Sport Fish Research Studies

Federal Aid Project F-394-R18

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Job Progress Report

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State: Colorado **Project No. F-394-R18**

Project Title: Sport Fish Research Studies

Period Covered: July 1, 2018 – June 30, 2019

Project Objective: Investigate methods to improve spawning, rearing, and survival of sport fish species in hatcheries and in the wild.

Job No. 1 Breeding and Maintenance of Whirling Disease-Resistant Rainbow Trout Stocks

Job Objective: Rear and maintain stocks of whirling disease-resistant Rainbow Trout.

Need

The Hofer 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 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 Hofer and Harrison Lake strains show increased resistance over the pure Harrison strain. Brood stocks of the Hofer and Harrison Lake strains, and their crosses, are maintained at the Colorado Parks and Wildlife (CPW) Bellvue Fish Research Hatchery for both research and stocking purposes. In addition to the Hofer and Harrison Lake strain fish, the Bellvue Fish Research Hatchery rears and distributes other *M. cerebralis*-resistant Rainbow Trout strains and crosses for research purposes.

Objectives

1. Spawn and rear brood stocks of *M. cerebralis*-resistant rainbow trout at the Bellvue Fish Research Hatchery through June 30, 2019.
2. Maintain genetic and disease integrity of brood stocks housed at the Bellvue Fish Research Hatchery through June 30, 2019.

Approach

Action #1:

- *Level 1 Action Category: Facilities and Areas (Operations and Maintenance)*
- *Level 2 Action Strategy: Hatcheries (recreational purposes)*
- *Level 3 Action Activity: N/A*

Hofer and Harrison Lake brood stocks will be spawned on-site at the Bellvue Fish Research Hatchery in November 2018 through January 2019, and reared through June 30, 2019. Brood stocks will be marked, identified, and maintained by strain or cross and year class.

Action #1 Accomplishments

The *Myxobolus cerebralis*-resistant Rainbow Trout brood stocks reared at the Bellvue Fish Research Hatchery (BFRH; Bellvue, Colorado) 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 brood stocks. All lots of fish are uniquely fin clipped and most 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 used for spawning.

Starting in the middle of October 2018, BFRH personnel checked all of the Hofer (GR)[1] and Harrison Lake (HL) brood fish (2 and 3 year-olds) weekly for ripeness. Maturation is indicated by eggs or milt flowing freely when slight pressure is applied to the abdomen of the fish. The first females usually maturate two to four weeks after the first group of males. As males are identified, they are moved into a separate section of the raceway to reduce handling and fighting injuries. On November 14, 2018, the first group of GR females was 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 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 individual strain separate. 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 11.1ºC (52ºF) 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 individual crosses 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 third day until eye-up.

Eggs reached the eyed stage of development after 14 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.

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[1] Hofer (H) is used interchangeably with German Rainbow (GR) throughout this document to describe the resistant strain of Rainbow Trout obtained in 2003 from facilities in Germany.

Action #2:

- *Level 1 Action* <u>*Category*</u>*: Data Collection and Analysis*
- *Level 2 Action* <u>*Strategy*</u>*: Techniques development*
- *Level 3 Action* <u>*Activity:*</u> *Artificial propagation studies*

Maintaining the genetic integrity of resistant rainbow trout brood stocks is imperative to the production, stocking, and management of Colorado's rainbow trout populations. Additionally, disease threats can interrupt production schedules and cause setbacks in the maintenance of important brood stocks. Spawning known individual male-female pairs and disease testing of parents and offspring preserves both the genetic and disease integrity of fish produced at the Bellvue Fish Research Hatchery to replace hatchery brood stocks and for stocking.

<u>Action #2 Accomplishments</u>

The Bellvue Fish Research Hatchery (BFRH) was found to be positive for *Renibacterium salmoninarum* during its annual inspection in 2016. Because the hatchery houses important *Myxobolus cerebralis*-resistant Rainbow Trout brood stocks for both production and research purposes, depopulation of the hatchery to eliminate the bacteria was not an option. Lethal spawn operations have therefore been conducted at the hatchery since 2016 to ensure that the unit is only producing offspring from *R. salmoninarum*-negative parents. During lethal spawn operations, ovarian fluid is collected from females and kidney samples are collected from males. All samples are tested for the presence of *R. salmoninarum* using a diagnostic direct fluorescent antibody test (DFAT) and confirmatory quantitative PCR test, both performed by the CPW Aquatic Animal Health Lab. Progeny used for future research projects and replenishing on-site brood stocks are created from single male-female pairs, consisting of a two-year-old male spawned with a three-year-old female or vice versa, and maintained separately until parental test results are obtained. Families testing positive are discarded, unless being used for a specific research purpose, such that future brood stocks are created from only those families of which both parents tested negative for *R. salmoninarum*. For research projects that require retention of all families created during the spawn, such as previous erythromycin injection or finishing feed experiments, positive families are moved to an on-site quarantine facility (FR2) where they are hatched and reared to the final endpoints of the experiment and then euthanized appropriately to prevent incorporation into that year's brood production.

A total of 72 families of Hofer Rainbow Trout were spawned at the BFRH in 2018, 12 on November 11, 28 on November 27, 20 on December 4, and 12 on December 11, 2018. All families produced good eggs, and none of the families were discarded due to bad or dead eggs. No Hofer males tested positive for *R. salmoninarum*, but five females tested positive, one two-year-old female spawned on November 27, and two two-year-old and two three-year-old females spawned on December 11, 2018. All five families from which the female tested positive were discarded, resulting in 67 families, with 10 fish from each for a total of 670 fish, used to create the next year's Hofer brood stock.

A total of 116 families of Harrison Lake Rainbow Trout were spawned at the BFRH in 2018/2019, 34 on December 18, 2018, 30 on January 2, 30 on January 9, and 22 on January 15, 2019. Six families, four spawned on December 18, 2018, and one each spawned on January 2 and January 9, 2019, produced bad eggs and were discarded. No Harrison Lake males tested positive for *R. salmoninarum*. However, 40 females tested positive for the bacteria, including seven three-year-old females and one two-year-old female spawned on December 18, 2018, 12 three-year-old females and one two-year-old female spawned on January 2, 2019, two three-year-old and 11 two-year-old females spawned on January 9, 2019, and three three-year-old and three two-year-old females spawned on January 15, 2019. All 40 families from which the female tested positive were discarded, and with the additional six families discarded due to bad eggs, a total of 70 families, 69 with 10 fish from each and three families that produced only five, four, and one eyed egg, were combined for a total of 690 fish used to create the next year's Harrison Lake brood stock.

