Sport Fish Research Studies

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

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BELLVUE FISH RESEARCH HATCHERY PRODUCTION AND RESEARCH UPDATES

The Hofer (GR or HOF; used interchangeably throughout 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 the middle of November 2019, BFRH personnel checked all of the two- and three-year-old GR and HL brood fish weekly for ripeness. Maturation was indicated by eggs or milt flowing freely when slight pressure was applied to the abdomen of the fish. The first females usually maturated two to four weeks after the first group of males. As males were identified, they are moved into a separate section of the raceway to reduce handling and fighting injuries. On November 13, 2019, the first group of GR females were ripe and ready to spawn.

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

Water-hardened fertilized (green) eggs from the GR and HL were moved to the BFRH main hatchery building. Extreme caution was used to keep each strain separate from the other. Upon reaching the hatchery, green eggs were tempered and disinfected (PVP Iodine, Western Chemical Inc., Ferndale, Washington; 100 ppm for 10 min at a pH of 7). Eggs were then put into vertical incubators (Heath Tray, Mari Source, Tacoma, Washington) with five gallons per

minute (gpm) of 12.2ºC (54ºF) of 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 min) 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 2019-2020 spawning season**.**

Strain	Date Spawned	No. Spawned Females	No. Green Eggs	No. Eyed Eggs	Destination
HL	11/13/19-1/14/20	121	1,210	968	BFRH
GR	11/13/20-12/10/20	100	1,000	850	BFRH
Total	11/13/19-1/14/20	221	2,210	1,818	

The BFRH 2019-2020 on-site Rainbow Trout production spawn started on November 13, 2019, with the last groups of HL females spawned on January 14, 2020 (Table 1.1). The goal was to produce 1,000 eggs per strain for brood stock replacement purposes. There were no GR or HL production requests for Colorado in 2019-2020.

On January 7, 2020, multiple strains and crosses were made for a bacterial coldwater disease experiment being conducted by Colorado State University Ph.D. student, Brian Avila (see Collaborative Research Projects with Colorado State University section of this report). These groups included: Pure GR, Pure HL, GR × fifth generation West Virginia *Flavobacterium psychrophilum*-resistant Rainbow Trout (WV5), HL×WV5, GR×CPW-reared *F. psychrophilum*-resistant Rainbow Trout (PRR), and HL×PRR. The BFRH also received pure WV5 eyed eggs from the USDA-ARS National Center for Cool and Cold Water Aquaculture and pure PRR eyed eggs from the CPW Crystal River Hatchery. One problem that arose during the spawn was a lack of motility in milt shipped from the other two facilities. This resulted in lower-than-usual fertilization rates, which in turn required a second spawn attempt to create more HL×PRR. The second attempt was successful at producing all of the HL×PRR eggs needed for the experiment.

Annual Disease Testing

Over the last five years, the BFRH has conducted a brood stock cull program to reduce and/or 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 by discarding eggs and progeny whose parents tested

positive for the bacteria. The annual BFRH disease inspection 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 florescent antibody test (DFAT) kidney tissue sampling protocols. Two follow-up inspections, with sampling dates yet to be set, will be used to determine if the BFRH will maintain a certified BKD-negative status. A negative status will allow the facility to stock fish into Colorado waters, which has not occurred since finding *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.

Three single male-female pair families of Brown Trout were spawned at North Delaney Lake on October 4, 2019, and the eggs were transported to the BFRH for use in the YY experiment. The first phase of the experiment currently being conducted at the BRFH involves the use of estradiol (E2)-coated feed to develop neo-females, which will subsequently be used to develop and produce the supermale brood stock (Schill et al. 2016). The 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 (total amount of E2 used in the study was 20 mL). Each treatment had four replicate tanks with 200 fish per tank, 800 fish total per treatment. The E2-coated feed was given to the fish immediately at swim-up which occurred on December 3, 2019. The 30 day treatment concluded on January 1, 2020, and the 60 day treatment concluded on January 31, 2020, at which time fish were switched over to a normal (no top coating) trout diet.

There were notable growth difference between the three treatments in the first 60 days. Control fish grew the largest on the ethanol-coated feed, and the fish exposed to E2 for 60 days were quite a bit smaller than the control fish, with the fish exposed to E2 for 30 days falling between the two. After concluding the E2 top-coated feed portion of the experiment, the two treatment groups started to catch up in growth to each other and the control group.

On March 24, 2020 each Brown Trout tank was reduced to 75 fish. Prior to reduction, five fish from each tank (60 total) were collected and a small incision made from the vent upward into the interperitoneal cavity to preserve fish in 10% neutral buffered formalin for later histological analyses. An additional five fish from the control and 60-day exposure treatments were uniquely fin-clipped and isolated to serve as "canary" fish, which will be used to examine gonadal development in the fall of 2020. The remaining 75 fish were measured (mm), weighed (g), PIT tagged using individual 12 mm pre-loaded tags, and a fin clip was taken for genetic analysis. Overall, tag loss was normal and mortality was negligible.

On April 21, 2020 all Brown Trout were moved into the main hatchery facility from FR2 were the feeding portion of the experiment had occurred. Fish will remain separated in troughs by treatment within the main hatchery facility until further testing can occur after the development of ovaries in the fall. The canary fish will be used to detect ovary ripeness and development prior to sampling the PIT-tagged fish to prevent a reduction in the experimental groups needed for further production beyond 2020.

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. Additionally, the methods for spawning and rearing sport fish are continuously evolving, especially as new strains or species are brought into the hatchery system. Experiments conducted under culture conditions can help improve hatchery survival, growth, the quality and quantity of fish stocked, and post-stocking survival.

2019 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 2019, with the mark run occurring on May 6, 2019, and the recapture run occurring on May 8, 2019. Two raft-mounted, fixed-boom electrofishing units were used to conduct the population estimates. All fish captured on the mark run were given a caudal fin punch for identification during the recapture run, measured (mm), and returned to the river. On the recapture run, fish were examined for the presence of a caudal fin punch, measured (mm), and weighed (g). Population estimates were calculated using the Lincoln-Peterson estimator with a Bailey (1951) modification, which accounted for fish being returned to the population following examination of marks on the recapture run, making them potentially available for subsequent recapture.

An estimated 7,305 (± 417) adult Brown Trout were present in the Chimney Rock/Sheriff Ranch study section in 2019, 1,200 less than in 2018 (Fetherman et al. 2018). Overall, 1,873 (± 107) Brown Trout were present per mile in the study section, averaging 318 (± 55) mm total length (TL) and 321 (± 124) 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 2.1).

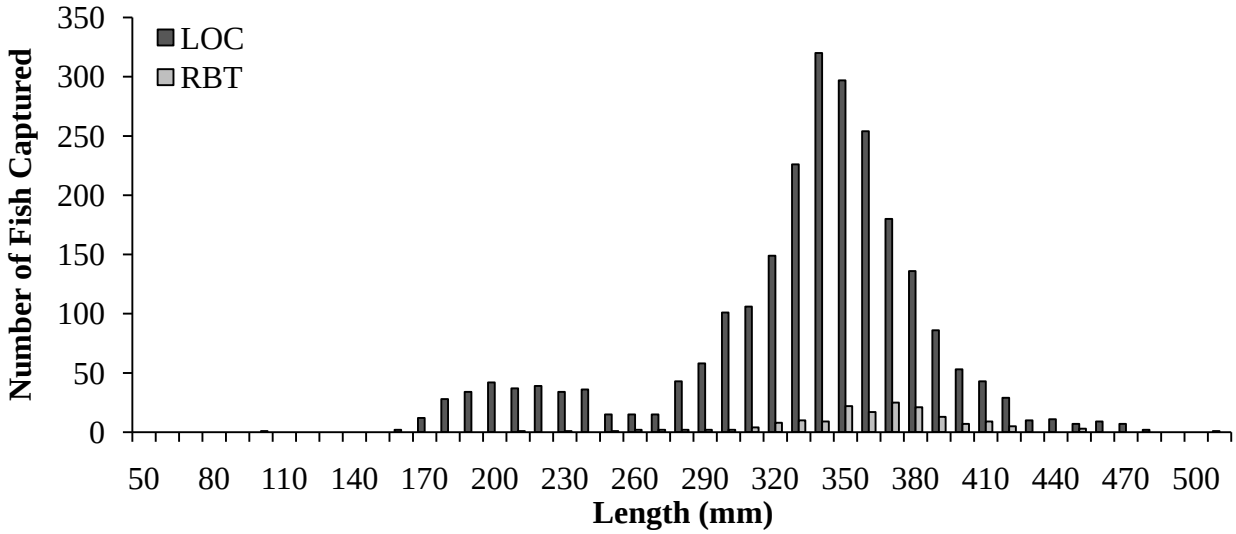

Figure 2.1. Number of Brown Trout (LOC) and Rainbow Trout (RBT) captured by total length (mm) during the 2019 adult salmonid population estimates in the Chimney Rock/Sheriff Ranch study section of the upper Colorado River.

Rainbow Trout densities decreased between 2018 and 2019, with an estimated 513 (± 65) adult Rainbow Trout present in the study section in 2018 (Fetherman et al. 2018), and 426 (± 79) present in 2019. Although the Rainbow Trout population in the upper Colorado River had exhibited an increase in abundance between 2013 and 2017, the lower survival rates exhibited by the Hofer (GR) fry (Fetherman et al 2018) resulted in fewer adult Rainbow Trout present in the study section, with an estimated 109 (± 20) present per mile in 2019 (Figure 2.2). Adult

Rainbow Trout averaged 354 (± 44) mm TL and 436 (± 115) g, larger than the average size Rainbow Trout encountered in 2018 (Fetherman et al. 2018), likely a result of the high number of age-3+ Rainbow Trout captured during the 2019 population estimates, relative to the other age classes (Figure 2.3). Very few fish were captured less than 260 mm, suggesting that in addition to low fry survival, GR fish were not recruiting to the adult population. To support this, age-2 fish (150-300 mm TL) were less prevalent in the population than in previous years following Hofer by Colorado River Rainbow (H×C) fry stocking. The age-3+ Rainbow Trout population also decreased in 2019, likely due to the lack of recruitment of GR fish to the adult population and loss of older H×C fish which were suspected to have constituted a large proportion of the adult population in previous years (Fetherman et al. 2018; Figure 2.4).

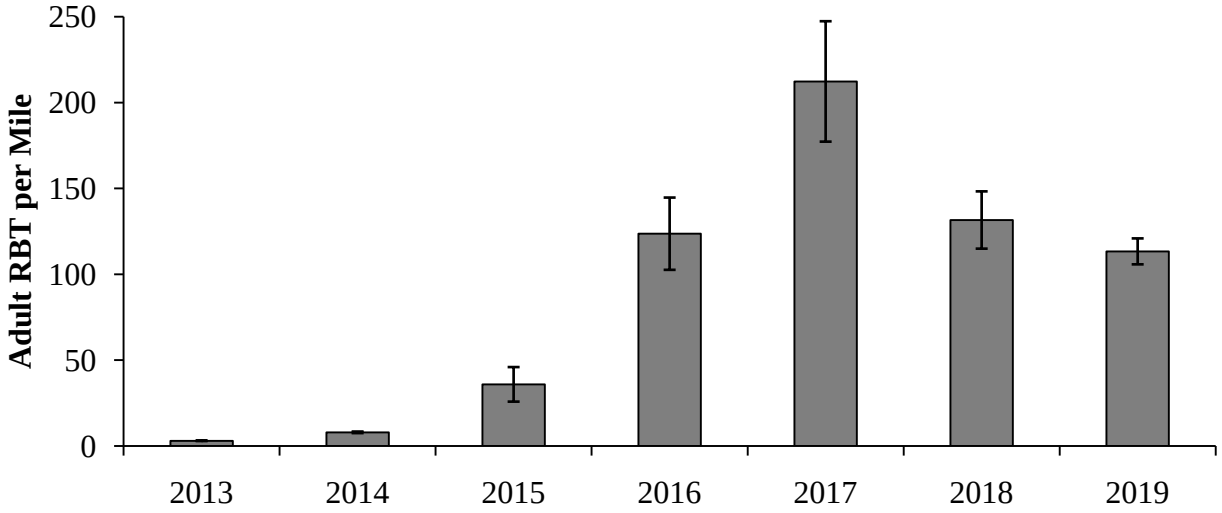

Figure 2.2. Estimated number of adult Rainbow Trout (RBT) per mile (SE bars) in the Chimney Rock/Sheriff Ranch study section of the upper Colorado River between 2013 and 2019.

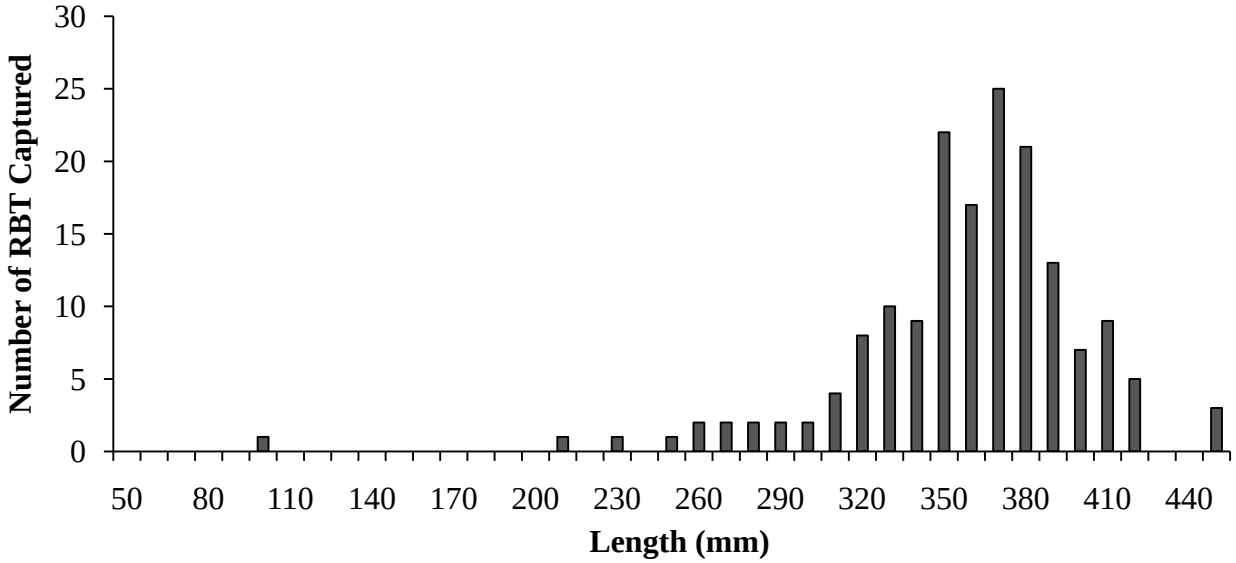

Figure 2.3. Number of Rainbow Trout (RBT) captured by total length (mm) during the 2019 adult salmonid population estimates in the Chimney Rock/Sheriff Ranch study section of the upper Colorado River.

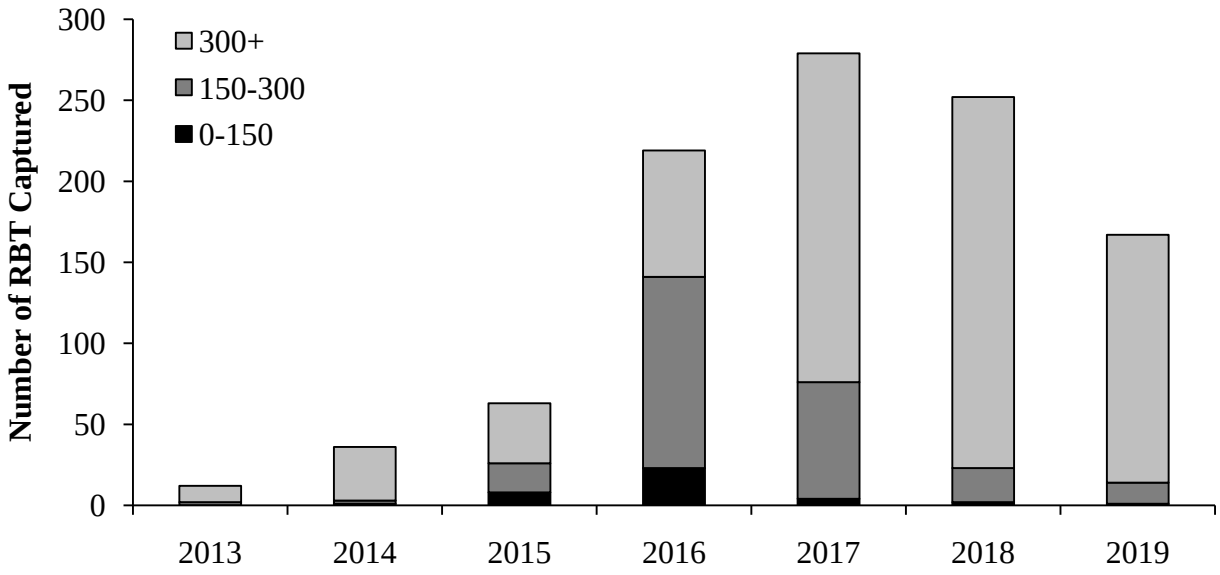

Figure 2.4. Number of age-1 (≤ 150 mm TL), age-2 (150-300 mm TL) and age-3+ (> 300 mm TL) Rainbow Trout (RBT) captured in the Chimney Rock/Sheriff Ranch study section of the upper Colorado River between 2013 and 2019.

The adult Rainbow Trout population in the upper Colorado River has exhibited a decline in abundance since 2017. The reduced number of age-2 fish, along with the lower observed fry abundances following GR fry stocking (Fetherman et al. 2018), suggest that the GR fry do not survive as well as the H×C fry in the upper Colorado River. This is in contrast to results from Avila et al. (2018) suggesting similar survival rates between GR and H×C fry, however, that study was conducted in smaller streams with less competition and predation. The Brown Trout population also experienced a decline in the previous two years, suggesting that environmental factors, possibly associated with river flows and/or temperatures, may have contributed to the declines in both species. The GR fry stocking study was concluded in 2018, and a new strain of Rainbow Trout, the Hofer by Gunnison River Rainbow (H×G) was stocked in 2019. The H×G fry exhibited high survival rates in its first year in the river (see 2019 Salmonid Fry Population Estimates section below). With survival rates of the H×G fry similar to those of the H×C fry stocked between 2013 and 2015, it is probable that the H×G will perform as well as the H×C, continue to persist in the river, recruit to the adult population, and potentially reverse the decline in the adult Rainbow Trout population observed in the past two years.

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

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

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

2019 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 2.5) was stocked with 100,000 to 250,000 H×C fry annually. Due to disease issues within CPW hatcheries in late 2015, H×C fry were not available for stocking in 2016. Recent studies showed that the 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. On August 5, 2019, approximately 46,000 H×G fry were stocked into the upper Colorado River between Hitching Post Bridge and Lower Red Barn on the Chimney Rock Ranch (Figure 2.5). 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 the irrigation diversion structure located at 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.

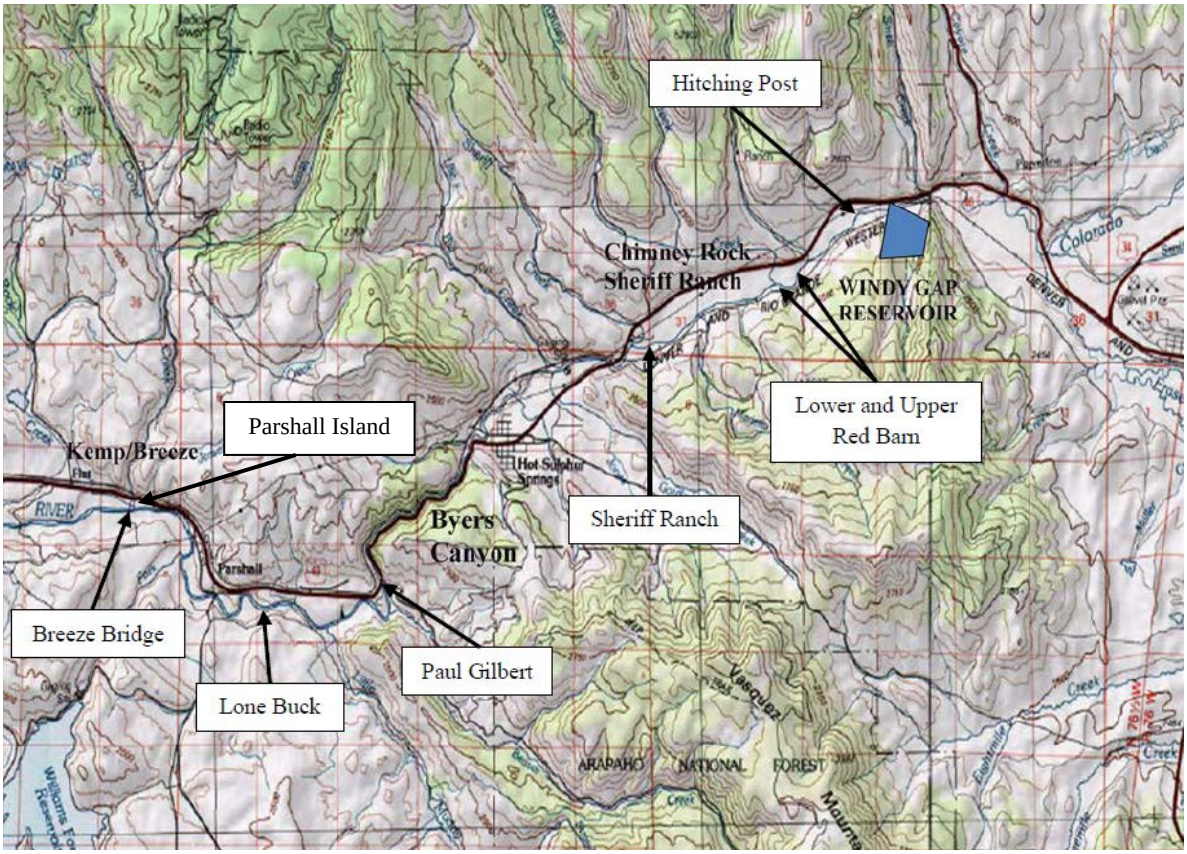

Figure 2.5. Upper Colorado River study area showing the eight sites at which salmonid fry population estimates were conducted in July, August, September, and October 2019.

Pre-stocking fry population estimates were conducted at eight sites in the upper Colorado River in early and late July, and post-stocking fry population estimates were conducted at the end of August, September, and October 2019. 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 August, September, and October provided information regarding the post-stocking survival of the H×G fry and survival of wild Brown Trout fry. 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 wild fry to those of the stocked H×G. Sampling sites (n = 4) below Byers Canyon include 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 2020 (Figure 2.5). The Colorado River below Byers Canyon had been stocked with H×C fry between 2010 and 2015, but no fry were stocked in 2019 to allow evaluation of natural reproduction and determine if there was evidence for a self-sustaining Rainbow Trout population in this section of the river. Sampling sites (n = 4) in the Chimney Rock/Sheriff Ranch study section include the Sheriff Ranch, Lower and Upper Red Barn, and the Hitching Post Bridge (Figure 2.5), historical sites used to evaluate fry production and survival in this section.

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 long study sites, and fry were removed on each pass. All salmonid fry encountered were measured (mm) and returned to the site. In October 2019, genetic samples were collected from up to five wild Rainbow Trout fry at each site, and five Brown Trout and up to five Rainbow Trout were also collected from each site to obtain myxospore counts. Myxospore enumeration was completed at the CPW Aquatic Animal Health Laboratory (Brush, Colorado). Fry density estimates were calculated using the three-pass removal equations of Seber and Whale (1970).

Brown Trout fry abundance estimates were lower in July as a result of high water affecting detection during the first two sampling occasions, increasing as flows were reduced later in the year. An estimated 2,577 (± 389) Brown Trout fry per mile were still present in October (Figure 2.6). Wild Rainbow Trout fry densities below Byers Canyon were highest in late August (143 ± 85), but dropped to an estimated 63 (± 51) Rainbow Trout fry per mile in October. Pre-stocking, wild Rainbow Trout fry densities above Byers Canyon were similar to those below Byers Canyon (Figure 2.6). Rainbow Trout fry densities above Byers Canyon, which were comprised mostly of stocked H×G fry, peaked at the end of August. Densities were reduced between August and October, but were significantly lower than Brown Trout fry densities only in October. By the end of October, stocked Rainbow Trout fry densities above Byers Canyon were much higher than those below Byers Canyon (Figure 2.6).

Brown Trout fry averaged 10,775 (± 2,583) myxospores per fish. None of the 40 Brown Trout collected for myxospore enumeration exhibited signs of disease. However, nine other Brown Trout encountered in October 2019 did exhibit signs of disease including opercular and spinal deformities, and black tail. Rainbow Trout fry averaged 1,805 (± 1,500) myxospores per fish, lower than in previous years (Fetherman et al. 2018). Disease signs were observed in 23% of the

21 Rainbow Trout fry collected for myxospore enumeration in October 2019. Signs of disease in rainbow trout included opercular, cranial, and lower jaw deformities, and exophthalmia. Two other Rainbow Trout fry encountered at Hitching Post Bridge exhibited opercular deformities.

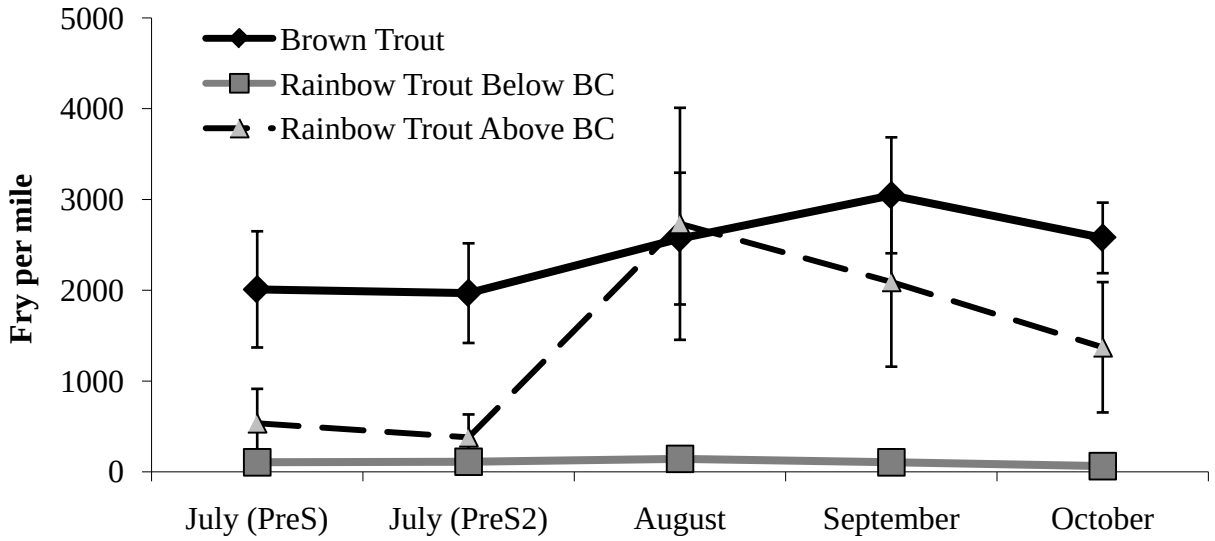

Figure 2.6. Upper Colorado River Brown Trout fry abundance estimates (fry per mile; SE bars) averaged across all seven sampling sites, and Rainbow Trout fry abundance estimates (fry per mile; SE bars) above and below Byers Canyon (BC) for the July pre-stocking sampling occasions (PreS and PreS2), as well as sampling occurring after the H×G fry had been stocked at the end of August, September, and October 2019.

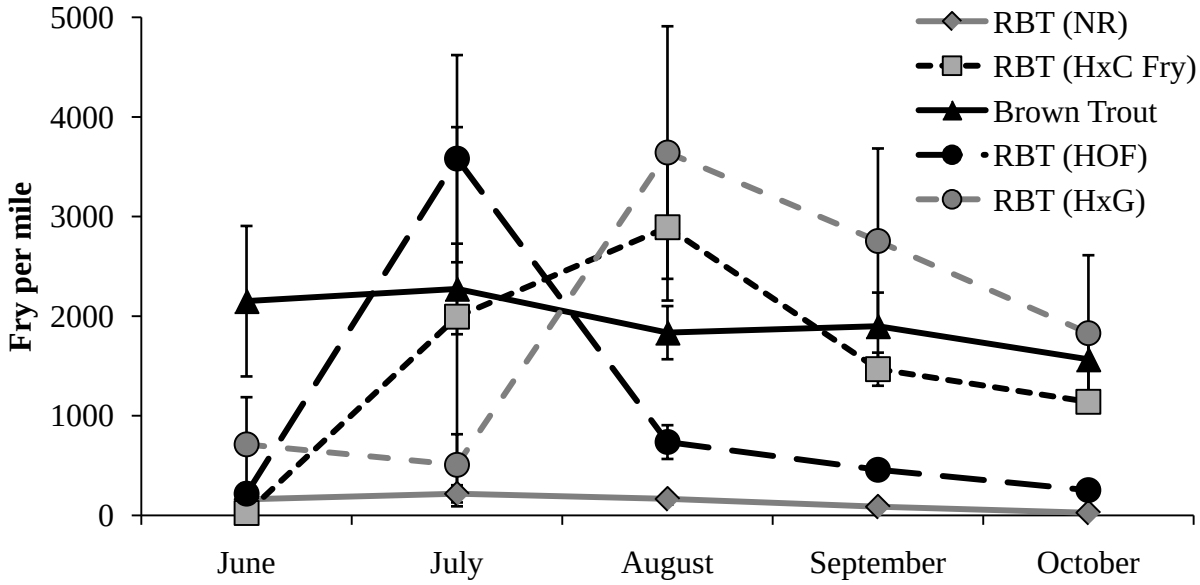

Figure 2.7. Upper Colorado River Brown Trout and wild Rainbow Trout (RBT [NR]) fry abundance estimates averaged between 2013 and 2019, H×C fry (RBT [H×C Fry]) abundance estimates averaged between 2013 and 2015, GR fry (RBT [HOF]) abundance estimates averaged between 2016 and 2018, and H×G fry (RBT [H×G]) abundance estimates from 2019 (fry per mile; SE bars).

H×G fry abundance was higher in 2019 than had been observed for the H×C fry between 2013-2015 and the GR fry between 2016-2018 (Figure 2.7). The abundance of H×G fry is especially notable when considering that fewer H×G fry were stocked in 2019 (~46,000) than either the GR (~60,000-70,000) or H×C fry (100,000-250,000) in previous years. Additionally, H×G fry abundance was similar to that of Brown Trout in 2019. Although the recruitment rate of the H×G fry to the adult population is still unknown, the results from the 2019 fry population estimates are promising, especially if the H×G perform similar to the H×C in future years. H×G fry stocking will continue in 2020 and 2021, with adult population estimates used to estimate fry recruitment rates continuing into 2022.

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

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

2020 Adult Salmonid Population Estimates

An adult salmonid population estimate was conducted in the Chimney Rock/Sheriff Ranch study section of the upper Colorado River in May 2020, with the mark run occurring on May 5, 2019, and the recapture run occurring on May 7, 2019. All fish marking, measuring, and abundance analyses were conducted in the same way as for the 2019 adult salmonid population estimates.

An estimated 7,045 (± 654) adult Brown Trout were present in the Chimney Rock/Sheriff Ranch study section in 2020, approximately 250 less than in 2019. Overall, 1,806 (± 168) Brown Trout were present per mile in the study section, averaging 325 (± 53) mm total length (TL) and 366 (± 151) g. All age classes of Brown Trout were represented in the sample, but the majority of the Brown Trout captured were age 3+ (Figure 2.8).

Rainbow Trout densities decreased between 2019 and 2020, with an estimated 426 (± 79) adult Rainbow Trout present in the study section in 2019, and 295 (± 95) present in 2020. Although the Rainbow Trout population in the upper Colorado River had exhibited an increase in abundance between 2013 and 2017, the lower survival rates exhibited by the GR fry (Fetherman et al 2018) resulted in fewer adult Rainbow Trout present in the study section, with an estimated 76 (± 25) present per mile in 2020 (Figure 2.9). Adult Rainbow Trout averaged 344 (± 101) mm TL and 485 (± 172) g, slightly smaller than in 2019, potentially due to the larger number of age-1 fish handled than had been captured in previous years (Figure 2.10). Although several age-1

H×G fish were caught during the estimates, age-2 fish (150-300 mm TL) were less prevalent in the population than in previous years, representing a similar, if not smaller proportion of the population compared to the age-1 fish. The age-3+ Rainbow Trout population also decreased in 2020, likely due to the lack of recruitment of GR fish to the adult population and loss of older H×C fish which were suspected to have constituted a large proportion of the adult population in previous years (Fetherman et al. 2018; Figure 2.11).

