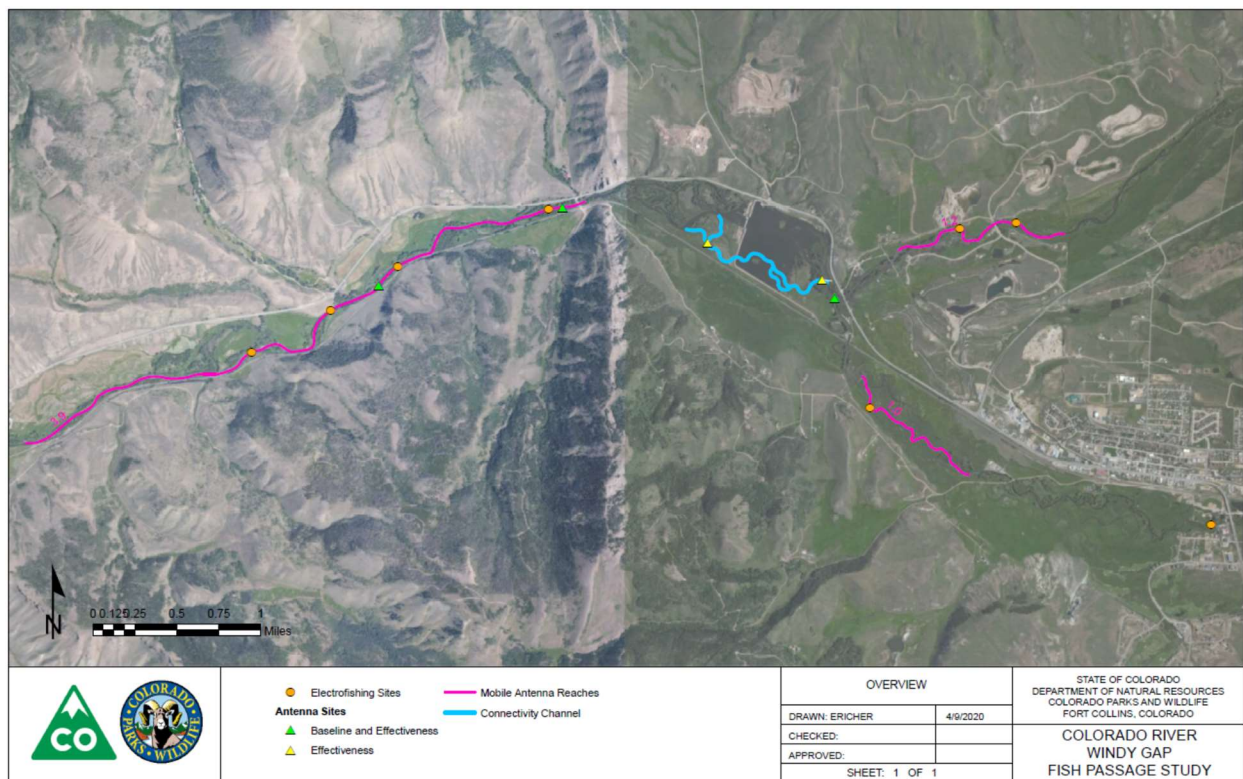


Figure 2.20. Portable antenna deployment reaches in the Colorado and Fraser rivers above Windy Gap Reservoir and the Colorado River below Windy Gap Reservoir (red lines). The blue line represents a future portable antenna deployment reach following completion of the Colorado River Connectivity Channel.

Portable antennas were deployed in three reaches in October 2020: 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 (3.9 miles; Figure 2.20). Both rafts are 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 remain about 100 yards apart during deployment to prevent reader interference. Portable antennas were deployed in the River Run reach on October 19, 2020. The starting location for deployment was located on the Miller Ranch just upstream of River Run, and the reach included portions of the Colorado River through the Miller Ranch, River Run, 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. Portable antennas were deployed in the Fraser River Ranch reach on October 20, 2020. The Fraser River splits just downstream of the starting location at the upstream-most access on the Fraser River Ranch. The north channel was run to avoid beaver dams and a water diversion structure located on the south channel. The rafts were pulled out upstream of the Fraser River gauge. Two passes were completed in the same day in both reaches. Rafts were deployed from the Hitching Post Bridge downstream to the Sheriff Ranch on October 21, 2020. Only one pass with both rafts was completed in the Chimney Rock Ranch reach due to the East Troublesome Fire preventing river access for the second pass on October 22, 2020.

Table 2.2. Number of tagged fish released at 11 sites in the Colorado and Fraser rivers above and below Windy Gap Reservoir, number of tagged fish detected in each portable antenna reach by release site, and total number of tags and percent of tags detected from each release site during portable antenna deployments in October 2020.

Release Site	Released Tags (#)	Mobile Detections				
		Chimney Rock	Fraser River Ranch	River Run	Total	% Detected
Kaibab Park	349	0	1	0	1	0%
Fraser River Ranch	181	0	64	3	67	37%
Upper Shorefox	172	0	0	74	74	43%
Lower Shorefox	185	0	0	85	85	46%
Below Confluence Antenna	37	0	0	1	1	3%
Windy Gap Dam	96	6	0	0	6	6%
Hitching Post	94	23	0	0	23	24%
Upper Red Barn Fry Site	126	11	0	0	11	9%
Below Red Barn Diversion #1	112	11	0	0	11	10%
Kinney Creek	90	22	0	0	22	24%
Sheriff Ranch Fry Site	97	7	0	0	7	7%
Total	1539	80	65	163	308	20%

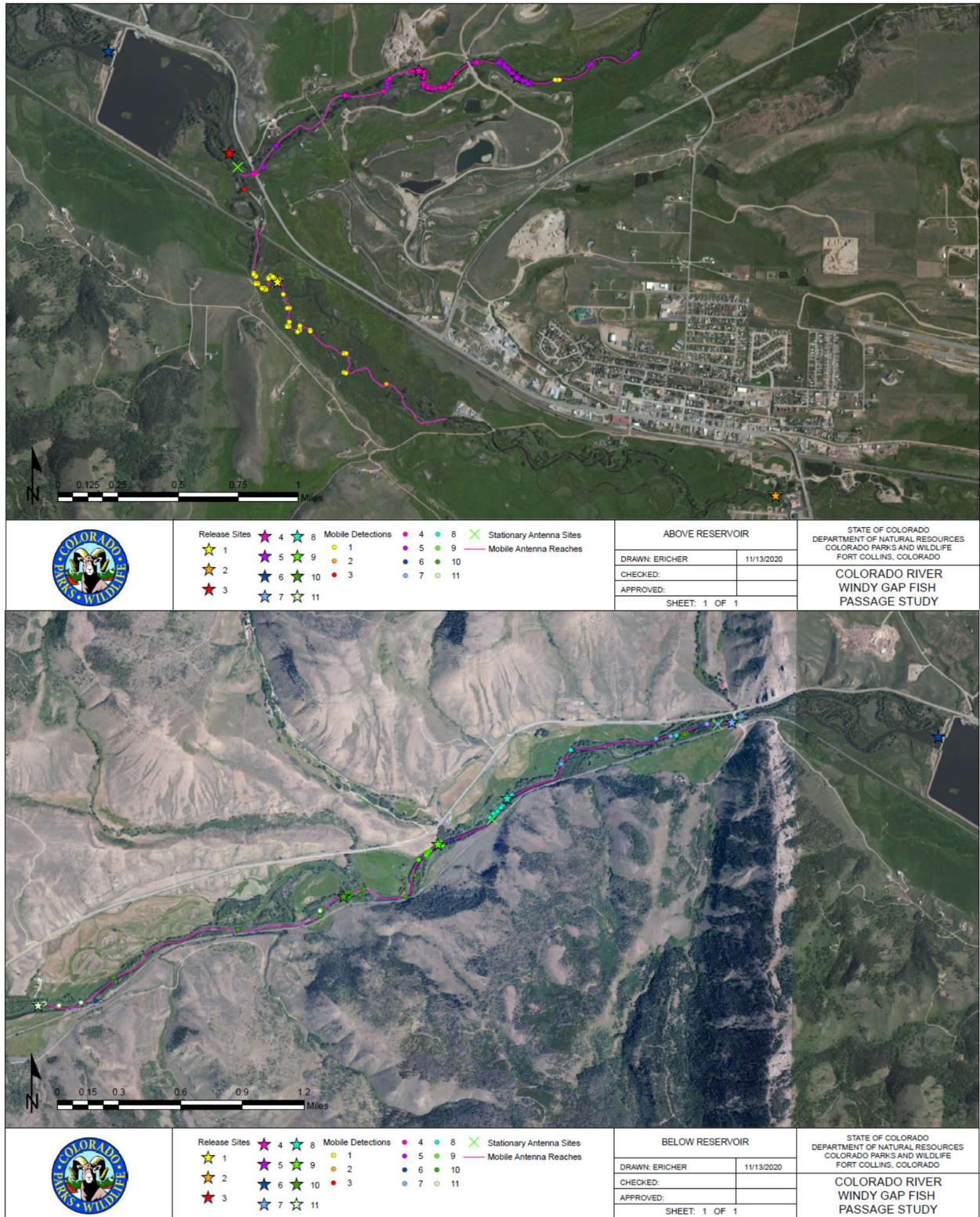


Figure 2.21. Location of detected PIT tags in the Fraser and Colorado rivers upstream of Windy Gap Reservoir (top panel) and the Colorado River downstream of Windy Gap Reservoir (bottom panel) in October 2020. Colored stars correspond to release locations to show distance moved.

Across the reaches, 308 PIT tags were detected during portable antenna deployments in October 2020, and at least one tag was detected from each release location (Table 2.2). The lowest number of tags detected were from releases in Kaibab Park and Mottled Sculpin released below the Confluence antenna station. Neither release site was included in a portable antenna reach. Although also not included in a portable antenna reach, six PIT-tagged fish were detected from the release site located immediately downstream of Windy Gap Reservoir. Seven to 85 PIT tags were detected from release sites located within the portable antenna reaches. On average, the portable antennas detected 20% of tags released in the Fraser and Colorado rivers in fall 2020 (Table 2.2). The GPS location for each detection was overlain on maps of the Fraser and Colorado rivers to visualize distance moved by release location (Figure 2.21), and this data was used to conduct the preliminary evaluation of distance moved presented in this report.

Table 2.3. Number of tagged fish released at 11 sites in the Colorado and Fraser rivers above and below Windy Gap Reservoir in September and October 2020, number of tagged fish detected in each mobile reach by release site, and total number of tags and percent of tags detected from each release site during portable antenna deployments in April 2021.

Release Site	Released Tags (#)	Mobile Detections				% Detected
		Chimney Rock	Fraser River Ranch	River Run	Total	
Kaibab Park	349	0	0	0	0	0%
Fraser River Ranch	181	2	47	1	50	28%
Upper Shorefox	172	0	1	41	42	24%
Lower Shorefox	185	0	0	61	61	33%
Below Confluence Antenna	37	0	0	2	2	5%
Windy Gap Dam	96	24	0	0	24	25%
Hitching Post	94	22	0	0	22	23%
Upper Red Barn Fry Site	126	24	0	0	24	19%
Below Red Barn Diversion #1	112	22	0	0	22	20%
Kinney Creek	90	31	0	0	31	34%
Sheriff Ranch Fry Site	97	6	0	0	6	6%
Total	1539	131	48	105	284	18%

Portable antennas were deployed again in all three reaches in April 2021. Deployments were conducted using the same methods described above for the River Run and Fraser River Ranch reaches, conducted on April 12 and April 13, 2021, respectively. Unlike fall 2020, two passes

were completed through the Chimney Rock Ranch reach on April 14, 2021. Additionally, the starting location for the deployment was moved upstream to immediately below Windy Gap dam so that this release location could be included in a portable antenna reach.

Across the reaches, 284 PIT tags were detected during the portable antenna deployments in April 2021. Kaibab Park was the only release location from which no tags were detected (Table 2.3). Otherwise, the lowest number of tags detected were from Mottled Sculpin released below Confluence, which was not included in a portable antenna reach. Six to 61 PIT tags were detected from release sites located within the portable antenna reaches. On average, the portable antennas detected 18% of the PIT tags released in the Fraser and Colorado rivers in fall 2020 (Table 2.3). Pit tagged fish from six different release sites were detected between Windy Gap and Hitching Post, indicating that moving the starting location of the Chimney Rock Ranch reach added to the data collected in spring 2021 compared to starting at Hitching Post Bridge in 2020. This will become the starting location for all future portable antenna deployments below Windy Gap Reservoir.

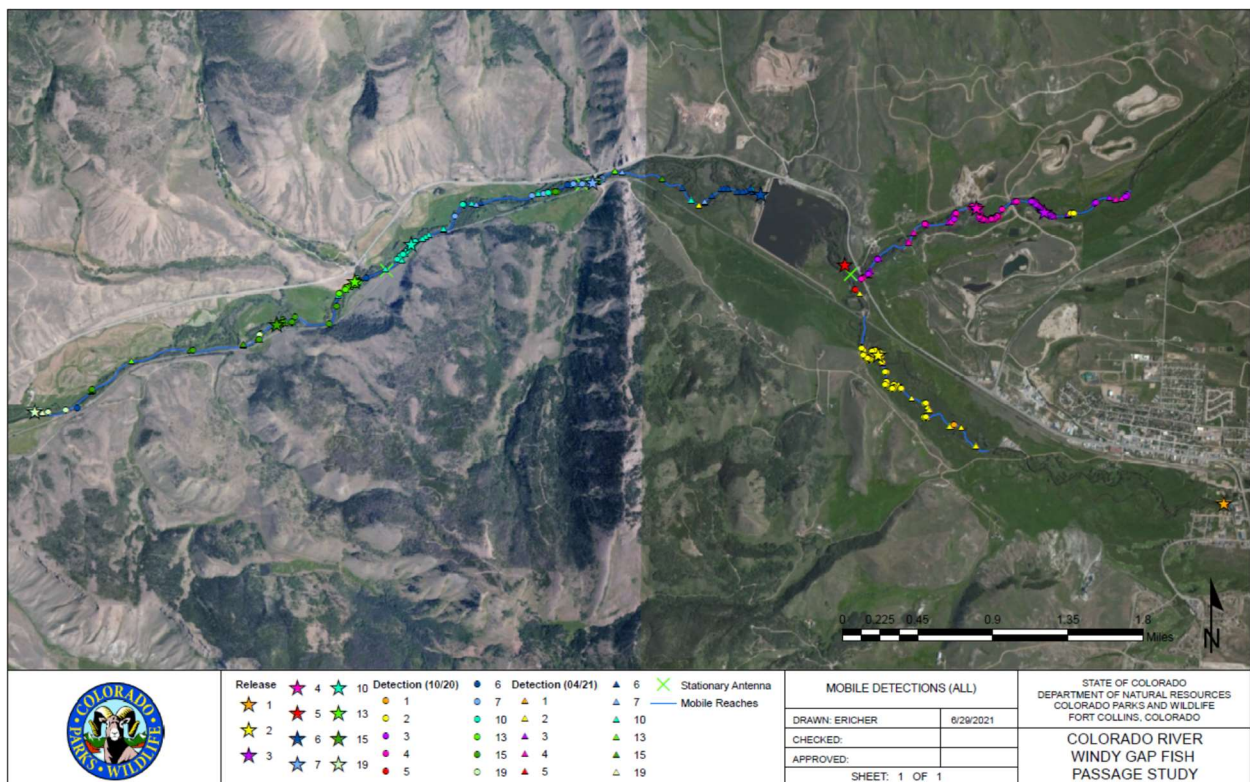


Figure 2.22. Location of detected PIT tags in the Fraser and Colorado rivers upstream of Windy Gap Reservoir and the Colorado River downstream of Windy Gap Reservoir in April 2020. Colored stars correspond to release locations to show distance moved.