Although there is some evidence that negative parents have the potential to produce positive offspring (Fetherman et al. 2018), lethal spawning, testing adults for the presence of *R. salmoninarum*, and retaining progeny originating from negative parents, continues to be the best method for producing *R. salmoninarum*-negative fish for future research projects and brood stock production at the BFRH. However, this method appears to be doing little to reduce the overall presence of the bacteria in spawning fish on the unit from year to year. Additional options, such as erythromycin injections to control bacteria in adult females, have also proven both time consuming and not entirely effective on a large scale (Fetherman and Schisler 2017; Fetherman et al. 2018). Antibiotic feeds administered prior to the spawn could be an option for reducing bacteria loads to below observable levels, allowing retention of a larger number of families for research and brood stock production purposes, and may be effective at reducing the bacterial loads on the facility enough to obtain an *R. salmoninarum*-negative status in the future. However, for the time being, it appears that *R. salmoninarum* will continue to be detected on hatchery units that are not depopulated following detection, and additional management strategies are needed to balance brood stock maintenance and production goals in the presence of *R. salmoninarum*.

Fetherman, E. R., and G. J. Schisler. 2017. Sport Fish Research Studies. Federal Aid Project F-394-R16. Federal Aid in Fish and Wildlife Restoration, Job Progress Report. Colorado Parks and Wildlife, Aquatic Wildlife Research Section. Fort Collins, Colorado.

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

Job No. 2 Improved Methods for Hatchery and Wild Spawning and Rearing of Sport Fish Species

Job Objective: Provide experimental support for both hatchery and wild spawning and rearing of sport fish species as they arise.

Need

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.

Objectives

1. Complete one hatchery feed study examining the growth and overall health of a split lot of Hofer by Harrison Lake Rainbow Trout by Snake River Cutthroat Trout (HHN) reared on two basic commercial diets by December 31, 2018.
2. Complete one experiment to examine the effects of rearing density and feed on post-stocking survival of Rainbow Trout by November 30, 2018.
3. Complete one *Flavobacterium psychrophilum* challenge experiment to develop protocols for future challenge experiments examining *F. psychrophilum* resistance in Rainbow Trout by December 31, 2018.

Approach

Action #1:

- *Level 1 Action Category: Data Collection and Analysis*
- *Level 2 Action Strategy: Techniques development*
- *Level 3 Action Activity: Artificial propagation studies*

Contracts for hatchery feed suppliers are often awarded to the lowest bidder. However, cheaper feeds may not provide the nutritional components necessary for effective growth or fish health, especially when rearing different strains than those for which a feed was developed. Similar to human foods, fish feeds can vary widely with regards to protein, lipids, vitamins, and additives such as astaxanthins, which can affect the shape, coloration, and, ultimately, angler satisfaction of the final product. This hatchery feed experiment, conducted at the CPW Bellvue-Watson Hatchery, will expand on those previously conducted in 2016 and 2017 by examining the growth and overall health of a split lot of HHN reared on the basic diet of two major commercial fish feed manufacturers. Endpoints include mortality, food conversion ratio, coefficient of variation in fish length and weight, fin wear rating, hepatosomatic index, viscerosomatic index, white blood cell counts as a measure of immune system health and function, and susceptibility to infection by Flavobacterium psychrophilum.

Action #1 Accomplishments

Hatchery feed experiments conducted in 2016 and 2017 examined growth and health metrics of Hofer Rainbow Trout reared on basic feeds produced by four feed manufacturers, EWOS, Skretting, Bio Oregon, and Rangen. In both experiments, the top performing feed in relation to both growth and health metrics was Bio Oregon, while Rangen was one of the lower performing feeds (Fetherman et al. 2019). However, these studies were conducted with small groups of fish under controlled hatchery conditions. Additionally, Hofer Rainbow Trout exhibit a voracious appetite and increased growth potential, which made the strain a good candidate for examining feed effects, but may not have been representative of results obtained from other strains produced by Colorado's hatchery system. The objective of this study was to examine the growth and health metrics of a split lot of Hofer × Harrison Lake Rainbow Trout × Snake River

Cutthroat Trout (HHN), with half of the lot fed Rangen and the other half fed Bio Oregon, from hatch to catchable-size fish at the CPW Bellvue-Watson Fish Hatchery (Bellvue, Colorado).

The HHN lot was produced by the CPW Crystal River Hatchery and received at the CPW Bellvue-Watson Fish Hatchery in December 2018 as eyed eggs. Eggs were hatched out, number of fish in the lot estimated, and split into troughs receiving either Rangen or Bio Oregon feed within the hatch house to begin the feeding trial. Throughout the entirety of the study, the manufacturer's recommendations for percent body weight per day at a given temperature were followed for both feed types. Additionally, feeding and sampling protocols for changing feed size or amount, splitting lots to reduce densities and crowding issues, and timing of moving fish from the hatch house to raceways, were the same typically used by the Bellvue-Watson hatchery staff, such that this study represented a real-world application of the use of these two feeds under normal operating conditions.

Fish size and health metrics, including hepatosomatic and viscerosomatic indices, were collected at two time periods associated with splitting the lot in the hatch house into additional troughs to reduce densities and crowding (February and March 2018), immediately prior to moving fish out of the hatch house to raceways located on the Bellvue Unit (April 2018), immediately before the fish were moved from the Bellvue raceways to the raceways located on the Watson Unit (September 2018), and immediately before being stocked out as catchable-size fish (April 2019). Sampling at these time periods allowed a comparison of fish size and health at the beginning and end of being held in each of three hatchery environments. At each sampling occasion, 100 fish each from the Rangen and Bio Oregon troughs or raceways were measured to the nearest mm and weighed to the nearest gram. If fish on a given feed were spread across multiple troughs or nurse basins, an equal number of fish were sampled from each to obtain the 100 fish sample. In addition to size comparisons, the coefficient of variation in length and weight, calculated as $CV = \frac{s}{\bar{y}}$, where s is the standard deviation in length or weight and $\bar{y}$ is the mean, was used to assess and compare differences in size variability within and among the two feed groups. Twenty fish from each feed group were also dissected at each sampling occasion, with the exception of the first at which the fish were too small to dissect, to obtain liver weights for calculation of the hepatosomatic index (relationship between liver weight and total weight; measure of stored energy in the liver) and viscera weights for calculation of the viscerosomatic index (relationship between viscera weight and total weight; measure of stored energy in primarily visceral fat). Similar to the fish size sample, if fish on a given feed were spread across multiple troughs or raceways, a subsample was taken from each to obtain the 20 fish sample.