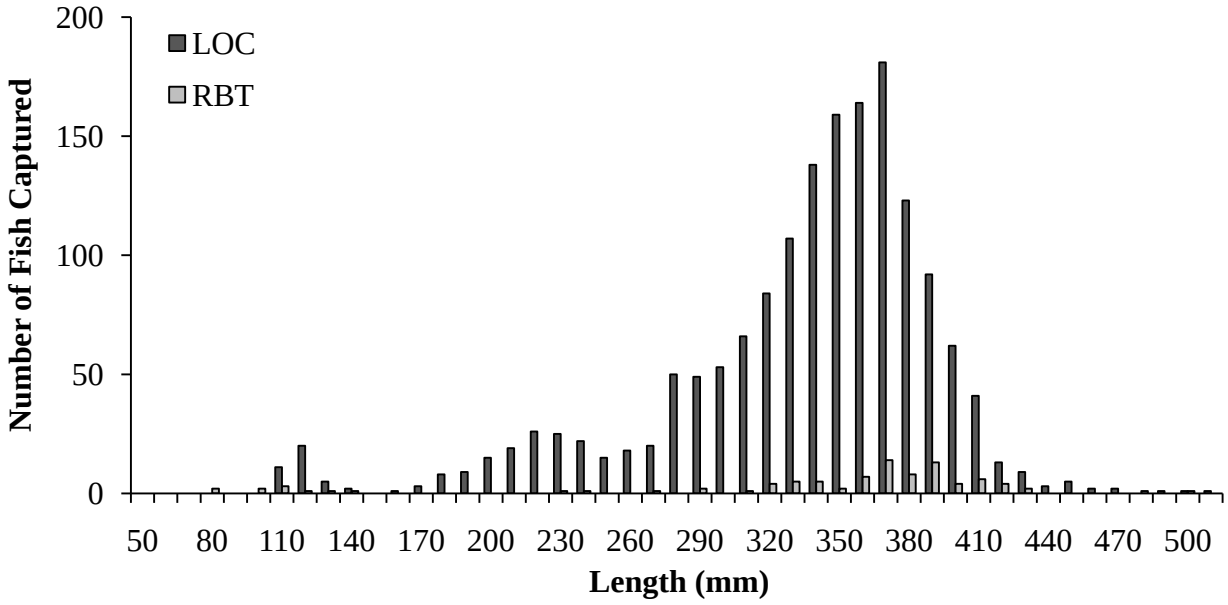

Figure 2.8. Number of Brown Trout (LOC) and Rainbow Trout (RBT) captured by total length (mm) during the 2020 adult salmonid population estimates in the Chimney Rock/Sheriff Ranch study section of the upper Colorado River.

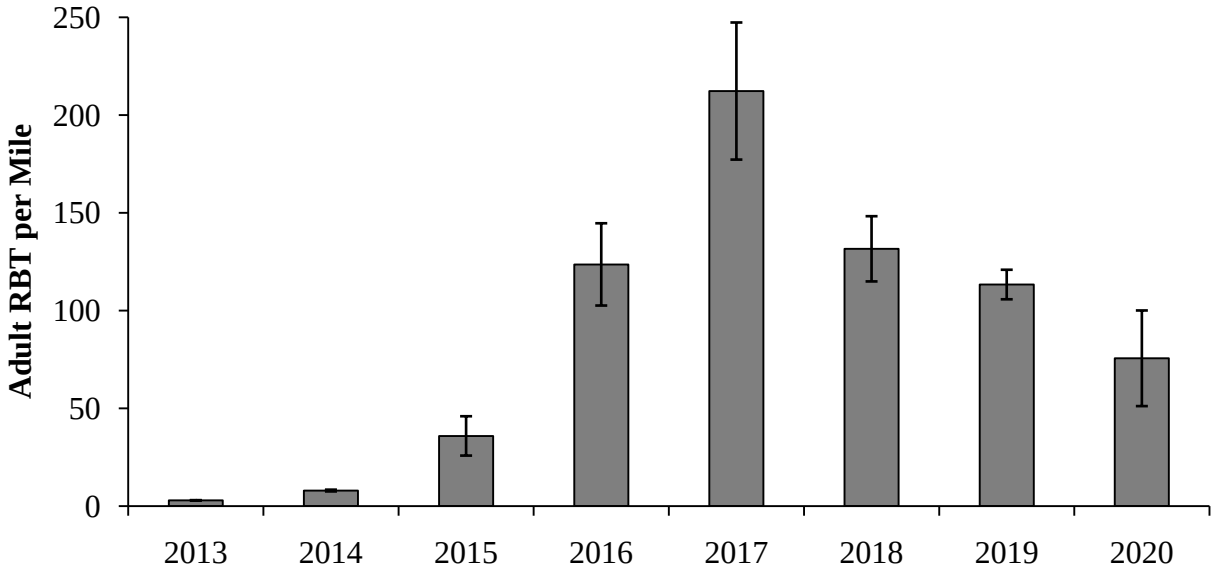

Figure 2.9. Estimated number of adult Rainbow Trout (RBT) per mile (SE bars) in the Chimney Rock/Sheriff Ranch study section of the upper Colorado River between 2013 and 2020.

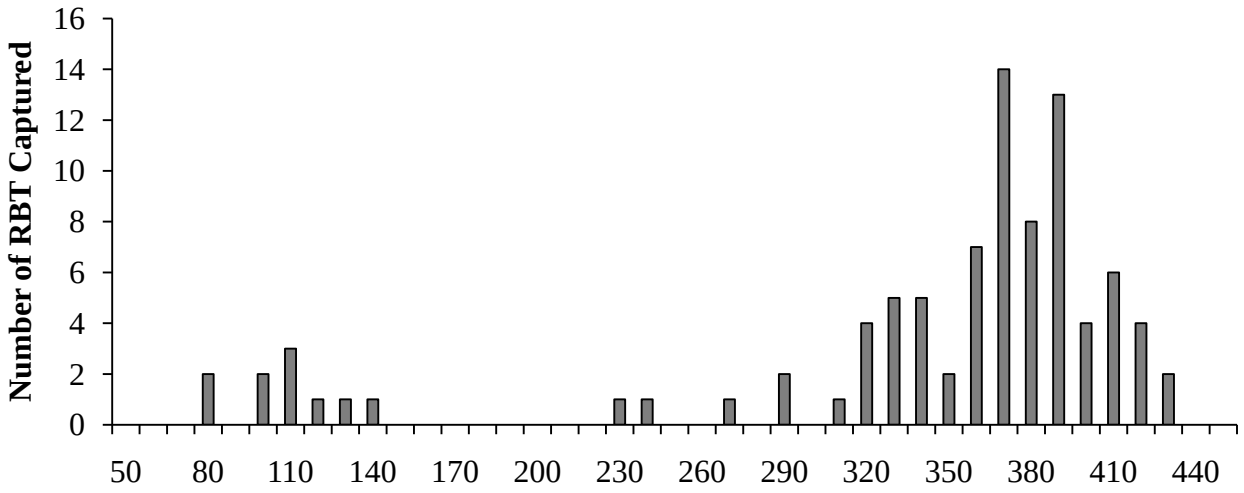

Figure 2.10. Number of Rainbow Trout (RBT) captured by total length (mm) during the 2020 adult salmonid population estimates in the Chimney Rock/Sheriff Ranch study section of the upper Colorado River.

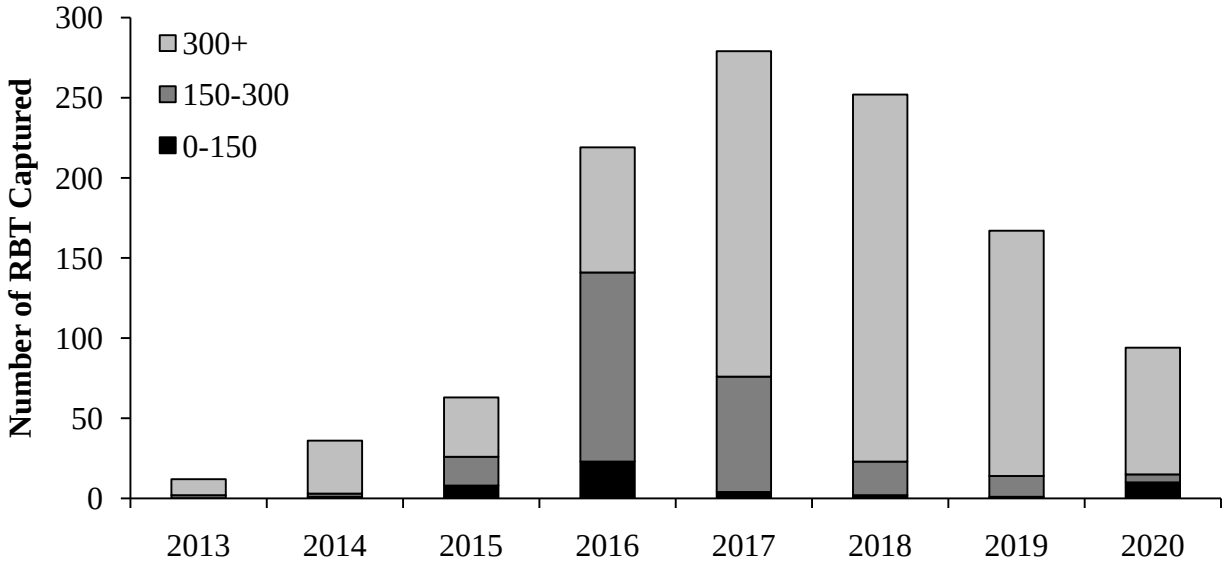

Figure 2.11. Number of age-1 (≤ 150 mm TL), age-2 (150-300 mm TL) and age-3+ (> 300 mm TL) Rainbow Trout (RBT) captured in the Chimney Rock/Sheriff Ranch study section of the upper Colorado River between 2013 and 2020.

The adult Rainbow Trout population in the upper Colorado River has exhibited a decline in abundance since 2017. The reduced number of age-2 fish, along with the lower observed fry abundances following GR fry stocking (Fetherman et al. 2018), suggest that the GR fry did not survive as well as the H×C fry in the upper Colorado River. The Brown Trout population also experienced a decline in the previous three years, suggesting that environmental factors, possibly associated with river flows and/or temperatures, may have contributed to the declines in both

species. The GR fry stocking study was concluded in 2018, and the Hofer by Gunnison River Rainbow (H×G) was stocked in 2019. The H×G fry exhibited high survival rates in its first year in the river (see 2019 Salmonid Fry Population Estimates section). Although fry are usually not targeted by raft electrofishing gear, several H×G fry were collected during the 2020 population estimates, suggesting the increased fry survival from 2019 may have resulted in recruitment to the juvenile size class in 2020. An increase in the age-2 portion of the Rainbow Trout population in 2021 would indicate that this is the case. It is probable that the H×G will continue to persist in the river, recruit to the adult population, and potentially reverse the decline in the adult Rainbow Trout population observed in the past three years. H×G fry will be stocked in the study section in larger numbers in summer 2020.

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

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 being 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 over 30 years. Experimental design and timelines for the study have been approved by all interested parties involved in the upper Colorado River Headwaters Monitoring Plan, and the final draft of the study proposal can be found in Appendix A of this report. The following describes the steps taken to implement the upper Colorado River fish movement study within the last year.

In August 2019, an intergovernmental implementation agreement (IGA) was entered into between CPW and the Windy Gap Firming Project Water Activity Enterprise (WGFP Enterprise), the purpose of which is to provide funds from the WGFP Enterprise to CPW for implementation of the fish movement study. In short, the IGA establishes a project timeline for completion of both the Windy Gap connectivity channel and the fish movement study, the project funding amount and timeline for distribution of funds, and that CPW will be responsible for implementation of the fish movement study. To that end, CPW employees maintained communication with all parties involved regarding updates to the fish movement study and coordination of field implementation, including obtaining site-specific approval for antenna installation sites on the Chimney Rock Ranch and Northern Water properties and coordinating access agreements for these properties. Strong communication from CPW is and will continue to be a key component for implementation of the fish movement study.

During the winter and spring 2019 and 2020, the primary focus of the fish movement study research team, which includes Dr. Eric Fetherman, Eric Richer, and Matt Kondratieff, was design and testing of the stationary antenna stations to be installed in the upper Colorado River in 2020. Three stationary antenna stations will be installed in the river, one located upstream of Windy Gap Reservoir immediately downstream of the Colorado River and Fraser River confluence, and two downstream of Windy Gap Reservoir at the Hitching Post and Red Barn

locations on the Chimney Rock Ranch. Each station will consist of paired river-spanning antennas installed on the bottom of the river. Antennas, which are made of copper wire run through 2-inch schedule-80 PVC pipe to maintain wire spacing and shape, will be anchored to the substrate using duckbill anchors, and held in place by river straps connecting the anchors to the PVC. Using river straps provides flexibility in anchor driving location in the substrate. Antenna wires are connected to tuner boxes, which maintain tuning for optimal read range and are in turn connected to Oregon RFID half-duplex ORSR single readers, which maintain tag detection records. One reader powers each of the paired antennas, and the two readers are connected and programmed to alternate pulse frequencies to prevent proximity detection errors. The readers are powered by a bank of five, 200-Ah lead-acid batteries, and additional power is supplied by solar panels to prevent the need for frequent battery changes. The majority of the equipment needed for these stations was purchased during the winter and spring of 2019 and 2020, test stations were built at the BFRH (Figure 2.12), and fabrication of additional stations occurred in Fort Collins prior to transport up to the Colorado River installation sites.

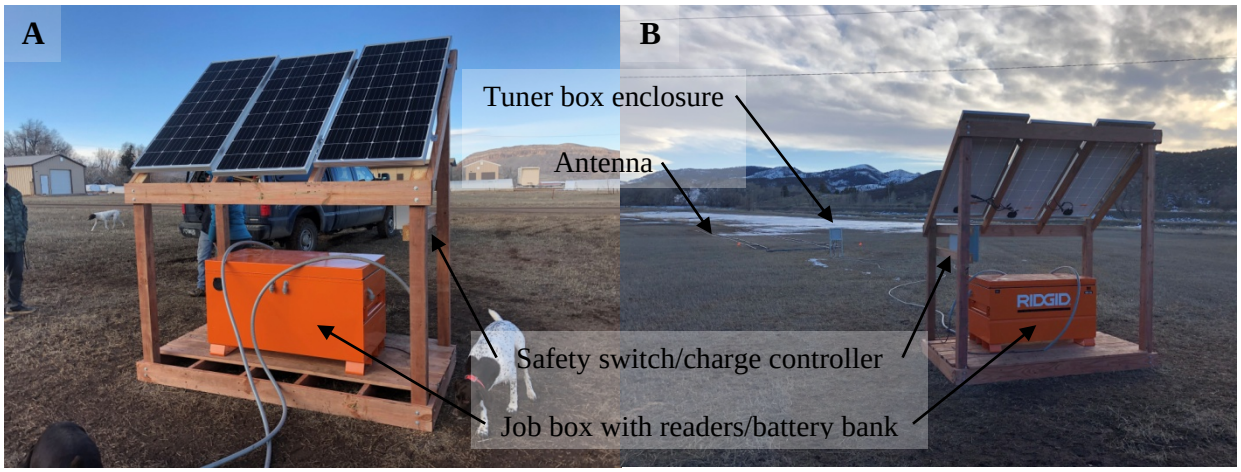

Figure 2.12. Primary stationary antenna station (panel A), composed of three solar panels mounted on a wooden frame and connected through a safety switch and charge controller to a battery bank, housed in the job box and used to power the antenna readers also housed in the job box. Twin axial cable connects the readers through the back of the job box to tuner boxes located in an enclosure about 70 feet from the primary antenna station (panel B). Six gauge or 1/0 welding cable connected to the tuner boxes and run through 2-inch schedule 80 PVC forms the antennas that will be installed on the bottom of the Colorado River.

Testing of the antenna stations was conducted in two phases. The first phase of testing was used to select the antenna wire gauge needed for optimal passive integrated transponder (PIT) tag read range. Larger antennas require larger wire due to resistance and the power needed to generate the pulsing electromagnetic field that reads the PIT tags and transmits their codes for storage in the reader database. Two of the river-spanning antenna stations being installed in the upper Colorado River are 100 feet long, while the third is 130 feet long. It was likely that the wire size requirements would differ between the 100 and 130 foot sites. Additionally, wire spacing can affect antenna read range as well as the detection field, the electromagnetic field generated by the antenna. Wire spacing of one, two, and three feet was tested with each wire size to determine optimal detection and antenna design criteria.

For the 100 foot antennas, 8 AWG copper speaker wire with a three foot width spacing and 6 AWG welding cable with a two foot width spacing produced similar results for average and maximum PIT tag read range (Table 2.1). However, the inductance for the 6 AWG configuration was lower than the 8 AWG configuration, and fell within a more tunable range using the Oregon RFID manual tuners. Therefore, the design criteria of 6 AWG welding cable with two foot width spacing was chosen for construction of the 100 foot antennas. For the 130 foot antennas, optimal average and maximum read ranges were obtained using 1/0 welding cable with two foot width spacing (Table 2.1).

Table 2.1. Testing results for antenna wire size (AWG), width spacing (feet), inductance (μH), max load (amps), average read range (feet ± SD), and maximum read range (feet ± SD) for 100 and 130 foot antennas. ND = PIT tag not detectable using the specified configuration.

AWG	Width	Inductance	Max Load	Avg Read Range	Max Read Range
100 Foot Antenna					
8	1	79.9	2.32	0.83 ± 0.64	0.93 ± 0.67
8	2	88.4	2.08	0.94 ± 0.74	1.05 ± 0.78
8	3	93.0	1.60	1.55 ± 0.03	1.75 ± 0.00
6	1	80.0	2.26	0.92 ± 0.44	1.03 ± 0.53
6	2	88.2	2.12	1.58 ± 0.08	1.73 ± 0.04
6	3	94.0	1.84	0.98 ± 0.85	1.13 ± 0.95
1/0	1	70.4	2.72	0.86 ± 0.29	1.03 ± 0.39
1/0	2	79.0	2.19	0.92 ± 0.45	1.00 ± 0.49
1/0	3	84.5	2.26	1.01 ± 0.55	1.13 ± 0.67
130 Foot Antenna					
8	1	95.5	1.92	0.92 ± 0.01	1.03 ± 0.04
8	2	105.2	0.50	0.43 ± 0.16	0.60 ± 0.16
8	3	111.5	0.42	ND	ND
6	1	94.1	1.97	1.12 ±0.01	1.23 ± 0.04
6	2	105.4	0.53	0.70 ± 0.07	0.75 ± 0.07
6	3	110.7	0.42	0.52 ± 0.04	0.58 ± 0.04
1/0	1	82.4	2.16	0.82 ± 0.50	0.90 ± 0.56
1/0	2	93.8	2.15	1.32 ± 0.13	1.46 ± 0.18
1/0	3	101.5	0.67	0.83 ± 0.16	1.00 ± 0.29

The second phase of testing investigated the effect of purchased solar panels on noise generation at the readers and PIT tag detection distance at the antennas. Solar panels were selected to generate enough power to recharge battery banks at the stationary antenna sites given the max load (amps) measured in the first phase of testing (Table 2.1). Noise generation from the solar panels was a concern given that panels used in previous experiments generated noise that affected read range. To test detection ranges at the antennas, solar panels were incorporated into the stationary antenna setup at the BFRH (Figure 2.12), and average maximum and continuous detection distances were measured vertically from the antennas under three different scenarios: 1) solar panels on and safety switch/charge controller on, representing a fully operational antenna site, 2) solar connected but switch off, representing antennas collecting solar energy but not charging the battery bank, and, 3) battery power only with solar power and switch disconnected.

Read ranges (feet ± SD) did not differ under the three scenarios (scenario 1: maximum = 0.7 ± 0.1, continuous = 0.6 ± 0.1; scenario 2: maximum = 0.6 ± 0.2, continuous = 0.5 ± 0.1; scenario 3: maximum = 0.7 ± 0.2, continuous = 0.6 ± 0.2), suggesting that the solar panels purchased for the experiment would adequately charge the battery bank while not generating noise that would affect PIT tag read range.

Table 2.2. Model selection results for factors influencing maximum detection distance of PIT tags along a 6 AWG, 100-foot antenna. The maximized log-likelihood (log[L]), the number of model parameters (K), and Akaike's information criterion corrected for small sample sizes (AIC_c) are shown for each model. Models are ranked based on the AIC_c difference (ΔAIC_c) relative to the best model in the set. Akaike weights (w_i) quantify the probability that a particular model is the best model in the set given the data and the model set.

Model	R^2	Log(L)	K	AIC_c	ΔAIC_c	w_i
Tag size + Orientation + Plane	0.69	302.31	8	-588.04	0.00	1.00
Tag size + Orientation	0.62	274.76	4	-541.37	46.67	0.00
Orientation + Plane	0.53	248.99	6	-485.63	102.41	0.00
Orientation	0.46	230.17	2	-456.30	131.74	0.00
Tag size + Plane	0.23	185.43	6	-358.53	229.51	0.00
Tag size	0.16	173.66	2	-343.27	244.77	0.00
Plane	0.07	161.31	4	-314.46	273.58	0.00
Intercept	0.00	151.48	1	-300.95	287.09	0.00

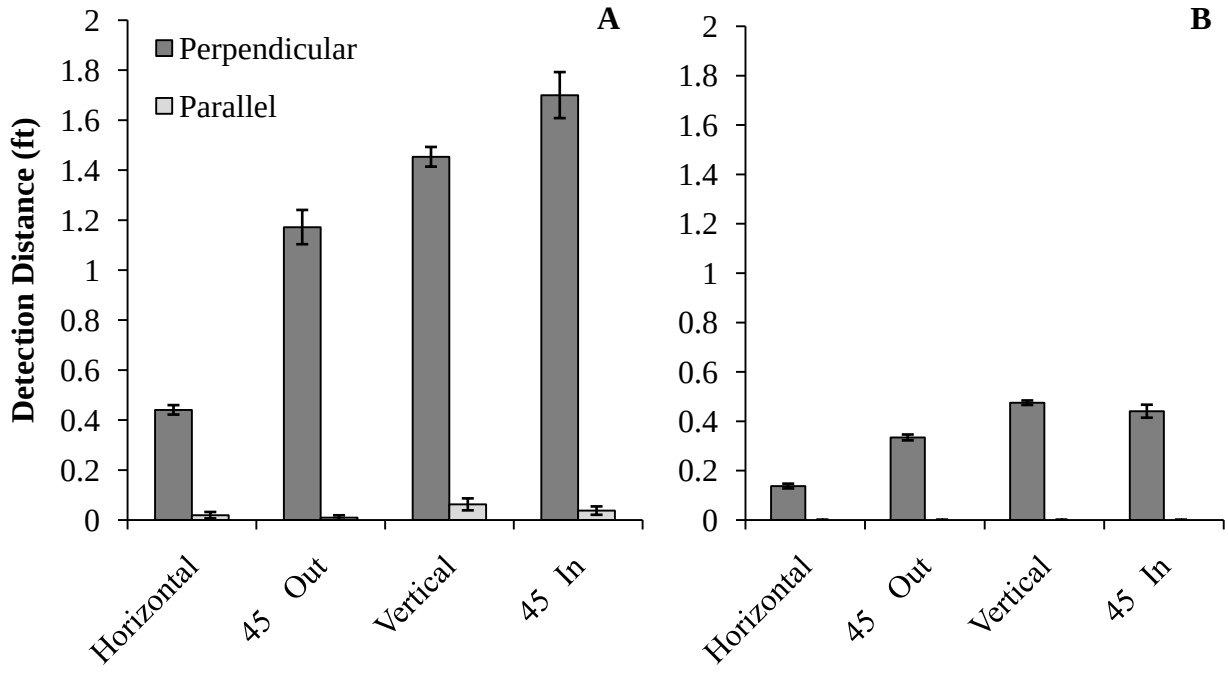

Figure 2.13. Average maximum detection distances (± SE) for the 6 AWG, 100-foot antenna with 32 mm (panel A) and 12 mm (panel B) PIT tags oriented perpendicular and parallel to the antenna wire in the horizontal, 45° outward, vertical, and 45° inward detection planes.

Final tests were conducted on the 6 AWG, 100-foot antenna using 32 and 12 mm PIT tags run at perpendicular and parallel orientations to the antenna wire along the horizontal, vertical and 45° detection planes following the testing and analysis methods of Fetherman et al. (2014) and Richer et al. (2017). Model results suggest that detection distance was influenced by tag size, orientation, and plane of detection (Table 2.2). Detection distances were greater for 32 mm (0.61 ± 0.06 feet) than 12 mm (0.17 ± 0.02 feet) tags, and when the tags of either size were oriented perpendicular (0.77 ± 0.05 feet) versus parallel (0.02 ± 0.01 feet) to the antenna wire, and this pattern held true for all detection planes. With the perpendicular orientation, the optimal orientation for detection, detection distances for the 32 mm tags were greatest for the 45° angle inward detection plane and lowest in the horizontal plane. For the 12 mm tags, there was little difference in detection distances in the vertical or 45° angle inward detection planes, which were both greater than either the horizontal or 45° angle outward detection planes (Figure 2.13).

The testing, development, and fabrication activities implemented by the research team in 2019 and 2020 should facilitate smoother and faster antenna installation in the Colorado River, and require less troubleshooting time to get stations up and running, when conditions allow later in 2020. Antenna installations are scheduled for early fall 2020. Fish tagging will occur following antenna installations as locations are sampled above and below the reservoir throughout the fall. In addition to stationary antenna data, portable antennas will be run in fall 2020 to confirm movements and increase recapture events for tagged fish. Initial movement data from both antenna types will be available in 2021.

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 37:1249-1264.

Post-stocking Survival of Rainbow Trout in the Yampa River

The comparative survival experiment being conducted in the Yampa River between Stagecoach Reservoir and Lake Catamount was initiated in 2017. The primary goal of the study is 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. The motivation and site description for the experiment can be found in Fetherman et al. 2018. The following describes the methods and results obtained during the third year of the study.

Catchable Rainbow Trout for stocking into the study section were reared at the CPW Rifle Falls Hatchery (Rifle, Colorado). A total of 2,014 Rainbow Trout, averaging 379 (± 40) mm total length (TL) and 676 (± 212) g, were tagged with 32 mm PIT tags on May 15-16, 2019. All fish were anesthetized using tricaine methanesulfonate (MS-222), and PIT tags were inserted into the intraperitoneal cavity using a large tagging needle. Nothing was used to close the insertion location on the fish; previous observations have shown that these locations usually close on their own within 48 hours. Fish were separated into four groups with known tag numbers in each

group: 1) Stagecoach Tailwater (171 fish), 2) Wellar Ranch (465 fish), 3) Service/BLM/Foster's Ranch (709 fish), and 4) Green Creek/Kuntz ranches (669 fish). The number of fish included in each group was standardized based on the length of the sections to which the fish were being stocked such that each section contained the same number of fish per linear foot of river. The four groups were secondarily fin clipped to identify stocking location in the event of tag loss, with a left pectoral clip used for fish stocked into the Stagecoach Tailwater, a left pelvic clip used for fish stocked on the Wellar Ranch, a right pelvic clip used for fish stocked into the Service Creek State Wildlife Area (SWA), BLM, and Foster's Ranch properties, and a right pectoral clip for fish stocked into the Green Creek and Kuntz ranches. PIT-tagged Rainbow Trout were stocked into the Yampa River on June 6, 2019. Groups with known tag numbers were maintained separately on the hatchery truck until stocked, and all fish were stocked by hand in small groups in an attempt to distribute them evenly throughout the entire study section.

Fingerling Rainbow Trout were reared at the CPW Finger Rock Hatchery (Yampa, Colorado). A total of 6,389 Rainbow Trout, averaging 72.4 (± 6.6) mm TL and 4.5 (± 1.3) g, were tagged with coded wire tags (CWT) on June 5, 2019. Numbers of fingerling Rainbow Trout were reduced in 2019 to determine if lower stocking densities would result in similar survival rates, especially given the Brown Trout removals conducted in 2017 and 2018. All fish were anesthetized using MS-222, and a Mark IV tag injector and handheld coded wire tagging guns (Northwest Marine Technology, Shaw Island, Washington) were used to insert tags into the snout of the fish. Additionally, fish were secondarily fin clipped using the same scheme as with the catchable Rainbow Trout. Fish were split into four groups during tagging, and the number of fish per group was calculated using the length of river the fish were being stocked into such that 699 fish were held for the Stagecoach Tailwater, 1,471 fish for the Wellar Ranch, 2,166 fish for the Service/BLM/Foster's Ranch section, and 2,053 fish for the Green Creek and Kuntz ranches. CWT Rainbow Trout were stocked into the Yampa River on June 6, 2019, and evenly distributed throughout the study section in the same manner as the catchable Rainbow Trout.

Two five-electrode catrafts were used to complete the Rainbow Trout recapture events in fall 2019. The Foster Ranch, BLM property, Service Creek SWA, Wellar Ranch, and the Stagecoach Tailwater were sampled using a continuous single pass removal September 9-13, 2019. 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 tags, measured and weighed. All CWT fish stocked and recaptured in 2019 were adipose fin clipped prior to returning them to the river so that a unique encounter history could be created following the next, and final, recapture event in 2020. Additionally, CWT fish stocked in 2017 and 2018 recaptured in 2019 were given a PIT tag to allow continued monitoring of survival beyond the first year, which would not have been possible using previous batch marks. Brown Trout from all sections, with the exception of the Foster Ranch, were removed and euthanized after being measured and weighed. Euthanization of the Brown Trout was necessary in 2019 because Lake Catamount and the Yampa River tested positive for *Renibacterium salmoninarum* earlier in 2019, restricting the ability to move fish out of the study reach. All Brown Trout captured on the Foster Ranch, as well as all other species encountered throughout the study section, including Brook Trout *Salvelinus fontinalis*, Mountain Whitefish *Prosopium williamsoni*, Mottled Sculpin *Cottus*

baridii, and Speckled Dace *Rhinichthys osculus*, 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 estimate the percent of the Brown Trout population removed from each section. All fish captured were removed from the river and held in net pens by pass until they could be processed. PIT-tagged and CWT Rainbow Trout, and all other species of fish captured, were treated in the same manner as described for the single pass removals. All wild Rainbow Trout captured in the standard sampling sites were tagged with 12 mm PIT tags, secondarily adipose clipped for later identification in the event that the tag was lost, and returned to the river. Brown Trout from all standard sampling sites were removed and euthanized. 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. Additionally, the length of the section was used to estimate number of Brown Trout present during the sampling efforts, and using the number of Brown Trout captured in each section, determine the percentage of the Brown Trout population that had been removed during the sampling efforts.

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 14-17, 2019. 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. To test assumptions related to closure of sections during the estimates, fish returned in the upper Green Creek section on the first mark day were upper caudal clipped, and fish returned in the Kuntz and lower Green Creek sections on the second day were lower caudal clipped. Brown Trout removal was also different in the three sections dependent upon study goals and landowner preference. The objective in 2019 was to determine if Brown Trout removal would differentially affect Rainbow Trout survival in the upper and lower sections of the Green Creek Ranch. As such, Brown Trout were not removed on the upper Green Creek Ranch, and all Brown Trout encountered were removed from the lower Green Creek Ranch. To minimize impacts to anglers on the Kuntz Ranch, by request of the landowner, only Brown Trout less than 350 mm were removed from that section of the river. All fish were handled similarly to the methods described above for the single and two-pass removal efforts described above. Wild Rainbow Trout were opportunistically tagged with 12-mm PIT tags and secondarily adipose clipped throughout the Green Creek and Kuntz ranches on both the mark and recapture runs. Population estimates for all fish with the exception of Brown Trout less than 350 mm from the Kuntz Ranch and all Brown Trout from the Lower Green Creek Ranch were calculated using the Lincoln-Peterson estimator with a Bailey (1951) modification. Population estimates for the two sections in which Brown Trout removal occurred were

calculated using the Huggins closed capture-recapture estimator (Huggins 1989, 1991) in program MARK (White and Burnham 1999).