The GPS location for each detection was overlain on a map of the Fraser and Colorado rivers to visualize distance moved by release location and deployment date (Figure 2.22). Dispersion of tagged fish appears to be greater below Windy Gap Reservoir compared to above. Additionally, detections appear to be grouped around release sites 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 portable antenna deployments, and protocols will be implemented to locate and confirm the identity of ghost tags and remove them from the dataset.

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.

Preliminary Evaluation of Distance Moved

Data collected from the stationary and portable antennas were used to conduct a preliminary evaluation of distance moved by PIT-tagged fish in the Fraser and Colorado rivers. Stationary antenna data collected between September 3 and October 21, 2020 was sorted by tag number and all repeat detections were filtered out of the dataset so that only initial and final detections at an antenna station were used in the evaluations. The same filtering process was used on the portable antenna data collected October 19-21, 2020 such that only the final detection for a fish detected on multiple passes was included in the dataset. The two datasets were then combined to compile a list of tag numbers detected by either or both the stationary and portable antennas within the evaluation period. Associated release location, species, length, and weight data for each tag was included in the dataset.

Distance moved (m) was measured in Google Earth by tracing a path along the thalweg of the river from the release location to known locations where each fish had been detected. A majority of the fish were only detected at one stationary antenna station or by the portable antennas. In these cases, a single path was drawn from the release location to the last known detection. However, several fish were detected at more than one stationary antenna station or by both stationary and portable antennas. In these cases, detection history was used to determine movement distance from one detection to another. For example, a 395 mm Brown Trout was released above the Red Barn antenna on October 6, 2020, was detected at Red Barn on October 12, and again using the portable antennas upstream of Red Barn on October 21. The first detection at Red Barn represented a 351 m downstream movement from the release location, and detection by the portable antennas represented a 195 m upstream movement to the detection location. The absolute distance moved was therefore 546 m. Note that the absolute distance (downstream, upstream, or both) represents the minimum distance a fish moved within the period as distance could only be evaluated if the fish was detected. Other movements may have occurred during that time which were not detectable.

Three hundred sixty-four fish were detected between September 3 and October 21, 2020. Of those, 164 were detected by stationary antennas, 182 were detected using the portable antennas, and 18 were detected by both. Movements were made in both directions, with 176 fish moving downstream and 199 moving upstream during the evaluation period. On average (\pm 2SE),

downstream and upstream distances moved were relatively similar, with fish moving an average of 915 ± 193 m downstream and 815 ± 176 m upstream from a release site. The average absolute distance moved across all tagged fish was 888 ± 135 m. Absolute distance moved differed by species, with Brown Trout moving $1,017 \pm 159$ m, Rainbow Trout moving 424 ± 198 m, and Mottled Sculpin moving 105 ± 35 m. Note that a movement of less than 100 m represents movement within the same pool that a fish was released or movement into the next nearest habitat feature (pool or riffle). Due to the short distances moved, Mottled Sculpin were removed from further evaluation.

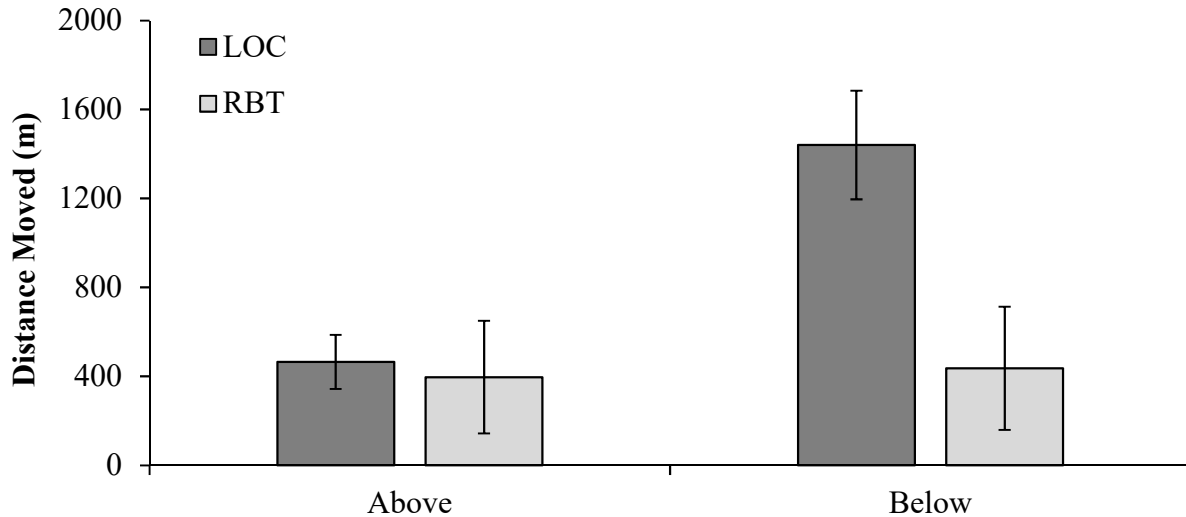


Figure 2.23. Average distance moved (m; 2SE bars) for Brown Trout (LOC) and Rainbow Trout (RBT) above and below Windy Gap Reservoir in fall 2020.

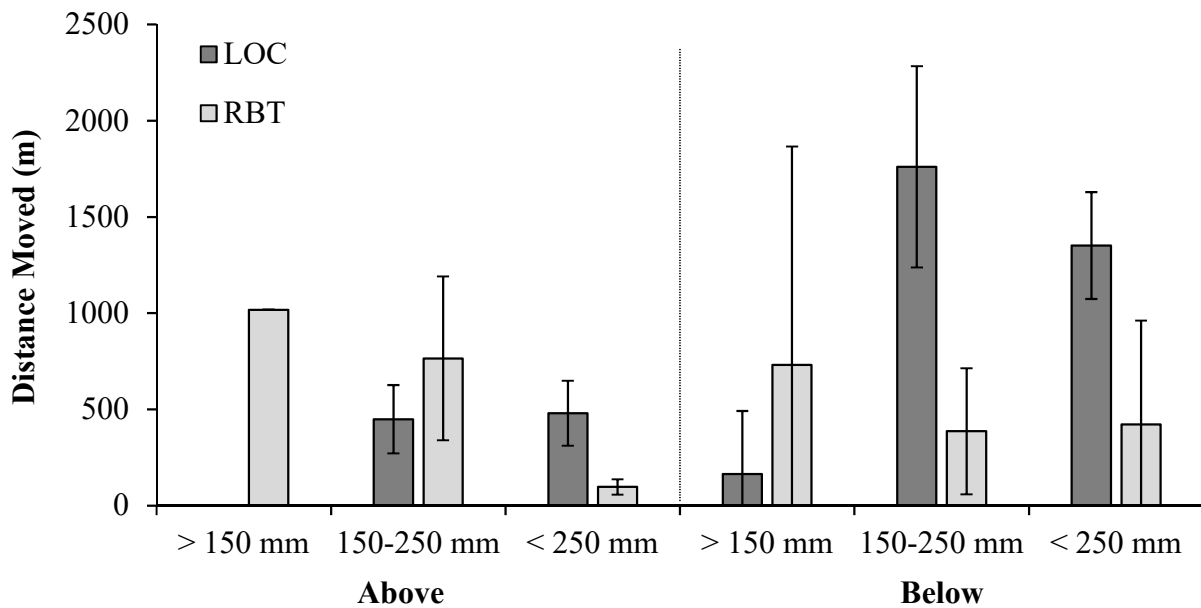


Figure 2.24. Average distance moved (m; 2SE bars) by Brown Trout (LOC) and Rainbow Trout (RBT) size class above and below Windy Gap Reservoir in fall 2020.

Movements above Windy Gap Reservoir were significantly shorter than below. Above the reservoir, fish moved an average of 459 ± 113 m, whereas below, fish moved an average of $1,305 \pm 220$ m. Brown Trout and Rainbow Trout distances were similar above the reservoir, but below, Brown Trout moved significantly greater distances than Rainbow Trout. Rainbow Trout moved similar distances above and below the reservoir. However, Brown Trout below the reservoir moved significantly greater distances than those above (Figure 2.23). Rainbow Trout > 250 mm TL moved greater distances than those < 250 mm TL above the reservoir, but there were no differences in distance moved among size classes below the reservoir (Figure 2.24). There was no difference in distance moved among Brown Trout size classes above the reservoir. However, below, Brown Trout < 150 mm TL moved significantly farther than Brown Trout > 150 mm TL, and farther than fish of the same size above the reservoir. Additionally, Brown Trout < 150 mm TL moved significantly farther than Rainbow Trout of the same size below the reservoir (Figure 2.24). The greater distances moved by larger Brown Trout are likely associated with locating appropriate spawning habitat. Proximity to preferred spawning locations and the need to move to those locations from where fish reside during other times of the year likely drove differences in Brown Trout distances moved above and below the reservoir.

Movement evaluations revealed some interesting patterns that were not captured in the summary statistics presented above. Unlike fish below Windy Gap Reservoir that can only move linearly up and down the Colorado River, fish above the reservoir can move between and utilize habitats in both the Fraser and Colorado rivers. Two Brown Trout released in the Fraser River Ranch in September were detected using the portable antennas in the River Run reach of the Colorado River, likely having moved to utilize spawning habitat in that section of the river. Two of the greatest distances moved downstream were made by Rainbow Trout released in the Fraser River Ranch (208 mm TL) and Kaibab Park (175 mm TL). Both of these fish were detected at Confluence, moved downstream through Windy Gap Reservoir while it was drained for survey work, and were subsequently detected at Hitching Post and Red Barn. Neither fish was detected with the portable antennas, and it is possible they continued to move downstream past the Sheriff Ranch and out of the study section. Lastly, an 89 mm TL Rainbow Trout released in the Fraser River Ranch was later detected at Red Barn, followed by detection at Confluence. The fish was not detected at Hitching Post moving either direction, although it was tagged with a 12 mm PIT tag, which has a relatively short detection range and could be missed by a stationary antenna. It is possible this fish was able to swim downstream and back upstream through the reservoir, though unlikely given its size. Alternatively, it is probable that this fish was consumed by a fish or bird predator that moved between these stations. Evaluation of stationary and portable antenna data will likely to continue to yield unique movement patterns, and provide more information regarding movements by species and size class in relation to spawning, season, and river conditions.

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

this 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 2020: 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 2020 to determine if TAM counts are correlated with myxospore counts in salmonid fry. At each location and on each sampling occasion, four consecutive 1-L samples were collected by placing the sample bottle at 0.67 the depth of the water column and removing the lid to quickly fill the bottle. Samples were kept on ice until filtering could occur. Water was vacuum filtered through 5 μm filters, one 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.

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 programming 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 are considered positive when two or three wells fluoresced, and considered “not detected” when zero or one well fluoresced. One sample from each location sampled in May 2020 was run in a separate assay (Hallett Cs assay with IPC) to confirm that no inhibition was present in the final samples, which may generate false negative results. This validated that a working dilution of 1:10 was sufficient for the environmental DNA samples. The *M. cerebralis* assay performed as expected. Positive control samples showed quantification to a level of one spore per L ($C_q \sim 32$; reported as number of TAMs/L), but with detection of DNA below this level (represented as <1 TAM/L).

Detected levels of *M. cerebralis* DNA were very low for all sites and dates (Table 2.4). However, using both the DNA quantification results and the number of wells fluoresced, there are patterns that emerge from the data. *M. cerebralis* DNA was detected in all sites in May 2020 when water was being released from Windy Gap Reservoir, with more replicates with detection at Hitching Post, the site closest to the reservoir. With the exception of August 2020, *M. cerebralis* was consistently detected at Hitching Post, but not always other sites downstream. Assuming Windy Gap Reservoir was the primary source of TAMs, detection at the upstream-most site was expected. In July, flows may not have been sufficient for transporting TAMs

further downstream to the other sites. No *M. cerebralis* DNA was detected in August 2020 suggesting either a seasonality in TAM production or insufficient flows to move TAMs downstream from the reservoir even to the closest site at Hitching Post. *M. cerebralis* DNA was detected at all sites in September 2020 (Table 2.4). Water samples in September were taken shortly after the reservoir had been drained for survey work, and it is likely that the increase in *M. cerebralis* DNA detection was related to this release.

Table 2.4. Number of triactinomyxons per liter (TAMs/L) and number of wells that fluoresced (F) for each sample collected on five dates and from five locations (Hitching Post, HP; upper Red Barn, URB; lower Red Barn, LRB; below the Red Barn diversion, BRBD; Sheriff Ranch, SR) in the upper Colorado River in 2020. ND indicates *M. cerebralis* DNA was not detected in a given replicate, and NA indicates that samples were not collected from a given location/date.

Site/ Replicate	5/5/20		7/7/20		7/30/20		8/26/20		9/28/20	
	TAMs/L	F	TAMs/L	F	TAMs/L	F	TAMs/L	F	TAMs/L	F
HP1	ND	0	ND	0	<1	2	ND	0	<1	3
HP2	<1	2	<1	2	<1	3	ND	1	ND	1
HP3	<1	3	ND	0	ND	0	ND	1	ND	1
URB1	NA	NA	ND	1	ND	0	ND	0	ND	0
URB2	NA	NA	ND	0	ND	0	ND	0	ND	1
URB3	NA	NA	ND	0	ND	0	ND	0	<1	3
LRB1	ND	0	<1	2	ND	0	ND	0	ND	0
LRB2	<1	3	ND	0	ND	0	ND	0	<1	3
LRB3	ND	1	ND	0	ND	0	ND	0	ND	1
BRBD1	<1	3	NA	NA	NA	NA	NA	NA	NA	NA
BRBD2	ND	0	NA	NA	NA	NA	NA	NA	NA	NA
BRBD3	ND	0	NA	NA	NA	NA	NA	NA	NA	NA
SR1	<1	3	ND	0	ND	0	ND	0	ND	0
SR2	ND	0	ND	0	ND	0	ND	0	ND	0
SR3	ND	0	ND	0	ND	0	ND	0	<1	2

Water samples were again collected in May 2021 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 2021, and water sampling will continue through two years post-construction of the Colorado River Connectivity Channel. These data will be used to not only 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.