In the Bellvue hatch house, HHN reared on Bio Oregon were longer than fish reared on Rangen at all occasions and heavier in March and April 2018. Increased growth rates in fish fed Bio Oregon required splitting more often and into more troughs than those used for fish fed Rangen of the same age. Overall, the fish fed Bio Oregon grew 26 mm and 2.5 g between February and April 2018, whereas the fish fed Rangen grew 21.8 mm and 1.8 g over the same time period. Although both groups exhibited variability in length and weight, the variability in both was higher in fish fed Rangen compared to those fed Bio Oregon, especially in March and April 2018. Similar HSI and VSI values were obtained for both groups by the end of the rearing period in the hatch house in April 2018 (Table 2.1.1).

Table 2.1.1. Hofer × Harrison Lake Rainbow Trout × Snake River Cutthroat Trout (HHN) length (mm), length coefficient of variation (CV), weight (g), CV weight, hepatosomatic index (HSI), and viscerosomatic index (VSI) by feed type [standard deviation], and at five sampling occasions relating to the three hatchery environments experienced between hatch and stocking as catchable-size fish.

	Bellvue Hatch House Troughs			Bellvue Raceways	Watson Raceways
	Feb 2018	March 2018	April 2018	Sept 2018	April 2019
			Bio Oregon		
Length	41.6 [5.1]	53.6 [5.6]	67.6 [6.2]	199.2 [21.3]	246.3 [24.4]
CV Length	0.12	0.10	0.09	0.11	0.10
Weight	0.6 [0.2]	1.6 [0.5]	3.1 [0.8]	93.8 [28.2]	153.2 [50.6]
CV Weight	0.35	0.33	0.27	0.30	0.33
HSI	N/A	0.015 [0.006]	0.013 [0.002]	0.013 [0.002]	0.009 [0.002]
VSI	N/A	0.171 [0.029]	0.115 [0.016]	0.114 [0.015]	0.087 [0.017]
			Rangen		
Length	39.1 [4.8]	48.1 [5.3]	60.9 [7.1]	183.7 [20.6]	221.9 [17.8]
CV Length	0.12	0.11	0.12	0.11	0.08
Weight	0.6 [0.2]	1.2 [0.4]	2.4 [0.9]	68.9 [22.9]	105.8 [31.8]
CV Weight	0.40	0.36	0.39	0.33	0.30
HSI	N/A	0.011 [0.007]	0.012 [0.003]	0.016 [0.004]	0.008 [0.001]
VSI	N/A	0.160 [0.023]	0.118 [0.024]	0.150 [0.025]	0.068 [0.018]

In September 2018, after five months in the Bellvue raceways, fish fed Bio Oregon were on average 15.5 mm longer and 24.9 g heavier than fish fed Rangen. Although the two groups exhibited similar variability in length, the fish fed Rangen were more variable in weight. Overall, the fish fed Bio Oregon grew 131.6 mm and 90.7 g in the Bellvue raceways, whereas fish fed Rangen grew 122.8 mm and 66.5 g over the same time period. However, fish fed Rangen had higher HSI and VSI values than fish fed Bio Oregon in September 2018. In April 2019, after seven months in the Watson Unit raceways, fish fed Bio Oregon were on average 24.4 mm longer and 47.4 g heavier than fish fed Rangen, and fish fed Bio Oregon were more variable in length and weight than fish fed Rangen. Overall, the fish fed Bio Oregon grew 47.1 mm and 59.4 g in the Watson Unit raceways, whereas fish fed Rangen grew 38.2 mm and 36.9 g over the same time period. Similar to previous time periods, with the exception of September 2018, fish fed Bio Oregon exhibited higher HSI and VSI in April 2019 than did fish fed Rangen (Table 2.1.1).

Due to growth differences between the Hofer and HHN, variable water temperatures experienced in the various hatchery environments, and larger lot sizes than those used in the previous growth experiments, HHNs took about five months longer to reach catchable size than the Hofers used in 2016 and 2017. Although the HHNs were slower growing compared to the Hofer strain (Fetherman et al. 2019), similar results were seen with respect to faster growth in the fish fed Bio Oregon than those fed Rangen at the Bellvue-Watson Fish Hatchery. Bio Oregon fish were longer and weighed more at the end of the hatchery rearing period, and it is likely that, similar to the previous experiments, three to four weeks of additional feeding would have been needed to achieve the same goal weight (in this case ~150 g) prior to stocking. Unlike previous feed trials, there did not appear to be an advantage of using Bio Oregon feed to increase energy storage in the liver and viscera as indicated by the relatively similar HSI and VSI values obtained from the two groups over the course of the study. Additionally, HSI and VSI values for the HHNs were generally lower than those observed in the Hofer strain. It is unknown whether this is a result of strain differences, e.g., feed conversion or consumption rates (not measured in this study), differences in how the strains assimilate the nutrients from the feeds, or environmental differences, e.g., temperature, water chemistry, or densities. Despite the lack of differences in HSI and VSI, results from this study suggest that Bio Oregon continues to be a good option for increased growth and obtaining larger fish in a shorter period of time than feeding Rangen, even at a larger production scale and for slower growing strains of Rainbow Trout.

Fetherman, E. R., B. Neuschwanger, T. Davis, C. Praamsma, and D. Karr. 2019. Colorado hatchery feed experiments. Colorado Parks and Wildlife, Aquatic Wildlife Research Section. Fort Collins, Colorado.

Action #2:

- *Level 1 Action <u>Category</u>: Data Collection and Analysis*
- *Level 2 Action <u>Strategy</u>: Research, survey or monitoring – fish and wildlife populations*
- *Level 3 Action <u>Activity</u>: N/A*

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. Although there are treatment options for the disease, treatment can be costly and time consuming, and effectiveness can vary. In addition, low quality feeds can cause health issues to arise, especially in overcrowded populations. These effects can carry over into stocked populations, causing lower survival rates. An experiment initiated in 2017 was conducted to examine the effects of rearing fish at multiple densities and on a high and low quality feed to determine if post-stocking survival rates differ with regard to feed type and rearing density. Fish from this experiment were PIT tagged and stocked into Parvin Lake, Red Feather Lakes, CO in August 2017. Post-stocking survival was assessed using bimonthly recapture events. Annual post-stocking survival rates will be assessed via electrofishing recaptures in summer and fall 2018.