Unique encounter histories for the PIT-tagged Rainbow Trout were created using individual tag numbers and recaptures, whereas batch encounter histories were created for all coded wire-tagged Rainbow Trout captured during the sampling efforts. All encounter histories included four occasions, a release occasion ("1" for all fish released in spring 2017, 2018, or 2019) and three encounter occasions ("1" for all fish encountered and "0" for all fish not encountered in fall 2017, 2018, and 2019). A Cormack-Jolly-Seber open capture-recapture estimator, implemented in Program MARK (White and Burnham 1999), was used to estimate survival and detection probability for both sizes of Rainbow Trout by year. Fish released above and below the Service Creek confluence with the Yampa River were treated as two separate groups in the analysis for fish released in 2017, whereas four release locations were used as groups for fish released in 2018 and 2019, and survival and detection probability was estimated for each location.

Overall, 121 PIT-tagged Rainbow Trout were captured in the Stagecoach Tailwater, 378 were captured on the Wellar Ranch, 448 were captured in the Service Creek SWA/BLM/Foster's Ranch section, and 553 were captured in the Green Creek/Kuntz Ranch section in 2019. PIT-tagged Rainbow Trout stocked in 2017, 2018, and 2019 contributed to the number of fish captured in each section, although very few fish from 2017 were encountered, and fish stocked in 2019 outnumbered fish stocked in 2018 about 2:1. Two hundred thirty-nine CWT Rainbow Trout were captured in the Stagecoach Tailwater, 96 were captured on the Wellar Ranch, 347 were captured in the Service Creek SWA/BLM/Foster's Ranch section, and 25 were captured in the Green Creek/Kuntz Ranch section in 2019. A large majority of the CWT fish captured were stocked in 2019, with 57, 4, 18, and 5 CWT Rainbow Trout stocked in 2018, and only 6, 5, 4, and 4 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.

Rainbow Trout in the Green Creek/Kuntz Ranch section appeared to meet the assumptions of closure during the mark-recapture estimates conducted in October 2019. No Rainbow Trout marked in the Kuntz or lower Green Creek Ranch reaches moved upstream into the upper Green Creek Ranch reach during the estimates. Only four fish marked in the upper Green Creek Ranch were recaptured downstream in the Kuntz Ranch reach (3.7% of fish captured on the Kuntz Ranch), while no fish tagged in upper Green Creek were found in the lower Green Creek Reach. The four individuals found on the Kuntz Ranch were likely processed at the final station on the upper Green Creek Ranch, making very short downstream movements from where they had been processed. Brown Trout moved amongst the reaches during the estimates more than the Rainbow Trout, with 19% of the Brown Trout recaptured in the upper Green Creek reach having moved upstream from the lower two reaches and 8% captured on the Kuntz Ranch having moved down from the upper Green Creek reach. Four fish were recaptured in the lower Green Creek Ranch (where full removal had occurred during the mark run), three of which moved down from the Kuntz Ranch, and one of which moved down from the upper Green Creek Ranch. Using PIT-tagged Rainbow Trout recaptures (known tag numbers for each individual marked and later recaptured), the absolute deviation in length and weight measurements were estimated. Length measurements on an individual fish deviated an average of 5 mm (range 0-25 mm) among repeated measurements, whereas weight deviated an average of 30 g (range 0-260 g).

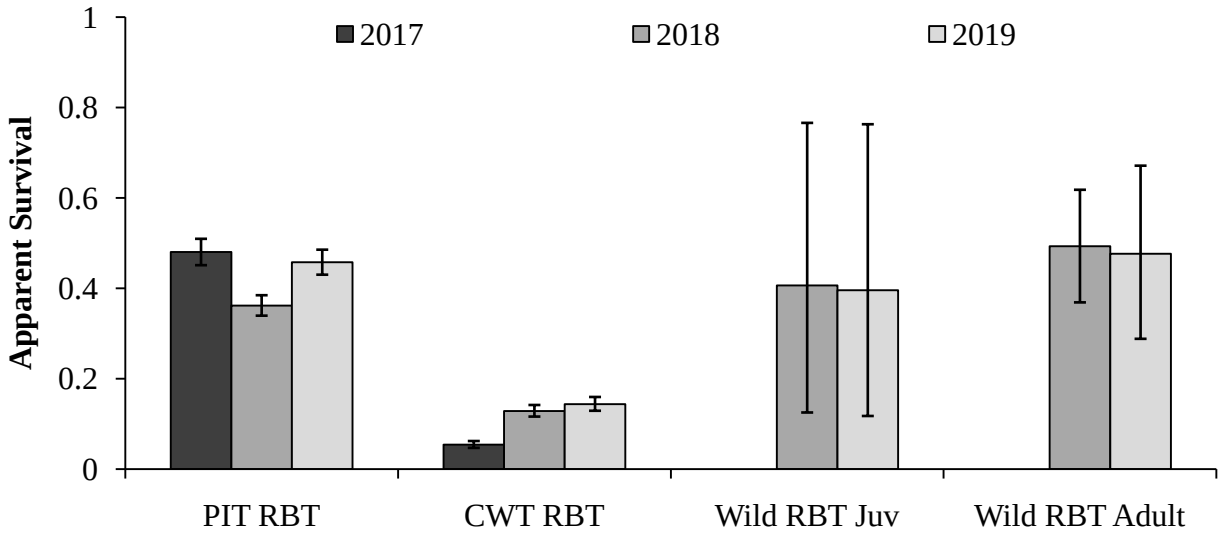

Figure 2.14. Apparent survival estimates (unconditional SE bars) for PIT- and coded wire-tagged (CWT) Rainbow Trout (RBT) stocked in the Yampa River in 2017, 2018, and 2019, and wild Rainbow Trout juveniles (Juv) and adults PIT tagged during population estimates conducted in 2017 and 2018 and recaptured in 2019.

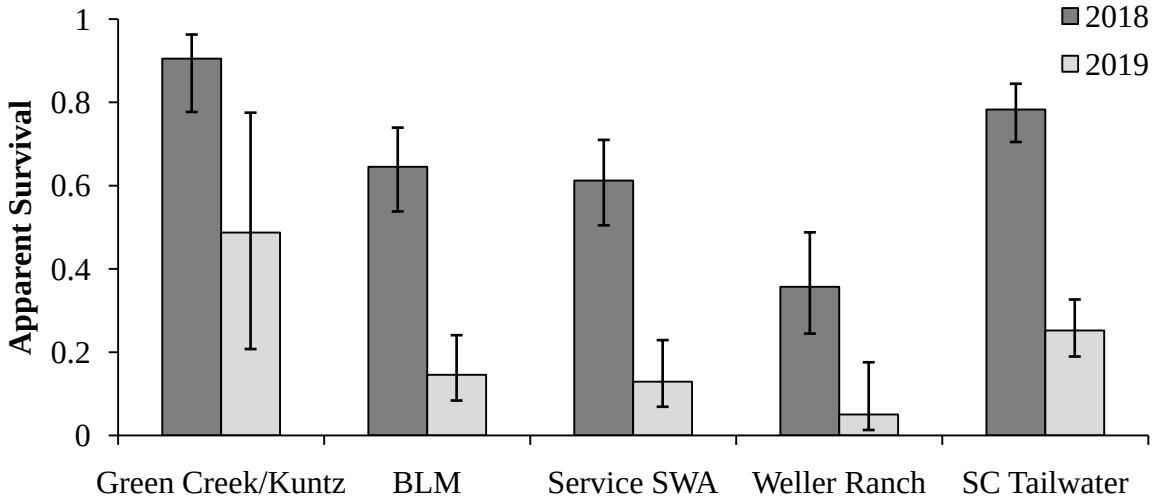

Figure 2.15. Apparent survival estimates (unconditional SE bars) for wild Rainbow Trout of all sizes PIT-tagged during population estimates in 2017 and 2018 and recaptured in fall 2019 in the Green Creek/Kuntz, BLM, Service Creek SWA, Wellar Ranch, and Stagecoach (SC) Tailwater sections of the Yampa River.

On average, PIT-tagged Rainbow Trout survival estimates were lower in 2018 than either 2017 or 2019, but higher in all years than CWT Rainbow Trout survival estimates (Figure 2.14). Coded wire-tagged Rainbow Trout stocked in 2018 and 2019 had higher survival estimates on average than did CWT Rainbow Trout stocked in 2017, with no differences in survival for fish stocked in 2018 and 2019 despite the lower stocking densities in 2019 (Figure 2.14). On average, wild Rainbow Trout juveniles, PIT-tagged in 2017 or 2018 at ≤ 150 mm total length (TL), exhibited similar survival estimates to wild Rainbow Trout adults, those PIT-tagged in

2017 and 2018 at > 150 mm TL, and both groups exhibited similar survival estimates to PIT-tagged Rainbow Trout, but higher than those of the CWT Rainbow Trout (Figure 2.14). Survival estimates were higher for wild Rainbow Trout through all sections of the Yampa River in 2018 compared to 2019, and in general survival was lowest in wild Rainbow Trout on the Wellar Ranch, especially compared to the Green Creek/Kuntz Ranch section (Figure 2.15), likely because of the large differences in habitat between these two sections.

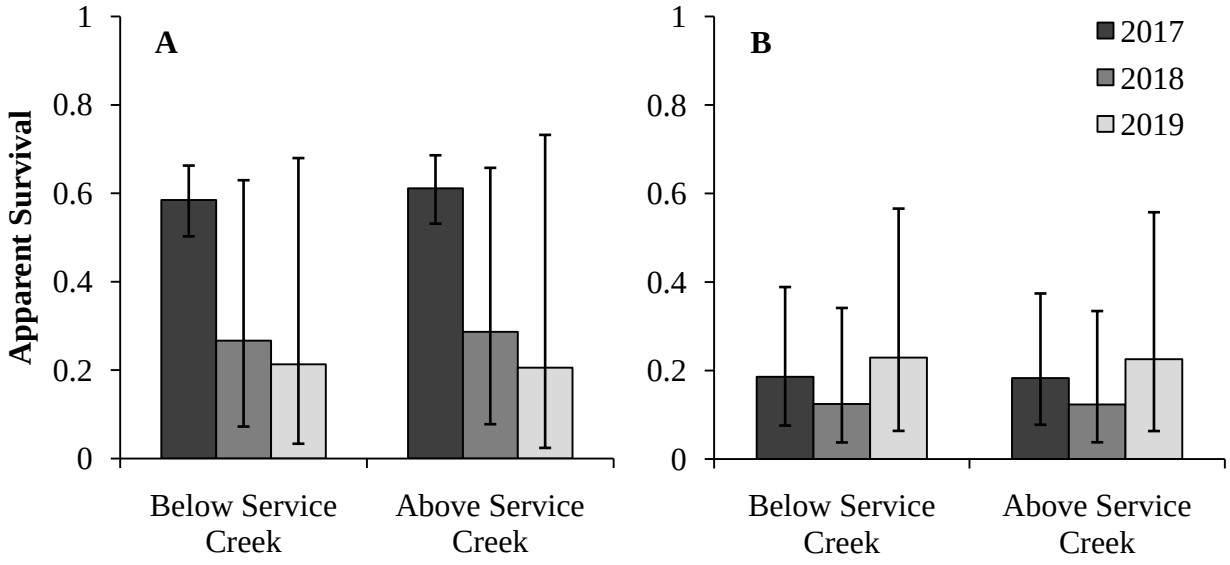

Figure 2.16. Short-term (May-September 2017) and annual (September 2017-September 2018 and September 2018-September 2019) apparent survival estimates (unconditional SE bars) for PIT-tagged (panel A) and CWT Rainbow Trout (panel B) stocked above or below the Service Creek confluence with the Yampa River in spring 2017.

PIT-tagged Rainbow Trout stocked in 2017 exhibited higher short term (May-September 2017) survival estimates in comparison to annual survival estimates (September 2017-September 2018 and September 2018-September 2019), and survival rates were similar for fish stocked above and below the Service Creek confluence with the Yampa River (Figure 2.16). Short-term survival estimates for CWT Rainbow Trout stocked in 2017 were similar to annual survival estimates, and similar above and below the Service Creek confluence (Figure 2.16). Given that these are apparent survival rates, i.e., the fish both survived and were retained and available for recapture in the Yampa River study section, estimates could represent actual survival in the system, or movements out of the system, perhaps to avoid competition or predation prior to maturity, either downstream into Lake Catamount or into one of the tributaries (Service Creek or Morrison Creek). The final year of recaptures in 2020 will help determine the fate of these individuals.

Short-term (June-September 2019) survival estimates were higher for PIT-tagged Rainbow Trout compared to CWT Rainbow Trout in all sampled sections of the Yampa River (Figure 2.17). Only a small number of CWT Rainbow Trout were encountered in the Green Creek/Kuntz Ranch section, which produced a lot of uncertainty in the survival estimates in that section, although lower apparent survival rates in this section were not unexpected since the deep pool habitat on the ranches favor larger fish. In contrast, the constructed riffles in the Stagecoach Tailwater

section favor smaller fish, which is reflected in the CWT Rainbow Trout survival estimates which were higher in the Stagecoach Tailwater than the other three sections (Figure 2.17).

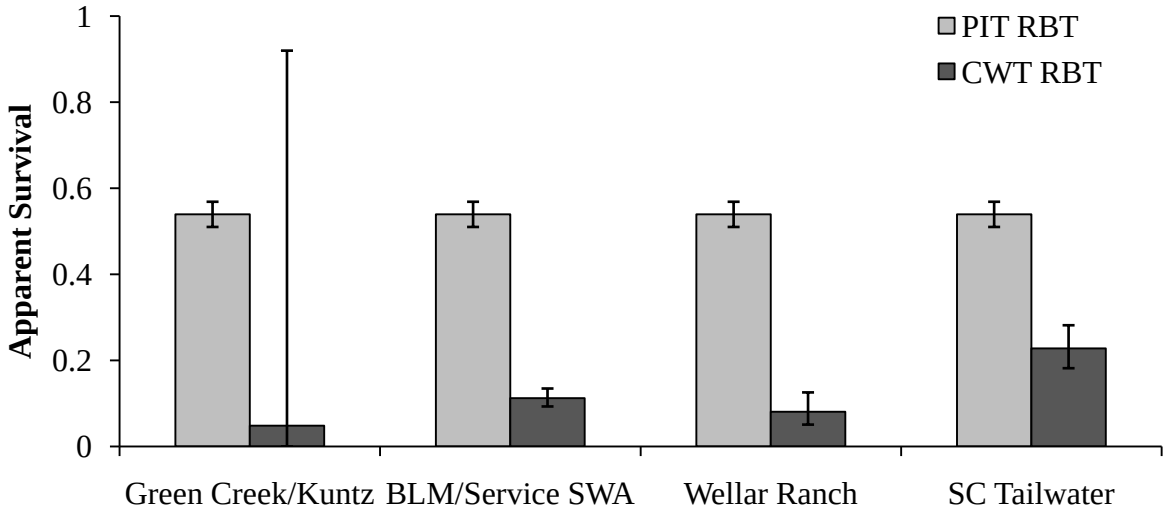

Figure 2.17. Short-term (June-September 2019) apparent survival estimates (unconditional SE bars) for PIT-tagged and CWT Rainbow Trout stocked in four sections of the Yampa River in spring 2018.

Coded wire-tagged Rainbow Trout stocked in spring 2019 grew well, averaging 180.7 (± 16.8) mm TL and 70.9 (± 22.9) g upon recapture in fall 2019. Coded-wire tagged Rainbow Trout stocked in spring 2018 continued to grow and were between 1.5 and two times larger than reported in 2019 (Fetherman et al. 2019), averaging 293.4 (± 20.6) mm TL and 263.9 (± 52.6) g upon recapture in fall 2019. Length for CWT Rainbow Trout stocked in 2017 slowed in 2019, although fish were larger, and weight nearly doubled from that reported in 2019 (Fetherman et al. 2019), averaging 355.0 (± 39.5) mm TL and 440 (± 148.4) g. Fetherman et al. (2018) showed that PIT-tagged Rainbow Trout growth in the Yampa River appeared to be correlated with land use and fishing pressure, with fish stocked into publicly accessible locations losing weight corresponding with fishing pressure in those locations between spring and fall 2017, and fish stocked on private land gaining weight over that same time period. In contrast, PIT-tagged Rainbow Trout stocked in spring 2018 and 2019 gained weight in all locations prior to recapture (Figure 2.18). Interestingly, weight gain continued to be correlated with land use and fishing pressure. During summer 2018, low water levels and higher than average water temperatures resulted in a fishing closure in the Stagecoach Tailwater, and fish stocked in the tailwater gained more weight in relation to the other public sections of the river that remained open (Service Creek SWA and BLM) and likely received greater than normal fishing pressure due to the closure. PIT-tagged Rainbow Trout stocked on private land gained more weight than fish in the Service Creek SWA and BLM sections, with growth on the Foster Ranch similar to that observed in the Stagecoach Tailwater. Fish on the Wellar Ranch, which is more heavily fished than the Foster Ranch, gained less weight than those stocked in the Foster Ranch. In 2019, when fishing pressure was more comparable to 2017 in that all public fishing access remained open, fish gained weight in all locations, but weight gain was greater on private land than public land, and weight gain on private land was greater on properties with less fishing pressure (e.g., Green Creek Ranch) than others (e.g., Wellar Ranch; Figure 2.18).

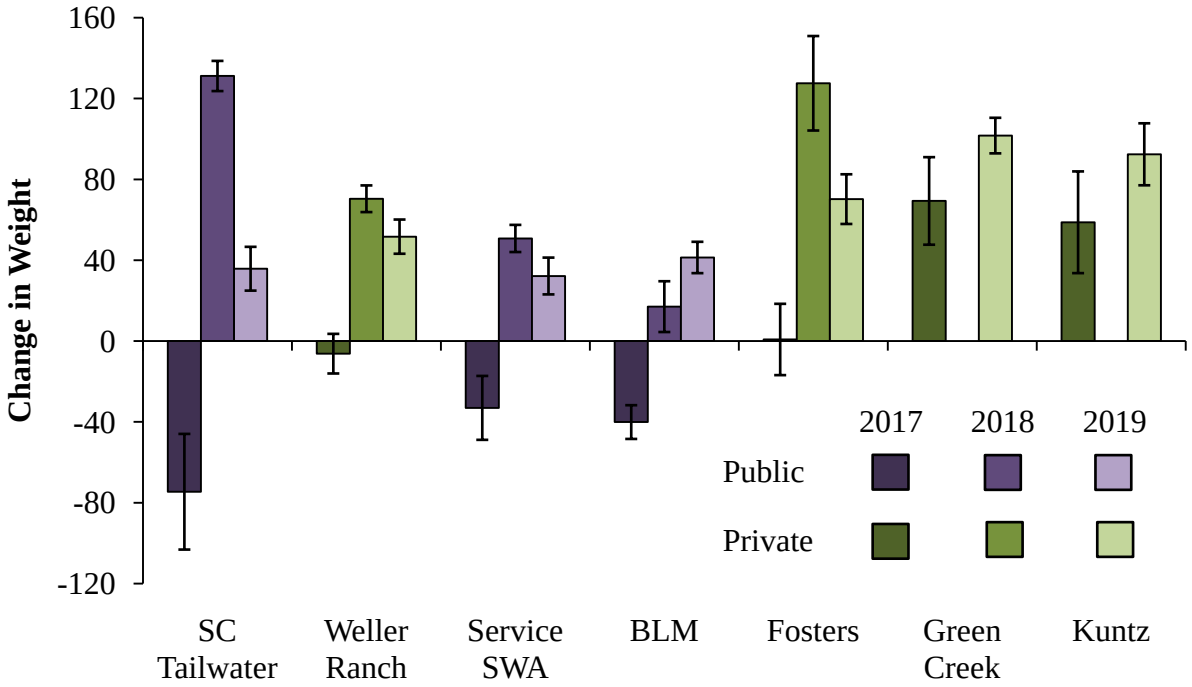

Figure 2.18. Short-term change in weight (g; SE bars) of PIT-tagged Rainbow Trout stocked and recovered in 2017, 2018, or 2019 in public and private land sections of the Yampa River.

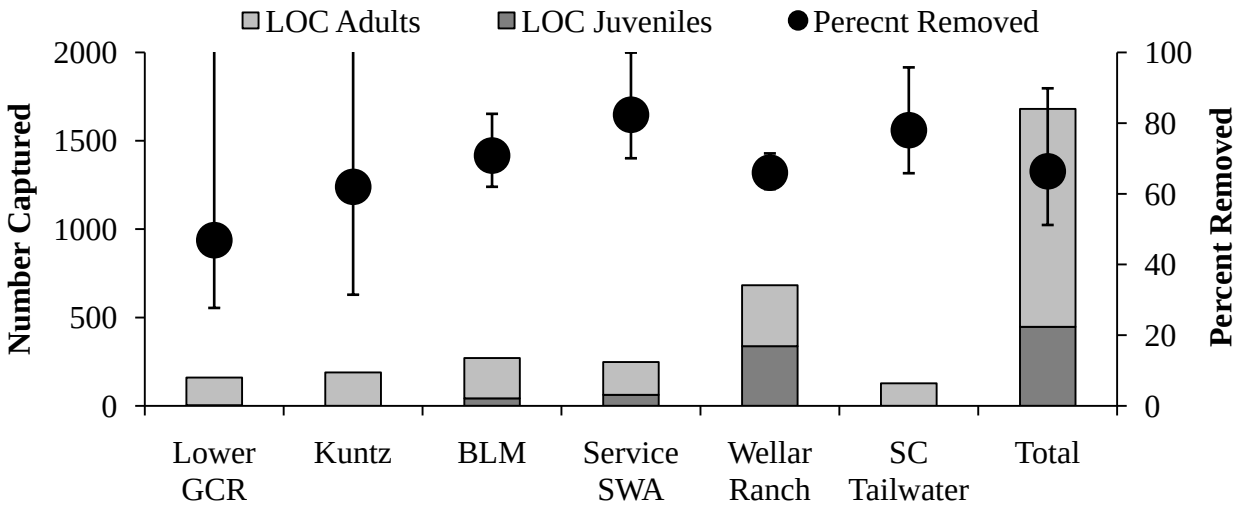

Figure 2.19. Number of juvenile and adult Brown Trout captured and removed in the Lower Green Creek Ranch (GCR), Kuntz Ranch, BLM, Service Creek SWA, Wellar Ranch, and Stagecoach (SC) Tailwater sections of the Yampa River in fall 2019, and corresponding estimated percent Brown Trout (SE bars) removed based on two-pass removal abundance estimates conducted in each section.

A total of 1,681 Brown Trout, 3,046 less than 2018 (Fetherman et al. 2019), were removed and euthanized, 66% of the estimated 2,532 Brown Trout present in the Yampa River in fall 2019.

The percentage of Brown Trout removed from the BLM, Service SWA, Wellar Ranch, and Stagecoach Tailwater ranged between 66 and 82%, whereas only 47% of the Brown Trout population present in the lower Green Creek Ranch reach was removed (Figure 2.19). The lower Green Creek Ranch habitat consists of more, deeper pools and constructed riffles than many of the other sections, which makes sampling more difficult and could result in lower capture probabilities in that section. Three quarters of the Brown Trout removed in 2019 were adults, differing greatly from 2018 where three quarters of the fish removed were juveniles (Fetherman et al. 2019), suggesting that previous years of Brown Trout removals had an effect on natural reproduction in the Yampa River in 2019, which can also be seen in the change in number of juvenile Brown Trout per mile from 2018 to 2019 (Figure 2.20). Previous years Brown Trout removal efforts, and lower numbers of Brown Trout present in the Yampa River in 2019, appeared to have an effect on CWT Rainbow Trout survival, which was similar in 2018 and 2019 despite stocking half as many CWT Rainbow Trout in 2019 (Figure 2.14), as well as the estimated number of CWT Rainbow Trout per mile (Figure 2.20).

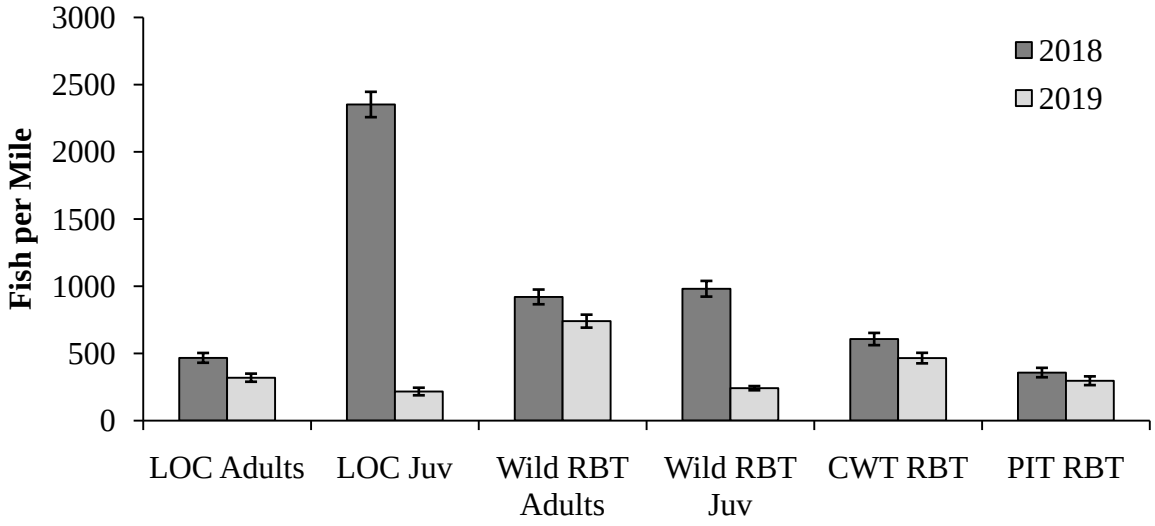

Figure 2.20. Adult and juvenile Brown Trout (LOC) and wild Rainbow Trout (RBT), CWT RBT, and PIT-tagged RBT abundance estimates (fish per mile; SE bars) averaged across the sites in which two-pass or mark-recapture estimates were conducted in fall 2018 and fall 2019.

Although the 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 the fish are distributed and what habitats are being used by which age classes or species of fish. For example, the Wellar Ranch, which is much wider, shallower, and contains more aquatic rooted 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 than juvenile salmonids. In addition to being good adult Brown Trout, Rainbow Trout, and juvenile Rainbow Trout habitat, the BLM section also supports a higher number of benthic species such as Mottled Sculpin than do other sections of the river (Table 2.3). Future multistate survival and movement analyses will focus on associating the habitat data collected in 2018 with population and survival estimates to determine

which factors are driving observed patterns in salmonid distribution, abundance, and survival in the Yampa River.

Table 2.3. 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, Speckled Dace, 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 2019.

Species/Type	Green Creek/Kuntz	BLM	Service Creek SWA	Wellar Ranch	Stagecoach Tailwater
LOC (Adults)	461 [225-737]	347 [309-385]	171 [140-201]	217 [196-238]	541 [441-642]
LOC (Juv)		44 [26-62]	24 [20-28]	610 [568-651]	
RBT (Wild Adults)	200 [158-243]	522 [470-574]	181 [149-212]	203 [183-223]	1,400 [1,354-1,446]
RBT (Wild Juv)	8 [0-19]	758 [664-852]	142 [113-170]	51 [41-61]	14 [10-18]
RBT (Wild Recaps)	6 [4-9]	38 [31-45]	30 [18-41]		587 [566-608]
RBT (2019 CWT)	18 [0-36]	269 [230-308]	171 [140-203]	51 [41-61]	959 [820-1,099]
RBT (2018 CWT)	2 [2-2]	10 [5-14]	20 [10-30]	22 [16-28]	314 [298-330]
RBT (2017 CWT)	1 [1-1]	9 [7-12]		22 [16-28]	14 [12-16]
RBT (PIT-tagged)	210 [187-232]	82 [77-88]	269 [250-342]	304 [277-330]	504 [487-521]
Mottled Sculpin		4,545 [4,163-4,928]	1,878 [1,705-2,053]	94 [80-109]	41 [34-49]
Speckled Dace			10 [3-17]		
Mountain Whitefish	36 [20-51]	19 [13-26]	30 [18-41]	51 [41-61]	14 [10-17]
Brook Trout	2 [0-5]	62 [44-79]	101 [77-124]	51 [21-82]	289 [269-309]
Fathead Minnow				116 [101-131]	
White Sucker	15 [4-26]				

The results from the third year of the study continue to suggest that catchable Rainbow Trout exhibit higher survival rates than fingerling Rainbow Trout stocked into the Yampa River. However, CWT Rainbow Trout survival estimates were higher in 2018 and 2019 than 2017, suggesting that a reduction in the adult Brown Trout population may have reduced predation and increased the survival of these fish. This is especially apparent in 2019 when half as many CWT Rainbow Trout were stocked compared to 2018. Patterns observed in the first year regarding growth of the PIT-tagged Rainbow Trout in public versus private land sections of the Yampa River continued to be observed in the second and third years of the study as well. Habitat data collected in 2018, and the final recapture data to be collected in 2020, will be used to determine if fishing pressure versus other habitat parameters of interest have an effect on survival and growth of the stocked Rainbow Trout, and what effects Brown Trout removal had on survival of the stocked fingerling Rainbow Trout.

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.

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MYXOBOLUS CEREBRALIS RESISTANCE IN GUNNISON RIVER RAINBOW TROUT

In the early 2010s, CPW researchers began an intensive investigation of the Rainbow Trout in the East Portal of the Gunnison River, a wild Rainbow Trout population that experienced a

smaller population-level decline following the statewide establishment of *Myxobolus cerebralis* relative to other populations. As a result of the lower infection prevalence and severity in the East Portal population, this location was also selected to be a Hofer by Colorado River Rainbow (H×C) wild brood stock location, with H×C stocking occurring between 2006 and 2013. However, fry and adult genetic samples collected in 2009 and 2011-2014 suggested that the H×C fish were not well established in the population (Fetherman and Schisler 2015). Genetic data also suggested that the East Portal Rainbow Trout, though genetically similar to pure Colorado River Rainbow (CRR), appeared notably different from CRR brood stocks previously maintained in CPW hatcheries, resulting in naming these fish the Gunnison River Rainbow (GRR).

Two *M. cerebralis* exposure experiments conducted in 2012 and 2014 showed that myxospore development was low in GRR fish originating from the East Portal of the Gunnison River, especially compared to Puget Sound Rainbow Trout controls (Figure 2.21). GRRs developed an average of 38,063 myxospores per fish, exhibited low mortality rates (4%), and a low incidence of deformities (16%) compared to CRRs tested in previous laboratory experiments (average myxospore count: 187,595, mortality: 13% [Fetherman et al. 2012]; incidence of deformities: 96% [Fetherman et al. 2011]). Results suggested that either the GRRs had developed resistance to *M. cerebralis* naturally over 20 years of continuous exposure, or that resistance had been imparted to the population by the repeated stocking of H×Cs. Because *M. cerebralis* prevalence is low in the East Portal, exposure levels were not high enough to cause a complete collapse of the Rainbow Trout population, and natural reproduction continued to occur annually, the combination of which may have resulted in the development of resistance. Alternatively, the H×C may have also reproduced in the East Portal, but the contribution of Hofer genetics to the spawning population may have been below detectable levels, as all adults and fry tested in the exposure experiments appeared to be genetically CRR.

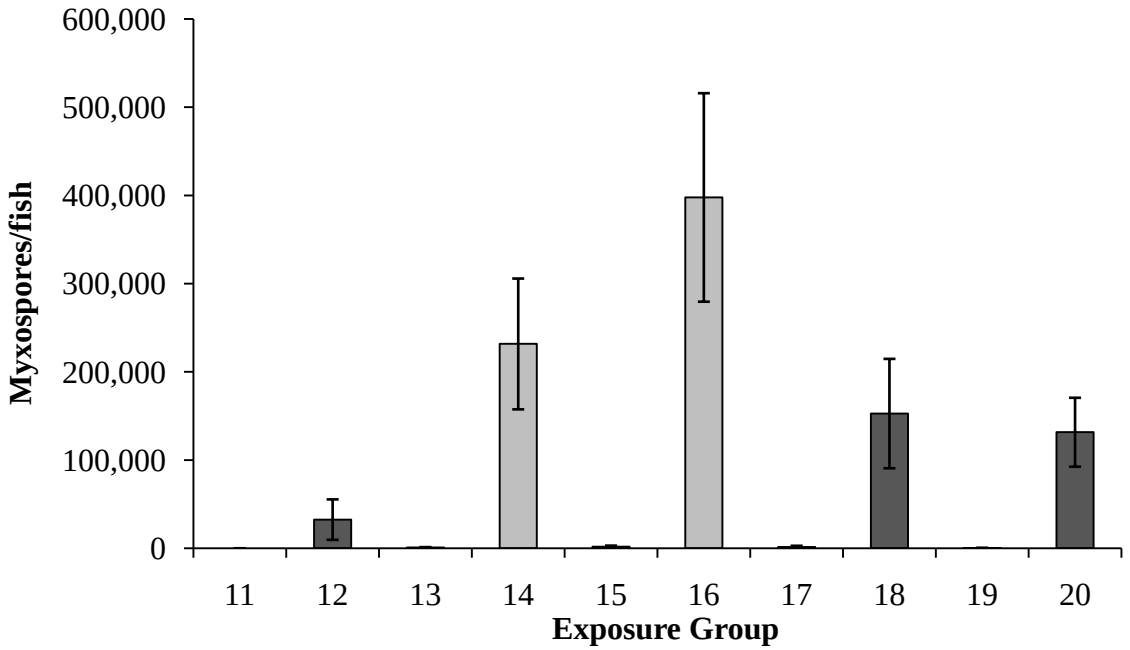

Figure 2.21. Average number of myxospores per fish (SE bars) in eight families of Rainbow Trout originating from the East Portal of the Gunnison River (GRR; dark bars; families 11-13, 15, and 17-20) and two families of Puget Sound Rainbow Trout (light bars; families 14 and 16).