POST-STOCKING SURVIVAL OF RAINBOW TROUT IN THE YAMPA RIVER

The comparative survival experiment conducted in the Yampa River between Stagecoach Reservoir and Lake Catamount was initiated in 2017. The goal of the study was to evaluate survival of H×H Rainbow Trout in the Yampa River through a range of habitat conditions, manipulations of the resident Brown Trout population, and stocking strategies (Fetherman et al. 2018). Fish were tagged and stocked in the first three years of the study (Fetherman et al. 2020), but not in this final year which was intended for population monitoring only. The following describes the methods used for monitoring population responses to previous management and stocking, and a summary of data collected during the fourth and final year of the study.

Two five-electrode catrafts were used to complete the fall 2020 sampling. The Foster Ranch, BLM property, Service Creek SWA, Wellar Ranch, and the Stagecoach Tailwater were sampled using a continuous single pass removal September 14-18, 2020. All fish captured during the electrofishing efforts were removed from the river and held in net pens until they could be processed. Rainbow Trout were examined for fin clips, indicating they had been stocked as part of the study, scanned for PIT or coded wire (CWT) tags, measured and weighed. Brown Trout \geq 250 mm total length (TL) were removed from all sections, with the exception of the Foster Ranch, and euthanized after being processed. Euthanization of the Brown Trout was necessary because Lake Catamount and the Yampa River are considered positive for *Renibacterium salmoninarum*, preventing relocation of fish out of the study reach. All Brown Trout captured on the Foster Ranch and those <250 mm TL, as well as all other species encountered, including Brook Trout, Mountain Whitefish, Mottled Sculpin, and Speckled Dace, were measured, weighed, and returned to the section from which they were captured.

Two pass removal estimates were conducted in four standard sampling sites on the BLM property, Service Creek SWA, Wellar Ranch, and Stagecoach Tailwater to estimate the number of fish per mile in each section, used to inform patterns of habitat use and evaluate stocking success. All fish captured were removed from the river and held in net pens by pass until they could be processed. Fish captured, were treated in the same manner as described for the single pass removals. Population abundance estimates were calculated using the Huggins closed capture-recapture estimator (Huggins 1989, 1991) in program MARK (White and Burnham 1999), which provided an estimate of the number of fish in the site, and standardized to number of fish per mile for comparison of abundance and habitat use among reaches. The Green Creek and Kuntz ranches were sampled using a two-pass mark-recapture effort with raft-mounted, throw-electrode electrofishing equipment to sample the deep holes formed by habitat restoration activities on October 12-15, 2020. Due to the length of the section and number of fish processed, the mark and recapture runs were split into two days. The upper Green Creek Ranch was sampled on the first day of the mark run, with the Kuntz Ranch and lower Green Creek Ranch sampled on the second day of the mark run. The upper Green Creek Ranch and Kuntz Ranch were sampled on the first day of the recapture run, with the lower Green Creek Ranch sampled on the second day of the recapture run. All fish were handled similarly to the methods described above for the single- and two-pass removal efforts. Population estimates were calculated using the Lincoln-Peterson estimator with a Bailey (1951) modification. The length of each reach was used to estimate number of Brown Trout present, and using the number of Brown Trout

captured, determine the percentage of the Brown Trout population that had been removed during the sampling efforts.

Overall, 31 PIT-tagged Rainbow Trout were captured in the Stagecoach Tailwater, 140 were captured on the Wellar Ranch, 103 were captured in the Service Creek SWA/BLM/Foster’s Ranch section, and 141 were captured in the Green Creek/Kuntz Ranch section in 2020. PIT-tagged Rainbow Trout stocked in 2019 dominated the catch in all sections. Very few PIT-tagged fish from 2017 and 2018 were encountered in 2020. One hundred forty-five CWT Rainbow Trout were captured in the Stagecoach Tailwater, 17 were captured on the Wellar Ranch, 26 were captured in the Service Creek SWA/BLM/Foster’s Ranch section, and 13 were captured in the Green Creek/Kuntz Ranch section in 2020. A large majority of the CWT fish captured were stocked in 2019, with only 35, 3, 4, and 0 CWT Rainbow Trout stocked in 2018 and 3, 3, 1, and 0 CWT Rainbow Trout stocked in 2017 captured in the Stagecoach Tailwater, Wellar Ranch, Service/BLM/Foster’s Ranch, and Green Creek/Kuntz Ranch sections, respectively.

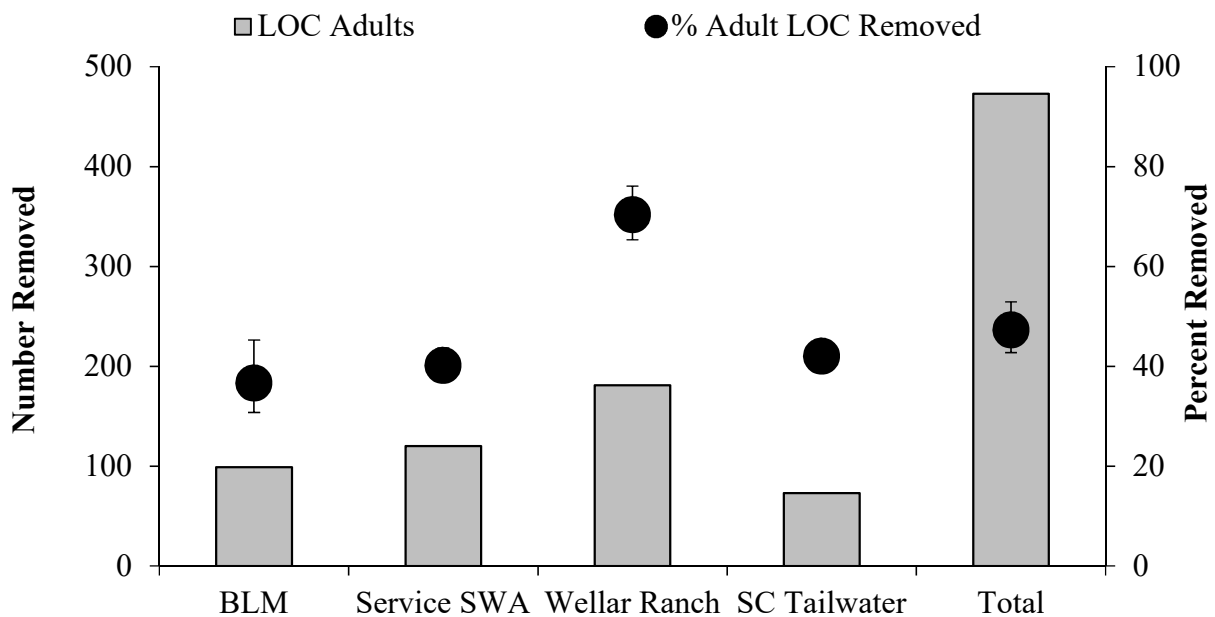


Figure 2.25. Number of adult Brown Trout ≥ 250 ml TL captured and removed in the BLM, Service Creek SWA, Wellar Ranch, and Stagecoach (SC) Tailwater sections of the Yampa River, and total number removed in fall 2020, and corresponding estimated percent adult Brown Trout (SE bars) removed based on two-pass removal abundance estimates conducted in each section.

A total of 473 adult Brown Trout ≥ 250 mm, 3,046 less than 2019 were removed and euthanized, 47% of the estimated 1,001 Brown Trout present in the Yampa River in fall 2020. The percentage of Brown Trout removed from the BLM, Service SWA, and Stagecoach Tailwater ranged between 37 and 42%, whereas 70% were removed from the Wellar Ranch (Figure 2.25). Brown Trout numbers in 2020 were significantly lower than in previous years, likely a result of previous removal efforts in 2017-2019 (Fetherman et al. 2018; Fetherman et al. 2019; Fetherman et al. 2020).

Table 2.5. Abundance estimates (fish per mile [95% CIs]) for adult and juvenile Brown Trout (LOC), wild adult and juvenile Rainbow Trout (RBT), PIT- and coded wire-tagged (CWT) RBT, Mottled Sculpin, Mountain Whitefish, Brook Trout, Fathead Minnow, and White Sucker, from two-pass removals conducted in the Green Creek/Kuntz Ranch, BLM, Service Creek SWA, Wellar Ranch, and Stagecoach Tailwater sections of the Yampa River in fall 2020.

Species (Type)	Green Creek/Kuntz	BLM	Service Creek SWA	Wellar Ranch	Stagecoach Tailwater
LOC (Adults)	248 [207-289]	276 [224-329]	380 [350-411]	206 [190-221]	574 [537-610]
LOC (Juv)		100 [71-129]	622 [576-670]	3,889 [3,723-4,054]	58 [42-73]
RBT (Wild Adults)	139 [107-170]	1,370 [1,241-1,553]	522 [484-560]	208 [197-220]	814 [780-849]
RBT (Wild Juv)		844 [736-952]	470 [430-511]	2,063 [1,959-2,167]	391 [339-442]
RBT (Wild Recaps)	19 [9-29]	55 [34-77]	27 [22-32]	34 [33-35]	480 [458-503]
RBT (2019 CWT)	4 [0-8]	11 [2-20]	9 [5-13]	7 [5-9]	629 [592-502]
RBT (2018 CWT)	5 [0-10]		9 [6-12]		152 [138-166]
RBT (2017 CWT)	1 [0-2]			7 [6-8]	14 [10-17]
RBT (PIT-tagged)	57 [44-70]	44 [25-63]	45 [38-52]	130 [126-133]	193 [178-209]
Mottled Sculpin		2,732 [2,460-3,004]	8,646 [8,370-8,923]	232 [206-257]	101 [81-120]
Mountain Whitefish	11 [4-18]	11 [2-20]	176 [156-195]	218 [193-243]	
Brook Trout	1 [0-2]	200 [157-242]	342 [311-375]	22 [15-30]	168 [149-187]
Fathead Minnow				23 [15-31]	60 [42-74]
White Sucker				7 [3-11]	14 [8-20]

Although habitat data collected in 2018 is still being compiled and analyzed (Fetherman et al. 2019), population abundance estimates can be used to provide an initial look at how fish are distributed and what habitats are being used by which age classes or species of fish. For example, the Wellar Ranch, which is wider, shallower, and contains more aquatic vegetation

than other sections, appears to be good juvenile salmonid rearing habitat. In comparison, the deep pools in the Stagecoach Tailwater and Green Creek Ranch reaches appear to support larger numbers of adult salmonids. In addition to being good salmonid habitat, the BLM and Service Creek SWA sections also supports a higher number of benthic species such as Mottled Sculpin than do other sections of the river (Table 2.5). Future multistate survival and movement analyses will focus on incorporating habitat data collected in 2018 to determine factors driving observed patterns in salmonid distribution, abundance, and survival in the Yampa River.

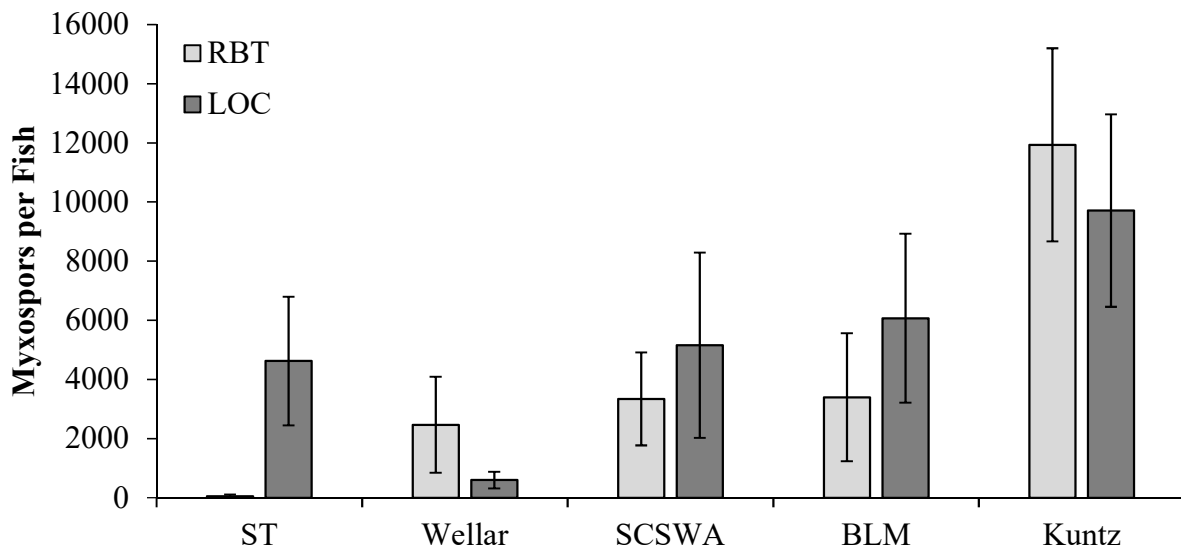


Figure 2.26. Myxospore counts (myxospores per fish) for Rainbow Trout (RBT) and Brown Trout (LOC) fry collected from the Stagecoach Tailwater (ST), Wellar Ranch, Service Creek SWA (SCSWA), BLM, and Kuntz sections of the Yampa River in November 2020.

In November 2020, ten Brown Trout and Rainbow Trout fry were collected from the Stagecoach Tailwater, Wellar Ranch, Service Creek SWA, BLM, and Kuntz sections of the Yampa River for myxospore enumeration. Myxospore counts were low (less than 20,000) in all five sections of the Yampa River (Figure 2.26). In general, average myxospore count increased moving downstream. Tributaries such as Morrison Creek, entering the Yampa River on the Wellar Ranch, and Service Creek, entering the Yampa River on the Service Creek SWA, likely contribute increased sediment and TAMs to the system, resulting in higher myxospore counts below the confluences with these tributaries. Rainbow Trout and Brown Trout myxospore counts did not differ in the Service Creek SWA, BLM, or Kuntz sections of the Yampa River. However, in the Stagecoach Tailwater, Brown Trout had higher myxospore counts than Rainbow Trout, and the opposite was observed on the Wellar Ranch (Figure 2.26). Overall, the establishment of *M. cerebralis*-resistant Rainbow Trout in the Yampa River has significantly decreased infection rates over time. Myxospore counts in Rainbow Trout fry collected from similar sites in the Yampa River in 2002 averaged $383,958 \pm 157,464$ myxospores per fish, and in 2010 averaged $55,566 \pm 19,014$ myxospores per fish. In comparison, Rainbow Trout fry averaged only $4,238 \pm 2,017$ myxospores per fish in 2020.