<u>Action #2 Accomplishments</u>

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*. In addition, low quality feeds can cause health issues to arise due to nutritional deficits or reductions in excess energy storage used to fight infections, as well as reduced water quality, especially in overcrowded populations. These effects can carry over into stocked populations, causing lower survival rates. This experiment examined the effects of rearing fish at multiple densities and on a high- and low-quality feed to determine if post-stocking survival rates differ with regard to feed type and rearing density.

Feeds from two commercial feed companies were evaluated, Bio Oregon and Rangen. To maintain low cost and consistency among the two feed companies, the basic feeds from each company were used in this experiment. Each company uses slightly different proportions of crude protein and crude fat in their diets, and proportions change with a change in feed size. Though proportions are similar among diets produced by the two companies, the type of ingredients used to produce the diets likely result in the differences in cost and proposed feed conversion ratios. Each company also has their own recommendations for feeding rates (Fetherman and Schisler 2017), which were followed in this experiment.

Hofer × 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. Twenty thousand Rainbow Trout eggs were spawned for this experiment at the BFRH. Eggs were held in egg cups within two experimental tanks, one for each feed company, and dead eggs were removed to prevent fungus growth. Upon hatching, fish were released into the experimental tanks, and cripples were removed from the tank through swim-up.

Fish were fed a starter diet for each feed type to which a tank had been assigned. Starter feed was fed to fish four times daily at the feeding rate (percent body weight per day [% BW/d]) recommended by the company producing each feed (Fetherman and Schisler 2017). Prior to feeding, a subset of 20 fish were removed from the tank and measured and weighed to determine growth rate differences in the two weeks prior to the start of the experiment. Mortality of swim up fry was recorded to determine the percent of fish that did not take to feed in each tank. Fish were fed a starter diet for two weeks post-swim-up to ensure that all fish included in the density and feed experiment were actively feeding prior to the start of the experiment.

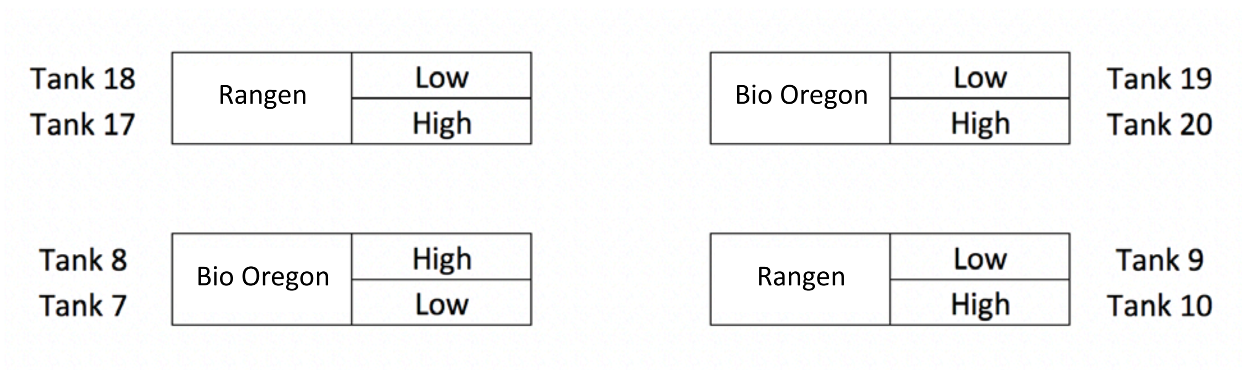

Figure 2.2.1. Paired experimental hatchery troughs used to rear the H×H at two densities (low and high) and on two feeds (Bio Oregon and Rangen) at the BRFH.

At two weeks post-swim-up, fish were counted and distributed into randomly-assigned fiberglass hatchery troughs (Figure 2.2.1). 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 often maintains fish at this density in its hatcheries 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 fed 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 experiment to keep the rearing density indexes for each density treatment from exceeding a rearing index of 0.5 or 2.0. 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 reaching the maximum density before volume was changed again. Flows also changed for each density treatment 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. Equal numbers of fish from each density and feed treatment were tagged, with 710-725 fish being tagged from each hatchery trough, resulting in 1,430-1,450 fish tagged per treatment type (Table 2.2.1). All fish (20,000), tagged (2,899 high density and 2,869 low density fish) and untagged extras (13,101 high density and 1,131 low density fish), were stocked into Parvin Lake (Red Feather Lakes, Colorado) in September 2017. Recaptures were conducted using a boat-mounted electrofishing unit every two weeks between September and November 2017, and one time per month in April, June, and September 2018. Only the survival results for the two-month post-stocking period between September and October 2017, seven-month post-stocking period between August 2017 and April 2018, and the annual survival (September 2017-September 2018) are shown here.

Survival analyses were conducted using a capture-recapture Cormack Jolly-Seber hierarchical Bayesian model. Vague prior information was used for the beta value parameters in the model with means equal to zero and the variance equal to 1.96 from a normal probability distribution. Posterior inference for model parameters and derived quantities (low rearing density survival, high rearing density survival, and both feed type survival) were based on the number of Markov chain Monte Carlo (MCMC) samples following convergence after 20% of an iteration burn-in period. Visual inspection of trace plots for model parameters indicated that the MCMC chains mixed and converged to the target distributions. Posterior distributions were examined and are presented for detection probability and rearing density.

More fish from the low density treatments were captured over the course of the experiment than fish from the high density treatments, but the number of fish captured that had been reared on the Bio Oregon versus Rangen feeds were similar (Table 2.2.1). Average detection probability (ρ) ranged between 0.05 and 0.50 among the seven sampling events (Figure 2.2.2). Feed did not appear to affect post-stocking survival (Table 2.2.2), and the 95% credible intervals of the beta value associated with feed overlapped with zero (Figure 2.2.3). As such, the posterior distributions related to feed are not presented. At two months post-stocking, fish reared at a lower density survived better than fish reared at a higher density (0.35 and 0.34, respectively; Figure 2.2.4). At seven months post-stocking, fish reared at a lower density survived better than fish reared at a higher density (0.29 and 0.27, respectively; Figure 2.2.4). At twelve months post-stocking, fish reared at lower density also survived better than fish reared at higher density (0.20 and 0.18, respectively; Figure 2.2.5).