A quantitative trait locus (QTL) was identified in F2-generation H×C, known as the WDRES-9 QTL, which was capable of explaining a large percentage of the phenotypic variance contributing to *M. cerebralis* resistance in these fish (Baerwald et al. 2011). It is likely, and assumed for this analysis, that the WDRES-9 QTL is unique to the Hofer strain, and that wild fish populations, even if they were to develop resistance to *M. cerebralis* naturally, would not have the same QTL involved in resistance. To determine if H×Cs reproduced and contributed to the resistance of the East Portal Rainbow Trout population, four GRR exposure groups from the 2014 exposure experiment were examined for the WDRES-9 QTL. If the WDRES-9 QTL was incorporated into the population and continuing to play a role in *M. cerebralis* resistance, it was suspected that it would be present in the families with lower myxospore counts and potentially absent in the families with higher myxospore counts. As such, ten individuals were tested for the WDRES-9 QTL from each of the two exposure groups that had the lowest average myxospore counts per fish and the two exposure groups that had the highest average myxospore counts per fish (Figure 2.22). Fin clips had been taken from each individual in these families during the 2014 exposure experiment, previously analyzed for percent Hofer (Fetherman et al. 2018), and were stored at the University of California, Davis Genomic Variation Laboratory. Reanalysis of these samples was conducted by the Genomic Variation Laboratory, and methods for genotyping and analysis for the WDRES-9 QTL followed those of Baerwald et al. 2011.

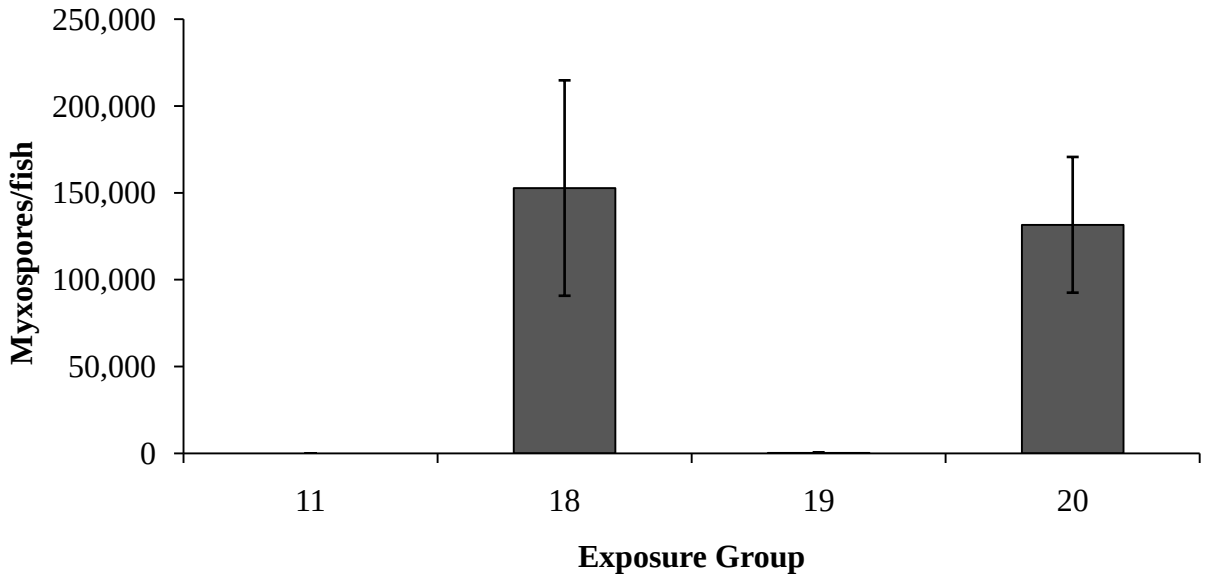

Figure 2.22. Average myxospores per fish (SE bars) for the East Portal exposure groups that exhibited the two highest (exposure groups 18 and 20) and two lowest (exposure groups 11 and 19) myxospore counts during the 2015 exposure experiment, and which were analyzed for the WDRES-9 quantitative trait loci (QTL).

The WDRES-9 QTL is 202 base pairs (bp) in length. At least one copy of the QTL was observed in 38 of the 40 individuals tested. Additional genes of varying length (175, 186, 0r 206 bp), which are assumed to not confer resistance to *M. cerebralis*, were identified in the same location in 18 of the individuals tested. Overall, gene expression in the offspring in each of the exposure groups was based on parental genetics. Both parents of exposure group 11 were homozygous (contained two copies) for the 202 bp Hofer resistance gene. As such, offspring in

exposure group 11 were also all homozygous for the 202 bp gene, and none of the offspring in exposure group 11 developed myxospores during the 2014 exposure experiment (Figure 2.22). One parent of exposure group 19 was homozygous for the 202 bp gene, but the other was heterozygous for the 202 and 175 bp genes. As such, 60% of the offspring in exposure group 19 were homozygous for the 202 bp gene, while the other 40% were heterozygous for the 202 and 175 bp genes, although due to the larger proportion of homozygous individuals in this group, myxospore counts remained low. A similar pattern was observed in the parents of exposure group 20, where one was homozygous for the 202 bp gene, and the other was heterozygous for the 202 and 186 bp genes. However, the proportion of offspring inheriting these genes differed from exposure group 19 in that only 40% of the offspring were homozygous for the 202 bp gene while the other 60% was heterozygous for the 202 and 186 bp genes, resulting in higher average myxospore counts per fish. Lastly, the parents of exposure group 18, which had the highest average myxospore count per fish in the offspring (Figure 2.22), were both heterozygous for the 202 bp gene, with one parent additionally expressing a 175 bp gene, and the other expressing a 206 bp gene. As a result, only 30% of the offspring were homozygous for the 202 bp gene, 50% were heterozygous for the 202 and either 175 or 206 bp genes, and 20% did not have the 202 bp gene, and both fish were heterozygous for the 175 and 206 bp genes.

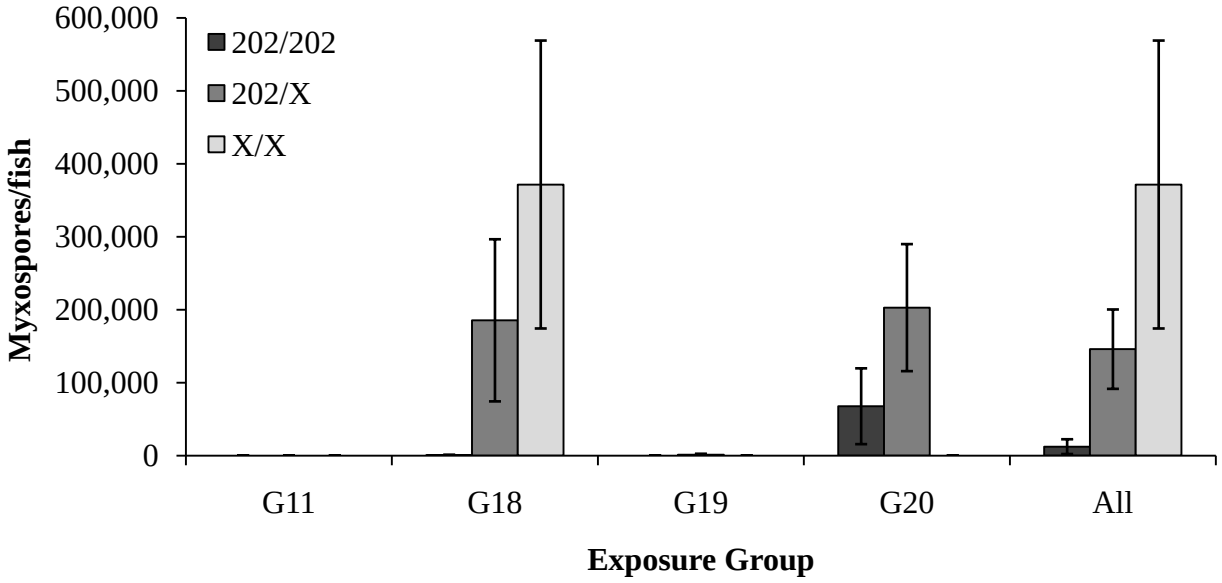

Figure 2.23. Average myxospores per fish (SE bars) within an exposure group or averaged across exposure groups (All) for individuals that were homozygous for the 202 base pair long WDRES-9 quantitative trait loci (QTL; 202/202), heterozygous for the WDRES-9 QTL (202/X), or did not have the WDRES-9 QTL (X/X).

Average myxospore appeared to be correlated with presence of the 202 bp gene, with individuals homozygous for the 202 bp gene developing fewer myxospores than individuals that were heterozygous for the 202 bp gene or did not have the gene. Additionally, individuals that did not have the gene exhibited higher myxospore counts than individuals that were heterozygous for the 202 bp gene (Figure 2.23). Given this pattern in the other exposure groups, the higher average myxospore count of the 202 bp homozygotes in exposure group 20 was unexpected if the 202 bp gene were the only one involved in resistance to *M. cerebralis*. However, Fetherman et al.

(2012) estimated that there were 9 ± 5 genes involved in resistance, and the effects of these genes were likely additive. This additive effect of resistance genes was also suggested by Baerwald et al. (2011). The homozygous individuals with higher myxospore counts in exposure group 20 support that there are likely more genes involved in resistance, and these genes must be present to confer the level of resistance and reduced myxospore counts observed in the 202 bp homozygotes of exposure groups 11 and 19.

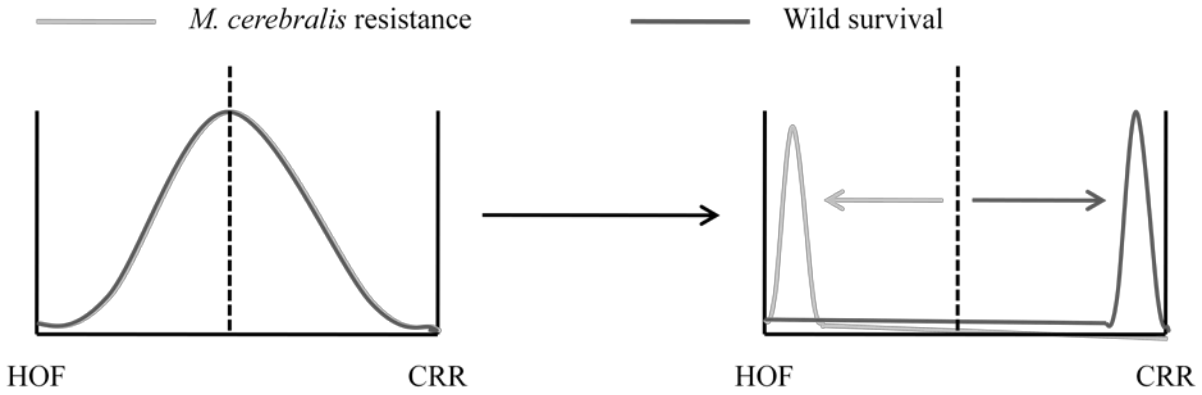

Figure 2.24. Example of the directional selection processes that likely occurred in the East Portal Rainbow Trout population following H×C stocking resulting in the genetic patterns observed in the population in the early 2010s. Panel A shows the distribution of *M. cerebralis* resistance and wild survival characteristics in a 50:50 H×C population, and panel B shows the distribution of those characteristics following directional selection for the two survival characteristics, reflecting the genetic structure of the East Portal Rainbow Trout population.

Overall, the detection of the 202 bp WDRES-9 QTL in the East Portal Rainbow Trout population suggests that the resistance characteristics of the Hofer were incorporated into the population through the natural reproduction of the stocked H×C. However, these characteristics were undetectable given previous genetic tests for specific markers differentiating the Hofer and CRR that did not look specifically for the WDRES-9 QTL, the only known QTL involved in resistance to *M. cerebralis* (Baerwald et al. 2011). It is likely that directional selection also played a role in the observed genetic structure of the East Portal Rainbow Trout population. Two characteristics are being selected for naturally in the East Portal and other stocked populations: 1) survival of exposure to *M. cerebralis*, for which the WDRES-9 QTL and small set of other genes are needed, and 2) wild survival, including swimming performance, feeding behavior, predator avoidance characteristics, etc. for which a suite of genes throughout the entire genome are likely needed. Given the history of domestication in the Hofer strain, and data from recent stocking experiments, the Hofer does not appear to have many of the genes needed for wild survival, while the CRR does not have the genes needed for surviving exposure to *M. cerebralis*. When a 50:50 cross such as the H×C is stocked, the distribution of these genes for both survival characteristics are distributed across a bell curve that peaks half way between the two pure strains (Figure 2.24). Over time, directional selection works on both survival characteristics such that only individuals that retain the resistance characteristics of the Hofer and the wild survival characteristics of the CRR survive and contribute to future generations in that population (Figure 2.24). It is likely that this selection occurred fairly rapidly in the East Portal Rainbow Trout

population, explaining the genetic patterns observed and the development of resistance following the stocking of H×C fish.

In the spring of 2017, a wild spawning operation in the East Portal produced fertilized eggs that were transported to the Glenwood Springs Hatchery to develop a new brood stock of GRRs. Hofer eggs were also brought to the Glenwood Springs Hatchery in 2017 to start a pure Hofer brood stock at that facility. The same process was used in 2018 and 2019 to produce three age classes pure GRR and pure Hofer on the unit. The Hofer and GRR were spawned together for the first time in 2017 to create 50:50 Hofer by Gunnison River Rainbow fish (H×Gs). The resistance of the H×G was compared to that of the GRR using a standard exposure experiment in 2017, and H×G were found to be more resistant to *M. cerebralis* than the pure GRR (Fetherman et al. 2018). Additionally, the H×G were found to be more resistant to *M. cerebralis* than the H×C evaluated in a standard *M. cerebralis* exposure experiment conducted in 2008 (Fetherman et al. 2011). As such, the H×G was determined to be a suitable replacement for the H×C, the brood stock of which was euthanized during depopulation of the CPW Glenwood Springs Hatchery following the discovery of *Renibacterium salmoninarum* in 2015. Currently, the Hofer and GRR brood stocks at the Glenwood Springs Hatchery are maintained separately, such that all H×G produced by the unit are a 50:50 cross of the two pure strains. However, in 2020, H×G eggs produced at the CPW Glenwood Springs Hatchery will be sent to the CPW Poudre Rearing Unit to establish an H×G brood stock, and create redundancy within Colorado's hatchery system should depopulation of a hatchery unit due to disease become necessary in the future. H×G fry were stocked into the Colorado River for the first time in 2019, and these fish exhibited higher rates of survival than H×Cs stocked in that same location between 2013 and 2015. Future population estimates conducted in the upper Colorado River will provide more information on the survival, recruitment, and reproduction of the H×G in the wild, data for which will be available in future reports.

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PROXIMATE COMPOSITION OF RAINBOW TROUT REARED ON FOUR COMMERCIAL DIETS

Hatchery feed experiments were conducted at the BFRH in 2016 and 2017 to evaluate the growth and health of, and angler preference for, Rainbow Trout reared on four commercially-available trout feeds. The results of these experiments were summarized in a Colorado Parks and Wildlife white paper (Fetherman et al. 2019), and made available for distribution upon request. After completion of that report, results of feed and proximate composition analyses were received from the U.S. Fish and Wildlife Service Bozeman Fish Technology Center (Bozeman, Montana). The following summarizes the results of that analysis for the four commercial diets, and the fish reared on those diets, during the 2016 hatchery feed experiment.

Feeds from four commercial trout feed companies were evaluated in the 2016 hatchery feed experiment, EWOS, Skretting, Bio Oregon, and Rangen. Details regarding the feed companies, sizes, and description of feeds used in that experiment can be found in Fetherman et al. (2019). During the experiment, two samples of 125 grams of feed each were retained from each feed size produced by the feed company. Diet samples were sent to the Bozeman Fish Technology Center (collaborators: Gibson Gaylord and Wendy Sealey) and analyzed for protein and fat content to ensure that these met or exceeded the minimum amount guaranteed on the label. Although percent protein and fat content were analyzed in both whole (wet) and dry form, only the results for the dry analysis are presented as most of the calculations for individual growth performance (Fetherman et al. 2019) are based on the dry feed weight. The energy content of the dry matter (cal/g) was also included as a part of this analysis. Differences in average protein, fat, and energy content of the feeds were compared across the feed companies using an analysis of variance (ANOVA) implemented in SAS Proc GLM. An additional ANOVA was run to compare the average deviation in percent protein and fat from that guaranteed on the label, calculated by subtracting the known protein or fat content from the amount stated on the label.

Fish meal is the “ideal protein” in commercial diets because the amino acid profile of the feed mimics the whole-body amino acid profile of the animal being fed (Trushenski et al. 2006). However, to reduce costs, alternative protein sources are continually being investigated, and many commercial feeds include several other sources of proteins as meals. The list of ingredients from each feed label was examined to 1) determine the number of ingredients in the feed listed prior to fish meal, and 2) determine other protein meals included in the feeds. An ANOVA was used to compare these two values across feed companies.

Fat, protein, and energy contents differed among the feed sizes within the feed companies (Tables 2.4, 2.5, 2.6, and 2.7), as well as among the feed companies. There was a significant difference in average fat content among the feed companies ($P = 0.013$). Bio Oregon feeds contained the highest fat content (21.4 ± 1.9%), which was significantly higher than Rangen ($P = 0.019$) or Skretting ($P = 0.035$), but not EWOS ($P = 0.159$). Average fat content did not differ among EWOS (18.7 ± 3.3%), Rangen (17.7 ± 2.4%), or Skretting (17.8 ± 2.6%; $P > 0.874$). There was not a significant difference in average protein content among the feed companies ($P = 0.078$), with Bio Oregon feeds containing 54.3 ± 4.7% protein, EWOS feeds containing 56.1 ± 3.7%, Rangen feeds containing 49.8 ± 6.0%, and Skretting feeds containing 52 ± 4.7%. Dry matter energy content differed significantly among the feed companies ($P < 0.001$). Bio Oregon feeds contained the highest energy content (5,688.5 ± 127.6 cal/g), significantly higher than that

of the other three companies ($P \leq 0.023$). EWOS (5,484.4 ± 120.2 cal/g), Rangen (5,335.8 ± 170.3 cal/g), and Skretting (5,351.7 ± 103.2 cal/g) did not differ in energy content ($P \geq 0.152$). The four feed companies did not differ in deviation of fat or protein content from the minimum contents listed on their labels (fat: $P = 0.743$; protein: $P = 0.249$), with the average deviation in fat content ranging from 2.1 ± 0.6% to 3.1 ± 1.4%, and the average deviation in protein content ranging from 3.9 ± 4.5% to 7.2 ± 3.8%. In general, feeds from all the feed companies contained more fat or protein than the guaranteed minimum on the label. EWOS size 0 feed was the only feed that contained less fat than the minimum (Table 2.4), and Rangen 3/32" and 5/32" feeds were the only ones to contain less protein than the minimum (Table 2.7).

Table 2.4. Dry matter (DM; %), dry fat (DM fat; %), dry protein (DM Pro; %), and dry energy content (DM energy; cal/g) of each feed size, the guaranteed minimum percent protein (Label Pro) and fat (Label Fat) content from the feed label, percent deviation in protein (Dev Pro) and fat (Dev Fat) content from the guaranteed minimum (positive, more than minimum; negative, less than minimum), the number of label ingredients prior to fish meal (Ing FM), and number of other protein meals (Other Pro) for the EWOS feeds used in the 2016 hatchery feed experiment.

Feed	DM	DM Fat	DM Pro	DM Energy	Label Pro	Label Fat	Dev Pro	Dev Fat	Ing FM	Other Pro
#0	93.7	12.1	62.1	5,239.0	54.0	16.0	8.1	-3.9	0	1
#1	93.3	21.3	56.3	5,533.3	54.0	16.0	2.3	5.3	0	1
#2	90.1	20.0	60.3	5,456.4	53.0	20.0	7.3	0.0	0	1
1.2 mm	93.7	22.1	53.8	5,573.7	52.0	18.0	1.8	4.1	0	2
1.5 mm	93.7	19.6	54.1	5,578.4	43.0	14.0	11.1	5.6	0	4
2.0 mm	93.2	17.4	52.7	5,451.5	43.0	14.0	9.7	3.4	0	4
3.0 mm	92.6	18.3	53.4	5,558.2	43.0	14.0	10.4	4.3	0	4

Table 2.5. Dry matter (DM; %), dry fat (DM fat; %), dry protein (DM Pro; %), and dry energy content (DM energy; cal/g) of each feed size, the guaranteed minimum percent protein (Label Pro) and fat (Label Fat) content from the feed label, percent deviation in protein (Dev Pro) and fat (Dev Fat) content from the guaranteed minimum (positive, more than minimum; negative, less than minimum), the number of label ingredients prior to fish meal (Ing FM), and number of other protein meals (Other Pro) for the Skretting feeds used in the 2016 hatchery feed experiment.

Feed	DM	DM Fat	DM Pro	DM Energy	Label Pro	Label Fat	Dev Pro	Dev Fat	ING FM	Other Pro
#0	95.0	17.3	56.1	5,230.3	52.0	16.0	4.1	1.3	0	3
#1	93.0	18.7	56.7	5,324.4	52.0	16.0	4.7	2.7	0	3
#2	93.4	18.0	56.3	5,337.7	52.0	16.0	4.3	2.0	0	3
1.0 mm	94.2	20.2	51.6	5,449.3	45.0	19.0	6.6	1.2	0	3
2.0 mm	91.9	21.3	51.4	5,514.7	45.0	19.0	6.4	2.3	1	3
3.0 mm	92.3	14.2	47.5	5,363.3	40.0	12.0	7.5	2.2	5	5
4.0 mm	93.1	14.8	44.8	5,242.0	40.0	12.0	4.8	2.8	4	5

Table 2.6. Dry matter (DM; %), dry fat (DM fat; %), dry protein (DM Pro; %), and dry energy content (DM energy; cal/g) of each feed size, the guaranteed minimum percent protein (Label Pro) and fat (Label Fat) content from the feed label, percent deviation in protein (Dev Pro) and fat (Dev Fat) content from the guaranteed minimum (positive, more than minimum; negative, less than minimum), the number of label ingredients prior to fish meal (Ing FM), and number of other protein meals (Other Pro) for the Bio Oregon feeds used in the 2016 hatchery feed experiment.

Feed	DM	DM Fat	DM Pro	DM Energy	Label Pro	Label Fat	Dev Pro	Dev Fat	Ing FM	Other Pro
Mash	94.3	19.3	59.8	5,516.0	53.0	18.0	6.8	1.3	0	0
#0	93.8	19.3	59.6	5,560.8	53.0	18.0	6.6	1.3	0	0
#1	94.3	22.7	57.1	5,722.4	52.0	20.0	5.1	2.7	0	0
#2	94.0	20.3	61.2	5,723.6	52.0	20.0	9.2	0.3	0	0
1.2 mm	94.3	21.7	52.5	5,774.8	47.0	18.0	5.5	3.7	0	4
1.5 mm	95.3	20.4	50.5	5,670.7	47.0	18.0	3.5	2.4	0	4
2.0 mm	93.8	21.1	50.7	5,613.1	47.0	18.0	3.7	3.1	0	4
2.5 mm	94.2	22.0	52.1	5,660.4	47.0	18.0	5.1	4.0	0	4
3.0 mm	92.8	21.1	50.7	5,665.6	47.0	18.0	3.7	3.1	0	4
4.0 mm	94.5	25.9	48.3	5,977.1	45.0	24.0	3.3	1.9	1	3

Table 2.7. Dry matter (DM; %), dry fat (DM fat; %), dry protein (DM Pro; %), and dry energy content (DM energy; cal/g) of each feed size, the guaranteed minimum percent protein (Label Pro) and fat (Label Fat) content from the feed label, percent deviation in protein (Dev Pro) and fat (Dev Fat) content from the guaranteed minimum (positive, more than minimum; negative, less than minimum), the number of label ingredients prior to fish meal (Ing FM), and number of other protein meals (Other Pro) for the Rangen feeds used in the 2016 hatchery feed experiment.

Feed	DM	DM Fat	DM Pro	DM Energy	Label Pro	Label Fat	Dev Pro	Dev Fat	Ing FM	Other Pro
#0	85.6	22.1	59.6	5,664.5	45	19	14.6	3.1	0	2
#1	92.6	19.9	54.4	5,415.5	52	16	2.4	3.9	0	3
#2	94.6	18.4	57.2	5,400.1	52	16	5.2	2.4	0	3
#3	92.7	17.8	48.4	5,357.2	45	15	3.4	2.8	0	4
#4	93.9	18.6	49.1	5,369.5	45	15	4.1	3.6	0	4
3/32"	92.1	15.2	44.5	5,153.9	45	15	-0.5	0.2	0	4
1/8"	91.6	16.0	48.1	5,061.9	43	12	5.1	4.0	0	4
5/32"	91.5	17.0	42.8	5,323.3	43	12	-0.2	5.0	0	4
3/16"	91.8	14.5	44.2	5,276.7	43	12	1.2	2.5	0	4

The feed companies differed in the number of ingredients listed prior to fish meal ($P = 0.025$), with Skretting having significantly more ingredients listed prior to fish meal than the other three companies ($P \leq 0.054$), which did not differ ($P \geq 0.997$). None of the EWOS or Rangen feeds had ingredients listed prior to fish meal (Tables 2.4 and 2.7), whereas Bio Oregon had one feed with one ingredient listed prior to fish meal (Table 2.6), and Skretting had three feeds with one to

five ingredients listed prior to fish meal (Table 2.5). The average number of other protein meals included in the feeds did not differ among the feed companies ($P = 0.142$). The number of other protein meal sources in EWOS ranged from 1 to 4 (Table 2.4). Blood meal was the primary additional protein source in smaller EWOS feeds, while corn gluten meal, poultry meal, feather meal, and meat meal were all found in the larger feeds. Skretting had three other protein sources listed in smaller feeds (sizes 0 to 2.0 mm), and five sources of protein in the two largest feeds (Table 2.5). Additional sources of protein included poultry by-product meal, feather meal, squid meal (smaller feeds only), blood meal, soybean meal, and corn gluten meal, the last two of which were only found in the two largest feeds. Bio Oregon was the only feed company in which multiple sizes of feed had only fish meal listed as a protein source (Table 2.6). Larger sizes of Bio Oregon feeds had 3 to four other protein sources, including feather meal, corn gluten meal, porcine blood meal, and poultry meal. The additional number of protein sources increased with an increase in feed size within the Rangen feeds (Table 2.7), with additional protein sources including blood meal, krill meal (size #0 only), poultry by-product meal, soybean meal, and feather meal.

Proximate analyses of individual fish were used to determine percent protein and percent lipid composition in the fish. Prior to the start of the 2016 experiment, fish were held in a single tank. At swim-up, 20 fish were collected, measured, weighed, and retained for proximate analysis to determine a baseline protein and lipid composition prior to feeding, and the remainder distributed to a single starter tank per feed company. At one week post-swim-up, a similar sample of 20 fish was collected, measured, weighed, and retained for proximate analysis. The hatchery feed experiment was started at two weeks post-swim-up, at which time 150 fish from each tank were counted out of the starter tank and distributed into three replicate tanks per feed company. Any remaining fish in the starter tanks were euthanized and retained for proximate analysis. Throughout the remainder of the experiment, once a given tank reached the maximum average individual weight of the range for a given feed size, a subset of 20 fish were individually measured and weighed to set the next feed rates, and five of the 20 fish were euthanized, dissected to obtain liver and viscera weights for calculation of the hepatosomatic and viscerosomatic indices (HSI and VSI, respectively; Fetherman et al. 2019), and retained for proximate analysis.

Proximate analyses were conducted using the homogenized tissue from whole fish samples. With the exception of the 20 fish (or greater) samples taken from the starter tank, which were homogenized as a group, all fish were processed individually. Tissues were homogenized by first cutting frozen whole fish into the smallest pieces that could be obtained with a knife. All pieces of the fish were then placed in a food processor and homogenized until individual tissues were no longer recognizable and tissue size was as fine as the processor could produce. The entirety of the homogenized sample was removed from the processor, put into a 50 ml Falcon tube, and frozen for later analysis. The processor was thoroughly cleaned between samples to prevent cross-contamination. The weight of the tube was taken before and after placing the sample in the tube, the difference of which, the weight of the ground sample, was used to calculate percent protein and lipid composition by weight. Samples were sent to and processed by the Bozeman Fish Technology Center. ANOVA tests, implemented in SAS Proc GLM, were used to compare the average protein and lipid content across feed companies as well as across feed sizes within a feed company. A correlation analysis, implemented in SAS Proc Corr, was

used to determine if correlations existed between the percent protein content in the feed versus proximate protein content in the fish, percent fat content in the feed versus proximate lipid content in the fish, and determine if there was a correlation between the HSI or VSI indices and the percent protein or fat content in the feed (Pearson Correlation Coefficient [PCC]). Lastly, a correlation analysis was used to determine if the percent protein, fat, or energy content of the feed was correlated with weight gain (%) or specific growth weight (SGR; % body weight per day) both within and across feed companies.

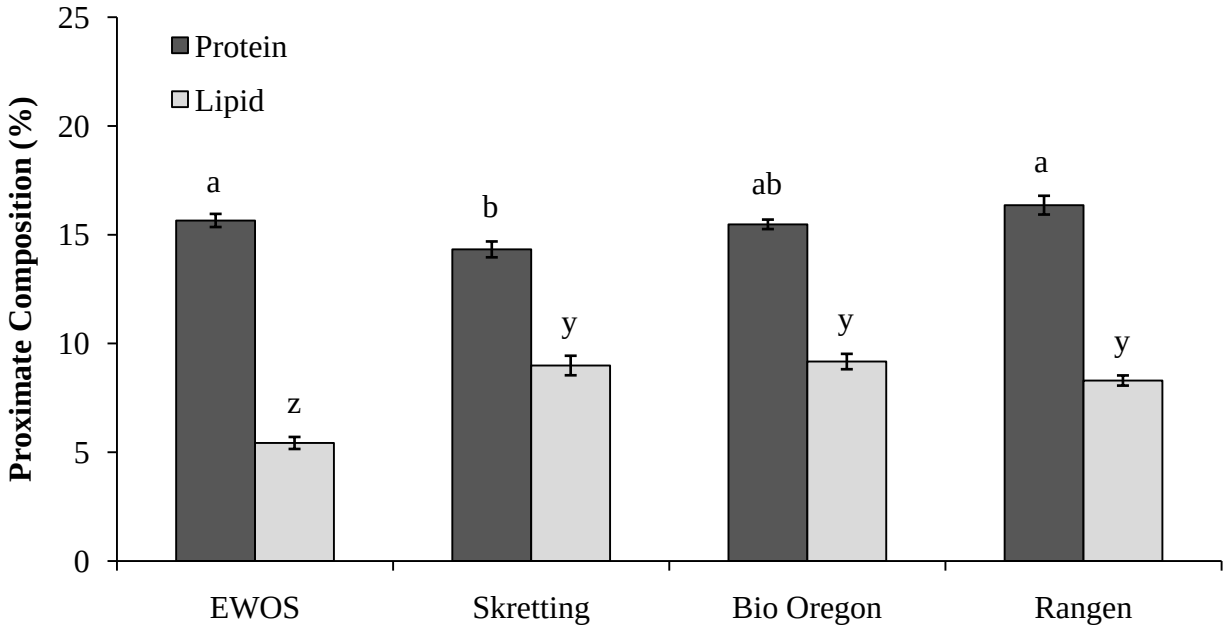

Figure 2.25. Proximate composition comparisons for average percent protein and lipid content (SE bars) in fish reared on EWOS, Skretting, Bio Oregon, or Rangen feeds in the 2016 hatchery feed experiment. Values with same letters indicate no significant difference in average percent protein (a,b) or lipid (y,z) content.