Genetic samples (upper caudal fin clip) were collected from 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. On average, stocked Rainbow Trout maintained a high percent Hofer genetics ($83.4 \pm 0.08\%$), and did not differ from genetic expectations for the current hatchery brood stocks (Fetherman et al. 2016; Figure 2.27). Percent Hofer was similar across age classes and years, and was significantly lower than that of the stocked fish (Figure 2.27). The assumption was that if stocked fish were reproducing and contributing to the population, we would see an increase in percent Hofer over time starting in the fry/juvenile age class, but this was not the case. We have seen similar patterns in genetics in other wild fish populations (e.g., Gunnison River Rainbow; Fetherman et al. 2020), where percent Hofer decreases over time and the fish display more wild-type genetic characteristics. However, these same fish retain Hofer *M. cerebralis* resistance quantitative trait loci (WDRES-9 QTL). Therefore, a lack of increase in percent Hofer in the wild fish in the Yampa River may not necessarily indicate a lack of reproduction and recruitment from the stocked fish, but that the fish recruiting in the Yampa River are genetically more wild-type fish but with Hofer genetic resistance. We hope to rerun these samples in 2021 to compare percent Hofer with the presence of the WDRES-9 QTL to determine if this is the case.

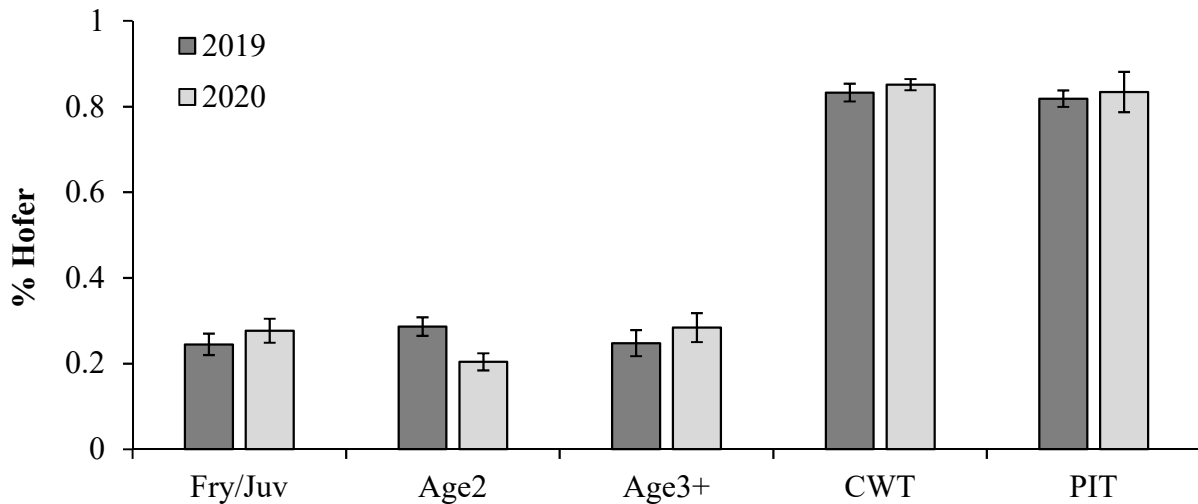


Figure 2.27. Percent Hofer genetics (SE bars) in coded wire-tagged (CWT) and PIT-tagged Rainbow Trout stocked in the Yampa River, and wild fry/juvenile, Age2, and Age3+ Rainbow Trout collected from the Yampa River in 2019 and 2020.

With the complete four-year dataset, we plan to use Rainbow Trout recapture data to create encounter histories and run a multistate analysis for probability of detection, movement, and survival over time. This analysis will include data from habitat surveys as explanatory variables affecting survival and movement. Potential effects of Brown Trout removal on survival and movement will also be incorporated. Overall, the results from this experiment are expected to help biologists and researchers understand the effects of river restoration activities and Brown Trout removal on the retention and survival of stocked and wild Rainbow Trout. Unique to this

study will be the knowledge gained regarding the length-specific effects of restoration activities on apparent survival of stocked fish, i.e., if restoration activities are more of a benefit to larger or smaller fish, or benefit both equally. Additionally, the effects of Brown Trout removal and stocking density are being evaluated. Stocking density effects on survival will be used to determine if biologists could reduce the number of fish requested for stocking to obtain similar returns, thereby reducing hatchery rearing densities and potential issues with disease that come with high-density culture. Two manuscripts are expected from this study, one with the results of the multistate model, and one showing the effects of land use and fishing pressure on patterns of weight loss in stocked catchable fish observed in previous sampling years (Fetherman et al. 2018; Fetherman et al. 2019; Fetherman et al. 2020).

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MYXOBOLUS CEREBRALIS RESISTANCE MECHANISMS IN GUNNISON RIVER RAINBOW TROUT

In May 2020, Eric Fetherman gave a keynote presentation on the *Myxobolus cerebralis* resistance of the Gunnison River Rainbow Trout (GRR) at the Alberta Environment and Parks Virtual Whirling Disease Symposium. The results shown in the talk were the same as those presented in Fetherman et al. (2020). The talk sparked interest in a collaborative research project designed to examine the transcriptomic response of the GRR following exposure to *M. cerebralis*. This collaborative research project between CPW and Patrick Hanington, associate

professor at the University of Alberta, was initiated in June 2020. CPWs role in the project was to obtain and rear control and exposure groups of GRR, expose the GRR to a known concentration of triactinomyxons, and collect fin clips for genetic analyses and kidney and spleen samples for transcriptomic analyses at set time points post-exposure. The GRR exhibit a range of genetic resistance among individuals, as measured by the presence of the 202 base pair WDRES-9 quantitative trait locus (QTL). Generally, individuals homozygous for the resistance allele develop fewer myxospore counts than those heterozygous for the resistance and susceptible alleles or homozygous for the susceptible alleles (Fetherman et al. 2020). The results of this experiment will allow researchers to determine what processes are activated following exposure to *M. cerebralis*, as well as how resistant individuals differ in their response to exposure compared to susceptible individuals. The following details the methods used and results obtained during the exposure experiments conducted by CPW.

GRR were spawned at the CPW Glenwood Springs Hatchery. Eggs from all groups of spawned fish were mixed together in a single batch of GRR produced in 2020. GRR eggs were reared at the CPW Glenwood Springs Hatchery until eyed. Approximately 450 eggs were transported to the CPW Salmonid Disease and Sport Fish Research lab (Fort Collins, Colorado) on June 10, 2020. Upon arrival, eggs were tempered by placing the egg cage containing the eggs wrapped in a wet paper towel into a 76-L flow-through tank (0.5 L/min) supplied by well water. After a 15-minute acclimation period, eggs were removed from the cage and distributed in a single layer on the bottom of the tank to facilitate oxygen exchange. Dead eggs were removed from the tank when found to prevent fungus from growing and killing the eggs. All eggs finished hatching on June 20, 2020. Fish began swimming up on June 29, 2020, at which time they were started on feed. All fish had swum up by July 4, 2020.

On August 10, 2020, fish were separated into two 76-L flow through tanks for the exposure experiment, a control tank and an exposure tank. Based on the experimental design and sample collection timeline, 60 control fish and 110 exposed fish were needed to complete the experiment. A buffer was applied to these numbers to account for any unintentional mortality prior to experiment completion. Therefore, the control tank contained 80 GRR and the exposure tank contained 140 GRR, with fish randomly distributed between the two tanks from a single source tank. The control tank was placed on the top shelf of a multi-tier shelving unit to avoid potential contamination or exposure due to overflow or spills from the exposure tank.

The 140 GRR in the exposure tank were exposed to *Myxobolus cerebralis* triactinomyxons (TAMs), the infectious waterborne stage of the parasite, at 709 degree-days ($^{\circ}\text{C}$), or 7.6 weeks, post-hatch (following Schisler et al. 2006, Fetherman et al. 2012). TAMs were produced by *Tubifex tubifex* worm cultures maintained at the CPW Parvin Lake Research Station (Red Feather Lakes, Colorado). The concentration of viable TAMs was estimated by mixing 1,000 μL of filtrate containing the TAMs and 60 μL of crystal violet; 84.6 μL of this mixture was then placed on a slide and the number of TAMs per slide was counted. This process was repeated 10 times to account for an uneven distribution of TAMs within the filtrate. An average of the ten counts was used to estimate TAMs per mL within the filtrate (~ 129 TAMs per ml) and determine the number of mL of filtrate needed to infect fish with an average of 2,000 TAMs per individual (280,000 TAMs total; 2,097 ml of filtrate).

Prior to the addition of TAMs, water flow to the exposure tank was stopped to provide a static bath exposure. The tank continued to receive aeration to maintain fish health, as well as ensure mixing of the TAMs and even exposure to all fish. A 50 ml bulb-operated pipette was used to deliver the correct amount of filtrate to deliver 280,000 TAMs to the aquarium, which required 42 doses from the filtrate. Between each dose, the filtrate was gently stirred to prevent TAMs from settling to the bottom of the filtrate jar and ensure a more even distribution in each 50 ml dose. After the addition of TAMs, flow to the tank remained stopped for one hour to allow complete exposure, after which time water flow was resumed. The control tank was not exposed to the pathogen, but was treated in the same manner as the exposure tank. After exposure, fish were reared for approximately five months to ensure full development of myxospores. During that time, tissue samples were collected at established post-exposure time periods for genomic and transcriptomic analyses.

Tissue samples, fin clips for genomic analysis and head kidney and spleen for transcriptomic analysis, were collected from five fish in the control tank and five fish in the exposure tank immediately prior to exposure of *M. cerebralis* TAMs (T0) to serve as a baseline for changes in transcriptomic activity due to pathogen exposure. The same tissues were collected from five control fish and five exposure fish immediately after the one-hour bath exposure to the TAMs (T1h). Tissues were then collected from ten control fish and 20 exposure fish at T1day (approximately 24 hours post-exposure), T2days (approximately 48 hours post-exposure), T4days (approximately 96 hours after exposure), T24days, and T5months (147 days post-exposure).

At the time of tissue sample collection, fish were individually sacrificed using an overdose of tricaine methanesulfonate (MS-222) so that tissue samples could be collected immediately upon death. Fin clips were collected first using tweezers and scissors to prevent transfer of human DNA to the sample. Small scissors were used to cut open the abdomen of the fish starting at the vent and ending just behind the operculum. Additional cuts were made up the body wall along the operculum and along the dorsal end of the intraperitoneal cavity to fully visualize internal organs. The spleen was located and removed using fine-pointed tweezers. The remainder of the organs were then moved out of the body cavity to provide access to the head kidney, which was scraped using blunted forceps. Each tissue was placed in an individually labeled tube filled with 250 μ L of RNAlater (three tubes per fish). After collection of samples from all fish in each time period, samples were placed in a laboratory refrigerator and later shipped on ice to the University of Alberta for analysis.

M. cerebralis exposure evaluations were completed when fish reached 2,136 degrees days ($^{\circ}$ C) post-exposure. Exposure evaluations were conducted on the same 10 control fish and 20 exposure fish from which tissue samples were collected at T5months. Fish were measured (mm), weighed (g), and examined for possible disease signs (skeletal deformities, exophthalmia, or blacktail). To ensure development of infection, heads were severed from the body just behind the pectoral fins and placed in individually labeled bags to allow information from the exposure evaluation to be paired with genomic and transcriptomic data for each individual. Heads were sent to the CPW Aquatic Animal Health Lab (Brush, Colorado) for myxospore enumeration (O'Grodnick 1975) using the pepsin-trypsin digest method (Markiw and Wolf 1974).

Control fish did not develop myxospores, suggesting that no accidental introduction of TAMs had occurred during the exposure process and ensuring that transcriptomic results represented fish in which an infection did not develop. Two out of ten control fish exhibited cranial deformities, suggesting that cranial deformities may be a commonly observed deformity within the GRR fish and not necessarily a sign of infection. Myxospores developed in six of 20 exposure fish ensuring that fish had been successfully exposed to *M. cerebralis* and developed infection. Myxospore counts averaged 24,428 myxospores per fish, ranging from 0 to 288,656 myxospores per fish, likely representing the genomic range of variability in susceptibility to infection that has been observed in previous studies (Fetherman et al. 2020). Deformities were more common in exposed fish, including cranial deformities, spinal deformities, opercular deformities, and exophthalmia, although they did not necessarily correlate to infection severity as measured by myxospore count. Blacktail, which is generally associated with higher infection severity (Hedrick et al. 1999; Fetherman et al. 2011), and whirling behavior were not observed in any fish. Along with the overall low average myxospore counts per fish, this confirmed that, though variable among individuals, the resistance to *M. cerebralis* observed in wild GRR (Fetherman et al. 2020) is being retained in subsequent generations of hatchery-reared GRR.

Transcriptomic and genomic samples are currently being analyzed by the University of Alberta. Results from these analyses should be available later in 2021, and a manuscript will be prepared for publication in 2022.

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RENIBACTERIUM SALMONINARUM IN YAMPA RIVER RAINBOW TROUT

In April 2019, the wild Rainbow Trout brood stock in Harrison Creek and Lake Catamount (Steamboat Springs, Colorado) tested positive for *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease (BKD), during a routine annual health inspection. Sixty fish were collected for the inspection from both Harrison Creek and Lake Catamount. Kidney and/or spleen tissues from five fish were pooled (n = 12) to test for viruses or bacteria. Fish were tested for infectious pancreatic necrosis virus (IPNV), viral hemorrhagic septicemia virus (VHSV), infectious hematopoietic necrosis virus (IHNV), and *Oncorhynchus masou* virus (OMV), and *R. salmoninarum*. All fish tested negative for IPNV, VHSV, IHNV, and OMV, but 25-50 suspect cells of *R. salmoninarum* were observed in one of the five-fish pooled samples (8%) via direct fluorescent antibody test (DFAT) and confirmed using single-round PCR (Fetherman et al. 2020). It is unknown how *R. salmoninarum* was introduced to the system. The Yampa River was stocked with thousands of fish that tested negative for *R. salmoninarum* from CPW hatcheries in 2017-2018 for the Yampa River Rainbow Trout post-stocking survival experiment. In addition, Rainbow Trout having an unconfirmed infection status were stocked within the system from a private hatchery in 2018. The brood stock in Harrison Creek and Lake Catamount was developed using Hofer x Harrison Lake Rainbow Trout (H×H) and was being maintained to conduct wild egg takes for hatchery supplementation if the need were to arise. State regulations prevent moving fish or collecting eggs from *R. salmoninarum* positive locations, preventing utilization of the brood stock after it tested positive for *R. salmoninarum*.

In April 2020, a wild spawn was conducted in Harrison Creek to collect several genetically unique male-female pair families for comparison of wild H×H genetics to those from fish reared in CPW hatcheries and utilized in the dual exposure experiment conducted by Colorado State University (CSU) Ph.D. student Brian Avila. Fish utilized in the wild spawn were kept in net pens, along with fish from Lake Catamount, and tested for IPNV, VHSV, IHNV, OMV, and *R. salmoninarum* during the annual disease inspection conducted on May 5, 2020. The eggs were maintained in separate egg containers and transported to the Bellvue Fish Research Hatchery (Bellvue, Colorado) to eye up. After the eggs had eyed, they were transported to the Salmonid Disease and Sport Fish Research lab (Fort Collins, Colorado) and placed in separate five-gallon flow-through tanks, by family, for hatching and rearing, and later genetic sample collection. After arriving at the lab, results of the annual inspection were received. At least two of the pooled samples were positive by DFAT, and several of the other samples collected had cross reactors that made it hard to determine their status. As such, all 12 of the five-fish pooled samples were tested using PCR. Six of the pooled samples (50%) were confirmed positive for *R. salmoninarum* DNA by single-round PCR.