Table 2.2.1. Number of tags stocked per treatment type (low density or high density; Bio Oregon or Rangen feed) and associated capture and recapture numbers ([ ]) at two, seven, and twelve months post-stocking.

Treatment	# Tags Stocked	2 Months	7 Months	12 Months
Low, Bio Oregon	1430	45 [10]	27 [6]	1 [1]
High, Bio Oregon	1449	20 [4]	7 [0]	1 [0]
Low, Rangen	1439	39 [6]	25 [3]	3 [1]
High, Rangen	1450	36 [4]	11 [3]	1 [0]

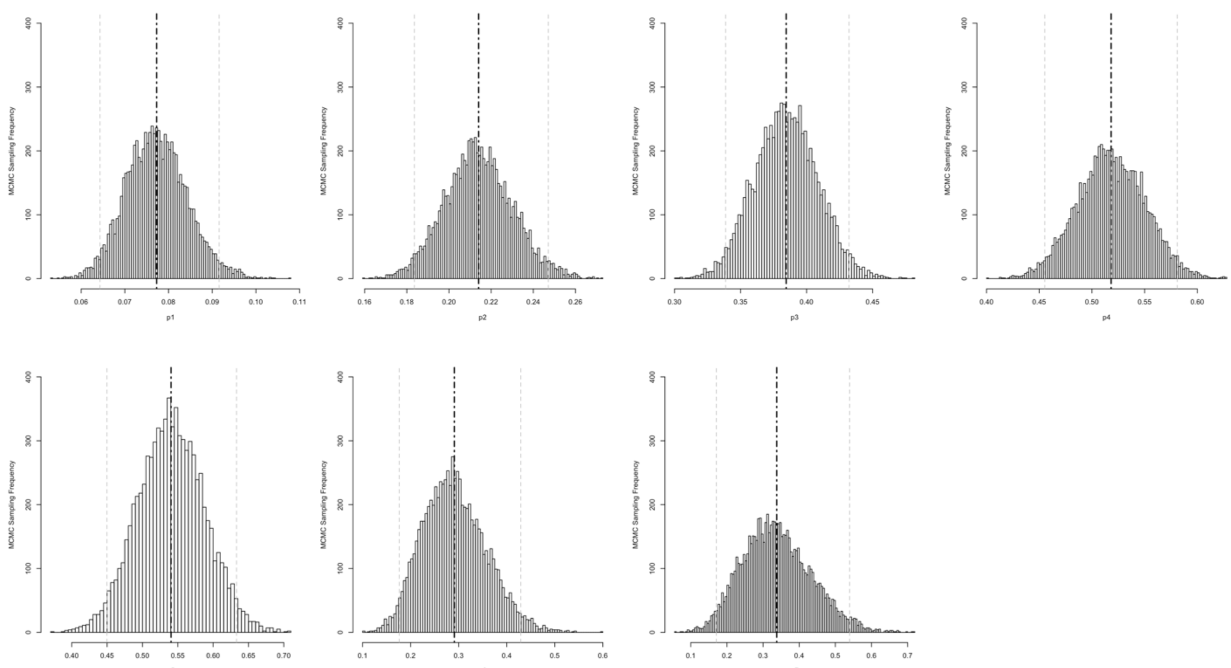

Figure 2.2.2. Posterior distributions for detection probabilities (ρ) associated with each recapture event, with ρ1-ρ4 representing biweekly sampling events occurring in September and October 2017, ρ5 representing the sampling event occurring in April 2018, ρ6 representing the sampling event occurring in June 2018, and ρ7 representing the sampling event occurring in September 2018. Black dotted black line denotes the mean of the distribution and the grey dotted lines denote the upper and lower bounds of the 95% credible intervals.

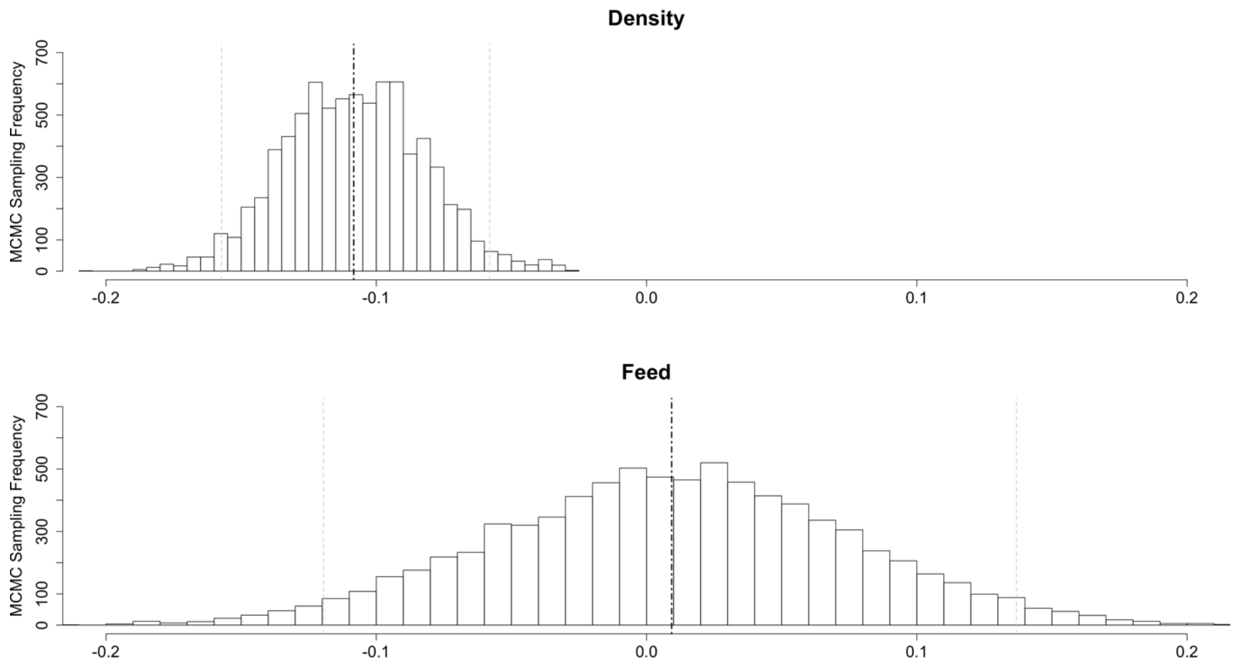

Figure 2.2.3. Posterior distributions for the regression coefficients associated with rearing density and feed type. Black dotted black line denotes the mean of the distribution and the grey dotted lines denote the upper and lower bounds of the 95% credible intervals.