Average proximate protein and lipid content differed by feed company. Fish fed Rangen and EWOS had significantly higher protein contents than fish fed Skretting, but neither differed from fish fed Bio Oregon. Fish fed Bio Oregon and Skretting did not differ in protein content. Fish fed EWOS had a significantly lower average lipid content then fish fed Skretting, Bio Oregon, or Rangen, potentially because fish grew more slowly on the EWOS diet (Fetherman et al. 2019). Lipid content did not differ among fish fed Skretting, Bio Oregon, or Rangen (Figure 2.25). Overall, there was a significant negative correlation between feed protein content and fish protein content (PCC = -0.466, $P < 0.001$), likely because fish protein content appeared to be additive as feed size increased (Figures 2.26, 2.27, 2.28, and 2.29), while feed protein content decreased in larger feed sizes (Tables 2.4, 2.5, 2.6, and 2.7). However, this same correlation was not observed between feed fat content and fish lipid content (PCC = 0.008, $P = 0.851$). There was not a significant correlation between feed protein content and HSI (PCC = -0.045, $P = 0.345$), but there was a significant positive correlation between feed protein content and VSI (PCC = 0.183, $P < 0.001$). Feed fat content was correlated with both HSI (PCC = 0.207, $P < 0.001$) and VSI (PCC = 0.212, $P < 0.001$), likely because energy reserves in the form of fat are

stored in the liver and viscera for later utilization. Lastly, there was not a significant correlation between feed protein or energy content with weight gain (protein: PCC = 0.053, P = 0.252; energy: PCC = 0.069, P = 0.130), but feed fat content was positively correlated with weight gain (PCC = 0.267, P < 0.001). Feed protein, fat, and energy content were all positively correlated with SGR (protein: PCC = 0.680, P < 0.001; fat: 0.234, P < 0.001; energy: PCC = 0.098, P = 0.033).

Protein and lipid composition appeared to be additive in fish fed on EWOS feeds, with individual protein and lipid compositions increasing with an increase in feed size. The one exception to this was feed size #2. Fish fed on size #2 feed had significantly higher protein content than fish fed on smaller feed sizes, and similar protein content to fish fed the largest feeds (Figure 2.26). There were no significant correlations between feed protein content and the fish protein content (PCC = -0.023, P = 0.824), nor between feed fat content and fish lipid content (PCC = -0.110, P = 0.294). Both feed protein and fat content were significantly correlated with HSI, albeit in different directions (protein: PCC = 0.440, P < 0.001; fat: PCC = -0.232, P = 0.028), but neither were correlated with VSI (Protein: PCC = -0.106, P = 0.321; fat: PCC = 0.005, P = 0.964). Feed protein and energy content were both significantly correlated with SGR, with a positive correlation between protein and SGR (PCC = 0.833, P < 0.001) and a negative correlation between energy and SGR (PCC = -0.644, P < 0.001), but feed fat content was not (PCC = -0.097, P = 0.343). The opposite was true for weight gain as feed fat content was significantly positively correlated with weight gain (PCC = 0.472, P < 0.001), whereas feed protein and energy content were not correlated with weight gain (protein: PCC = -0.086, P = 0.401; energy: PCC = 0.017, P = 0.870).

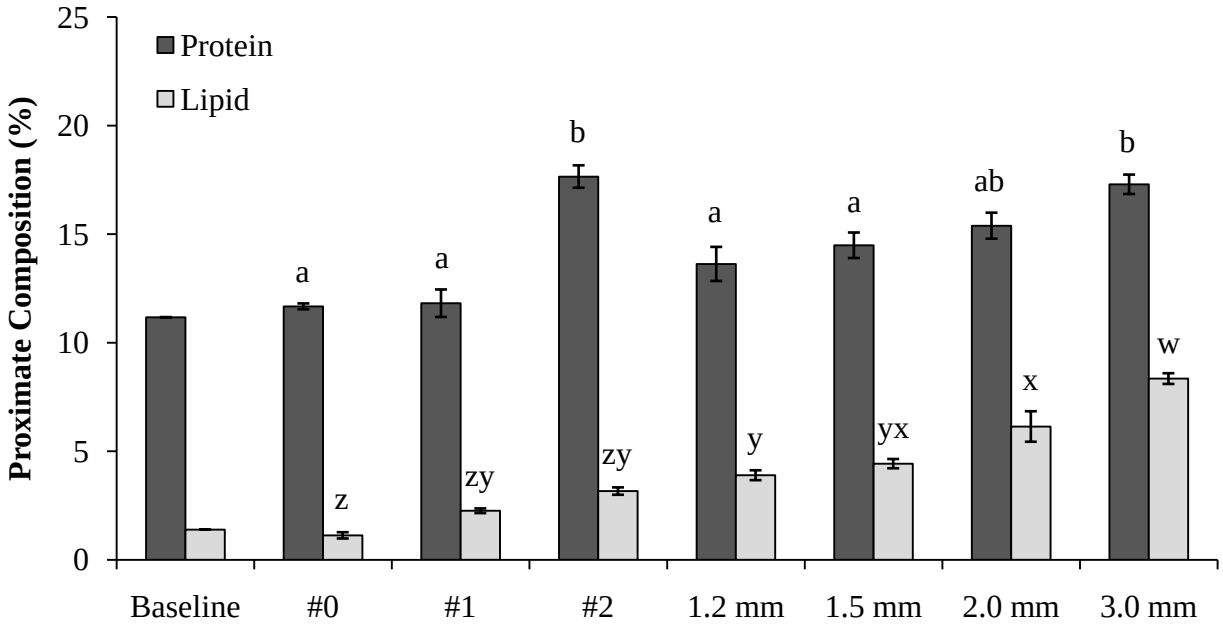

Figure 2.26. Proximate composition comparisons by feed size for average percent protein and lipid content (SE bars) in fish reared on EWOS feeds in the 2016 hatchery feed experiment. Values with same letters indicate no significant difference in average percent protein (a,b) or lipid (w-z) content.

Protein and lipid composition followed different patterns in fish fed Skretting feeds. Protein content was lowest in fish fed smaller feeds, and dipped from the baseline composition before increasing in fish fed larger feeds. Unlike EWOS, which showed a continuous increase in fish protein content with an increase in feed size, protein content of fish fed larger sizes of Skretting feeds appeared to reach a maximum, with similar protein contents across the three largest feeds (Figure 2.27). Lipid composition increased with an increase in feed size, but also appeared to reach a maximum at the larger sizes. Additionally, fish lipid content was lower in fish fed size 3.0 mm feed (Figure 2.27), likely because the 3.0 mm feed had the lowest fat content of the three sizes (Table 2.5). There was a significant negative correlation between feed protein content and fish protein content (PCC = -0.835, $P < 0.001$), as well as feed fat content and fish lipid content (PCC = -0.294, $P = 0.002$). Feed protein content showed a significant negative correlation with HSI (PCC = -0.314, $P = 0.001$) and a significant positive correlation with VSI (PCC = 0.449, $P < 0.001$), whereas feed fat content showed a significant positive correlation with VSI (PCC = 0.484, $P < 0.001$), but there was no correlation between feed fat content and HSI (PCC = -0.076, $P = 0.441$). There was a significant positive correlation between feed fat, protein, and energy content and SGR (fat: PCC = 0.667, $P < 0.001$; protein: PCC = 0950, $P < 0.001$; energy: PCC = 0.270, $P = 0.004$), whereas feed fat and energy content showed a significant positive correlation with weight gain (fat: PCC = 0.621, $P < 0.001$; energy: PCC = 0.635, $P < 0.001$), but there was not a correlation between feed protein content and weight gain (PCC = 0.091, $P = 0.344$).

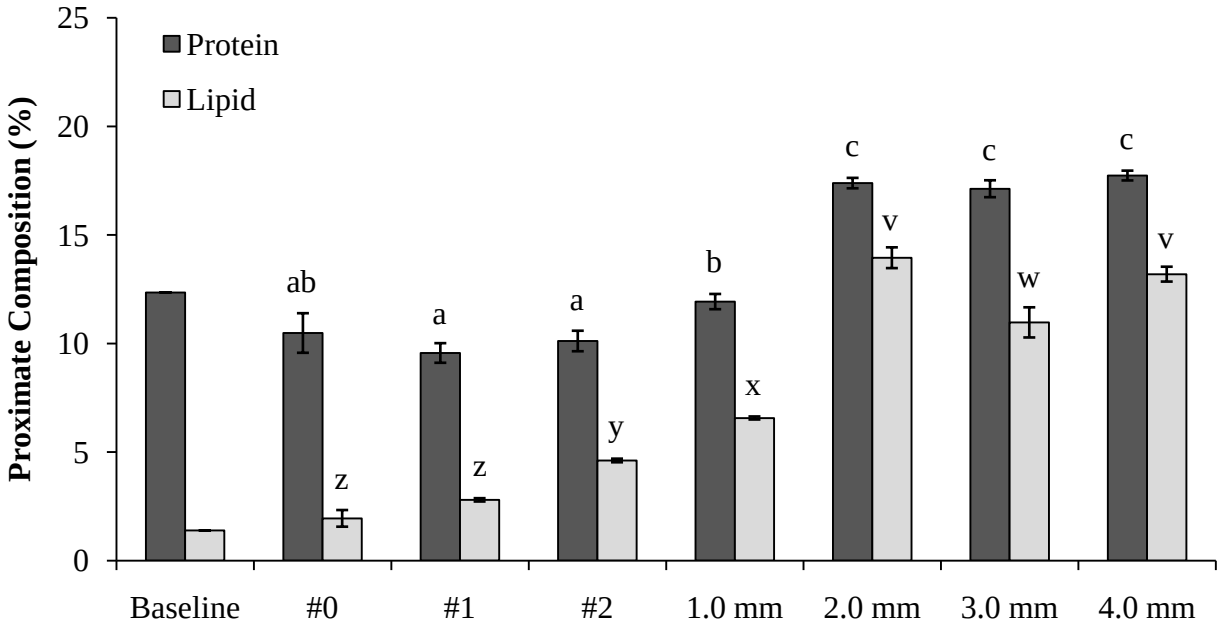

Figure 2.27. Proximate composition comparisons by feed size for average percent protein and lipid content (SE bars) in fish reared on Skretting feeds in the 2016 hatchery feed experiment. Values with same letters indicate no significant difference in average percent protein (a-c) or lipid (v-z) content.

Lipid composition appeared to be additive in fish fed Bio Oregon feeds, with increasing lipid content with an increase in feed size. Protein composition reached an apparent maximum in fish fed 1.5 mm or larger feeds, with the exception of fish fed 2.0 mm feed (Figure 2.28). The 2.0

mm feed did not differ greatly in feed protein or fat content compared to similar sizes (Table 2.6), so the reason for reduction in fish protein content is unknown, but could be a result of individual variation and sample size. Feed protein content was significantly negatively correlated with fish protein composition (PCC = -0.742, P < 0.001), whereas there was a significant positive correlation between feed fat content and fish lipid composition (PCC = 0.599, P < 0.001). There was a significant negative correlation between feed protein content and HSI (PCC = - 0.253, P = 0.003), and a significant positive correlation between feed protein content and VSI (PCC = 0.246, P = 0.004), whereas there was a significant negative correlation between feed fat content and both HSI (PCC = -0.408, P < 0.001) and VSI (PCC = -0.575, P < 0.001). Feed fat and energy content were both significantly positively correlated with weight gain (fat: PCC = 0.457, P < 0.001; energy: PCC = 0.243, P = 0.004), whereas there was not a correlation between feed protein content and weight gain (PCC = -0.125, P = 0.141). There was a significant negative correlation between both feed fat and energy content and SGR (fat: PCC = -0.623, P < 0.001; energy: PCC = -0.505, P < 0.001), and a significant positive correlation between feed protein content and SGR (PCC = 0.863, P < 0.001).

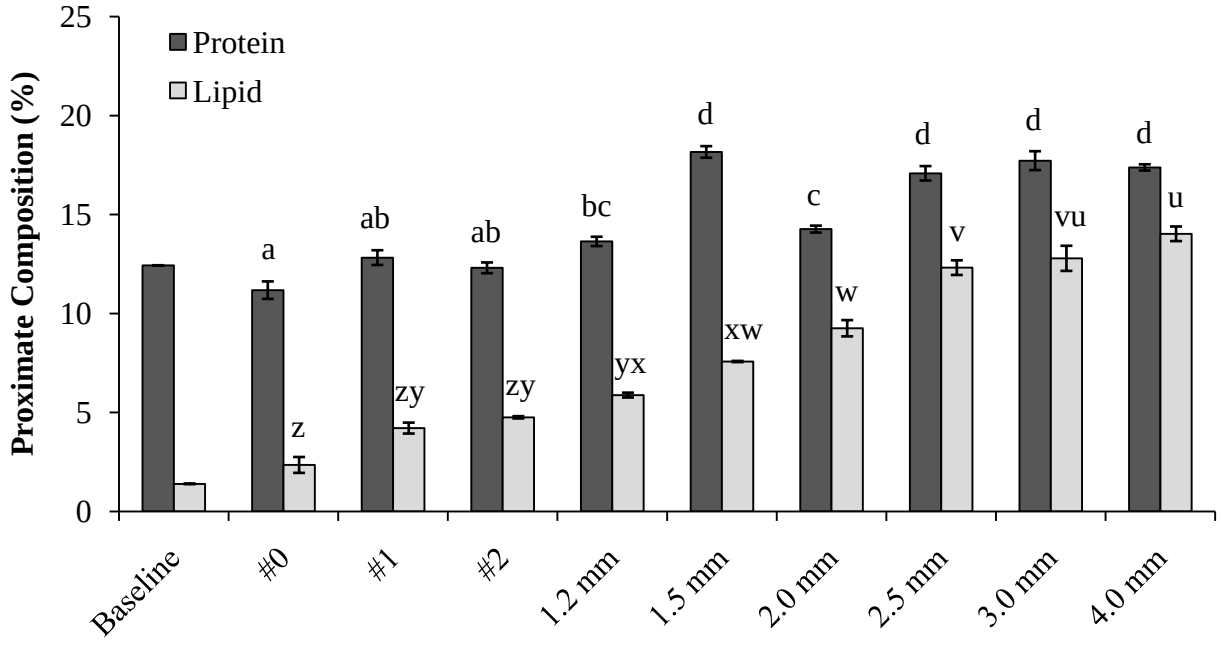

Figure 2.28. Proximate composition comparisons by feed size for average percent protein and lipid content (SE bars) in fish reared on Bio Oregon feeds in the 2016 hatchery feed experiment. Values with same letters indicate no significant difference in average percent protein (a-d) or lipid (u-z) content.

In the fish fed Rangen feeds, lipid composition appeared to be additive, with an increase in lipid content with an increase in feed size up to an apparent maximum reached at the larger sizes. Fish protein composition had two peaks, one at the mid-range of smaller feed sizes, and one towards the upper end of the larger feed sizes (Figure 2.29). This is reflective of the feed protein content which increases and decreases in a similar fashion across feed sizes (Table 2.7). Feed protein content was significantly negatively correlated with fish protein content (PCC = -0.371, P < 0.001), as was feed fat content and fish lipid content (PCC = -0.773, P < 0.001). There was a

significant positive correlation between feed protein content and HSI (PCC = 0.248, P = 0.006) and VSI (PCC = 0.628, P < 0.001), as well as between feed fat content and VSI (PCC = 0.663, P < 0.001), but no correlation between feed fat content and HSI (PCC = 0.126, P = 0.171). Feed fat, protein, and energy content were all significantly positively correlated with weight gain (fat: PCC = 0.414, P < 0.001; protein: PCC = 0.285, P = 0.001; energy: PCC = 0.247, P = 0.005) and SGR (fat: PCC = 0.847, P < 0.001; protein: PCC = 0.939, P < 0.001; energy: PCC = 0.677, P < 0.001).

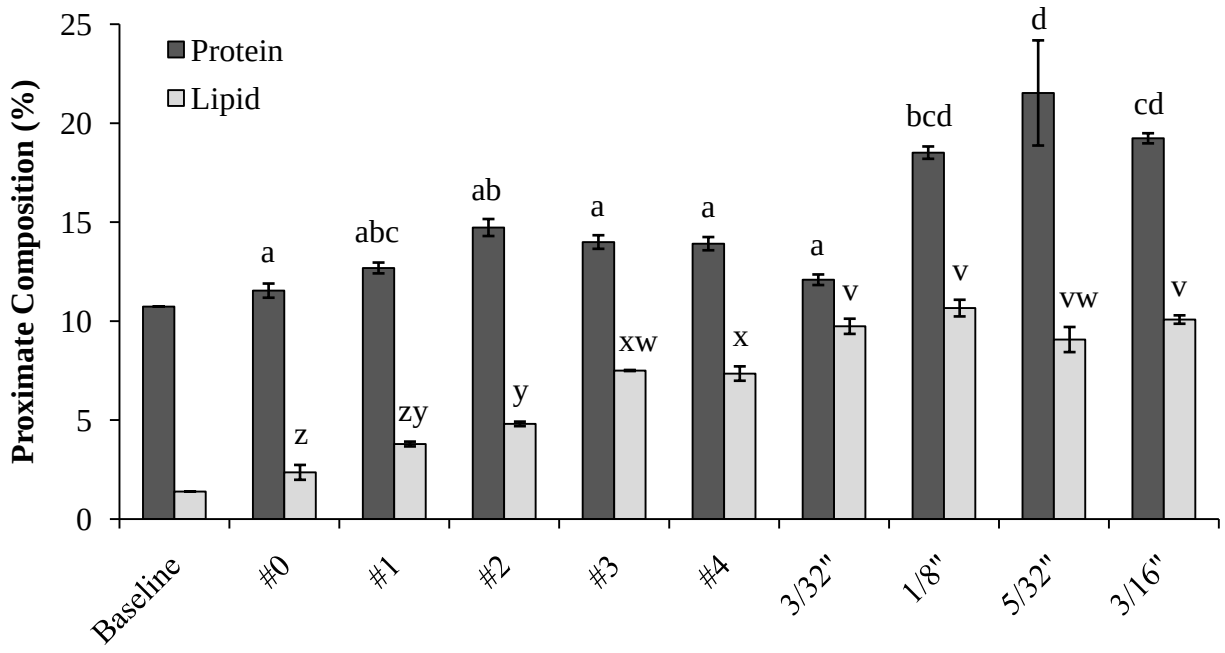

Figure 2.29. Proximate composition comparisons by feed size for average percent protein and lipid content (SE bars) in fish reared on Rangen feeds in the 2016 hatchery feed experiment. Values with same letters indicate no significant difference in average percent protein (a-d) or lipid (v-z) content.

Overall, the feed and proximate analyses highlight the differences observed in the fish, as well as the difference in costs among the feed companies, during the 2016 hatchery feed experiments (Fetherman et al. 2019). Bio Oregon, the most expensive diet, is the only diet in which the first four feed sizes have only fish meal as a protein source, and even when additional protein sources are added, it has the smallest number of other protein sources used (four total). The other feed companies used 5-6 other protein sources that appear in various combinations throughout the feed sizes. Although the majority of the feeds contained higher than minimum protein and fat content, EWOS had a lower fat content than the minimum for its smallest size feed, which may have affected early growth in the those fish, and Rangen had lower protein content for two of its larger feeds. In general, similar patterns of additive fish protein and lipid composition across feed sizes was observed across feed companies, although fish reached different apparent maximums within each company. Bio Oregon was the only feed company in which there was a negative correlation between feed protein and fish protein content, indicating that the feed was correctly formulated, reducing protein amount in larger feeds while increasing protein content of the fish. Additionally, the feed fat content was positively correlated such that if more fat was

present in the feed, the fish were able retain and store that as lipid content for a later energy source. This same pattern of fat storage was observed in the higher HSI and VSI values of Bio Oregon fish during the hatchery feed experiments (Fetherman et al. 2019), and highlights the benefits associated with the higher costs of Bio Oregon feed. These analyses will be further used to compare and explain patterns observed in the hatchery feed experiments.

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COLLABORATIVE RESEARCH PROJECTS WITH COLORADO STATE UNIVERSITY

Collaborations with graduate students at Colorado State University (CSU) provide an opportunity to expand on management and research questions of interest to the State of Colorado. 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. Candidate) and Dana L. Winkelman, Ph.D.

Effects of Rearing Density on Post-stocking Survival of Rainbow Trout

Hatchery rearing densities often result in stressful, overcrowded conditions to meet specific stocking objectives. Overcrowded conditions can lead to disease outbreaks, specifically bacterial coldwater disease (BCWD), caused by *Flavobacterium psychrophilum*. The effects of rearing density and disease could carry over into stocked populations, causing lower survival rates. This experiment examined the effects of rearing density on the post-stocking survival rates of Rainbow Trout in Parvin Lake (Red Feather Lakes, Colorado).

In the first of two rearing density experiments, all fish reared at two densities were stocked into Parvin Lake (Fetherman et al. 2019). Since all fish were stocked, more fish were stocked from

the high-density treatment (14,777 fish total) than the low-density treatment (3,096 fish total). As such, post-stocking survival may have been influenced by stocking density (total number of fish stocked) rather than rearing density (the number of fish reared in each treatment). In contrast, this second experiment was designed such that fish were reared at the same densities as the first experiment, but equal numbers of fish from each density treatment were stocked into Parvin Lake, thereby controlling for the potential effect of stocking density.

Hofer by Harrison Lake (H×H) Rainbow Trout were used for this experiment because they are one of the most common strains affected by BCWD outbreaks in Colorado hatcheries. H×H eggs (20,000) were obtained from the CPW Crystal River Hatchery and transported to the BFRH. Upon arrival, eggs were held in egg cups within two experimental tanks, and dead eggs were removed to prevent fungal growth. After hatching, fish were released into the experimental tanks, and cripples were removed from the tank daily through swim-up.

At two weeks post-swim-up, fish were counted and distributed into randomly assigned fiberglass hatchery troughs. The number of fish in each trough corresponded to the density assigned to that tank, with low density tanks containing 1,000 fish and high-density tanks containing 4,000 fish. Rearing density in the low-density treatment was chosen such that the rearing index did not exceed 0.5, a density (pounds per cubic foot) no greater than one-half the fish's length in inches (Piper et al. 1982). A rearing index of 2.0 was chosen for the high-density treatment because CPW hatcheries often maintain fish at this density to meet production goals and stocking requests, and BCWD outbreaks often occur at theses densities.

An initial sample weight was taken from fish in each tank by placing a known number of fish in a tared water bucket on a scale, obtaining individual weights by dividing the total weight by the known number of fish, and calculating the number of fish per pound. This known weight was used to assign a feeding rate (% BW/d) and calculate total amount of feed per day (g) based on fish number for each tank. Batch weights were taken on a weekly basis and amount of feed per day was adjusted based on these weights. Feeding occurred six to eight times daily.

Once a given tank reached the maximum average individual weight of the range for a given feed size, the fish were switched to the next size of feed and/or to a different feeding rate. Each tank was treated as an independent unit so that the time it took to switch feed sizes or feeding rates was known. Additionally, the volume for each rearing trough was manipulated throughout the three-month rearing period to keep from exceeding a rearing index of 0.5 or 2.0 in the low- and high-density treatments, respectively. As fish grew, tank volumes changed three times: 1) 2.7 cubic feet, 2) 5.4 cubic feet, and 3) 10.8 cubic feet. Upon volume change, densities were reduced to nearly half of the maximum, and fish were allowed to grow up to and held until the tank reached the maximum density before volume was changed again. Flows also changed to maintain appropriate dissolved oxygen concentrations and water exchange.

After the three-month rearing period, fish were Passive Integrated Transponder (PIT) tagged using 12 mm tags. Although individual tags, such as PIT tags, are more expensive than traditional batch marking techniques used to mark large numbers of fish, such as coded-wire or Visual Implant Elastomer (VIE) tags, they provide a better estimate of survival when multiple, individual recapture events are used. Roughly equal numbers of fish from each density treatment

were tagged (Table 3.1). All tagged fish were stocked into Parvin Lake in May 2019. Recapture events were conducted using a boat-mounted electrofishing unit every month between June and November 2019, with a final recapture event occurring in May 2020. All fish captured during the recapture events were scanned for a PIT tag, measured, and weighed, and capture and recapture numbers will be used to determine if post-stocking survival differed by rearing density.

Table 3.1. Number of PIT-tagged fish stocked per treatment (low density or high density) with associated capture and recapture [ ] numbers at one, two, three, four, five, and 12 months post-stocking.

Density	# Fish	1 Month	2 Months	3 Months	4 Months	5 Months	12 Months
Low	3,664	10	9 [1]	8 [4]	14 [6]	17 [5]	6 [3]
High	3,661	8	1 [0]	2 [0]	18 [0]	14 [4]	7 [2]

A full statistical analysis had not been conducted at the time of writing. The planned analysis will include a suite of models (Table 3.2) in a Cormack Jolly-Seber capture-recapture framework, which will be analyzed using hierarchical Bayesian modeling and model selection techniques. Vague prior information will be used for the all parameters values within each model set. Widely applicable information criterion (WAIC) will be used to determine the best model for inference. Posterior inference for model parameters and derived quantities (low density survival, high density survival) will be based on the number of Markov chain Monte Carlo (MCMC) samples following convergence after 20% of an iteration burn-in period.

Table 3.2. Models associated with survival (φ) and detection probability (p) for the capture-recapture events conducted in Parvin Lake between June 2019 and May 2020. Intercept models (.) indicate constant values. Int_var indicates a variable intercept term for each density treatment. Time accounts for potential differences in parameter value for each sampling period.

Model Number	Survival (φ)	Detection (p)
1	φ(.)	p(.)
2	φ(time)	p(.)
3	φ(.)	p(time)
4	φ(time)	p(time)
5	φ(density)	p(.)
6	φ(density)	p(time)
7	φ(density+time)	p(.)
8	φ(density+time)	p(time)
9	φ(int_var +density)	p(.)
10	φ(int_var+density+time)	p(.)
11	φ(int_var +density)	p(time)
12	φ(in_var+density+time)	p(time)

A brief inspection of sampled numbers (Table 3.1) indicates a steep decline in the overall number of captured fish at each sampling event compared to the first experiment. This could

lead to potential model convergence problems during analysis. Preliminary analysis of the simplest model, φ(.)p(.), indicated a very low detection probability, ranging between 0.3% and 0.6%, and a difficulty to converge on a survival value after 50,000 model iterations (Figure 3.1). Based on the low number of both captured and recaptured fish, a very large number of iterations within the Bayesian models will be needed. Analyses will proceed using the predetermined model set (Table 3.2), and model selection will be implemented. The final results will be reported in the 2021 annual report.

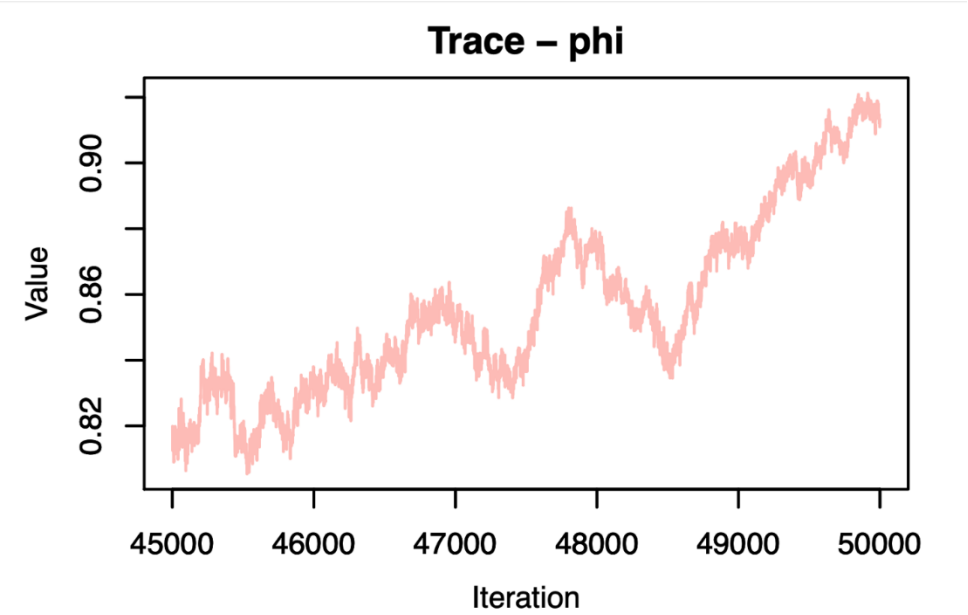

Figure 3.1. Trace plot for survival (φ) values estimated by iteration number. The trace plot indicates that full mixing had not yet occurred at 50,000 iterations, and that more iterations are needed to construct a full posterior distribution.

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Flavobacterium psychrophilum- and Myxobolus cerebralis-Resistant Rainbow Trout Strain Development

The use of *Flavobacterium psychrophilum*-resistant fish in Colorado's state hatchery system may prove to be a useful tool for reducing morality due to outbreaks of bacterial coldwater disease (BCWD). However, it is unknown if these fish are resistant to *Myxobolus cerebralis*, the parasite that causes whirling disease (WD). For *F. psychrophilum*-resistant fish to be a viable management tool, it is imperative to determine if resistance to BCWD and WD are compatible and how they might interact. Stocking *F. psychrophilum*-resistant fish that do not have resistance to *M. cerebralis* into Colorado's streams and rivers could result in failure due to

mortality associated with WD as well as increase the prevalence of *M. cerebralis* in waters where these fish are stocked. The overall goal for this dual exposure experiment was to determine if genetically resistant fish are suitable for use in Colorado's state hatchery system. By utilizing these fish, CPW could manage for two environmentally present pathogens, *F. psychrophilum* and *M. cerebralis*. Currently, CPW is utilizing *M. cerebralis*-resistant Hofer Rainbow Trout and crosses to manage for WD. Determining if the Hofer or Hofer-cross fish have resistance to *F. psychrophilum*, and the potential survival and disease prevalence benefits or consequences of dual exposure to both pathogens, is beneficial to future management practices.

Three varieties of Rainbow Trout were used for this dual exposure experiment. The first was a strain that is resistant to *F. psychrophilum* known as the Psychrophilum-Resistant Rainbow (PRR). The second was an *M. cerebralis*-resistant cross known as the Hofer by Harrison Lake Rainbow Trout (H×H). The third was a cross of the PRR and H×H (HHP). The varieties were produced at the CPW Crystal River Hatchery in January 2020 and transported to the BFRH for hatching and rearing.

The dual exposure experiment contained six treatments: 1) no pathogen exposure (control), 2) exposure to *M. cerebralis* only, 3) exposure to *F. psychrophilum* only, 4) a mock injection control, 5) exposure to *F. psychrophilum* followed by exposure to *M. cerebralis* four days later (*F. psychrophilum + M. cerebralis*), and 6) exposure to *M. cerebralis* followed by exposure to *F. psychrophilum* four days later (*M. cerebralis + F. psychrophilum*). A factorial design resulted in six treatments containing six replicate tanks per variety. Each rearing tank contained 35 fish, for a total of 108 tanks and 3,780 fish used in the experiment.

Fish were moved from the BFRH to the wet lab located on the CSU main campus in the basement of the Anatomy/Zoology building. An initial sample weight was taken for each variety prior to moving the fish and used to calculate the total amount of feed per day (g) for each tank, as well as the *F. psychrophilum* exposure dosage by weight. Control fish (both no exposure and mock injection) were randomly assigned to tanks located on the top shelf of a three-tier shelving unit to prevent cross-contamination of *F. psychrophilum* or *M. cerebralis* from above. The remaining treatment fish were randomly assigned to tanks on the lower two shelves.

For treatments involving *M. cerebralis* exposure, fish were exposed using previously established protocols (Fetherman et al. 2011). Fish were exposed to 2,000 triactinomyxons (TAMs) per individual for a total of 50,000 TAMs per tank. Prior to the addition of TAMs, water flow to each tank was stopped for one hour and each tank received aeration to ensure mixing of the TAMs. The amount of filtrate needed to deliver 2,000 TAMs per fish was delivered to each tank in two doses, each dose containing half of the necessary filtrate. Using two doses helped ensure equal distribution of TAMs in the tank and accounted for a possible unequal distribution of TAMs within the filtrate.