R. salmoninarum can be transmitted in two ways, horizontally (fish-to-fish), and vertically (intra-ovum, from the female through the egg). The primary concern when collecting eggs from a positive brood stock is vertical transmission and introduction of *R. salmoninarum* to the receiving hatchery. Given that 50% of the five-fish pooled samples from the annual disease

inspection, which included the parents of the families in the research lab, tested positive for *R. salmoninarum*, researchers and staff from the CPW Aquatic Animal Health Lab decided to maintain fish in the lab after genetic samples were collected to determine the risk of vertical transmission from the wild brood stock to a CPW hatchery. Fish were reared in five-gallon tanks in relatively high density, high stress conditions for five months prior to being tested on November 16, 2020. The goal was to test 60 fish from each family in five-fish pooled samples following standard disease inspection protocols. To reduce densities and utilize tanks in which only a small number of fish had survived to genetic sample collection, 10 fish from seven families containing more than 60 fish were combined into an additional four mixed-group tanks. In an effort to determine the infection prevalence by individual, fish from these tanks were individually tested. Eleven individual fish from the mixed-group tanks and four five-fish pooled samples from the tanks containing single families were positive by DFAT. However, all 15 samples were found negative for *R. salmoninarum* DNA by single-round PCR. A positive with DFAT followed by a negative confirmatory PCR test is considered a negative result. Therefore, the risk of vertical transmission from the *R. salmoninarum*-positive brood stock, even when the parents were known to be positive, was presumed to be low.

Rainbow Trout from Harrison Creek and Catamount Lake are tested in the spring to maintain a disease history during the time of year from which eggs would be collected via a wild spawn. The fish are generally stressed during the spawn, weakening the immune system and making it more likely for disease to occur and pathogens to be detected during this time. Results from past fish health inspections and research projects in other systems have suggested that there is a seasonality to *R. salmoninarum* detection, with fewer or no fish testing positive outside of the spawning season. Additionally, it is suspected that in systems with salmonids that spawn at different times of the year (spring versus fall), the bacteria may be present but below detectable limits in the species that is not spawning at the time of testing. Lastly, Rainbow Trout, Brook Trout, and Cutthroat Trout are known to develop *R. salmoninarum* infections in Colorado, but little is known about Brown Trout infection status or if they play a role in host-to-host transmission in systems where Rainbow Trout and Brown Trout coexist. To try to answer these questions, 60 adult Rainbow Trout and 60 adult Brown Trout were collected on September 16, 2020 from the Yampa River in the Sarvis Creek State Wildlife Area during the annual Yampa River post-stocking survival experiment sampling. Additionally, 60 fry and juvenile Rainbow Trout were collected for comparison to the research lab fish to determine the risk of vertical transmission within the Yampa River. All 120 fry, juvenile, and adult Rainbow Trout tested negative for *R. salmoninarum* by DFAT. The results suggested that, if present, *R. salmoninarum* may not be detectable in the Rainbow Trout outside of the spawning season. Nine of the 60 Brown Trout samples were positive by DFAT. However, all nine were negative for *R. salmoninarum* DNA by single-round PCR. These results suggested that Brown Trout were negative for *R. salmoninarum*, although the fish were tested about a month prior to peak spawn, so infection may not yet have occurred or *R. salmoninarum* could have been present in below-detectable levels.

With other pathogens such as *Myxobolus cerebralis*, once established in a portion of a connected system, the entire system is considered positive for that pathogen. However, recent field experiments and risk mapping suggest that *R. salmoninarum* may not have a continuous distribution within a system depending on species distributions, spawning locations, habitat, and

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

All of the Rainbow Trout collected from the Yampa River tested negative for *R. salmoninarum* by DFAT. The majority of the fish collected were of spawning age and *R. salmoninarum* should have been detectable, if present. These results suggest that *R. salmoninarum* may not be distributed throughout the entire system and egg takes from the Weller Ranch or Stagecoach Tailwater may be possible in the future. The Yampa River between Lake Catamount and Stagecoach Reservoir is a different water code than Lake Catamount and Harrison Creek. Therefore, a full disease inspection must occur and a three-year disease history needs to be developed for this site before taking eggs. Fish will be collected again during the annual disease inspection in 2022. Interestingly, all 57 fish collected from Lake Catamount also tested negative for *R. salmoninarum* by DFAT in 2021. However, it is important to note that no fish were collected from Harrison Creek as they had been in previous years because discharge in the creek was lower than usual and very few fish had entered the creek to spawn. Because previous collections have grouped fish from Harrison Creek and Lake Catamount, it is unknown which individuals from each location previously tested positive. It is possible that fish that remain in the lake are not spawning, and therefore have lower-than-detectable concentrations or no bacteria compared to the fish spawning in Harrison Creek. This question may still need to be answered during future disease inspections. The negative result for Lake Catamount in 2021 is promising, and a step in the right direction for returning to a negative status. However, per state regulations, before that can occur, the site must be tested two more times at least three months apart, obtaining a negative status during each test, and 12 months must pass since the first negative result was obtained, so Harrison Creek and Lake Catamount will not return to negative status until at least spring 2022.

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.

Fetherman, E. R., B. Neuschwanger, T. Davis, C. L. Wells, and A. Kraft. 2020. Efficacy of Erymicin 200 injections for reducing *Renibacterium salmoninarum* and controlling vertical transmission in an inland Rainbow Trout brood stock. *Pathogens* 2020, 9(7), 547. DOI: <https://doi.org/10.3390/pathogens9070547>.

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. Two such projects are currently being conducted by CSU graduate students in conjunction with sport fish research, one focused on bacterial coldwater disease and one focused on bacterial kidney disease. Bacterial coldwater disease is a major disease of concern in Colorado, sometimes causing high losses of salmonid species in many state hatcheries. Understanding the susceptibility of Colorado's Rainbow Trout strains to the disease, as well as incorporating strains that are resistant to the bacteria, *Flavobacterium psychrophilum*, can help prevent major losses under culture conditions. Bacterial kidney disease, caused by *Renibacterium salmoninarum*, is also 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 COLDWATER DISEASE RESEARCH

Project Collaborators: Brian W. Avila, Ph.D. and Dana L. Winkelman, Ph.D.

Genetics of Laboratory, Hatchery, and Wild H×H Rainbow Trout

Resistance to *Myxobolus cerebralis* is highly variable in wild, hatchery, and laboratory populations of Rainbow Trout, even when individuals of the same strain (e.g., H×H) are evaluated in the different settings. Differences in resistance may be due to spawning procedures, selective pressures, or variability in laboratory exposures to *M. cerebralis*. For example, in hatcheries negative for *M. cerebralis*, fish do not experience the same selective pressures requiring maintenance of genetic resistance as may be experienced in wild populations with continuous exposure to the parasite. As such, susceptible fish that would have otherwise died from exposure to *M. cerebralis* in the wild can be retained in hatchery brood stocks of resistant Rainbow Trout strains. Selecting individuals to spawn without knowledge of their genetic resistance can increase the proportion of susceptible fish in the brood stock over time. Additionally, only a small proportion of the progeny from these brood stocks are evaluated in laboratory exposure experiments, which could result in varying levels of resistance or susceptibility compared to the brood stock as a whole. Exposure to *M. cerebralis* in the lab occurs under controlled conditions, and may result in differences in mortality than would otherwise be observed through natural exposure to the parasite in the wild. Periodic genetic evaluation in these different populations is therefore needed to determine changes in genetic resistance over time.

Results from the *M. cerebralis* and *Flavobacterium psychrophilum* dual exposure experiment conducted by Colorado State University Ph.D. student Brian Avila (Fetherman et al. 2020) showed that the H×Hs evaluated in that experiment developed higher than expected myxospore counts compared to previous evaluations of resistant Rainbow Trout strains. Myxospore counts from the lab experiment were similar to those observed in second-generation backcrosses of the Hofer and Colorado River Rainbow Trout (Fetherman et al. 2012). It was unknown whether this was a result of the experimental conditions experienced by these fish or represented a change in the resistance of the H×H brood stock at the CPW Crystal River Hatchery.

Genetic samples were collected from fish that died prior to the end of the dual exposure experiment to compare to hatchery H×H progeny. Unfortunately, genetic samples could not be collected from fish that survived experimental exposures to *M. cerebralis*. H×H progeny produced by the Crystal River Hatchery and reared at the Bellvue Fish Research Hatchery were used for comparison to the laboratory H×H, and were the next subsequent generation from fish used in the dual exposure experiment. The H×H wild brood stock in Lake Catamount was established at roughly the same time as the Crystal River brood stock. To determine if selective pressures resulted in a difference in genetic expression of *M. cerebralis* resistance genes, single male-female pairs were spawned out of Harrison Creek (tributary to Lake Catamount) in May 2020. Genetic samples were collected from the progeny of these spawns, representing a similar generational group as the hatchery progeny, once fish had reached a large enough size for genetic material collection. All fish were tested for the presence of the WDRES-9 quantitative trait loci (QTL) as had been previously used to determine resistance in wild populations of Rainbow Trout (Fetherman et al. 2020). 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.

The results of the genetic testing revealed that 59% of the Crystal River progeny were RR, while 37% were RS and 4% were SS (Figure 3.1). 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. As such, 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 individuals 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 experiencing differential selection pressures from exposure to *M. cerebralis*. 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 the other resistance loci.

Given the high myxospore counts observed in the dual exposure experiment, a higher proportion of SS individuals was expected, however, most of the individuals tested were RR (Figure 3.1). Additionally, proportions differed from the hatchery progeny. The fish tested originated from all

six exposure and control treatments used in the experiment. These results show that deviations from expectations may occur due to random selection of individuals when a small proportion of the population produced by the hatchery is used in an experiment such as the dual exposure experiment. Some of the fish that died during the dual exposure experiment originated from *M. cerebralis* exposure treatments. Experimental exposure conditions may have been ideal for causing increased mortality, even in fish that were homozygous RR. However, Fetherman et al. 2020 showed that high myxospore counts could develop in RR fish, potentially because of disassociation of other resistance alleles. Therefore, it is also possible that fish that were RR in the dual exposure experiment may have been susceptible to infection and mortality because of missing alleles at other loci.

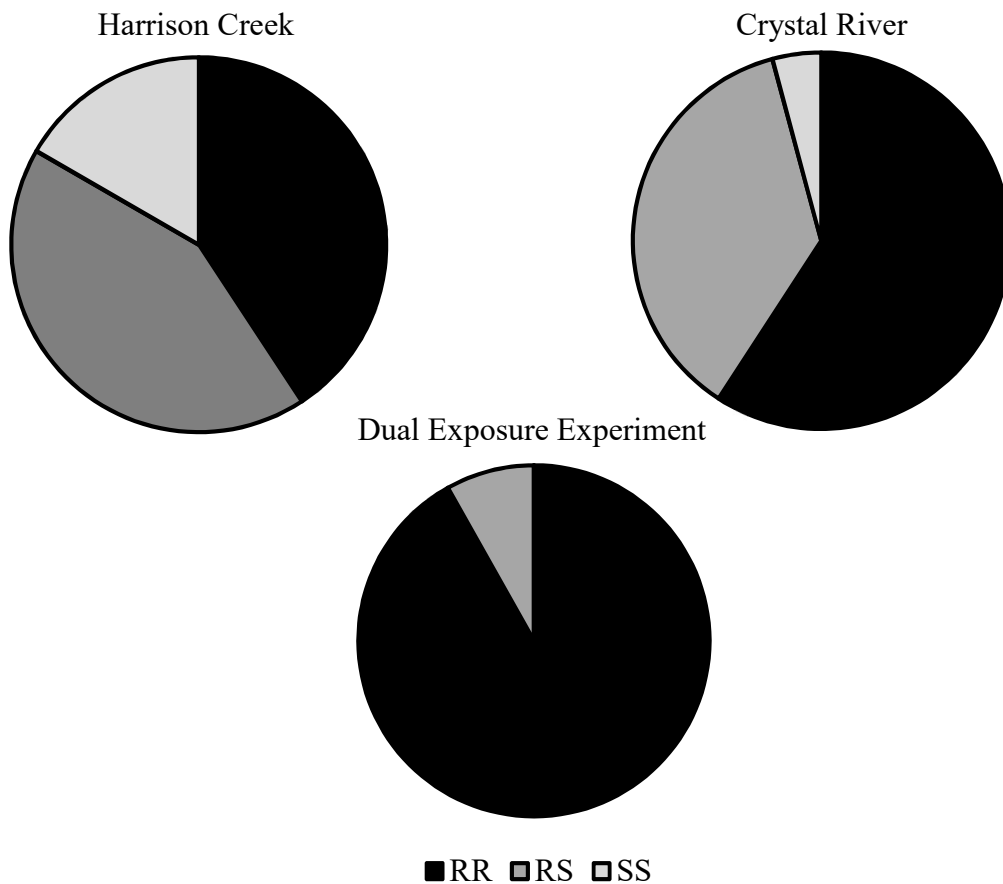


Figure 3.1. Percent of HxH Rainbow Trout tested from Harrison Creek, the Crystal River Hatchery, and the Colorado State University dual exposure experiment containing two copies of the WDRES-9 QTL resistance allele (RR), one copy of the WDRES-9 QTL allele and one copy of a susceptible allele (RS), or two copies of a susceptible allele (SS).

A smaller proportion of RR individuals were obtained from the wild HxH brood stock in Harrison Creek than from the hatchery HxH, which was also unexpected given selection pressures and continuous exposure to *M. cerebralis*. There are a number of reasons why these fish may have differed from genetic expectations. First, the progeny tested from Harrison Creek originated from 11 single male-female pairs, representing a very small proportion of the overall

spawning population. Therefore, one or two RS or SS parents could have a large influence on the genetic outcome in the progeny. Second, Fetherman et al. 2018 showed that there was a large number of high proportion wild-type fish in the Lake Catamount brood stock. Therefore, there may still be fish that are not H×Hs surviving in reproducing in Harrison Creek. This would be true if, third, selective pressures and exposure to *M. cerebralis* were not as high as expected. The progeny for this testing were collected from fish that had moved into Harrison Creek to spawn. However, after fish hatch in Harrison Creek, they likely move into Lake Catamount for the majority of their life span before returning to spawn years later. Therefore, they may not be retained in the creek long enough to be exposed to high numbers of triactinomyxons (TAMs) in early life stages when they are most susceptible to infection. Additionally, with few fish remaining in Harrison Creek, there may not be enough mortality occurring in that system to release high number of myxospores to perpetuate infection. The lentic waters of Lake Catamount may additionally cause TAMs from the Yampa River to fall out of suspension before encountering susceptible life stages. Finally, myxospore counts obtained in fall 2020 showed that overall infection rates in the Yampa River were lower than observed over the past two decades. Any one of these possibilities would reduce selection pressure and allow SS individuals to survive and reproduce in the system. Wild fish from other locations in the Yampa River will be tested for the presence of the WDRES-9 QTL in 2021/2022 to determine if there is a larger proportion of RR individuals in reaches where *M. cerebralis* exposure may be higher.