Table 2.2.2. Derived mean survival [95% credible interval bounds] of Rainbow Trout reared at low and high densities, and on Bio Oregon and Rangen, at two, seven, and twelve months post-stocking into Parvin Lake.

Treatment		**Months Post-Stocking**	**Mean Survival**
Density	**Feed Company**		
Low	Bio Oregon	2	0.35 [0.34, 0.37]
High	Bio Oregon	2	0.34 [0.33, 0.35]
Low	Rangen	2	0.35 [0.34, 0.36]
High	Rangen	2	0.34 [0.33, 0.35]
Low	Bio Oregon	7	0.29 [0.28, 0.30]
High	Bio Oregon	7	0.27 [0.26, 0.28]
Low	Rangen	7	0.28 [0.27, 0.29]
High	Rangen	7	0.26 [0.26, 0.27]
Low	Bio Oregon	12	0.20 [0.19, 0.21]
High	Bio Oregon	12	0.18 [0.18, 0.19]
Low	Rangen	12	0.20 [0.19, 0.21]
High	Rangen	12	0.18 [0.17, 0.19]

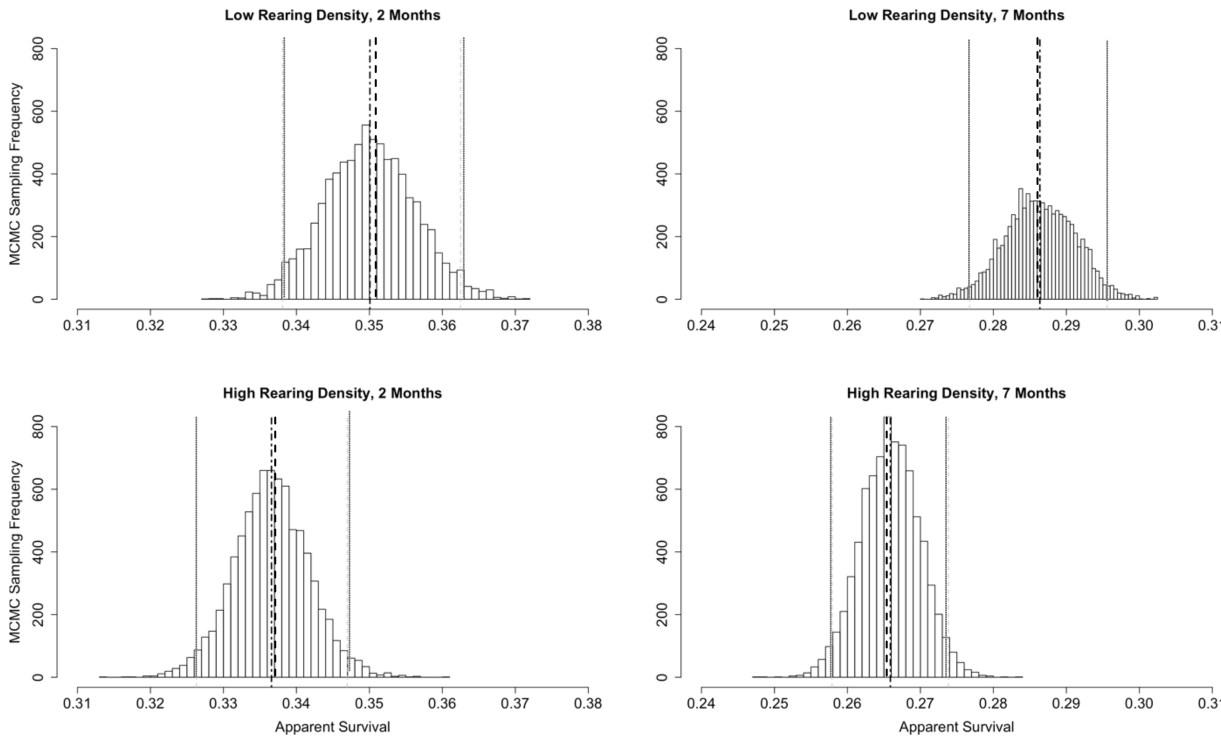

Figure 2.2.4. Posterior distributions for the average apparent survival for fish reared at low and high densities at two and seven months post-stocking. Black dotted black line denotes the mean of the distribution and the grey dotted lines denote the upper and lower bounds of the 95% credible intervals. These values are derived quantities within the analysis and are constructed by lumping feed types together and separating values into low and high rearing density treatments.

The capture-recapture data indicated that rearing density affected survival but feed type did not. Overall, survival was higher for fish reared at low densities. However, because all fish from each treatment were stocked, tagged and untagged extras, stocking density may be a confounding factor within the analysis. Although statistically different, biologically, there was no difference between the survival between the low-density and high-density fish at two, seven or twelve months post-stocking. Raising fish at higher densities did not result in bacterial coldwater disease outbreaks in the hatchery, although outbreaks have been known to occur when fish are reared at the high densities used in this experiment. Accounting for the number of fish stocked per density, the number of tagged and untagged fish that were estimated to be alive from the high versus low density treatments differed considerably after a year (2,880 and 800 fish, respectively) despite survival differences, primarily due to the number of high versus low density fish stocked. As such, it may still be advantageous to maximize stocking density within a system, even if it means rearing fish at higher densities to do so. Further research is needed to separate stocking density from rearing density to estimate the true effect of rearing density on post-stocking survival.