For *F. psychrophilum* exposures, we followed the same injection protocol described by Fetherman et al. (2019) using a dosage that was expected to cause 50% cumulative mortality in *F. psychrophilum*-susceptible varieties based off of previous data. Rainbow Trout were injected subcutaneously at the dorsal midline posterior to the dorsal fin with an 8.8×10^6 CFU/ml virulent *F. psychrophilum* (CSF259-93, 25 μL). For the mock injection controls, Rainbow Trout were

subcutaneously injected at the dorsal midline posterior to the dorsal fin with 25 μL of tryptone yeast extracts and salt (TYES).

After pathogen exposure, fish were monitored and reared for 2,000 degree-days (°C) to ensure full development of myxospores in the *M. cerebralis* exposure treatments. Tanks were cleaned on a rotating schedule once every two weeks. Throughout the rearing process, all tanks were monitored twice daily, and moribund and dead fish in each tank were removed. Cumulative percent mortality (CPM), the number of dead fish divided by total number of fish, was calculated for each tank for the first 28 days post-exposure per previous *F. psychrophilum* exposure experiments (Fetherman et al 2019). After 2,000 degree-days, all surviving fish were euthanized, weighed, measured and inspected for clinical signs of infection from *M. cerebralis* or *F. psychrophilum*. Fish were then individually bagged, frozen, and transported to the CPW Aquatic Animal Health Laboratory (AAHL; Brush, Colorado) for further processing. With the help of the AAHL staff, all fish are currently being processed and myxospore counts will be quantified using the pepsin–trypsin digest (PTD) method (Markiw and Wolf, 1974a,b). At the time of writing, only 15 of the 108 tanks (*M. cerebralis* only) had been processed using PTD.

The analysis included in this report was broken up into two separate sections: 1) the CPM in the first 28 days for all tanks (Fetherman et al. 2019), and 2) myxospore count in tanks for which PTD was completed at the time of writing. A two-factor ANOVA ($\alpha = 0.05$), with CPM as the response and variety, treatment, and the interaction between variety and treatment as the predictor variables, was used to determine if variety, treatment, or their interaction predicted CPM. If there was a significant p-value associated with a predictor variable, then a pairwise comparison with a Tukey adjustment was used to compare between varieties and treatments. A single-factor ANOVA ($\alpha = 0.05$), with average myxospore count per tank as the response and variety as the predictor, was used to determine if there was a difference in average myxospore count between the varieties. If there was a significant p-value associated with variety, then a pairwise comparison with a Tukey adjustment was used to compare between the varieties.

Mortalities within the *F. psychrophilum* treatments started within two and ten days post-injection (Figure 3.2). The delay in mortality in the *M. cerebralis* + *F. psychrophilum* treatment can be explained by the four-day delay in injections due to the experimental design. The mortality trends (shape and duration) are similar throughout the 28-day period, indicating that there were no differences in how the varieties responded to the injection methods.

The two-factor ANOVA indicated that there was a significant difference among variety and treatment, and the interaction between variety and treatment was also significant ($p < 0.001$). Pairwise comparisons indicate that there are no differences between the H×H and HHP when exposed to *F. psychrophilum* only ($p = 0.400$), with an average (± SD) CPM of 90 ± 10.2% for the H×H and 67.6 ± 27.9% for the HHP. The broad distribution within the HHP indicates that it may be possible to obtain HHPs that are genetically resistant to *F. psychrophilum* as the lower range of CPM values approached those of the PPR (Figure 3.3). However, this would require ongoing *F. psychrophilum* exposure challenges and selection for families that exhibit resistance to the bacteria. All treatment CPM values for both the HHP and H×H are relatively high compared to the resistant PRR (Figure 3.3) indicating that the HHP may not be a suitable option for *F. psychrophilum* management in CPW hatcheries.

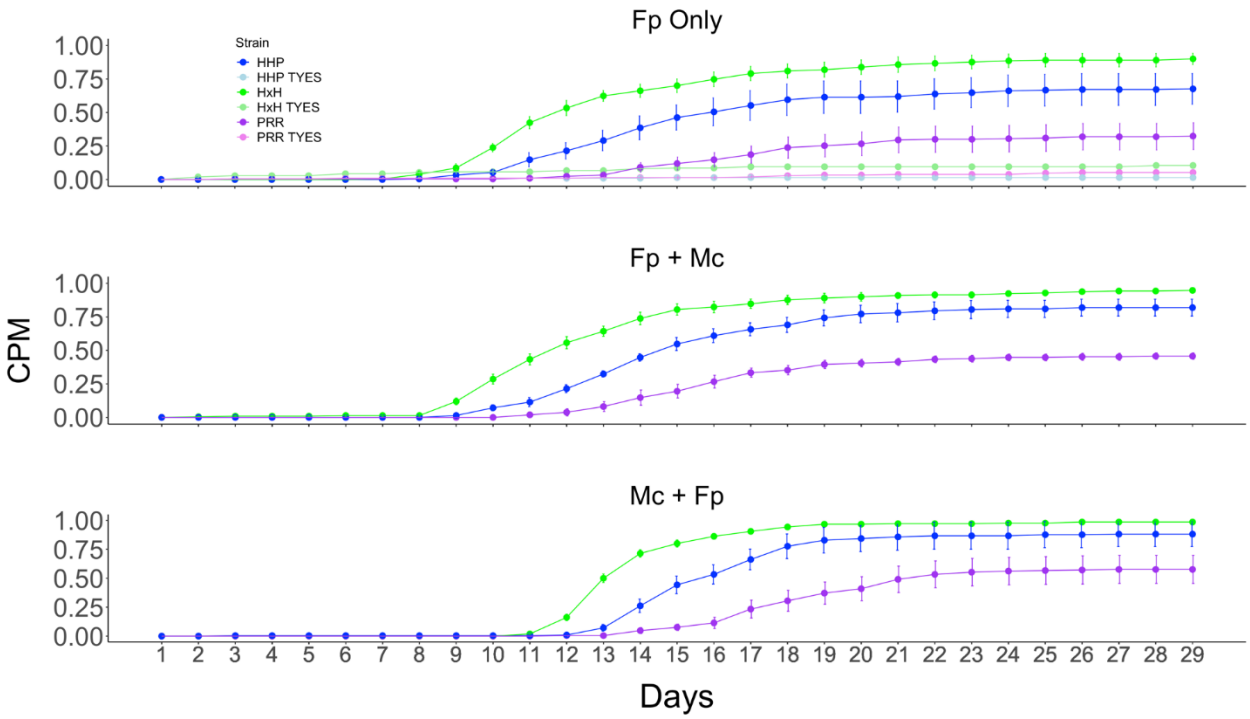

Figure 3.2. Cumulative percent mortality (CPM; SE bars) over the 28-day monitoring period for each variety of Rainbow Trout in the *F. psychrophilum* only, *F. psychrophilum* + *M. cerebralis* (Fp + Mc), or *M. cerebralis* + *F. psychrophilum* (Mc + Fp) treatments.

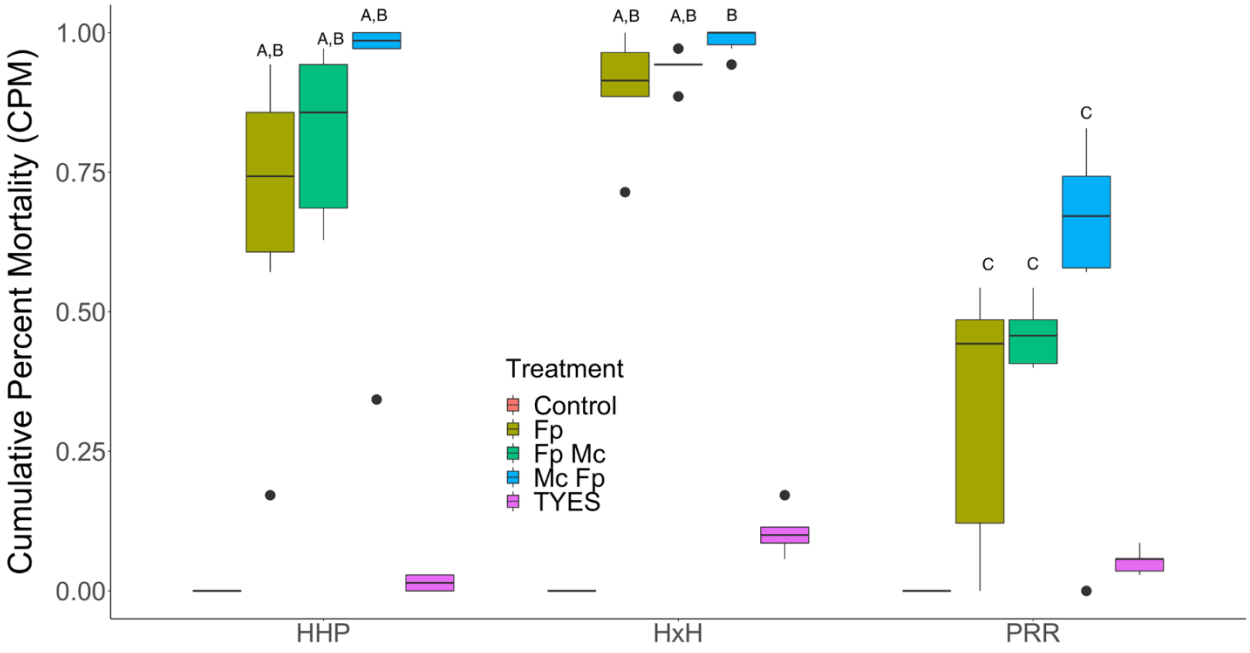

Figure 3.3. Cumulative percent mortality (CPM) among the Rainbow Trout varieties in five of the six treatments. Black lines within the boxes indicate the median of the distribution. Capital letters indicate statistical differences, with the same letter indicating no statistical difference within or among treatments.

Co-infection, or the exposure to both *F. psychrophilum* and *M. cerebralis*, resulted in an increase in CPM for all varieties (Figure 3.3). A similar increase was seen with a co-infection of *F. psychrophilum* and infectious hematopoietic necrosis virus (IHNV; Ma et al. 2019). Currently we do not know the driving factors for increased mortality due to co-infection or the specific interactions between *M. cerebralis* and *F. psychrophilum*. However, the increase in mortality with coinfection could be important to survival dynamics in a hatchery or wild setting where both pathogens are present.

At the time of writing, myxospores had been enumerated for 15 of the 108 tanks. All 15 tanks originated from the *M. cerebralis* only treatment. Of the three varieties, the H×H had the lowest average myxospore count (75,383 ± 24,001), the HHP were intermediate (203,268 ± 11,619), and the PRR had the highest myxospore count (568,014 ± 377,249 SD). The single-factor ANOVA showed that there was a significant difference in myxospore count among the varieties ($p = 0.010$). Pairwise comparisons indicated that there was not a significant difference between the H×H and HHP ($p = 0.635$) or the PRR and HHP ($p = 0.052$), however, there was a significant difference between the PRR and H×H ($p = 0.010$; Figure 3.4).

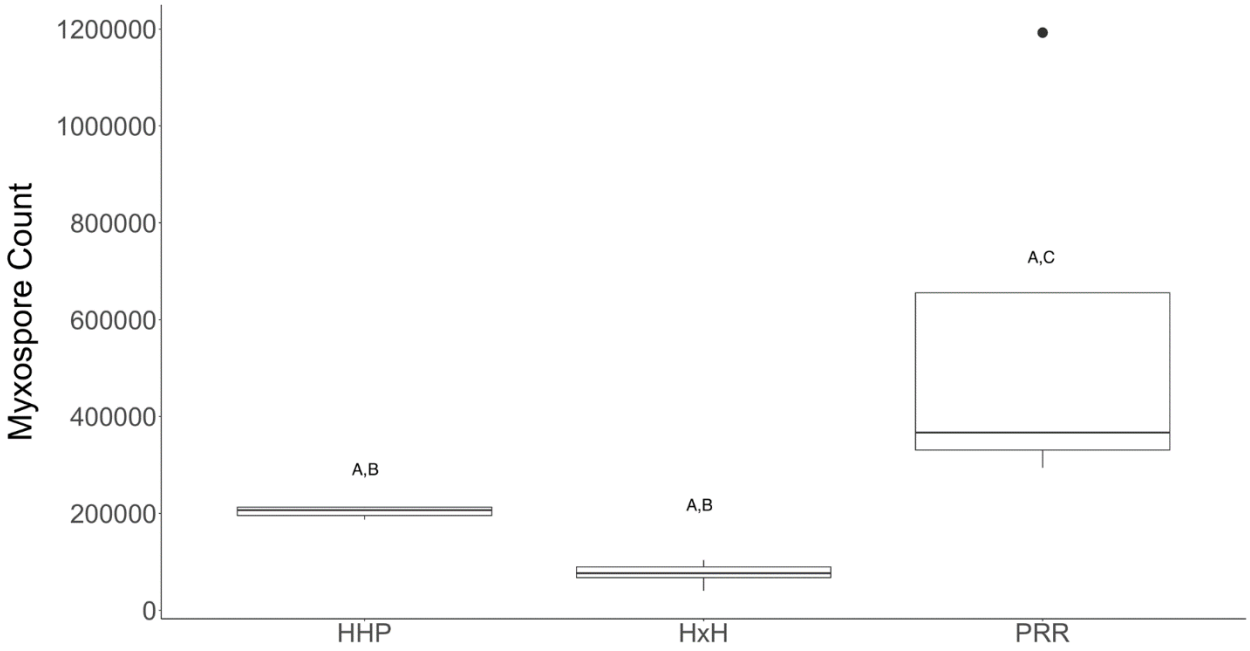

Figure 3.4. Myxospore count by variety in the *M. cerebralis* only treatment. Black lines within the boxes indicate the median of the distribution. Capital letters indicate statistical differences, with the same letter indicating no statistical difference among varieties.

These preliminary results suggest that the H×H, HHP, and PPR do not currently exhibit resistance to *M. cerebralis*. The Colorado River Rainbow Trout (CRR) investigated by Fetherman et al. (2011) had mean myxospore count of roughly 187,000 myxospores per fish. The PRR developed three times that amount, and the HHP exhibited similar counts to the CRR. The H×H had unusually high myxospore counts for a cross that is considered to be resistant to *M. cerebralis*. The high number of myxospores found in the H×H could be attributed to: 1)

outcrossing in the hatchery, which was shown by Fetherman et al. (2011) to increase myxospore count; 2) a significant release from *M. cerebralis* selection pressures in *M. cerebralis*-negative hatcheries, which could result in loss of resistance; 3) a combination of both outcrossing and loss of resistance due to selection; or 4) optimal exposure conditions, including water temperature, water quality, age of fish, and fresh TAMs. PTD analysis will continue for the remaining tanks. Myxospore counts of the dual exposure treatments should provide more insight on co-infection with *F. psychrophilum* and *M. cerebralis*.

The higher myxospore counts observed in the H×Hs may lead to future management issues. Stocking of fish with a similar susceptibility as the CRR could result in lower post-stocking survival and increased infection prevalence in systems where *M. cerebralis* is established. To determine if outcrossing and selection have resulted in a loss of resistance, genetic samples will be collected form H×Hs produced by the CPW Crystal River Hatchery and compared to wild stocks that have sustained exposure to *M. cerebralis* and, theoretically, continued selection for resistance. Genetic data should provide more information regarding how to proceed with producing *M. cerebralis*-resistant Rainbow Trout in *M. cerebralis*-negative hatchery environments.

The loss of resistance in the HHP to both *M. cerebralis* and *F. psychrophilum* may also be the result of outcrossing as this variety originated from spawning an H×H (already outcrossed multiple times to obtain a 7/8 Hofer, 1/8 Harrison Lake cross) and a PRR. Collaborators within the USDA and Utah Division of Wildlife Resources suggest that an F1 generation cross between an *M. cerebralis*-resistant strain and an *F. psychrophilum*-resistant strain could maintain dual resistance to both pathogens. Another *F. psychrophilum* challenge experiment was designed and conducted to determine if F1 crosses were genetically resistant to at least *F. psychrophilum* (see *Flavobacterium psychrophilum* Resistance of Various Rainbow Trout Strains and Crosses section below).

Fetherman, E. R., G. J. Schisler, and B. W. Avila. 2019. Sport Fish Research Studies. Federal Aid Project F-394-R18. Federal Aid in Fish and Wildlife Restoration, Job Progress Report. Colorado Parks and Wildlife, Aquatic Wildlife Research Section. Fort Collins, Colorado.

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Flavobacterium psychrophilum Resistance of Various Rainbow Trout Strains and Crosses

Results from the dual exposure experiment with *Flavobacterium psychrophilum* and *Myxobolus cerebralis* indicated that the cross between the H×H and the PRR (HHP), did not have genetic resistance to *F. psychrophilum.* Collaborators within the USDA and Utah Division of Wildlife Resources suggested that an F1 generation cross between an *M. cerebralis*-resistant strain and an *F. psychrophilum*-resistant strain could maintain dual resistance to both pathogens. In addition, the USDA currently has a 5th generation *F. psychrophilum*-resistant strain of Rainbow Trout (WV5) that has exhibited increased resistance to *F. psychrophilum* compared to the 3rd generation fish used in the first experiment (PRR) which are currently being utilized within the CPW hatchery system.

To determine if creating a fish that maintains resistance to both *M. cerebralis* and *F. psychrophilum* was an obtainable goal, we ran an additional 28-day *F. psychrophilum* exposure experiment with various strains and F1 crosses. The F1 crosses were created using Hofer Rainbow Trout (HOF; CPW) and Harrison Lake Rainbow Trout (HAR; CPW), which are resistant to *M. cerebralis*, WV5 (USDA), and PRR (CPW). As such, eight varieties were evaluated for Fp resistance in this experiment: 1) HOF, 2) HAR, 3) WV5, 4) PRR, 5) HOF×WV5, 6) HAR×WV5, 7) HOF×PRR, and 8) HAR×PRR. We also incorporated the USDA *F. psychrophilum*-susceptible line of Rainbow Trout (S-Line) as a positive control.

Due to time constrains and water use restrictions we exposed fish to *F. psychrophilum* only (no *M. cerebralis* exposure). A power analysis, performed in Program R (version 3.6.2) using data from the dual exposure experiment, provided an estimate of the minimum number of tanks needed to detect a difference in mortality among varieties with 80% confidence (10 tanks; Table 3.3). To account for response variation and increase replication given space constraints, we chose to only use five of the eight varieties for injection controls (HOF, HAR, WV5, HAR×WV5, and S-Line), with two tanks for each. As such, the experiment ultimately contained ten tanks each for the eight Rainbow Trout varieties exposed to *F. psychrophilum*, and two tanks each for the five varieties used as a mock injection control (90 tanks total).

Table 3.3. Power analysis results for number of tanks needed to detect a difference in mortality among the Rainbow Trout varieties, performed in Program R (version 3.6.2) using data from the dual exposure experiment (standard deviation of 0.1).

Power	# of Tanks
0.55	6.00
0.60	6.61
0.65	7.27
0.70	8.02
0.75	8.88
0.80	9.89
0.85	11.15
0.90	12.86
0.95	15.65
0.99	21.68

All of the above varieties were created in collaboration with the USDA-ARS National Center for Cool and Cold Water Aquaculture, CPW Crystal River Hatchery and the BFRH. The USDA provided WV5 milt and the Crystal River Hatchery provided PRR milt so the BFRH could create the desired Rainbow Trout crosses using HOF and HAR maintained onsite in January 2020. The Crystal River Hatchery produced pure PRRs and shipped the eggs to the BFRH in January 2020, and the USDA produced pure WV5s and S-Line and shipped them to the BFRH in February 2020. All fish were hatched and reared at the BFRH.

Fish were moved from the BFRH to the wet lab located on the CSU main campus in the basement of the Anatomy/Zoology building. An initial sample weight was taken for each variety prior to moving the fish (Figure 3.5) and used to calculate the total amount of feed per day (g) for each tank, as well as the *F. psychrophilum* exposure dosage by weight. Control fish were randomly assigned to tanks located on the top shelf of a three-tier shelving unit to prevent cross-contamination of *F. psychrophilum*. The remaining treatment fish were randomly assigned to tanks after control tanks were established. Each 20-gallon tank contained 25 fish of a given variety.

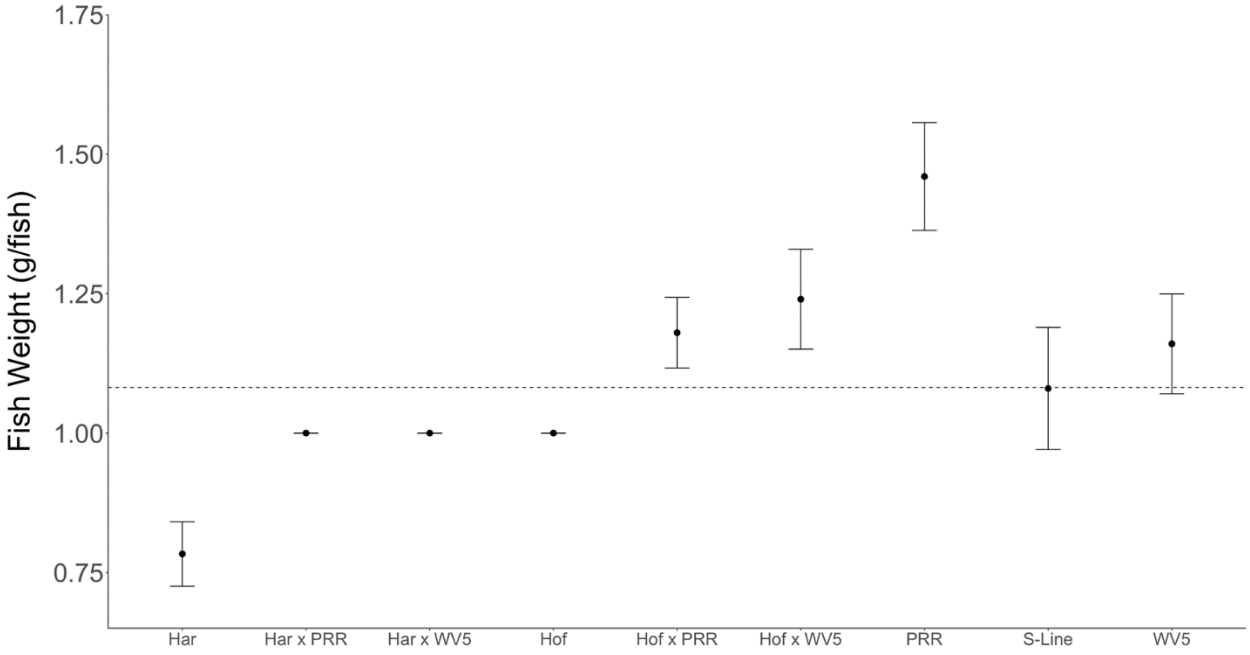

Figure 3.5. Average weight (g; SD bars) for each of the Rainbow Trout varieties at the time of injection with *F. psychrophilum*. The dotted black line indicates the average weight (g) across all varieties.

All treatment Rainbow Trout for were injected subcutaneously posterior to the dorsal fin above the midline with virulent 8.8×10^6 CFU/ml of *F. psychrophilum* (CSF259-93, 25 μL), whereas fish serving as mock injection controls were injected with tryptone yeast extract and salts (TYES, 25 μL). Fish were monitored twice a day for 28 days post-injection. Moribund and dead fish were removed and recorded. At the end the rearing period, all remaining fish were

euthanized, and cumulative percent mortality (CPM) was calculated for each variety and treatment.

A single-factor ANOVA ($\alpha = 0.05$), with weight at the time of exposure as the response and variety as the predictor, was used to determine if there was a difference in weight at the time of injections, which could potentially be used to explain differences in CPM among the varieties. A two-factor ANOVA ($\alpha = 0.05$), with CPM as the response and variety, treatment, and the interaction between variety and treatment as the predictor variables, was used to determine if variety, treatment, or their interaction predicted CPM. For both analyses, if there was a significant p-value associated with a predictor variable, then a pairwise comparison with a Tukey adjustment was used to compare between varieties and/or treatments.

Mortalities occurred within the first week post-injection (Figures 3.6 and 3.7), starting within the first two days post-injection for the WV5 and S-Line and within three to five days for the other varieties. Previous experimental exposures resulted in mortality between seven and ten days post-exposure (Fetherman et al. 2019), but for different dose and weight ranges. The mortality trends (shape and duration) for the varieties were similar across the 28-day experiment (Figure 3.6), indicating that there were no large differences in how the varieties responded to the injection method.

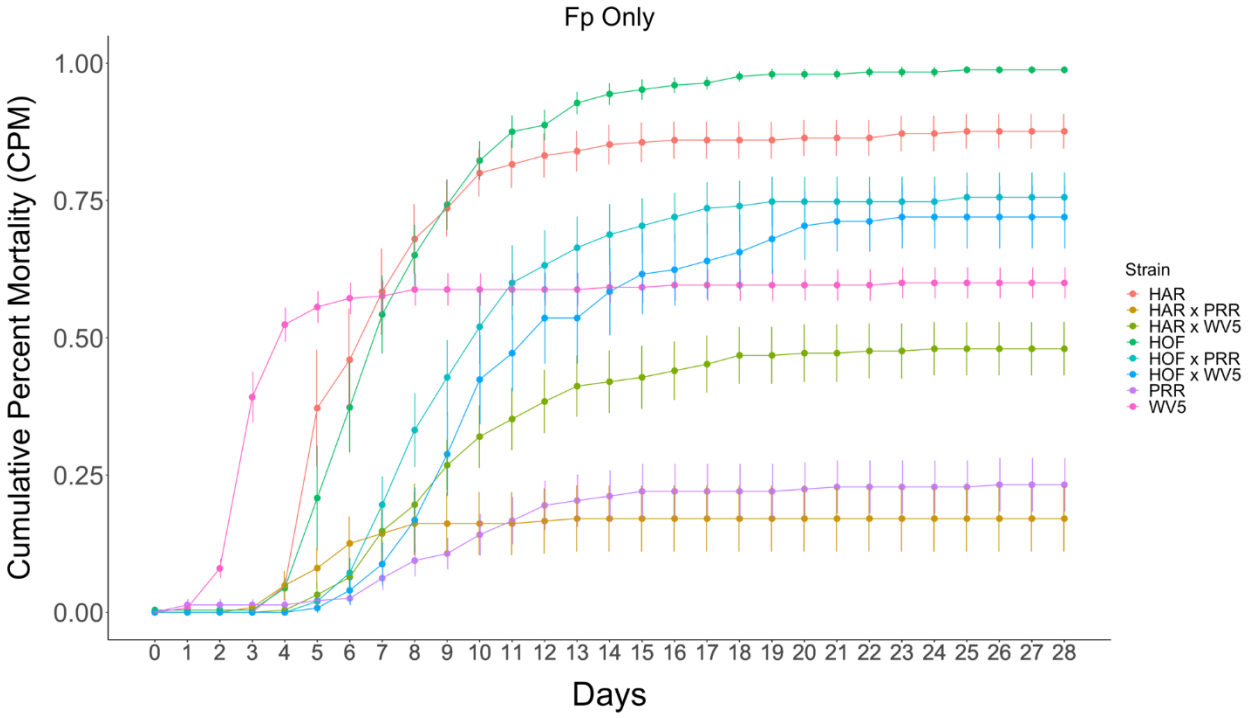

Figure 3.6. Cumulative percent mortality (CPM, SD bars) for the Rainbow Trout varieties over the 28-day *F. psychrophilum* exposure experiment.

The single-factor ANOVA analysis indicated that there was a significant difference in weight among varieties at the time of injection ($p < 0.001$). Average weights for the varieties ranged from 0.78 to 1.46 g, with a mean weight of all varieties of 1.1 g (Figure 3.5). Differences in

weight could result in larger fish having lower CPM values than smaller fish. However, biologically, the differences in weight were small (± 0.3 g) relative to the mean weight of all varieties. Results suggest that the small differences in weight did not play a role in CPM, and CPM was more likely driven by susceptibility to *F. psychrophilum*. For example, although the HAR×PRR were smaller than the HOF×PRR at the time of injection, they exhibited significantly lower CPM (Figure 3.8).

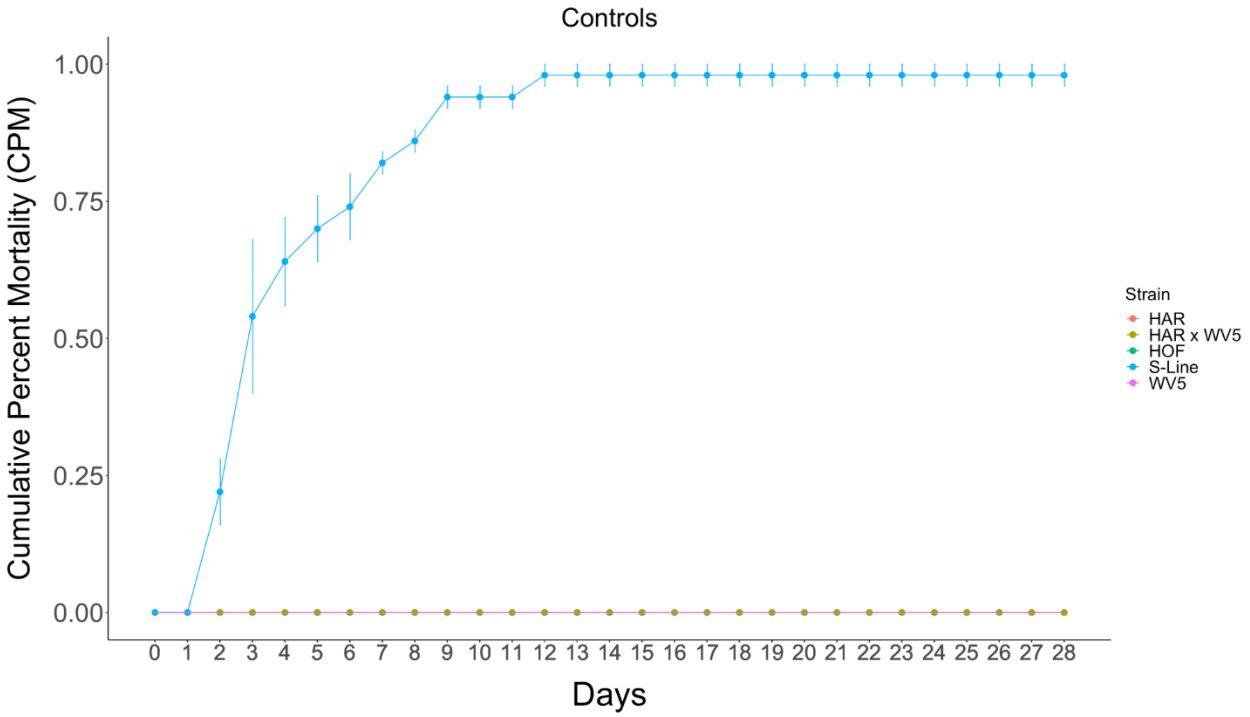

Figure 3.7. Cumulative percent mortality (CPM; SD bars) for the *F. psychrophilum*-susceptible line of Rainbow Trout (S-Line) and four other varieties used as mock injection controls over the 28-day *F. psychrophilum* exposure experiment.

The two-factor ANOVA analysis indicated that there was a significant effect of variety, treatment, and their interaction ($p < 0.001$). The TYES mock injection control fish exhibited little to no mortality associated with the injection procedure (Figure 3.9). The S-Line, used as a positive control, exhibited an expected high CPM that did not differ from the HOF or HAR ($p \geq 0.990$), which also exhibited high susceptibility to *F. psychrophilum*. The PRR had a significantly lower CPM than the WV5 ($p < 0.001$). The WV5 fish were included in the experiment to determine if two additional generations of selection for *F. psychrophilum* resistance resulted in lower CPM relative to the PRR (three generations of selection). The lower CPM of the PRR indicates that there is no need to replace the current PRR brood stock with a "newer" *F. psychrophilum*-resistant brood stock in the CPW hatchery system at this time. Continuous exposure to *F. psychrophilum* in CPW hatcheries may have resulted in the maintenance of or increase in resistance to *F. psychrophilum* in the PRR relative to the WV5.