Overall, the results of this genetic testing were inconclusive when it comes to determining patterns of selection on resistance. However, the results provide a baseline for future testing in both the Crystal River brood stock and H×Hs in the Yampa River. More testing is needed to determine if the proportion of RR individuals continues to change over time. Additional exposure experiments may also be needed to determine if fish retaining the resistance alleles continue to maintain resistance or if disassociation of other alleles causes a loss of resistance despite homozygosity at the WDRES-9 QTL.

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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.

Fetherman, E. R., D. L. Winkelman, G. J. Schisler, and M. F. Antolin. 2012. Genetic basis of differences in myxospore count between whirling disease-resistant and -susceptible strains of Rainbow Trout. *Diseases of Aquatic Organisms* 102:97-106.

Bacterial Coldwater Disease Investigations

Brian Avila completed his dissertation entitled “Bacterial Coldwater Disease Investigations”, which was accepted by the CSU Graduate School on June 7, 2021. An electronic version of the dissertation will be available from the CSU Library in fall 2021. The following is the extended abstract from the dissertation describing the Bacterial Coldwater Disease research conducted by

Dr. Avila in conjunction with Dr. Dana Winkelman and CPW Aquatic Research and Aquatic Animal Health Lab staff.

Bacterial Coldwater Disease Investigations (Brian W. Avila, Ph.D., Extended Abstract)

Global fish production has increased steadily since the 1950s, with the number of wild-captured fish reaching a plateau at an all-time high and the number of fish produced by aquaculture increasing in the early 2000s. In recent years, aquaculture accounts for over 60% of global fish production and is valued at about \$250 billion US dollars. Within the United States, 169 million tons of trout are produced for food, restoration, and conservation practices and are worth more than \$230 million US dollars. Trout production losses within the United States have averaged roughly 30 million trout per year and disease explains upwards of 90% of these losses.

Flavobacterium psychrophilum, the causative agent of bacterial coldwater disease (BCWD), is found in cultured and wild fishes worldwide and causes significant infection in captive salmonid populations. Mortality associated with infections can be as high as 90% depending on water temperature and developmental stage of the host. Due to mortalities associated with BCWD, outbreaks can result in massive economic losses to producers of salmon and Rainbow Trout *Oncorhynchus mykiss*. As a result, BCWD is considered one of the most important hatchery diseases in the world. Infections typically affect age-0 salmonids but can also affect larger and older fish. Infected fish show a broad range of clinical signs of disease such as discoloration of the adipose fin, lesions, spiral swimming behavior, “blacktail”, spinal deformities, and pale or necrotic gills.

Management options to treat infection due to *F. psychrophilum* consist of preventing infections by reducing crowding, handling, and physical damage, and by maintaining high water quality, as well as use of antibiotics, vaccines, or resistant fish hosts. The most common management option is the use of antibiotics. Oxytetracycline has been used worldwide, and amoxicillin and oxolinic acid have been used throughout Europe. However, several studies suggest that antimicrobial resistance is occurring in treated populations. Vaccines are a possible management option and though development of a vaccine has been attempted, none are currently commercially available. Due to concerns about antibiotic resistance and the lack of a vaccine, other strategies to prevent *F. psychrophilum* infections warranted investigation such as using genetically resistant hosts. In 2005, the US Department of Agriculture-Agricultural Research Service’s (USDA-ARS) National Center for Cool and Cold Water Aquaculture (NCCWA) developed a program to create a Rainbow Trout strain that was genetically resistant to *F. psychrophilum*. The resulting Rainbow Trout exhibit genetic resistance to *F. psychrophilum* and are currently being used within various aquaculture settings.

A similar approach of using genetically resistant hosts has been used to manage Rainbow Trout in the presence of the whirling disease parasite, *Myxobolus cerebralis*. Researchers at Colorado Parks and Wildlife (CPW) developed *M. cerebralis*-resistant strains of Rainbow Trout by crossing a domesticated, *M. cerebralis*-resistant strain with wild strains of Rainbow Trout. These crosses result in Rainbow Trout that are resistant to *M. cerebralis* and retain important wild characteristics, including the ability to survive and breed in the wild.

Currently in Colorado hatcheries, it is common practice to rear fish at high densities to maximize the number of fish available to stock for recreational fishing. *Flavobacterium psychrophilum* is managed by using *F. psychrophilum*-resistant Rainbow Trout and antibiotics and *M. cerebralis* is managed by using *M. cerebralis*-resistant Rainbow Trout. This results in Rainbow Trout that are resistant to *F. psychrophilum* in the hatchery but not *M. cerebralis* in the wild or Rainbow Trout that are resistant to *M. cerebralis* but not *F. psychrophilum*. Currently there is no Rainbow Trout that is resistant to both *F. psychrophilum* and *M. cerebralis*. Therefore, it is important to understand the ecological effects or benefits of rearing fish at lower densities to manage *F. psychrophilum* infections and determine if it is possible to develop dual resistance to both *F. psychrophilum* and *M. cerebralis*.

Three chapters comprise my dissertation with the goal of investigating Rainbow Trout management options in the face of *F. psychrophilum*. Chapter 1 describes the factors and the differences among those factors that affect mortality when Rainbow Trout are exposed to *F. psychrophilum* within a laboratory setting. Chapter 2 investigates genetic resistance of Rainbow Trout first-generation and multi-generation crosses to both *F. psychrophilum* and *M. cerebralis* to determine if resistance to both pathogens is possible. In Chapter 3, I investigated the ecological implications of rearing Rainbow Trout at high rearing densities and on different feed types to determine their effects on Rainbow Trout survival post-stocking.

In Chapter 1, I reviewed and conducted a Bayesian meta-analysis of the *F. psychrophilum* literature to determine what factors affect mortality when Rainbow Trout are exposed to *F. psychrophilum* in the laboratory. I examined how bacterial dose, culture time, exposure method, bacterial isolate, and fish weight impacted mortality. I identified studies published in peer reviewed journals utilizing Web of Science, Academic Search Premier, and Google Scholar and mortality was analyzed using a hierarchical Bayesian binomial beta regression model using Just Another Gibbs Sampler (JAGS) within program R. Review of the literature resulted in 22 manuscripts that contributed a total of 132 data points with 24 covariates used in the analysis. The meta-analysis shows that mortality from *F. psychrophilum* is variable among studies published since 1999, despite advances in culture methods and individual laboratory standardization of experimental methods. Injection produces more mortality than bath immersion, bacterial isolates differ in their effect on mortality, and bacterial dose is an important aspect affecting mortality due to *F. psychrophilum* exposures.

In Chapter 2, I conducted two experiments to assess dual pathogen resistance of first-generation and multi-generation crosses (F1) of Rainbow Trout created by crossing *M. cerebralis*- and *F. psychrophilum*-resistant strains. In the first experiment, I exposed two Rainbow Trout strains and one Rainbow Trout cross (German Rainbow x Harrison Lake Rainbow, GR x HL; *psychrophilum*-resistant Rainbow Trout, PRR; and GR x HL x PRR, GHP) to six different treatments: control (no exposure), mock injection, *F. psychrophilum* only, *M. cerebralis* only, *F. psychrophilum* followed by *M. cerebralis*, and *M. cerebralis* followed by *F. psychrophilum*. Rainbow Trout were exposed to *F. psychrophilum* with a dose of 8.8×10^6 colony forming units per milliliter (CFU/mL) using subcutaneous injections and exposed to *M. cerebralis* using a static bath of 2,000 triactinomyxons per fish. Results indicated that GHP fish were not resistant to either pathogen. Dual resistance may be achieved if different parent strains are used to create different F1 crosses. In the second experiment I exposed five Rainbow Trout strains and four

Rainbow Trout crosses (GR; HL; PRR; USDA-ARS *F. psychrophilum*-resistant Rainbow Trout, ARS-Fp-R; USDA-ARS susceptible line, S-Line; HL x PRR; HL x ARS-Fp-R; GR x PRR; and GR x ARS-Fp-R) to *F. psychrophilum*. The second experiment indicated that there was at least one Rainbow Trout F1 cross, HL x PRR, which is *F. psychrophilum*-resistant. Although it appears that dual resistance may be possible with some strains, the lack of response in others indicates that dual resistance may be difficult to develop. However, some strains may be good candidates and a combination of crossing and selective breeding may be capable of achieving dual resistance.

In Chapter 3, my goal was to experimentally determine whether and to what extent rearing density and feed affect post-stocking survival of Rainbow Trout fry when stocked into a put-grow-and take fishery. German Rainbow x Harrison Lake Rainbow Trout, GR x HL, were raised for three months in the hatchery at two densities (high = 1,400 fish/ft³ – 350 fish/ft³, rearing index 2.0; and low = 350 fish/ft³ – 87.5 fish/ft³, rearing index 0.5) and fed two commercially available feeds (Bio Oregon and Rangen). Each treatment (4) had two replicates. Fish were tagged with passive integrated transponder (PIT) tags and stocked into Parvin Lake, Red Feather Lakes, Colorado. Recaptures of tagged fish occurred every two weeks for the first two-months, and again at seven- and 12-months post-stocking. At the time of stocking, there were no differences in Fulton's Condition Factor, total length, or weight for each treatment. However, at the time of stocking, hepatosomatic index (HSI) was higher for the fish raised at low density and fed Bio Oregon feed. Recapture data indicated that there was no difference in Fulton's condition factor and HSI post-stocking and apparent survival of stocked fish was higher for fish raised at low density in the hatchery but was not affected by feed type. My study suggest that rearing density affects fingerling Rainbow Trout post-stocking survival. The higher number of fish stocked due to fish being reared at higher densities within the hatchery did not result in more total fish after a year in the lake compared to the low-density treatment. Raising fish at high density uses increased resources and may not provide any additional catchable fish for anglers.

In summary, exposure experiments are an important aspect to BCWD research. There are important factors such as bacterial dose, which bacterial isolate should be used and how fish are exposed to the bacteria that affects mortality. Moving forward, *F. psychrophilum* resistance is a promising management option, however, depending on the desired traits, the correct parental strains need to be used to achieve *F. psychrophilum* resistance. Finally, preventative measures such as rearing Rainbow Trout at lower densities could have profound ecological impacts on survival post-stocking and reduce factors associated with BCWD outbreaks.

BACTERIAL KIDNEY DISEASE RESEARCH

Project Collaborators: Tawni B. Riepe (Ph.D. Student) 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 since 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 are expected to 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.

Optimization of Quantitative PCR for Detection of Renibacterium salmoninarum

The results of current detection assays used to screen for *R. salmoninarum* do not provide a quantitative relationship between the amount of bacteria present and the output value of the assay. Correlating infection intensity with the amount of bacteria present in a sample may provide insight into the probability of transmission and disease occurrence in individual fish. Therefore, we quantified the number of bacterial cells resulting in various qPCR threshold values (C_q) to provide a standardized measure of cells present in a fish tissue sample.

An isolate of *R. salmoninarum* was obtained from the CPW BFRH. The bacteria was cultured on inoculated selective kidney disease medium (SKDM) designated for *R. salmoninarum* (Evelyn 1977; Evelyn et al. 1990; Jansson et al. 1996). After three passes of cultured growth, KDM-2 broth was inoculated. *R. salmoninarum* was collected from the broth after nine days and confirmed for morphological characteristics with DFAT (USFWS and AFS-FHS 2014). Ten serial dilutions were prepared from the broth culture to count the number of bacteria present in each sample using a membrane-FAT method (Elliott et al. 2015). This was repeated ten times to obtain an average of bacterial cells present in each serial dilution to be used for the qPCR positive controls. Bacterial dilutions were suspended in PBS for DNA extraction following the Qiagen DNeasy Blood and Tissue Kit for gram-positive bacteria to quantitatively measure the amplification of the gene encoding the p57 protein of *R. salmoninarum*. We used the specific

forward primer RS1238 5' – GTG ACC AAC ACC CAG ATA TCC A-3', reverse primer RS1307 5' -TCGCCAGAGCCACCATTTACC – 3', and an internal specific probe (RS1262 6FAM –5'– CAC CAG ATG GAG CAA C– 3') with a 3' MGBNFQ Quencher, and added PCR reagents with Taqman Gene Expression Master Mix (GenEx) to be used in an Applied Biosystems qPCR instrument (following Chase et al. 2006). Incubation times for each qPCR assay included an initial incubation at 50°C for 2 minutes, incubation at 90°C for 10 minutes, 40 cycles of denaturing at 95°C for 15 seconds, and annealing at 60°C for 90 seconds.

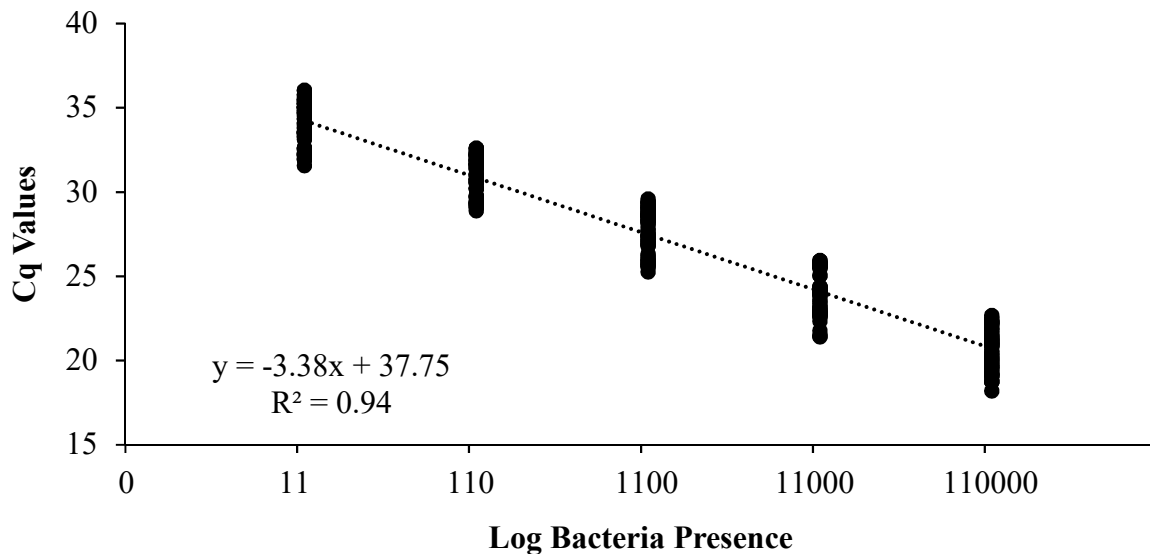


Figure 3.2. Standard curve of qPCR threshold values (Cq) as a function of the amount of bacteria present in 58 serial dilutions.