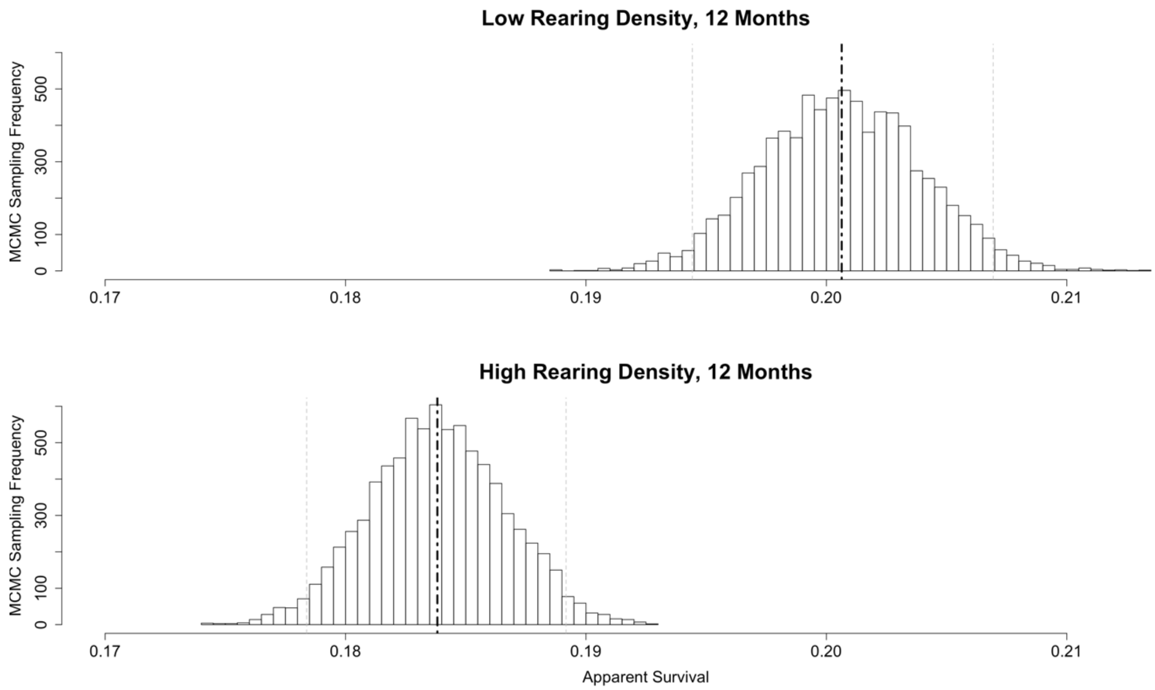

Figure 2.2.5. Posterior distributions for the average apparent survival for fish reared at low and high densities at twelve months post-stocking. Black dotted black line denotes the mean of the distribution and the grey dotted lines denote the upper and lower bounds of the 95% credible intervals. These values are derived quantities within the analysis and are constructed by lumping feed types together and separating values into low and high rearing density treatments.

Fetherman, E. R., and G. J. Schisler. 2017. Sport Fish Research Studies. Federal Aid Project F-394-R16. Federal Aid in Fish and Wildlife Restoration, Job Progress Report. Colorado Parks and Wildlife, Aquatic Wildlife Research Section. Fort Collins, Colorado.

Piper, R. G., I. B. McElwain, L. E. Orme, J. P. McCaren, L. G. Fowler, and J. R. Leonard. 1982. Fish hatchery management. U. S. Fish and Wildlife Service, Washington, D. C.

Action #3:

- *Level 1 Action Category: Data Collection and Analysis*
- *Level 2 Action Strategy: Techniques development*
- *Level 3 Action Activity: Fish and wildlife research, survey and management techniques*

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. In this experiment, rainbow trout will be experimentally injected with varying concentrations of F. psychrophilum to determine the optimal concentration needed for developing infection but preventing complete loss of the experimental group. The results from this challenge experiment will be used to inform the design and implementation of future challenge experiments examining F. psychrophilum resistance in rainbow trout.

Action #3 Accomplishments

Despite the large number of *Flavobacterium psychrophilum* challenge experiments that have been conducted relative to species susceptibility, development of resistant strains, and testing vaccine effectiveness, no standardized challenge protocol exists. The dosages in most published challenge experiments vary widely for both bacterial isolates and *F. psychrophilum* exposure. Therefore, it was necessary to develop a standardized protocol for exposure to *F. psychrophilum* prior to conducting additional experiments to examine control strategies. We developed an alternative method for culturing the bacteria and harvesting them at their maximum population growth rate. Our method maximizes the number of live bacteria fish are exposed to in challenge experiments. Our new culture method will allow us and others to expose fish to *F. psychrophilum* in a more repeatable and quantitative manner.

The CSF259-93 isolate of *F. psychrophilum* used to standardize culture and challenge methods was obtained from Dr. Kenneth Cain's lab located at the University of Idaho. CSF259-93 is the isolate that has been most widely used for challenge experiments in the literature. The bacterium was initially cultured on tryptone yeast extract salt (TYES; 0.4% tryptone, 0.04% yeast extract, 0.02% calcium chloride, 0.05% magnesium sulfate; PH between 7.1 and 7.3) agar plates. Once a pure colony was isolated, 100 ml of modified TYES (0.4% tryptone, 0.04% yeast extract, 0.02% calcium chloride, 0.05% magnesium sulfate, 0.2% dextrose; PH between 7.1 and 7.3) was inoculated in a baffled flask and placed on a shaker table. Inoculation of new 100-mL flasks of TYES + 0.2% dextrose broth was continued every 24 – 36 hours to maintain a culture at log phase growth. Optical density (OD) measurements were taken at a wavelength of 595 nm, in addition to ten-fold serial dilutions with plate counts every two hours, starting at 22 hours post inoculation (PI) through 32 hours PI (Figure 2.3.1). An additional OD measurement was taken at 45 hours PI to determine if bacteria had reached stationary phase and was no longer replicating.

Once a growth curve was constructed (Figure 2.3.1), it was determined that maximum replication was obtained at an OD of 0.3. Harvest and bacterial concentration for injection occurred around the maximum. Two large-baffled flasks with 1,500 mL of TYES + 0.2% dextrose were inoculated from a previously cultured broth at log phase growth, with 3000 mL of broth culture harvested at 0.34 OD. Bacteria were harvested by placing 40 ml of broth culture from the 1,500-ml flasks into each of six 50-ml centrifuge tubes. The tubes were then placed in a centrifuge and spun at 8,000 g (8,873 relative centrifugal forces) for 10 minutes. The remaining liquid was decanted and poured into a discard flask, leaving a concentrated pellet of *F. psychrophilum* bacteria. Forty mL of culture broth was then placed back into the same 50-mL centrifuge tubes. The process continued until all 3000 mL of the culture was concentrated into bacteria pellets, resulting in 12 50-mL centrifuge tubes with concentrated bacteria pellets.