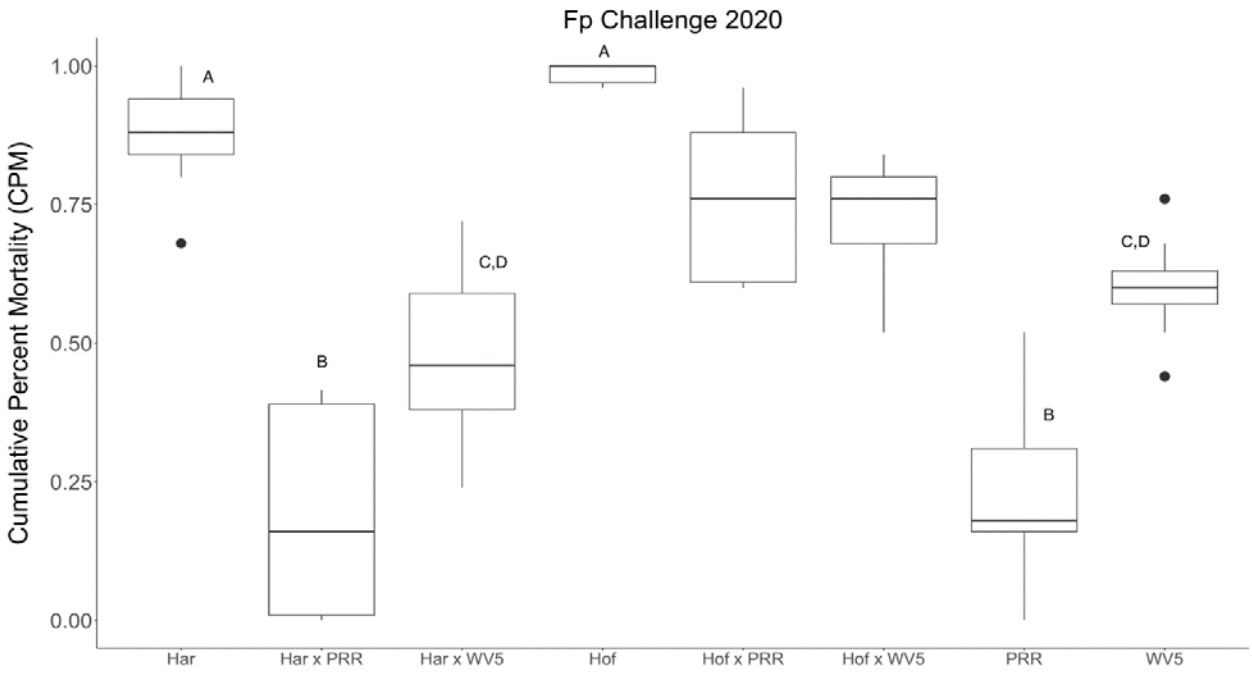

Figure 3.8. Cumulative percent mortality (CPM) for the Rainbow Trout varieties included in the 28-day *F. psychrophilum* exposure experiment. Black lines within the boxes indicate the median of the distribution. Capital letters indicate statistical differences, with the same letter indicating no statistical difference among varieties.

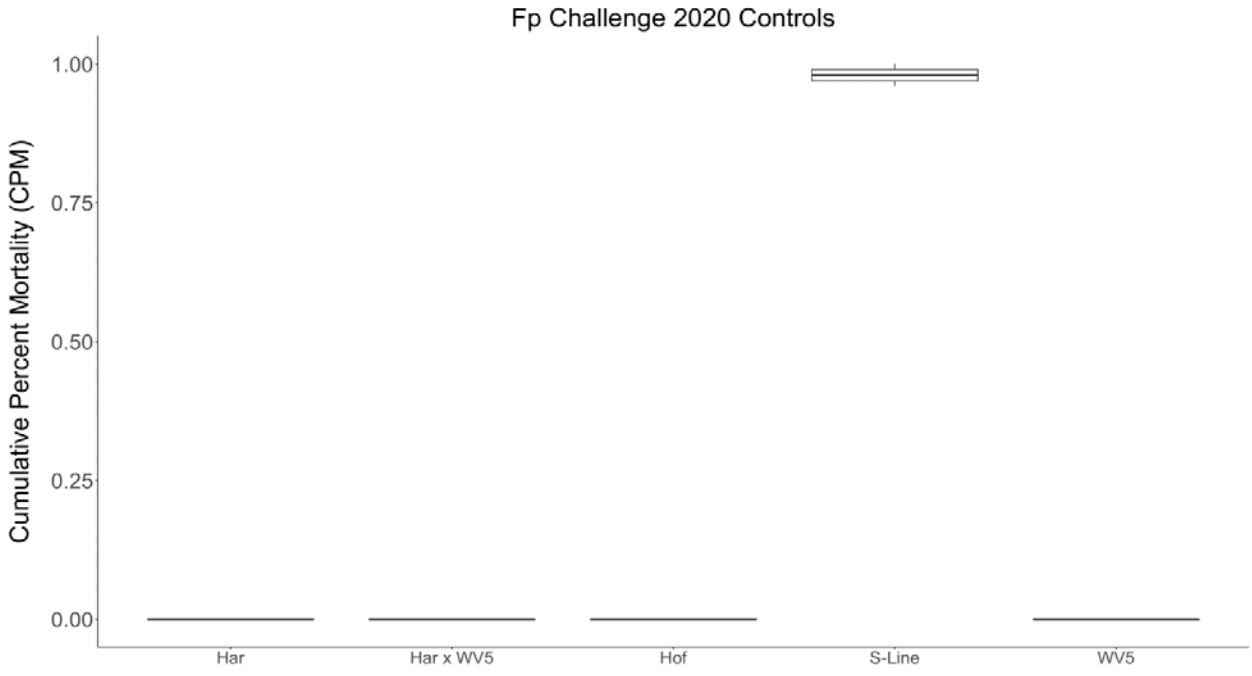

Figure 3.9. Cumulative percent mortality (CPM) for the *F. psychrophilum*-susceptible line of Rainbow Trout (S-Line) and four other varieties included as mock injection controls in the 28-day *F. psychrophilum* exposure experiment. Black lines within the boxes indicate the median of the distribution.

The F1 cross with the lowest average CPM was the HAR×PRR. The HAR×PRR CPM distribution had the largest variance (3.6%), however, the distribution included lower CPM values compared to the majority of the other Rainbow Trout varieties exposed to *F. psychrophilum*. The F1 cross with the second lowest CPM was the HAR×WV5 (Figure 3.8). Even though there is a significant difference between the HAR×PRR and HAR×WV5 ($p < 0.001$), the CPM of both crosses compared to the HAR, HOF, or H×H (dual exposure experiment) indicates the PRR and WV5 are potential options for obtaining genetic resistance to *F. psychrophilum* when crossed with the HAR.

This experiment was an extension of the dual *M. cerebralis* and *F. psychrophilum* exposure experiment. The ultimate goal was to determine if it was possible to create a cross of Rainbow Trout that is genetically resistant to both pathogens. The results of this experiment indicate that it is possible to create an F1 cross that is genetically resistant to *F. psychrophilum*, and that an F1 cross will likely maintain a higher resistance to *F. psychrophilum* than other filial generations such as the HHP tested in the dual exposure experiment. However, the resistance to *M. cerebralis* of the HAR×PRR, HAR×WV5, and the current generation of the HAR maintained in CPW needs to be evaluated. Additional *M. cerebralis* exposure and/or dual exposure experiments are needed to determine if these F1 crosses can be used to manage for both bacterial coldwater disease and whirling disease.

Fetherman, E. R., G. J. Schisler, and B. W. Avila. 2019. Sport Fish Research Studies. Federal Aid Project F-394-R18. Federal Aid in Fish and Wildlife Restoration, Job Progress Report. Colorado Parks and Wildlife, Aquatic Wildlife Research Section. Fort Collins, Colorado.

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 symptoms 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.

Comparison of Tissue Sampling for the Detection of Renibacterium salmoninarum

In recent years, diagnostic methods for detecting *R. salmoninarum* in fish tissues have increased to prevent outbreaks of BKD. Disease outbreaks can reduce the availability of brood fish and/or the number of fish stocked for conservation efforts and angling opportunities. Fish health inspections serve as a critical step in preventing and managing *R. salmoninarum* outbreaks, however, the lack of a gold standard assay increases the likelihood of missed detections, which increases the opportunity for transmission to uninfected fish. True states of infection within a given species can be difficult to determine depending on assay type, the developmental stage of the bacteria, and the tissue it resides in. The American Fisheries Blue Book (USFWS and AFS-FHS 2014) currently suggests testing kidney tissues by DFAT, nested PCR (nPCR), or ELISA, and non-lethally collecting ovarian fluid to determine the presence of *R. salmoninarum* during fish health inspections, but these protocols have been developed using primarily anadromous species (Elliott 2012). Here, we tested six tissues collected from Greenback Cutthroat Trout (*Oncorhynchus clarkii*), three lethal and three non-lethal, to offer specific diagnostic suggestions that may be used for other inland salmonid species.

In May 2019, we collected 788 Greenback Cutthroat Trout from the CPW Poudre Rearing Unit to test for *R. salmoninarum* using three non-lethal methods (blood, mucus swabs, and ovarian fluid) and three lethal methods (kidney, liver, and spleen tissue). Each fish was swabbed along the lateral line (mucus swab), euthanized using tricaine methanesulfonate (MS-222), and then ovarian fluid, blood, kidney, liver, and spleen tissues were collected and frozen immediately on dry ice for further laboratory processing. Upon arrival at the laboratory, tissues were homogenized and prepared in duplicate for either DFAT or qPCR. Samples were prepared for DFAT by smearing a kidney tissue on a non-coated glass slide, stained with a FITC-conjugated *R. salmoninarum* antisera, and counter-stained using Eriochrome Black (USFWS and AFS-FHS 2014). All DFAT samples were analyzed using a Nikon compound microscope fitted with a green fluorescent emission high-pressure mercury lamp and examined at 1000X magnification. Any bacterial cells that fluoresced and measured 1.0×0.5 μm were considered *R. salmoninarum*. DNA extraction of all tissues was carried out as described in the AFS-Blue Book (USFWS and AFS-FHS 2014) with an additional elution step. Analyses of DNA were completed using qPCR

with previously described primer sets (RS 1238 F, RS 1307 R, and RS 1262 MGB probe; Chase et al. 2006; Elliott et al. 2012). Samples were determined positive when greater than 1.99 copies of *R. salmoninarum* DNA were present, equal to 1.99 bacterial cells per mg of tissue. Positive DFAT samples were generally obtained when the qPCR reaction contained greater than 1.99 copies, but not less. Ten-fold serial dilutions from an *R. salmoninarum* culture were also run with each qPCR test to confirm quantities ranging from 1.1×10^5 to 1.1×10^1, with an average ± SD R^2 value of 0.99 ± 0.01 and an average slope of -3.32 ± 0.39. Critical threshold (Ct) values ranged from 12 to 38, with an average around 34. Average bacterial loads among all tissues ranged from 3.63×10^7 to 1.99.

At the time of writing, DFAT screening was not completed and all results presented herein come from the qPCR analysis only. Of the 788 fish tested by screening all tissues (blood, mucus swabs, ovarian fluid, kidney, liver, and spleen), 364 fish were determined positive by any method. Of the 364 fish, 11 fish were determined positive by blood, 135 by mucus swabs, 16 by ovarian fluid (out of 190 positive female fish), 134 by kidney tissue, 212 by liver tissue, and 22 by spleen tissue. Surprisingly, no fish were positive across all six tissues. Correlations between kidney and liver tissue were the highest among all paired tissue correlations, although only 62 fish were determined positive by both kidney and liver tissue (Table 3.4).

Table 3.4. Correlation between positives obtained by each tissue individually or paired using quantitative polymerase chain reaction (qPCR).

Tissue	Non-lethal Methods			Lethal Methods		
	Blood	Mucus Swabs	Ovarian Fluid	Kidney	Liver	Spleen
Blood	11	1	1	5	3	3
Mucus Swabs		135	4	51	44	8
Ovarian Fluid			16	7	9	4
Kidney				134	62	22
Liver					212	27
Spleen						34

Previous researchers have noted a lack of agreement between tissues testing positive for *R. salmoninarum* and our data is consistent with these findings. In our data, we observed that many positive tissues had low levels of bacteria, which could indicate a new infection or clearing of an older infection. Most of the time, tissues had less than 10 bacterial cells present, which is at or lower than the threshold needed for detection by qPCR (Elliott et al. 2013). Alternatively, non-uniform distribution of *R. salmoninarum* throughout the tissues could inhibit detection of bacteria via qPCR, which uses a small amount of the tissue overall for diagnostics (Elliott et al. 2013). While results are still being analyzed, we suggest testing hematopoietic tissues (liver and kidney) versus non-lethal tissues (blood, ovarian fluid, mucus swabs) for future diagnostic tests in inland salmonids such as the Greenback Cutthroat Trout.

Chase, D. M., D. G. Elliott, and R. Pascho. 2006. Detection and quantification of *Renibacterium salmoninarum* DNA in salmonid tissues by real-time quantitative polymerase chain reaction analysis. Journal of Veterinary Diagnostic Investigation 18(4):375-380.

Elliott, D. G. 2012. Bacterial kidney disease. *In* AFS-FHS. FHS Blue Book: Suggested procedures for the detection and identification of certain finfish and shellfish pathogens, 2016 edition.

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., D. M. Chase, and M. K. Purcell. 2012. Bacterial kidney disease Appendix 2: quantitative PCR for detection of *Renibacterium salmoninarum*. *In* AFS-FHS. FHS Blue Book: Suggested procedures for the detection and identification of certain finfish and shellfish pathogens, 2016 edition.

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.

Horizontal Transmission of Renibacterium salmoninarum

Renibacterium salmoninarum is a highly virulent pathogen that is slow to progress through its host and can utilize two modes of transmission. Through horizontal transmission, the fish can be infected by ingesting contaminated fecal matter, direct contact with an infected fish, or indirectly by bacteria in the water. Details of how or when horizontal transmission occurs is currently lacking in the literature, but this transmission route is suggested to play an important role in infection within and among populations. In a previous study, fish with a known infection intensity of 5% were later tested and an additional 75% of the population was infected with *R. salmoninarum* suggesting horizontal transmission may occur rapidly (Evelyn et al. 1984; Balfry et al. 1996). Unfortunately, studying and quantifying horizontal transmission in a laboratory can be difficult. Often times, inference of transmission can only be derived following successful inoculations by feeding fish *R. salmoninarum*-contaminated fecal matter (Balfry et al. 1996). In addition, horizontal transmission is often density dependent and factors contributing to host and pathogen interactions can be difficult to manipulate in wild populations. Due to the lack of investigational studies in wild populations of salmonids, we designed a naturalized experiment to observe horizontal transmission of *R. salmoninarum* in a hatchery-reared Greenback Cutthroat Trout (*Oncorhynchus clarkii*) population naturally infected with the bacteria.

Colorado hatcheries often utilize small rearing ponds or concrete raceways fed by surface water. These facilities often maintain high densities while rearing fish from juveniles to catchables or permanently retaining fish for brood stock replacement. High horizontal transmission rates are predicted to occur among fish held at high densities. Additionally, *R. salmoninarum* can persist in the water at optimal temperatures (10-18°C) for 4-21 days (Balfry et al. 1996). Surface water temperatures at the CPW Poudre Rearing Unit generally reach this optimum between May and September annually. In this study, we deployed two sentinel cages containing ten, 12-month-old Greenback Cutthroat Trout at each of six sites and during three, 30-day deployment periods in

May through August 2019. The six sites included: 1) above the hatchery in the Cache la Poudre River (surface water source); 2) in the north raceway, which contained positive two- and three-year-old Greenback Cutthroat Trout; 3) in the south raceway, which contained positive three-year-old Rainbow Trout (*Oncorhynchus mykiss*); 4) in settling pond number one, where the north raceway discharges; 5) in settling pond number two, where the south raceway discharges; and, 6) in the hatchery effluent (Figure 3.10). Cages were built using 10 inch, schedule 40 PVC pipes with removable end caps and a large cut out section (4 in x 10 in) covered with galvanized screen mesh to prevent fish from escaping. Our cages prevented direct contact between infected and non-infected fish. Therefore, infections were assumed to occur through indirect contact (ingestion of contaminated fecal matter from infected fish or through the presence of bacteria in the water).

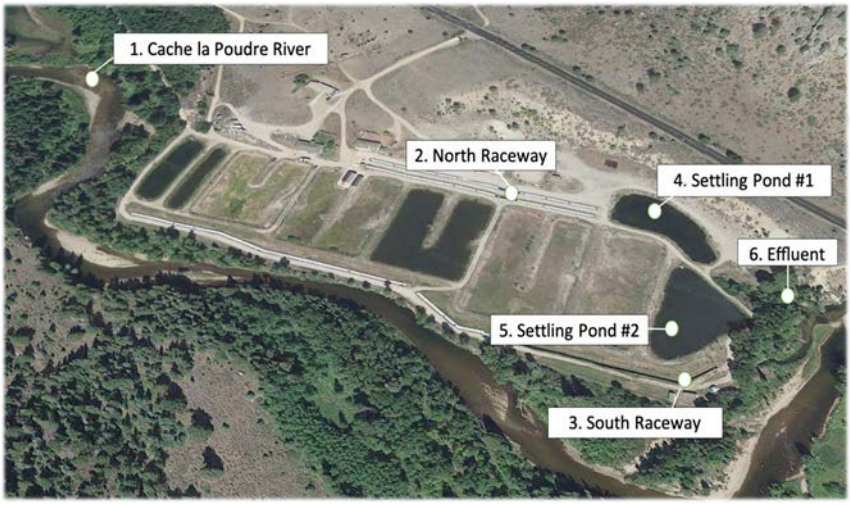

Figure 3.10. Locations of sentinel cages deployed at the CPW Poudre Rearing Unit.

A Thermochron high-resolution iButton was included in one cage at each site and programmed to log temperatures roughly five times per day. Cage deployments started in May 2019 and lasted 30 days each. After 30 days, fish were collected and replaced with a new set of ten sentinel fish for another 30 days until all three deployments were concluded in mid-August. Following each 30-day deployment, iButton temperature loggers were collected from the cage, temperatures downloaded, and then replaced. Fish from each cage were removed, weighed, measured, euthanized (MS-222), and frozen on dry ice for transport to the laboratory. Upon arrival, liver, spleen, and kidney tissues were collected and homogenized together for DNA extraction. All extractions were completed using a DNeasy blood and tissue kit (Qiagen, Inc.) following suggestions in the AFS Blue Book (USFWS and AFS-FHS 2014). *Renibacterium salmoninarum* was identified using qPCR targeting the *msa* p57 gene (Chase et al. 2006).

Horizontal transmission of *R. salmoninarum* has been predicted to follow the host spawning season, typically February through May for CPW-reared Greenback Cutthroat Trout and Rainbow Trout, concurrent with when water temperatures reach the optimal range for survival without a host. Although other studies have shown high rates of horizontal transmission, our study suggests transmission may be low when only indirect contact of *R. salmoninarum* occurs. Analysis of tissues with qPCR indicated only one fish was positive for the presence of the

bacteria. This fish was located in the south raceway near highly infected Rainbow Trout during the second deployment, with an average ± SD site temperature of 10.74 ± 1.77°C. No other fish tested positive for *R. salmoninarum* by qPCR in the other sites or other deployment periods.

Our study represents one of the first efforts to investigate *R. salmoninarum* transmission rates in inland salmonids. Inconsistent with other studies, our data suggest horizontal transmission through indirect contact may be low and direct contact may play a more significant role in maintaining infection. Transmission of *R. salmoninarum* most likely occurs when increased densities and suboptimal rearing conditions occur. The behavior of hatchery salmonids, particularly the tendency to crowd during feeding and cleaning times and increased handling, may also facilitate high horizontal transmission rates (Larson et al. 2020), conditions that were not replicated in our study. Horizontal transmission of *R. salmoninarum* through spawning and social behaviors, as seen in other cultured salmonid populations in the Pacific Northwest (Evelyn et al. 1984; Balfry et al. 1996), may explain the high probability of established infections through direct contacts. In conclusion, our study shows that while transmission of *R. salmoninarum* between infected and non-infected fish may be poorly understood, transmission through indirect contact on a hatchery unit is low, and future experiments should focus on the role of direct contact to guide future regulation and management efforts.

Balfry, S. K., L. J. Albright, and T. P. T. Evelyn. 1996. Horizontal transfer of *Renibacterium salmoninarum* among farmed salmonids via the fecal-oral route. Diseases of Aquatic Organisms 25:63-69.

Chase, D. M., D. G. Elliott, and R. Pascho. 2006. Detection and quantification of *Renibacterium salmoninarum* DNA in salmonid tissues by real-time quantitative polymerase chain reaction analysis. Journal of Veterinary Diagnostic Investigation 18(4):375-380.

Evelyn, T. P. T., J. E. Ketcheson, and L. Prosperi-Porta. 1984. Further evidence for the presence of *Renibacterium salmoninarum* in salmonid eggs and for the failure of povidone-iodine to reduce the intra-ovum infection rate in water-hardened eggs. Journal of Fish Diseases 7(3):173-182.

Larson, D. L., M. Faisal, R. J. Tempelman, H. Yu, and K. T. Scribner. 2020. Effects of hatchery rearing density, handling, and nutrition on *Renibacterium salmoninarum* infection prevalence in juvenile Chinook Salmon (*Oncorhynchus tshawytscha*). Journal of Aquatic Animal Health. DOI:10.1002/AAH.10103.

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.

Vertical Transmission of Renibacterium salmoninarum

Vertical transmission of *R. salmoninarum* in salmonid species has been previously documented and is known to cause intra-ovum infections that develop within the embryo (Evelyn et al. 1984,

1986; Brown et al. 1990; Evenden et al. 1993). Since vertical transmission can play a key role in infections, management efforts have been implemented at hatcheries to reduce the transfer of *R. salmoninarum* to offspring during spawning. One such strategy has been lethal culling operations where adult fish are euthanized after being spawned to collect kidney tissue to screen for the presence of *R. salmoninarum* through serological and molecular diagnostic methods. If parent fish are determined positive, the eggs from those fish are culled to limit the dissemination of potentially positive fish. While such management efforts can limit future infections, other studies have shown that progeny originating from negative adult fish can be positive for *R. salmoninarum* (Armstrong et al. 1989; Fetherman et al. 2020). Discrepancies between test results have been observed throughout the literature, and the best tissue for determining the probability of transmission to progeny is unknown. Our study focused on vertical transmission of *R. salmoninarum* from spawning adult Greenback Cutthroat Trout to progeny. The objectives were to determine: 1) at what rate vertical transmission occurs, 2) what level of infection in the adult is needed to successfully transmit the bacteria, 3) if only female fish contribute to vertical transmission, and 4) which tissue(s) better predict the occurrence of vertical transmission.

In May 2019, over five weeks, we collected 788 Greenback Cutthroat Trout (394 females and 394 males) from the CPW Poudre Rearing Unit during the annual spawning period. Males and females were spawned together in a 1:1 ratio, euthanized, and then we collected three non-lethal tissues (blood, ovarian fluid, and mucus swabs) and three lethal tissues (kidney, liver, and spleen tissues) to determine the presence of *R. salmoninarum* in the adult fish. All kidney tissues were tested first using qPCR, in duplicate, to determine the initial status of the adult fish. Once kidney tissues were determined positive or negative, eggs were either retained for use in the experiment or discarded. After testing all fish kidney tissues, the following treatments were assigned given the status of the adult fish (one 1:1 family per tank): 11 tanks of positive adult female and male, 8 tanks of negative adult female and positive adult male, 5 tanks of positive adult female and negative adult male, and 8 tanks of negative adult female and male. After this initial assignment, the remaining five tissues were also tested, resulting in a change of assignment for the treatments to 21, 6, 4, and 1 tanks, respectively.

After swim-up, tanks were reduced to 100 fish per tank, and reared for 6 months. Upon reaching 6 months old, each tank was further reduced to 50 fish and tissue samples were collected from all fish removed from the tanks. Tissues collected included mucus swabs, and kidney, liver, and spleen tissues, which are currently being processed for *R. salmoninarum* by ELISA and qPCR. The same tissues were collected from the remaining fish once they reached one year old in June 2020. Additionally, serum was collected from each one-year-old fish to test for antibody production, indicating an immune response towards *R. salmoninarum* had occurred prior to sampling.

Results from this study will contribute to our understanding of vertical transmission in Greenback Cutthroat Trout. In addition, determining the rate of vertical transmission on a hatchery unit will be useful for deciding if management strategies such as lethal culling continue to be necessary to prevent the production of positive progeny. Understanding which tissues better predict vertical transmission to progeny will also be useful for future health screenings and setting regulations for preventing further spread of *R. salmoninarum* within a unit or to wild fish populations.

Armstrong, R. D., W. Martin, T. P. T. Evelyn, B. Hicks, W. J. Dorward, and H. W. Ferguson. 1989. A field evaluation of an indirect fluorescent antibody-based broodstock screening test used to control the vertical transmission of *Renibacterium salmoninarum* in Chinook Salmon (*Oncorhynchus tshawytscha*). Canadian Journal of Veterinary Research 53:385-389.

Brown, L. L., R. Ricks, T. P. T. Evelyn, and L. Albright. 1990. Experimental intra-ovum infection of Coho Salmon (*Oncorhynchus kisutch*) eggs with *Renibacterium salmoninarum* using a microinjection technique. Diseases of Aquatic Organisms 8:7-11.

Evelyn, T. P. T., J. E. Ketcheson, and L. Prosperi-Porta. 1984. Further evidence for the presence of *Renibacterium salmoninarum* in salmonid eggs and for the failure of povidone-iodine to reduce the intra-ovum infection rate in water-hardened eggs. Journal of Fish Diseases 7(3):173-182.

Evelyn, T. P. T., J. E. Ketcheson, and L. Prosperi-Porta. 1986. Experimental intra-ovum infection of salmonid eggs with *Renibacterium salmoninarum* and vertical transmission of the pathogen with such eggs despite their treatment with erythromycin. Diseases of Aquatic Organisms 1:197-202.

Evenden, A. J., T. H. Grayson, M. L. Gilpin, and C. Munn. 1993. *Renibacterium salmoninarum* and bacterial kidney disease - the unfinished jigsaw. Annual Review of Fish Diseases 3:87-104.

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.

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. Two presentations were given at the CPW aquatic biologist meeting, one at a CPW Aquatic Senior Staff Meeting, one at a CPW Aquatic Animal Health Lab staff meeting, one at a CPW hatchery staff meeting, and one at the CPW conservation biology days meeting:

- Fetherman, E. R., E. Gardunio, D. Kowalski, and G. Schisler. 2020. *Myxobolus cerebralis* resistance in the Gunnison River Rainbow and resistance and survival evaluations of the H×G. 2020 Colorado Parks and Wildlife Aquatic Biologist Meeting. Evergreen, Colorado. January 22, 2020.

- Atkinson, B., E. R. Fetherman, and M. Kondratieff. 2020. Comparative survival of catchable and fingerling Rainbow Trout stocked in the Yampa River – 2020 update. 2020 Colorado Parks and Wildlife Aquatic Biologist Meeting. Evergreen, Colorado. January 22, 2020.
- Riepe, T. B., E. R. Fetherman, G. J. Schisler, and D. L. Winkelman. 2020. Current results of CSU *Renibacterium salmoninarum* experiments, and thoughts on future projects. CPW Aquatic Senior Staff Meeting. Virtual. April 14, 2020.
- Fetherman, E. R., E. Gardunio, D. Kowalski, and G. J. Schisler. 2020. Whirling disease resistance in the Gunnison River Rainbow Trout. CPW Conservation over Virtual Interface Days 2020. Virtual. April 28, 2020.
- Riepe, T. B., E. R. Fetherman, G. J. Schisler, and D. L. Winkelman. 2020. Current results of CSU *Renibacterium salmoninarum* experiments, and thoughts on future projects. CPW Aquatic Animal Health Lab Staff Meeting. Virtual. April 29, 2020.
- Riepe, T. B., E. R. Fetherman, G. J. Schisler, and D. L. Winkelman. 2020. Current results of CSU *Renibacterium salmoninarum* experiments, and thoughts on future projects. CPW Hatchery Staff Meeting. Virtual. May 5, 2020.

External presentations and posters provided an opportunity to give research updates to managers both within and outside Colorado. One talk was presented at the American Fisheries Society Fish Health Section Meeting and Western Fish Disease Workshop, one at the Annual Meeting of the Animal Behavior Society, two at the Joint Annual Conference of The Wildlife Society and American Fisheries Society, including an invited talk in the session *Use of Autonomous PIT Tag Antennas for Modeling Vital Rates and Movement*, one at the CSU Graduate Student Showcase, two at the Colorado/Wyoming Chapter of the American Fisheries Society Meeting, and a keynote address for the Alberta Environment and Parks Whirling Disease Symposium:

- Avila, B. W., D. L. Winkelman, E. R. Fetherman, and J. Drennan. 2019. Assessment of potential disease resistance to *Flavobacterium psychrophilum* and *Myxobolus cerebralis* in Rainbow Trout. 2019 Annual Meeting of the American Fisheries Society Fish Health Section and 60th Annual Western Fish Disease Workshop. Ogden, Utah. June 18, 2019.
- Kopack, C., E. R. Fetherman, R. M. Fitzpatrick, E. D. Broder, and L. Angeloni. 2019. Can training species of conservation concern prior to release increase survival? 2019 Annual Meeting of the Animal Behavior Society. University of Illinois. Chicago, Illinois. July 26, 2019.
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- Richer, E. E., E. R. Fetherman (presenter), and M. C. Kondratieff. 2019. Haunted rivers: Application of mobile RFID-GPS systems to evaluate the prevalence of ghost PIT tags. American Fisheries Society and The Wildlife Society 2019 Joint Annual Conference. Reno, Nevada. October 3, 2019.
- Riepe, T. B., V. Vincent, V. Milano, E. R. Fetherman, and D. L. Winkelman. 2019. Non-lethal methods used to detect *Renibacterium salmoninarum* in Brook Trout. Poster. Colorado State University, Graduate Student Showcase. Fort Collins, Colorado. November 12, 2019.
    - Resulted in student receiving the Colorado State University 2020 Vice President for Research Graduate Fellowship.

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- Riepe, T., E. R. Fetherman, and D. L. Winkelman. 2020. Horizontal transmission of *Renibacterium salmoninarum* among inland hatchery-reared trout. 2020 Annual Meeting of the Colorado/Wyoming Chapter of the American Fisheries Society. Laramie, Wyoming. February 26, 2020.
- Fetherman, E. R., E. Gardunio, D. Kowalski, and G. J. Schisler. 2020. Whirling disease in the East Portal of the Gunnison River and *Myxobolus cerebralis* resistance in the Gunnison River Rainbow Trout. Keynote Address. Alberta Environment and Parks Whirling Disease Virtual Symposium. Virtual. May 8, 2020.

In addition to public and professional meeting presentations, two presentations were given to the fisheries management class at Front Range Community College in Fort Collins, Colorado. The first, an informal presentation/laboratory, was presented at the BFRH. During this lab, students learned about the various tagging methods used in research and management across Colorado, and were given a chance to try the tagging methods on live fish. The second, a formal presentation, was given to the class in March 2020:

- Fetherman, E. R. 2020. Salmonid disease research in Colorado. Guest lecture, Introduction to Fisheries. Front Range Community College. Fort Collins, Colorado. March 12, 2020.

Manuscripts published in peer-reviewed scientific journals help to inform fisheries management decisions locally, nationally, and internationally. One manuscript was published in Pathogens special issue *Pathogen Characterization, Host Immune Response and Development Strategies to Reduce Losses to Disease in Aquaculture*:

- 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.

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

- Happel, A., E. R. Fetherman, B. Neuschwanger, T. Davis, and D. Karr. *In review*. Switching to a higher-cost commercial feed enhances growth but not reproductive success of Rainbow Trout. Aquaculture Research.
- Richer, E. E., E. R. Fetherman, E. A. Krone, F. B. Wright III, and M. C. Kondratieff. *In review*. Evaluation of a rock-ramp fishway for a transition zone stream in Colorado. North American Journal of Fisheries Management.

One other manuscript is being prepared for publication, but has not yet been submitted:

- Fetherman, E. R., and B. W. Avila. *In preparation*. Habitat associations of Rainbow Trout *Oncorhynchus mykiss* and Brown Trout *Salmo trutta* fry in the upper Colorado River. Intended for submission to North American Journal of Fisheries Management.

CPW is in the process of writing a book entitled *Fishes of Colorado* intended to be a complete guide to Colorado's diverse fish species and current management status. Two chapters have been written for the book by Sport Fish Research staff, the Rainbow Trout and cutbow chapters.

Social media has increasingly been used to inform the public about management and research activities being conducted by CPW. Sport Fish Research staff developed a story board and took over 30 short (30 seconds or less) videos of fry shocking estimates in the upper Colorado River in August 2019. In total, 14 videos of various lengths were combined and a script for the video was produced explaining the background of whirling disease in the Colorado River, how we conduct fry stocking, why we fry shock, how we obtain estimates, and how the data is used (total length: 2 minutes, 37 seconds). Sport Fish Research staff also reviewed and made suggestions on a video formatted as *Notes from the Field* showing the production of new fish by the BFRH, starting with the spawn and research data collection, following eggs through eye up, picking, hatch, swim-up, and finishing with the first feeding of these fish.