Analysis of qPCR outputs from 58 serial dilutions ranging from 1.1×10^5 – 1.1×10 were used to generate a standard linear curve relating Cq values to the number of bacteria present in each serial dilution (Figure 3.2). Unknown samples can be compared against this curve to quantify individual infection levels and relate these results to the probability of transmission and disease occurrence in a population. Additionally, we determined the cutoff value for determining if *R. salmoninarum* is present in a tissue sample is 37.75, which is appropriate (Riepe et al. 2021). Samples above this threshold will not be considered positive for *R. salmoninarum*. This cutoff value will be used throughout the remaining tissue testing needed to meet our research objectives for the vertical transmission and wild fish experiments.

Chase, D. M., D. G. Elliott, and R. J. Pascho. 2006. Detection and quantification of *Renibacterium salmoninarum* DNA in salmonid tissues by real-time quantitative polymerase chain reaction analysis. *Journal of Veterinary Diagnostic Investigations* 18:375–380.

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- Riepe, T. B., V. Vincent, V. Milano, E. R. Fetherman, and D. L. Winkelman. 2021. Evidence for the use of mucus swabs to detect *Renibacterium salmoninarum* in Brook Trout. *Pathogens* 2021, 10(4), 460. DOI: <https://doi.org/10.3390/pathogens10040460>.
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Vertical Transmission of Renibacterium salmoninarum

Management of *R. salmoninarum* has been focused on decreasing the risk of transmission to other fish on a hatchery unit and to progeny from infected adults. One management strategy is depopulation of the unit once *R. salmoninarum* has been detected. However, it is unclear if depopulation is necessary to eliminate the bacterium and, in many instances, critical and sometimes irreplaceable broodstocks, such as the Colorado Native Greenback Cutthroat Trout (GBN), may be lost. Another management effort in recent years has been the lethal spawning of fish and collection of samples to screen for *R. salmoninarum*. If parental fish were determined positive, the corresponding eggs were destroyed. This process alone resulted in 43% total loss of greenback eggs in 2017 and 2018 at the CPW Poudre Rearing Unit, and can be harmful to important conservation species in recovery or stocking programs.

Vertical transmission is thought to occur maternally through an intra-ovum infection originating in the ovarian fluid or tissues (Evelyn et al. 1986; Industry 2010; Elliott et al. 2014). However, a great deal of uncertainty about vertical transmission of the bacteria in inland salmonids and whether males can transmit the bacteria vertically remains. Previous studies have demonstrated higher prevalence of *R. salmoninarum* infections in progeny of heavily infected adult Chinook Salmon females (Pascho et al. 1991; Elliot et al. 1995; Munson et al 2010). Transmission of *R. salmoninarum* in inland Rainbow Trout progeny from infected adults has also been reported (Fetherman et al. 2020). Nonetheless, there is still much that is unknown regarding the probability of vertical transmission in Greenback Cutthroat trout, how the bacterial load and infection status of the parental fish effects transmission, and if males can contribute to transmission.

During the GBN spawn at the CPW Poudre Rearing Unit in May 2019, we sampled 788 adult fish to create unique male:female pair families. Adult fish used to create each family were euthanized after being spawned and mucus, blood, ovarian fluid, liver, spleen, and kidney tissue samples were collected. We initially tested kidney tissues from each spawning pair using standard qPCR method (USFWS and AFS-FHS 2014) to assess *R. salmoninarum* infections in each family. After samples were processed, each spawned family was determined positive or negative for *R. salmoninarum*. Eggs from 32 families containing combinations of positive and negative parents were initially placed in four treatment groups based on these results (Table 3.1).

Table 3.1. Tank treatment assigned initially by kidney tissue testing and then refined using results from liver tissue testing, and the number of progeny fish tested for *R. salmoninarum* from each treatment following final assignment.

Treatment	Number of Tanks	Progeny
Initial Assignment - Kidney		
Male (-) Female (-)	8	
Male (+) Female (-)	6	
Male (-) Female (+)	2	
Male (+) Female (+)	16	
Final Assignment - Liver		
Male (-) Female (-)	6	539
Male (+) Female (-)	4	372
Male (-) Female (+)	2	193
Male (+) Female (+)	20	1850

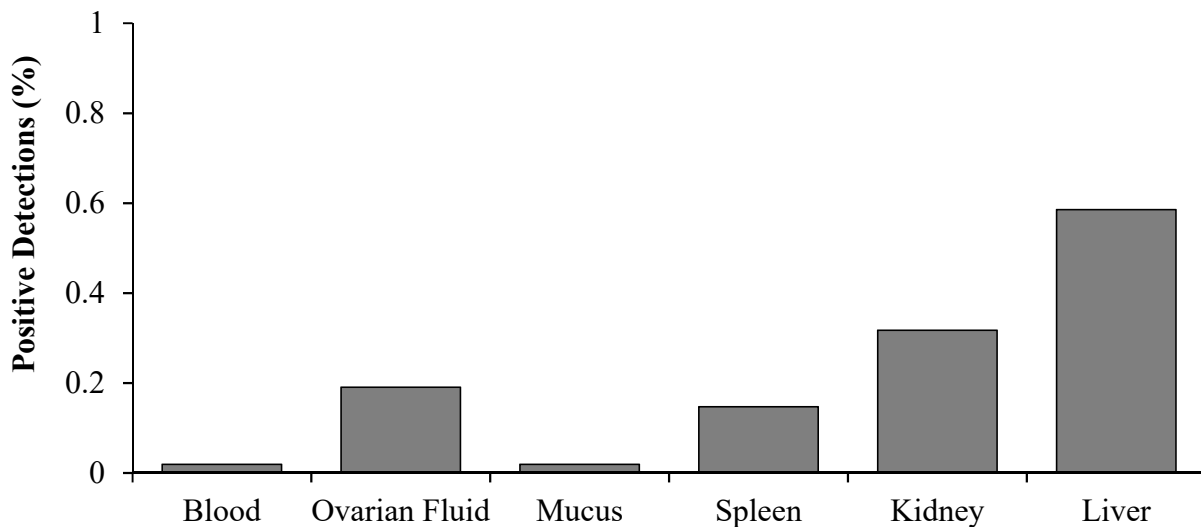


Figure 3.3. Percent of tissues testing positive by qPCR for the presence of *Renibacterium salmoninarum* out of a total of 788 adult fish, 394 males and 394 females. Note that ovarian fluid percent positive is out of 394 females only.

CPW requires testing kidney tissues for the presence of *R. salmoninarum* based on suggestions in the American Fisheries Society Fish Health Blue Book (USFWS and AFS-FHS 2014).

However, updates to the AFS Blue Book state that other hematopoietic tissues such as the liver and spleen can be tested for the presence of the bacteria. Additionally, testing all other tissues collected from the adult GBNs indicated that the liver tissue might provide a more accurate detection probability than the kidney tissue (Figure 3.3). Therefore, the final treatments were determined using liver tissue results from adult spawning fish (Table 3.1). One hundred progeny were reared per family for a total of 3,200 progeny included in the experiment. Half of the fish in each tank were tested for the presence of *R. salmoninarum* at 6 months old and the other half at 12 months old. Kidney, spleen, and liver tissues were collected and tested from all progeny. Samples of blood were also collected from the 12-month-old fish to test for the presence of antibody production against the bacteria.

Tissue samples were collected from 2,954 progeny due to mortality during the experiment. To date, 2,094 tissue samples have been tested with qPCR and 2,459 tissues have been tested for the presence of bacterial surface antigen with a sensitive enzyme-linked immunosorbent assay (ELISA). We will be screening the blood collected from the 12-month-old fish for antibody production against the bacteria in fall 2021. We are currently processing the qPCR and ELISA results to determine the probability of vertical transmission, which tissues may best predict infections within progeny, if males contribute to vertical transmission, and if bacterial load in adult fish affects the probability of transmission. These analyses will be completed in fall 2021 and results will be available in the next reporting cycle.

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Fetherman, E. R., B. Neuschwanger, T. Davis, C. L. Wells, and A. Kraft. 2020. Efficacy of Erymicin 200 injections for reducing *Renibacterium salmoninarum* and controlling vertical transmission in an inland Rainbow Trout brood stock. *Pathogens* 2020, 9(7), 547. DOI: <https://doi.org/10.3390/pathogens9070547>.

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USFWS and AFS-FHS (U.S. Fish and Wildlife Service and American Fisheries Society-Fish Health Section). 2014. Standard procedures for aquatic animal health inspections. *In* AFS-FHS. FHS blue book: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, 2016 edition.

Renibacterium salmoninarum in Wild Fish Populations

Fish health may pose major challenges outside of an aquaculture facility or laboratory setting when little is known about the pathogenic effects on wild populations (Frandsen et al. 1989; Harvel et al. 2004; Fenichel et al. 2009). *R. salmoninarum* infection prevalence in wild fish populations are generally unknown, with only intensity of infection or susceptibility among species noted in the literature (Souter et al. 1989; Fenichel et al. 2009). Prevalence of *R. salmoninarum* was surveyed across Colorado in 2016 and 2017, and results showed that the bacteria is nearly ubiquitous but bacterial load is low and clinical disease is rare (Kowalski 2018). To determine if infections in wild fish populations cause population-level effects, we began evaluations of the demography of populations with a high infection prevalence compared to populations with a low infection prevalence, as determined by Kowalski (2018). Measurable attributes include length-at-age relationships, fish size and age class structure, and annual survival/mortality rates across and within age classes. We are also assessing the use of non-lethal methods to detect the bacteria for potential use in future field studies.

Table 3.2. Number of fish collected by species from six sites with low and high *Renibacterium salmoninarum* infection prevalence in fall 2020.

Site	Prevalence	Species	Number Collected
Fall Creek	High	Brook Trout	40
		Cutthroat Trout	20
Fraser River – Winter Park	High	Brook Trout	185
Cunningham Creek	High	Brook Trout	304
		Brown Trout	19
Fraser River – Robber’s Roost	Low	Brook Trout	130
		Cutthroat Trout	58
Lost Creek	Low	Brook Trout	70
Mosquito Creek	Low	Brook Trout	256

In fall 2020, we began our first field season to assess infections, age structure, fish abundance, and annual survival at three low prevalence sites, Mosquito Creek, Lost Creek, and Fraser River-Robber’s Roost and four high prevalence sites, Fraser River-Winter Park, Cunningham Creek, Fall Creek, and Eagle Lake. Electrofishing and three-pass removals were used to capture fish for

population assessments at all sites except Eagle Lake, at which we used gill nets to sample only the adult population. Each captured fish was measured, weighed, and either used for tissue and otolith collection or returned back to the water. Sixty Brook Trout from each site were collected to age fish via the otoliths and to test for *R. salmoninarum* in hematopoietic tissues and mucus from the lateral line. To get an accurate representation of the age structure, we collected fish from multiple size classes, which were determined by the total length of the fish (e.g., 15 fish with lengths 0-50 mm, 15 fish with lengths 51-100 mm, etc.).

Total fish collected at each site ranged from 60 – 304 (Table 3.2). To date we have analyzed the otoliths from 60 Brook Trout collected at each site to estimate ages. Cutthroat Trout were sampled non-lethally (mucus only), and will not be aged as part of this experiment. Length frequencies will be created and used to determine age classes and a Von Bertalanffy length-age relationship will be constructed for each site. Modeling will be completed after the fall 2021 sampling season so that all the fish collected for this study are included. We will then use our predicted ages to construct a catch curve analysis. This will allow us to determine the annual mortality in each population and age class. We will be able to determine potential population-level effects of infection by comparing infection prevalence to annual mortality and age-class distributions. Field sampling for the second year of the project will occur in fall 2021 and data will be analyzed in winter 2022.

Fenichel, E. P., J. I. Tsao, and M. L. Jones. 2009. Modeling fish health to inform research and management: *Renibacterium salmoninarum* dynamics in Lake Michigan. *Ecological Applications* 19(3):747–760.

Frandsen, F., H. J. Malmquist and S. Snorrason. 1989. Ecological parasitology of polymorphic Arctic Charr, *Salvelinus alpinus* (L.), in Thingvallavatn, Iceland. *Journal of Fish Biology* 34:281–297.

Harvell, D., R. Aronson, N. Baron, J. Connell, A. Dobson, S. Ellner, L. Gerber, K. Kim, A. Kuris, H. McCallum, K. Lafferty, B. McKay, J. Porter, M. Pascual, G. Smith, K. Sutherland, and J. Ward. 2004. The rising tide of ocean diseases: Unsolved problems and research priorities. *Frontiers in Ecology and the Environment* 2(7):375–382.

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Souter, B. W., A. G. Dwilow, and K. Knight. 1987. *Renibacterium salmoninarum* in wild Arctic Charr *Salvelinus alpinus* and Lake Trout *S. namaycush* from the Northwest Territories, Canada. *Diseases of Aquatic Organisms* 3:151–154.

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 regarding stocking and use of *Myxobolus cerebralis*-resistant Rainbow Trout in Colorado. One presentation was given at a CPW hatchery staff meeting:

- Avila, B. W., D. L. Winkelman, and E. R. Fetherman. 2021. Management of rainbow trout in the face of two pathogens: Assessment of disease resistance to *Flavobacterium psychrophilum* and *Myxobolus cerebralis*. Colorado Parks and Wildlife Hatchery Staff Meeting. Virtual. April 29, 2021.

External presentations and posters provided an opportunity to give research updates to managers both within and outside Colorado. One poster was presented at the Annual Meeting of the Colorado/Wyoming Chapter of the American Fisheries Society, one poster was presented at the Annual Meeting of the Western Division of the American Fisheries Society, and a presentation was given to the Fraser River Ranch Fishing Club:

- Riepe, T. B., V. Vincent, V. Milano, E. R. Fetherman, and D. L. Winkleman. 2021. Non-lethal detection of *Renibacterium salmoninarum* (causing bacterial kidney disease) in Brook Trout (*Salvelinus fontinalis*). Poster. 2021 Annual Meeting of the Colorado/Wyoming Chapter of the American Fisheries Society. Virtual. February 24, 2021.
 - Awarded Best Student Poster.
- Fetherman, E. R., E. E. Richer, M. C. Kondratieff, J. Ewert, and D. A. Kowalski. 2021. Update on Fraser River Ranch salmonid population, PIT tagging, and the Windy Gap fish movement study. Fraser River Ranch Fishing Club Party. Virtual. March 23, 2021.
- Riepe, T. B., V. Vincent, V. Milano, E. R. Fetherman, and D. L. Winkleman. 2021. Non-lethal detection of *Renibacterium salmoninarum* (causing bacterial kidney disease) in Brook Trout (*Salvelinus fontinalis*). Poster. 2021 Annual Meeting of the Western Division of the American Fisheries Society. Virtual. May 13, 2021.

In addition to public and professional meeting presentations, two presentations were given to the fisheries management class at Front Range Community College in Fort Collins, Colorado. The first, an informal presentation/laboratory, was presented at the BFRH. During this lab, students learned about the various tagging methods used in research and management across Colorado, and were given a chance to try the tagging methods on live fish. The second, a formal presentation, was given to the class virtually in March 2021:

- Fetherman, E. R. 2021. Salmonid disease research in Colorado. Guest lecture, Introduction to Fisheries. Virtual, Front Range Community College. Fort Collins, Colorado. March 23, 2021.

Manuscripts published in peer-reviewed scientific journals help to inform fisheries management decisions locally, nationally, and internationally. One manuscript was published in North American Journal of Fisheries Management, and one in Pathogens:

- Richer, E. E., E. R. Fetherman, E. A. Krone, F. B. Wright III, and M. C. Kondratieff. 2020. Multispecies fish passage evaluation at a rock-ramp fishway in a Colorado transition zone stream. North American Journal of Fisheries Management 40:1510-1522. DOI: 10.1002/najfm.10516.

- Riepe, T. B., V. Vincent, V. Milano, E. R. Fetherman, and D. L. Winkelman. 2021. Evidence for the use of mucus swabs to detect *Renibacterium salmoninarum* in brook trout. *Pathogens* 2021, 10(4), 460. DOI: <https://doi.org/10.3390/pathogens10040460>.

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

- Fetherman, E. R., and B. W. Avila. *In review*. Habitat associations of rainbow trout *Oncorhynchus mykiss* and brown trout *Salmo trutta* fry. Submitted to *Journal of Fish Biology*.
- 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 *Biological Conservation*.

Sport Fish Research staff participated in two video interviews with Northern Water video production staff regarding antenna installations, electrofishing, and PIT tagging efforts for the fish movement study. Both videos were posted to the Northern Water YouTube channel, and one of the videos, along with additional interviews from Northern Water staff, was submitted to the Sustaining Watersheds virtual conference. Graduate student Tawni Riepe and Sport Fish Research staff were also interviewed while collecting *Renibacterium salmoninarum* samples at Robber's Roost in the Fraser River, and photos and the article were featured on the front page of the Winter Park Times on September 4, 2020. Eric Fetherman and Brad Neuschwanger participated in an educational video produced by the Colorado Wildlife Council focusing on CPW's research section, and specifically, the Bellvue Fish Research Hatchery. Lastly, Eric Fetherman and Tawni Riepe provided quotes and reviewed drafts of a CPW press release produced by Dan Kowalski and CPW PIOs as an update on the progress of CPW and CSU bacterial kidney disease research. The release was published in The Fort Morgan Times and online by CBS Denver in December 2020.

Technical assistance milestones included providing information and discussion for internal management decisions regarding bacterial kidney disease in Colorado's hatchery system and wild populations; fry shocking protocols, analysis, and presentation of fry population data; sampling options for *Myxobolus cerebralis* monitoring in the Arkansas River; filtering large amounts of water from a whirling disease positive source being transferred to a whirling disease negative source; and, genetic testing and identification of candidate wild Rainbow Trout brood stocks. Additional external technical assistance milestones included comprehensive exam preparation and consultation on Program MARK analyses with CSU graduate students not associated with Sport Fish Research Studies; counting viable and non-viable TAMs in filtrate sent to Utah Department of Wildlife Resources (DWR) for use in a lab diagnostic protocol standardization study; and, compiling stocking policies, Federal Aid and Annual Reports, and *M. cerebralis*-related publications for Utah DWR to inform potential management options and changes to regulations regarding the movement of *M. cerebralis*-positive fish.

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

- Collected dace from multiple fry sites in the upper Colorado River. Dace were Floy tagged and labeled for later genetic analysis.

- Data consultation and analysis for study examining the efficacy of eDNA sampling for *M. cerebralis* in relation to population reclamation and barrier construction in George and Cornelius creeks.
- Collected heads from control and exposed Gunnison River Rainbow for myxospore enumeration and detection technique validation with the Aquatic Animal Health Lab.
- Assisting with additional population sampling efforts in the Fraser River.
- Incorporating a mark-recapture assumption study into tagging efforts conducted as part of the fish movement study on the upper Colorado River.

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

- Fetherman et al.: anesthetizing and tagging fish in Fraser River for Fish Movement Study (opened August 2020; closed October 2020).
- Fetherman et al.: anesthetizing and tagging fish in Colorado River for Fish Movement Study (opened September 2020; closed November 2020).
- Fetherman et al.: PIT tagging fish in Colorado River (opened April 2021; closed May 2021).

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

- Richer, E. E., M. C. Kondratieff, G. Policky, M. D. Robinson, M. Atwood, and M. R. Myers. *In review*. From gold mining to gold medal fishery: evaluating the fishery response to stream restoration in the upper Arkansas River, Colorado. Submitted to *North American Journal of Fisheries Management*.
- Bushon, A. M., and J. M. Rash. *In review*. Retention of postocular visual implant elastomer in two sizes of adult brown trout and rainbow trout. Submitted to *North American Journal of Fisheries Management*.
- Research proposal entitled “Multistress effects on fish pathogen interactions”. Submitted to and reviewed for the French National Research Agency (ANR).
- As a member of the Review Board for the journal *Animals*:
 - Roh, H., J. Park, A. Kim, N. Kim, Y. Lee, B. S. Kim, J. Vijayan, M. K. Lee, C-I. Park, and D-H. Kim. *In review*. Overfeeding stress could cause potential immuno-physiological disorders in rainbow trout (*Oncorhynchus mykiss*). Submitted to *Animals*.
 - Fossati, A. A., R. B. Rodrigues, D. C. Fornari, A. F. da Silva, L. S. Marquesa, A. B. de S. Farias, S. P. C. Bentes, L. A. M. França, L. U. Gonçalves, and D. P. Striet, Jr. *In review*. Partial or total replacement of fish meal by soybean meal in diets for *Brycon amazonicus*: growth performance and white muscle cellularity. Submitted to *Fishes*.
 - Labonne, J., A. Manicki, L. Chevalier, M. Tétillon, F. Guéraud, and A. P. Hendry. *In review*. Unequal gene flow during reciprocal transplant between newly established populations. Submitted to *Genes*.
 - Rashidian, G., J. T. Boldaji, S. Rainis, M. D. Prokić, and C. Faggio. *In review*. Oregano (*Origanum vulgare*) extract enhances zebrafish (*Danio rerio*) growth performance, serum and mucus innate immune responses, and resistance against *Aeromonas hydrophila* challenge. Submitted to *Animals*.
 - McCormick, M., E. Dillon, I. O’Connor, and E. MacCarthy. *In review*. Investigation of the host response of naïve Atlantic salmon (*Salmo salar*) inoculated with *Paramoeba pururans* prior to the onset of clinical symptoms. Submitted to *Microorganisms*.

Internal reviews were also conducted upon request:

- Fitzpatrick, R. Large Scale Demographic Analysis of a Cyprinid.
- FAMILY SALMONIDAE for Fishes of Colorado Book.

Service outside of CPW:

- Member of the Colorado/Wyoming Chapter of the American Fisheries Society (AFS) Budget Review Committee. 2020, 2021.
- Member of the Colorado/Wyoming Chapter of AFS Arrangements Committee. 2020-2021.
- Member of the Western Division of AFS Resource Policy and Environmental Concerns Committee. 2020-2021.
- Member of the North American Journal of Aquaculture subcommittee of the AFS Publication Awards Committee. 2020-2021.

Highlights:

- Eric Fetherman received the 2021 Outstanding Mentor Award from the Colorado/Wyoming Chapter of the American Fisheries Society.

Dual Disease Resistance in Rainbow Trout



EVALUATING RESISTANCE TO BACTERIAL COLDWATER DISEASE AND WHIRLING DISEASE IN COLORADO'S TROUT

Bacterial Coldwater Disease (BCWD)



Lesion caused by *F. psychrophilum* infection

Bacterial coldwater disease (BCWD) is caused by the bacterium *Flavobacterium psychrophilum*. Found worldwide, BCWD causes significant complications and death in hatchery trout populations. Outbreaks typically occur at temperatures between 39 and 50°F. Infected fish show a broad range of clinical disease signs including lesions, spiral swimming, “black tail”, spinal deformities, and pale or necrotic gills. Mortality can be high if left untreated, and antibiotics are commonly used to treat BCWD. As an alternative, the

USDA National Center for Cool and Cold Water Aquaculture (NCCWA) developed a Rainbow Trout strain that is resistant to *F. psychrophilum*. With the help of Utah Division of Wildlife Resources, *psychrophilum*-resistant Rainbow Trout (PRR) were incorporated into the CPW hatchery system to help manage BCWD outbreaks.

Whirling Disease (WD)

Whirling disease (WD) is caused by the parasite *Myxobolus cerebralis*. Signs of infection include skeletal deformities, “black tail”, and “whirling” or spiral swimming. WD cannot be treated, and susceptible fish typically die within their first year. *M. cerebralis* has a complex multi-stage life cycle, making it extremely difficult to remove from aquatic environments. One option for management is to use *M. cerebralis*-resistant fish. The Hofer strain is genetically resistant to *M. cerebralis*, however, it is domesticated and shows reduced survival in the wild. To increase survival, CPW crossed the Hofer with wild Rainbow Trout strains. The resulting crosses (HxC, Hofer by Colorado River Rainbow; HxH, Hofer by Harrison Lake Rainbow) are resistant to *M. cerebralis*, and survive and reproduce in the wild. Stocking *M. cerebralis*-resistant Rainbow Trout has helped reduce WD in aquatic systems throughout Colorado.



Spinal deformity, *M. cerebralis* infection

Evaluating Dual Resistance via Dual Exposure to BCWD and WD

CPW uses the PRR to reduce mortality in the hatchery due to *F. psychrophilum* outbreaks. However, it is unknown if the PRR are resistant to *M. cerebralis*. Stocking PRRs with no resistance to *M. cerebralis* could result in high losses from WD, as well as increased *M. cerebralis* prevalence. Conversely, although resistant to *M. cerebralis*, the HxH shows increased mortality in the hatchery during BCWD outbreaks. This study examined if crossing the PRR with the HxH resulted in fish that are genetically resistant to both *F. psychrophilum* and *M. cerebralis*.

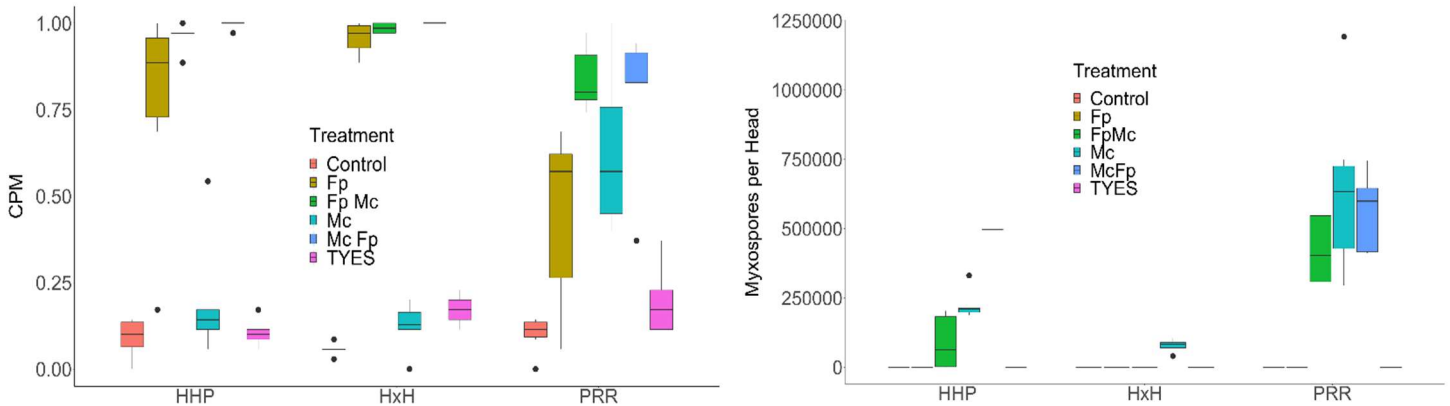


Dual exposure laboratory, Colorado State University



The PRR, HxH, and HHP, the first generation cross between the PRR and HxH, were used for the dual exposure experiment. To test genetic resistance to both pathogens, fish were exposed to *F. psychrophilum*, *M. cerebralis*, or both. Fish were exposed to *F. psychrophilum* using injections under the skin, and to *M. cerebralis* using bath exposure to triactinomyxons, the waterborne infectious stage of the parasite. Mortality from *F. psychrophilum* occurs within 28 days of exposure, and was the first measureable endpoint of the experiment. Fish were then reared for six months to allow development of myxospores, the countable form of *M. cerebralis* in fish, and disease signs and myxospore counts were obtained from the fish remaining at the end of the experiment.

The PRR experienced the lowest cumulative mortality when exposed to *F. psychrophilum*, showing that it was more resistant to *F. psychrophilum* than either the HxH or HHP. However, with higher myxospore counts than either the HxH or HHP, the PRR did not show any resistance to *M. cerebralis*. The HxH had much lower myxospore counts and was more resistant to *M. cerebralis* than the PRR. However, as had been observed in the hatchery, the HxH did not exhibit any resistance to *F. psychrophilum*. The myxospore counts for the HHP were intermediate to those of the HxH and PRR, and the HHP experienced high cumulative mortality when exposed to *F. psychrophilum*, showing that it had not gained resistance to *F. psychrophilum* from the PRR. Coinfection with *F. psychrophilum* and *M. cerebralis* increased mortality in the PRR, HxH, and HHP compared to single-pathogen exposure.



Left: Cumulative percent mortality (CPM) for the HHP, HxH, and PRR across six treatments, 1) control (no pathogen exposure), 2) *F. psychrophilum* only (Fp), 3) exposure to *F. psychrophilum* followed by exposure to *M. cerebralis* (Fp Mc), 4) *M. cerebralis* only (Mc), 5) exposure to *M. cerebralis* followed by exposure to *F. psychrophilum* (Mc Fp), and 6) mock injection with TYES media (TYES). Right: Myxospores per fish head as a measure of *M. cerebralis* infection for the HHP, HxH, and PRR in each of the six treatments at the end of the experiment.

Management Implications

The results of this experiment suggest that it was not possible to create fish that are resistant to both *F. psychrophilum* and *M. cerebralis* using the HxH and PRR. In a follow up experiment, we exposed pure strains and their crosses to *F. psychrophilum* and found that the first generation cross between the Harrison Lake Rainbow Trout and the PRR showed reduced mortality and resistance to *F. psychrophilum*. Therefore, it may be possible to produce Rainbow Trout that are resistant to both pathogens, though their resistance to *M. cerebralis* still needs to be evaluated. More research is needed to determine if other strains not included in these experiments can be used to create fish resistant to both pathogens. Until then, hatchery outbreaks of BCWD in susceptible fish such as the HxH can be reduced by maintaining high water quality, flows, and reduced densities to prevent stressful rearing conditions. Using PRRs in hatcheries where *F. psychrophilum* outbreaks are common will help reduce mortality from BCWD on the unit, but due to their susceptibility to WD, these fish should not be stocked in aquatic systems in which *M. cerebralis* is established.

Associated Literature

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.