An interview was provided for a video production effort by Ben Bortner, Hog Leg Fly Fishing, who put together a video on whirling disease and its history in the Gunnison Gorge. The video was accepted into the F3T Fly Fishing Film Tour and shown around the country during the 2020 film tour. Additionally, Sport Fish Research staff provided relevant research information, reviewed and helped correct a press release regarding the development of *Myxobolus cerebralis* resistance in the Gunnison River Rainbow.

Sport Fish Research staff participated in interview for an article regarding the current state of whirling disease research in Colorado and provided pictures of whirling disease infected rainbow trout and population sampling for article published in The Colorado Sun entitled "Colorado researchers spent decades trying to save disappearing rainbow trout. Finally, they are making progress" on June 4, 2020 (Author: Kevin Simpson).

Technical assistance milestones included providing information and discussion for internal management decisions regarding bacterial kidney disease in Colorado's hatchery system; fry habitat associations in the upper Colorado River, for use with habitat restoration efforts being conducted on CPW State Wildlife Areas; fry shocking protocols, analysis and presentation of fry population data; hatchery feed selection and use; and, quaternary ammonium compound utility as disinfectants for both internal and public use, and ways to properly dilute and dispose of QACs. Additional external technical assistance milestones included discussing Colorado's approach to incorporating wild characteristics into domesticated Rainbow Trout strains, experimental design procedures used to evaluate strains in the laboratory and field, potential management options for wild brood stock formation and egg takes, and providing relevant research materials on these topics to Idaho Fish and Game; discussing New Zealand Mudsnail disinfection techniques with California Department of Fish and Wildlife; discussing triploid Walleye production with Utah Department of Natural Resources and faculty at Karadeniz Technical University in Turkey; and, discussing potential changes to the AFS Fish Health Section Blue Book regarding tissues and sampling techniques for *Renibacterium salmoninarum*.

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

- Reared and collected data from groups of Rainbow Trout held in Square Top Lake to determine if the life cycle of whirling disease has been broken by making the lake fishless.
- Collected large dace from multiple fry sites in the upper Colorado River. Dace were Floy tagged and labeled for later genetic analysis.
- Assisted with experimental design and visual implant elastomer tagging of Plains Minnow for sunfish predation experiment with the Aquatic Toxicology Laboratory.
- Reviewed input files, model construction in Program MARK design matrices, beta and real parameter estimates, and discussed additional model construction and presentation of results for data collected from Fountain Creek on Flathead Chub.
- Advised on use and construction of repeated measures analysis for before-after-control-impact data collected from the upper Arkansas River.
- Reviewed proposal and provided design advice for study examining the effects of population reclamation and barrier erection on eradicating *Myxobolus cerebralis* in George and Cornelius creeks.

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

- Rachel Jones (Colorado State University; Masters Project) - tagging fish for fish passage evaluations (opened July 2019; closed December 2019). New protocol submitted in 2020 (opened March 2020).
- Fetherman et al. - tagging fish for the Colorado River Fish Movement Study (opened March 2020; closed April 2020).
- Fetherman and Atkinson - sedating fish to handleable for spawning in Harrison Creek (started April 2020; closed May 2020).

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

- Polivka, C. M., and S. M. Claeson. *In review*. Beyond redistribution: In-stream habitat restoration increases capacity for young-of-the-year salmon and trout in the Entiat River, WA. Submitted to North American Journal of Fisheries Management.
    - Two reviews: original (April 2019) and resubmission (December 2019).
- Anonymous. *In Review*. We ain't afraid of no ghosts: Tracking habitat interactions and movement dynamics of ghost PIT tags under differing flow conditions in a sand bed river. Submitted to North American Journal of Fisheries Management.
- Americus, B., B. Austin, T. Lotan, J. Bartholomew, and S. Atkinson. *In review*. *In vitro* and *in vivo* assays reveal that cations affect nematocyst discharge in *Myxobolus cerebralis* (Cnidaria: Myxozoa). Submitted to Parasitology.

Internal reviews were also conducted upon request:

- Kowalski, D. A., and E. E. Richer. *In preparation*. Quantifying the habitat preferences of the stonefly *Pteronarcys californica* in Colorado. Colorado Parks and Wildlife.

APPENDIX A: COLORADO RIVER FISH MOVEMENT STUDY PROPOSAL

A.1 INTRODUCTION

Connectivity is a fundamental element of landscape structure and ecological processes, and longitudinal connectivity is especially important in rivers (Taylor et al. 1993; Fausch et al. 2002). Loss of free passage due to artificial barriers can lead to habitat fragmentation and limit fish distributions, such as those of Mottled Sculpin (*Cottus bairdii*) in the Colorado River, by reducing access to key habitats (Fausch et al. 2002; Lucas et al. 2009). In general, fish require three major habitat types: 1) feeding habitats with favorable growth conditions, 2) refugia from harsh environmental conditions with unfavorable growth conditions, and 3) spawning habitat with necessary flow conditions for egg incubation. Movements among these various habitat types occurs continuously throughout the year based on spawn timing (e.g., spring for Rainbow Trout [*Oncorhynchus mykiss*] and fall for Brown Trout [*Salmo trutta*]), environmental and flow conditions, physical or habitat conditions and availability, and growth or life stage (Schlosser and Angermeier 1995). The construction of the connectivity channel around Windy Gap Reservoir will help restore connectivity for these types of movements, and provide access to favorable habitats, such as upstream spawning locations, that had been previously unavailable for fish populations in the Colorado River downstream of Windy Gap Reservoir. Conversely, fish such as Mottled Sculpin, which are currently absent immediately downstream of Windy Gap Reservoir, will be able to distribute downstream increasing the diversity of the riverine ecosystem downstream of the reservoir. This study will be focused on evaluating fish movement rates through the connectivity channel and validating that the connectivity channel is being used for fish passage in both the upstream and downstream directions.

Passive integrated transponder (PIT) tags are an important tool for evaluating fish growth, movement, and mortality due to their relatively low cost, longevity, ability to identify unique individuals, ease of application, and minimal effects on fish survival, growth, feeding behavior, and swimming performance (Zydlewski et al. 2006; Newby et al. 2007; Ficke et al 2012). Antennas constructed of copper wire anchored to the bottom of the river (stationary antenna) or actively passing over the fish (portable antennas) are used to detect PIT tags that have been inserted internally in target fish species. The antennas create an electromagnetic field that activates the tag and records the unique identification number returned from the tag to an interrogation system or reader. Since the tags are activated by an electromagnetic field rather than battery, they are not only considered passive, but also have an infinite life. In recent years, Colorado Parks and Wildlife (CPW) has utilized PIT technology to monitor movement of salmonids in and out of specific management sections (Fetherman et al. 2014; Fetherman et al. 2015), passage of salmonids, suckers, and dace at whitewater park structures (Fox et al. 2016), evaluate Brown Trout habitat utilization in mountain streams (Richer et al. 2017), and evaluate the effectiveness of fishways for restoring passage of various salmonid and non-salmonid species (Ficke 2015; Hodge et al. 2017; Richer et al. 2018). The focus species for this study include Rainbow Trout and Brown Trout, the dominant sport fish species in the Fraser River and Colorado River upstream and downstream of Windy Gap Reservoir. Mottled Sculpin distribution in the downstream direction through the connectivity channel will also be an important focus of the fish movement monitoring efforts. Although most Mottled Sculpin are

relatively sedentary, recent research has shown that a small percentage of these fish exhibit considerable movement capability (Breen et al. 2009; Hudy and Schiflet 2009).

Using PIT tags and stationary and portable PIT tag antennas, the overall objectives of this study are to evaluate Rainbow Trout, Brown Trout, and Mottled Sculpin movement both upstream and downstream through the connectivity channel, adequacy of attraction flows from the connectivity channel, and large-scale movement patterns of various age classes of target fish species throughout the upper Colorado River.

Goals
● Restore longitudinal connectivity for fish populations around Windy Gap Reservoir
Primary Objectives
● Evaluate fish movement patterns under existing conditions upstream and downstream of Windy Gap Reservoir ● Validate that the connectivity channel is being utilized for fish passage by Brown Trout, Rainbow Trout, and Mottled Sculpin
Secondary Objective
● Evaluate fish movement patterns at other structures located throughout the study area including the stream gage on the Fraser River upstream of Windy Gap Reservoir, the diversion structure on the Fraser River below Highway 40, and the diversion structure on the Chimney Rock Ranch.

A.2 METHODS

The Colorado River fish movement study will include two distinct phases. First, a baseline study of fish movement patterns and rates will be conducted for reaches upstream and downstream of Windy Gap Reservoir. The baseline phase will be conducted in two years prior to the completion of the Windy Gap connectivity channel (2020-2021). Second, the baseline study design will be expanded to evaluate the efficiency of fish movement through the Windy Gap connectivity channel two years post-construction (connectivity phase; 2023-2024 dependent upon completion of connectivity channel construction).

Stationary Antenna Stations

To evaluate fish movement patterns under existing conditions, stationary antennas will be installed in the Colorado River on the Chimney Rock Ranch upstream of the Hitching Post Bridge and upstream of Windy Gap Reservoir downstream of the confluence with the Fraser River in spring 2020 to monitor baseline movement rates. An additional stationary antenna will be installed on the Chimney Rock Ranch just upstream of the confluence with Drowsy Water Creek to determine distances moved from downstream fish tagging locations (Figure A.1). Installation of the two antenna stations located on the Chimney Rock Ranch and the antenna station located upstream of Windy Gap Reservoir will occur prior to tag releases in spring 2020. All three antenna locations will consist of paired antenna loops constructed from 8-gauge copper speaker wire and anchored to the substrate to prevent movement or change in shape, maximizing read range. Paired antenna loops allow researchers to determine directionality of movement (upstream versus downstream) when it occurs at each antenna location. Antenna stations will be

powered by multiple 12-V, 120-Ah marine deep cycle batteries housed in a job box along with the reader(s). Additional power for each antenna station will be supplied by solar panels installed near each antenna location. Readers will run continuously after installation to capture fish movements during all times of the day and throughout the entire time that they are installed. Integration of the Campbell Scientific equipment and Bluetooth connections at each antenna station will allow remote downloading of the data, as well as providing a periodic check of antenna function. Baseline fish movement rates will be monitored in spring 2020 through 2021, prior to the construction of the Windy Gap connectivity channel.

Following the completion of the Windy Gap connectivity channel, additional antenna stations will be installed upstream and downstream of the connectivity channel to validate that the connectivity channel is being used by Brown Trout, Rainbow Trout, and Mottled Sculpin. The downstream antenna will be installed in the connectivity channel just upstream of the confluence with the Colorado River downstream of Windy Gap Reservoir. The upstream antenna will be incorporated into the diversion structure located at the upstream end of the connectivity channel. Similar to the antenna design described above, each antenna location will have paired antenna loops to determine directionality of movement into or out of the connectivity channel. Additionally, successful movement in either direction through the connectivity channel will be evaluated during the data analysis phase of the project by quantifying the proportion of fish that were detected at both antenna locations within the connectivity channel. A third antenna station will be installed in the existing bypass channel originating from Windy Gap Reservoir just upstream of the confluence with the river to determine if flows attract fish and prevent them from finding or using the connectivity channel during certain times of the year. Efficiency of the connectivity channel will be monitored using these three antenna stations, along with the stations installed during the baseline monitoring phase, in 2023 and 2024, dependent upon completion of connectivity channel construction.

Stationary antenna stations will not be installed to specifically address the secondary study objective. However, the secondary objective of the study may be achievable through electrofishing and portable antenna recaptures occurring upstream and downstream of structures located throughout the study area (see below).

Antenna detection distances and efficiencies will be checked at least four times a year, once per season. Additional evaluations for detection distance and efficiency may be warranted depending on the change in flow conditions within a given season. Detection distances and efficiencies will be monitored using the stick-test method (Nunnallee et al. 1998; Compton et al. 2008). Velocity measurements will also be collected at the time of efficiency evaluations to calculate discharge, which will be included as a covariate affecting detection probability and/or probability of movement during analysis (Fetherman et al. 2015). Additionally, to determine if antenna detection distances meet or exceed the river surface, marker tags, which are programmed to “appear” every 15 minutes during continuous antenna operation, will be housed in floating PVC casings and deployed on the river surface over each antenna loop. Antenna tuning, function, and maintenance (including clearing of debris that could inhibit antenna function) will occur in conjunction with efficiency evaluation, other data collection periods such as portable antenna deployment or electrofishing recaptures, and as needed when indicated by the remote data download process.

Fish Tagging

Target species for tagging include Rainbow Trout, Brown Trout, and Mottled Sculpin. Multiple age classes will be tagged of all three target species using 12 mm, 23 mm, and 32 mm tags as appropriate for fish body size and desired detection distance. The number of tags used will be dependent upon target species and age class abundances. The goal is to tag at least 250 Rainbow Trout and 250 Brown Trout (500 fish total) in the Chimney Rock Ranch reach, with an additional 250 fish of each species tagged in the Colorado and Fraser rivers upstream of Windy Gap Reservoir (combined) annually between 2020 and 2021. In the Fraser and Colorado rivers upstream of Windy Gap Reservoir, up to 250 Mottled Sculpin will be tagged annually. Additional numbers of fish of each species will be tagged opportunistically dependent upon tag availability and number of fish captured during electrofishing efforts in each reach. All fish will be anesthetized using AQUI-S 20E (clove oil), tagged in the interperitoneal cavity using a tagging gun and needle, and secondarily fin clipped to estimate tag loss. For salmonids, a different fin will be clipped (e.g., adipose fin, left pelvic fin, or right pelvic fin) to indicate which size of tag had been used since different size tags are likely to have different retention or expulsion rates. Fish will be held in net pens following tag insertion to ensure recovery from tagging and anesthetization prior to release back into the river.

Fish will be tagged in multiple reaches upstream and downstream of stationary antenna locations. In fall 2019, bank and backpack electrofishing units will be used to capture fish for tagging at multiple locations throughout the study reach. A large portion of the tagging on the Chimney Rock and Sheriff ranches will occur during the spring adult population estimates conducted in May 2020-2021. Additional tagging events will be needed to tag fish in the Colorado and Fraser rivers upstream of Windy Gap Reservoir, likely concurrent with standard sampling sites conducted in these locations. Other tagging locations closer to Windy Gap Reservoir, both upstream and downstream of the reservoir, may be utilized to monitor fish movement rates for both the baseline and connectivity phases of the study.

Portable Antennas/Electrofishing Recaptures

Portable antennas, antennas mounted in or on rafts and floated on the surface of the river, will be deployed during both the baseline and connectivity phases of the study to improve detection probability of tagged fish and determine the fate of fish that are never detected at a stationary antenna site. GPS technology will be incorporated into portable antenna designs to get more precise locations on fish throughout the study reach and to estimate average distance moved by fish species and size. Portable antennas will be deployed in three reaches: 1) on the Colorado River between Hitching Post Bridge and the Sheriff Ranch; 2) on the Colorado River upstream of Windy Gap Reservoir and the confluence with the Fraser River; and 3) on the Fraser River between the Highway 40 diversion structure and Fraser Canyon (Figure A.1). An additional portable antenna reach will be added within the connectivity channel following its completion, which will be monitored during the connectivity phase of the study to determine location and fate of fish that entered but did not exit the channel. During deployment, portable antennas will be run down the thalweg, as well as the left and right sides of the river channel, to increase detection probabilities of fish throughout the channel. The combination of the three runs will constitute a single detection or “mark” pass in any given reach. A second set of three runs

(thalweg, left and right sides of the river) will be used as a "recapture" pass to estimate detection probability, tagged fish abundance, and change in detection location in the event that a fish moved between passes. To maximize the chance of capturing fish movements during portable antenna surveys, surveys will be conducted in the spring (March-April), summer (July-August) and fall (September-October) during times when fish are most likely to be moving to spawning sites.

PIT-tagged fish will be captured during annual and biannual electrofishing population estimates in the Colorado and Fraser rivers. All fish captured that have a fin clip, indicating that they had been tagged as part of this study, will be scanned for the presence of a PIT tag using a handheld reader, and the PIT tag number will be recorded along with fin clip type. If the PIT tag is absent, fin clip type will be recorded so that tag loss can be estimated for each size of tag used.

Portable antenna and electrofishing recaptures will be incorporated into multistate models, along with the data obtained from the stationary antenna stations, contributing information to both estimates of the probability of survival and movement in the Colorado and Fraser rivers. Additionally, recapture data from both sources will be used for the secondary objective to evaluate fish movement patterns at the stream gage on the Fraser River upstream of Windy Gap Reservoir, the diversion structure on the Fraser River below Highway 40, and the diversion structure on the Chimney Rock Ranch. Although recapture data cannot be used to determine when a fish passed any of these structures (i.e., under what flow conditions or time of year), the recapture of fish upstream of these structures that had been previously released below or moved downstream over these structures will be considered confirmation that the structure was passable at some point over the course of the study.

Data Management and Analysis

Data from the stationary antennas will be downloaded once monthly using the remote data collection provided by the integration of the Campbell Scientific dataloggers with Bluetooth capabilities at the stationary antenna sites. Monthly downloads of the data from each antenna site will ensure that data is not being lost, and will provide the ability to determine if the data collection capabilities at a stationary antenna site have been compromised and need to be fixed. Data collected during portable antenna and electrofishing recaptures will be collected and compiled upon completion. Encounter histories for the analyses will be created progressively over the course of the experiment.

Fish movement data will be analyzed using a Markov chain multistate model implemented in Program MARK (White and Burnham 1999). The Markov chain version of the multistate model allows incorporation of data from both the stationary antenna stations and the portable antenna and electrofishing recaptures, providing estimates of the probability of movement and survival, as well as improving estimates of detection probability at stationary antenna sites which can affect estimates of movement and survival. Individual covariates, including fish species and length, date tagged and released, temperature, stream discharge, and other environmental characteristics of interest will be included in the analysis to determine their effects on movement, survival, and detection probabilities. Data from the baseline phase of the study will be compiled and analyzed following the completion of the construction of the bypass channel, and data from

the connectivity phase of the study will be completed following the removal of the stationary antenna stations two years post-construction of the connectivity channel. Movement and survival probabilities obtained from the two analyses will be compared to determine if these probabilities differed prior to and after construction of the Windy Gap connectivity channel.

A.3 Study Sites

The project study area will extend from the Fraser and Colorado rivers upstream of the connectivity channel downstream through the Chimney Rock Ranch to the Sheriff Ranch, incorporating stationary and portable antenna deployment locations described above to meet the objectives of the study. Primary fish tagging reaches will include the Chimney Rock Ranch, the Colorado River upstream of the confluence with the Fraser River, and the Fraser River near Highway 40, and tagging will occur during annual or biannual electrofishing population estimates conducted in these locations. Additional tagging locations and occasions may be utilized to increase the number of tagged salmonids and Mottled Sculpin, if needed. An overview of stationary antenna locations, mobile antenna reaches, and electrofishing sites is presented in Figure A.1.

A.4 Monitoring Schedule

Pre-construction baseline movement rates will be monitored in the Colorado and Fraser rivers upstream of Windy Gap Reservoir and in Chimney Rock Ranch beginning in spring 2020 and continuing through 2021 concurrent with the initiation of construction of the connectivity channel. Tagging will occur on the Chimney Rock Ranch during adult population estimates conducted in spring 2020 and 2021. Tagging of salmonids and Mottled Sculpin in the Fraser and Colorado rivers upstream of Windy Gap Reservoir will begin in the summer/fall of 2020 at select sites that can be accessed with bank and backpack electrofishing units, and will continue in conjunction with fall population estimates conducted in these rivers through 2021. Antenna installation for baseline monitoring will occur in spring 2020 prior to the release of PIT-tagged fish, with additional antennas being installed in and around the connectivity channel for monitoring the efficiency of the channel to pass fish in 2022 or 2023, dependent upon when construction of the channel is complete. Antennas will remain in place two years post-construction of the connectivity channel. Portable antennas will be deployed in the spring (March-April) and fall (September-October) during the baseline and connectivity channel monitoring phases of the study to locate tagged fish in locations outside the range of detection by the stationary antennas.

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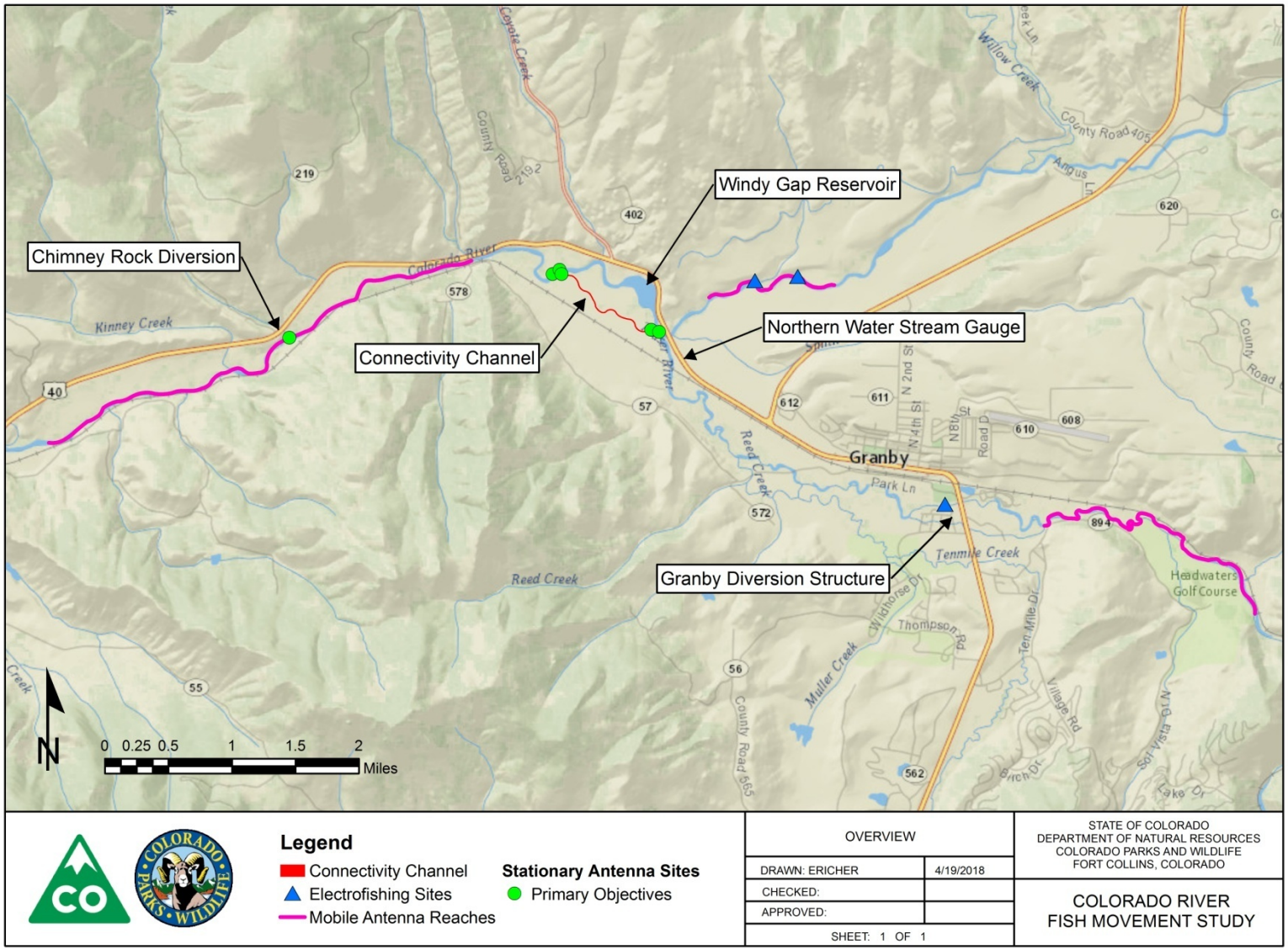
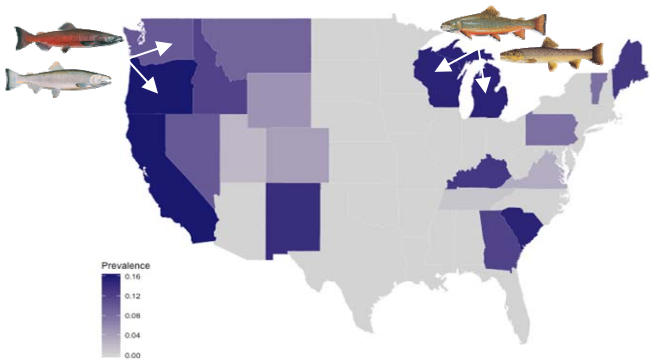


Figure A.1. Location of stationary antenna and electrofishing sites, and mobile antenna reaches, for the fish movement study.

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

Renibacterium salmoninarum Transmission Research

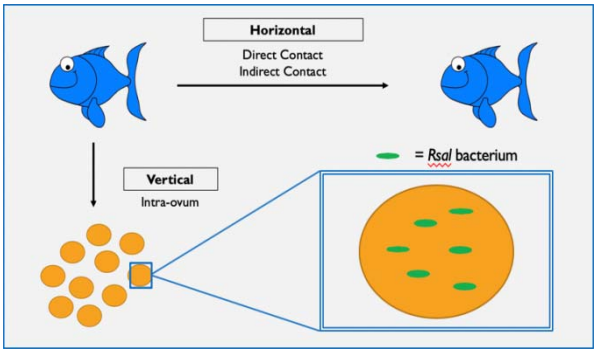
TRANSMISSION OF *RENIBACTERIUM SALMONINARUM* (BACTERIAL KIDNEY DISEASE) IN HATCHERY-REARED FISH



Prevalence of *Renibacterium salmoninarum* in the US. Highest detections occur in the Pacific Northwest and Midwest.

Renibacterium salmoninarum, the bacterium that causes bacterial kidney disease (BKD), was first described in Scotland in 1930, and first detected in the US in 1934. BKD is now found in trout and salmon throughout the US and worldwide, and can cause significant mortality in infected populations. In the US, the bacterium is most commonly detected in Coho and Chinook Salmon in the Pacific Northwest, and Brook Trout and Brown Trout in the Midwest. The transfer of infected eggs and the practice of pasteurizing fish by-products for fish feed (now discontinued) are suspected to have contributed to the worldwide spread of the pathogen.

R. salmoninarum can be transmitted in two ways, presenting challenges for prevention and management. Horizontal transmission of the bacteria is not completely understood. However, fish may become infected through direct contact with another infected fish, ingestion of contaminated fecal matter, or contact with a contaminated water source from which the bacteria enters through the gills, eyes, gut, and external injuries of the fish. Vertical transmission occurs during the spawn. Bacteria in the female find their way into the eggs during egg development. There is no method for safely disinfecting the inside of the eggs, and using iodine to disinfect the egg surface during hatchery spawns is not effective for preventing vertical transmission.

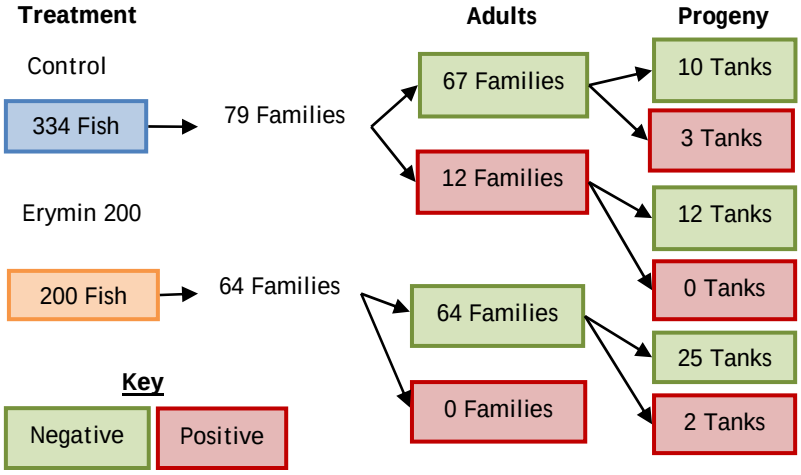


Horizontal and vertical transmission of *R. salmoninarum.*

R. salmoninarum is a regulated pathogen in Colorado. Current regulations prevent the transfer or stocking of infected fish. While infrequently found in Colorado since the 1960's, it has recently been detected in six state and federal hatcheries. In 2015, *R. salmoninarum* was detected in the CPW Glenwood Springs Hatchery. To eradicate the pathogen and prevent stocking infected fish, the hatchery was temporarily depopulated. *R. salmoninarum* was also detected in other CPW hatcheries rearing valuable brood stocks of whirling disease-resistant Rainbow Trout and Native Greenback Cutthroat Trout, and depopulation of these hatcheries was not an option. Recent management options have focused on methods to prevent vertical transmission during fish spawning, although other approaches are also being examined.

Management Options for Controlling Bacteria Transmission

Lethal spawning has been evaluated in Rainbow Trout and Cutthroat Trout brood stocks at the CPW Poudre Rearing Unit (PRU) and Fish Research Hatchery (FRH). Eggs are fertilized and collected as normal from brood fish during the spawn. After being spawned, adult fish are euthanized and tested for *R. salmoninarum*. Eggs from positive parents are then culled so that only eggs from negative parents are retained on the unit for stocking and management purposes. This technique has shown promise in preventing vertical transmission. However, more research was needed to understand the transmission rates of *R. salmoninarum*, develop management and regulatory protocols to decrease the prevalence of the pathogen within hatchery brood stocks, and limit the dissemination of positive progeny to other waters and hatcheries in Colorado.



Antibiotics administered orally or by injection have been shown to reduce bacterial concentrations in fish. Erythromycin (Erymin 200), an antibiotic approved for use in fish, was injected into Rainbow Trout at the CPW FRH to determine if its use could reduce *R. salmoninarum* levels in spawning fish and control vertical transmission to progeny. Male and female brood fish were injected with the antibiotic three times prior to spawning. The injections reduced bacteria levels in the injected fish to below detectable levels. However, progeny that tested positive for *R. salmoninarum* were produced by both treated and control fish that had tested negative for the bacteria. Additionally, positive control adults produced negative progeny. Results also indicated that the male may play a role in vertical transmission in inland trout, a significant finding as only females were previously known to contribute to vertical transmission of the bacteria. This study revealed several unknowns regarding pathogen transmission that needed to be answered for effective management in Cutthroat Trout. CPW and Colorado State University are currently conducting two studies to better understand transmission rates in Native Greenback Cutthroat Trout.

Transmission Studies in Native Greenback Cutthroat Trout

Horizontal Transmission

Horizontal transmission of *R. salmoninarum* within a hatchery may pose a risk to fish throughout the unit, as well as feral fish in the river below a unit if the bacteria are present in the hatchery effluent. In summer 2019, two sentinel cages were deployed in six locations at PRU and at three time periods encompassing a range of optimal temperatures for bacterial growth (10-18°C). Each cage housed 10 Cutthroat Trout. After 30 days, fish were collected and tested for *R. salmoninarum*. Only one fish out of a total of 360 tested positive. The cage containing the positive fish had been deployed near highly-positive Rainbow Trout. These results suggest the rate of horizontal transmission to fish both on, and in the river below a positive unit may be insignificant, even when conditions are optimal for the bacteria to persist.

Vertical Transmission

Progeny collected from positive adults

Eggs from unique male-female pairs of Cutthroat Trout were collected during the spawn at PRU in May 2019. Adult fish were tested for *R. salmoninarum* after being spawned to determine the potential infection status of the progeny created from these spawns. Thirty-two tanks of progeny are being reared at the FRH: one containing progeny from a male negative × female negative pair, six from a male positive × female negative pair, four from a male negative × female positive pair, and 21 from a male positive × female positive pair. Progeny from each tank will be tested for *R. salmoninarum* and associated antibodies, indicating that the progeny had mounted an immune response to the bacteria at some point prior to the testing, at six months and one year of age. The results will be used to understand vertical transmission rates, male and female contributions to vertical transmission, and the bacterial load at which transmission occurs from parent to offspring. Overall, the results from the transmission studies will be used to inform future management options. Results from these transmission studies will be available in 2021.

Associated Literature

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

Fetherman, E. R., B. Neuschwanger, T. Davis, C. L. Wells, and A. Kraft. *In review*. Use of Erymin 200 injections for reducing vertical transmission of *Renibacterium salmoninarum* in a Rainbow Trout brood stock. Diseases of Aquatic Organisms.