Each bacteria pellet was then re-suspended into 10 mL of TYES + 0.2% dextrose broth using a pipet. All concentrated and re-suspended media was pooled together, and 50 mL of TYES + 0.2% broth and 10% glycol was added. To allow for easy dosage manipulation, potential future bacteria propagation, and to reduce freeze-thaw events, the bacterium was placed into ten 1.2-mL vials and 31 15-mL vials containing 5 mL of bacteria. All vials were kept in a -80°C freezer. Final concentration of the bacteria was 8.1×10^9 colony forming units/mL (CFU/mL).

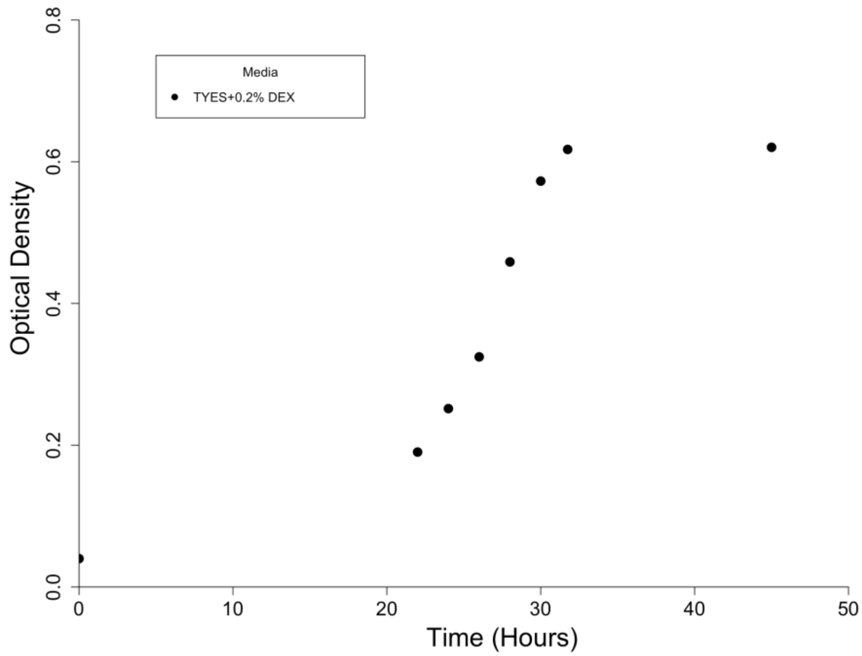

Figure 2.3.1. Growth curve for *Flavobacterium psychrophilum* (CSF 259-93) produced using optical density measurements taken every two hours as an index of bacteria grow over time.

Table 2.3.1. *Flavobacterium psychrophilum* exposure doses (CFU/mL) and average fish weights (g ± SD) associated with the second and third challenge experiments.

Experiment	Dose	Fish Weight
2	5.00×10^5 5.00×10^6 5.00×10^7 5.00×10^8	10.5 ± 0.05
3	6.85×10^3 1.40×10^4 4.80×10^5 1.10×10^6 1.19×10^7 1.90×10^8	14.0 ± 0.8

Rainbow Trout (Hofer × Harrison Lake; H×H) *F. psychrophilum* challenge experiments (three total) were conducted in the Colorado Cooperative Fish and Wildlife Research Unit Quonset hut wet lab (Fort Collins, Colorado). Each challenge experiment consisted of five treatments replicated in triplicate (15 experimental tanks): low, medium, and high bacterial dosages, a mock saline injection, and a naïve control (no injection). Bacterial dosages in the first experiment consisted of 1×10^5 CFU/mL (low), 1×10^6 CFU/mL (medium), and 1×10^7 CFU/mL (high). Dosages used in the two subsequent experiments were adaptively determined based on results from the previous experiment. For example, if the high dose in the first experiment resulted in 90-100% mortality, then we reduced the maximum dosage, and proportionally the low and medium dosages, in the following experiments (Table 2.3.1). Twenty-five fish were held in each tank to allow assessment of mortality, and to standardize and compare to other published studies.

To start an experiment, fish were moved from the BFRH to the Quonset hut wet lab. After a two-day acclimation period, Rainbow Trout in the bacterial challenge tanks were injected subcutaneously at the dorsal midline posterior to the dorsal fin with virulent *F. psychrophilum* (CSF259-93; 25 μl). Rainbow Trout in mock injection tanks were subcutaneously injected at the dorsal midline posterior to the dorsal fin with 25 μl of TYES broth media to ensure that exposure to the bacteria and not the physical damage from injection caused observed mortality. No injections occurred for fish in the control tanks. At the time of injection, fish were weighed to determine fish size effects on mortality. Fish were monitored at least twice a day, and moribund and dead were removed and recorded per tank daily during the 28 day experiment. Raw mortality data was transformed into cumulative percent mortality curves to examine trends in mortality over time. Upon review of the results from the first challenge experiment, it was determined data from that experiment should not be included in the analyses due to bacterial preparation differences between the first versus second and third challenge experiments.

Analyses using only the data originating from the second and third challenge experiments were conducted using a Bayesian logistic regression model, with data consisting of the number of fish dead in each tank, with bacterial dose and fish weight as predictor variables. Posterior inference for model parameters and derived quantities were based on the number of Markov chain Monte Carlo (MCMC) samples following convergence after 20% of an iteration burn-in period. Visual inspection of trace plots for model parameters indicated that the MCMC chains mixed and converged to the target distributions. Posterior distributions were examined and are presented for both predictor variables and their interaction. Model selection was completed utilizing deviance information criterion (DIC) to select the top model.

Mortality typically started 7-10 days post-exposure to *F. psychrophilum*, and that mortality generally concluded 7-12 days after it began. In addition, mortality appeared to be dose dependent. Mock injections did not result in increased mortality, suggesting that exposure to *F. psychrophilum* versus the injection method was the cause of the observed patterns in mortality in the two challenge experiments (Figure 2.3.2). The top predictive model consisted of a linear combination of dose, weight, and the interaction of dose and weight (Table 2.3.2). All Bayesian R-squared values were between 0.44 and 0.48. Of the three predictor variables, dose had the largest impact on mortality (Figure 2.3.3). Data generated from the Bayesian analyses was used to develop a predictive equation of mortality for future exposure experiments (Equation 2.3.1).

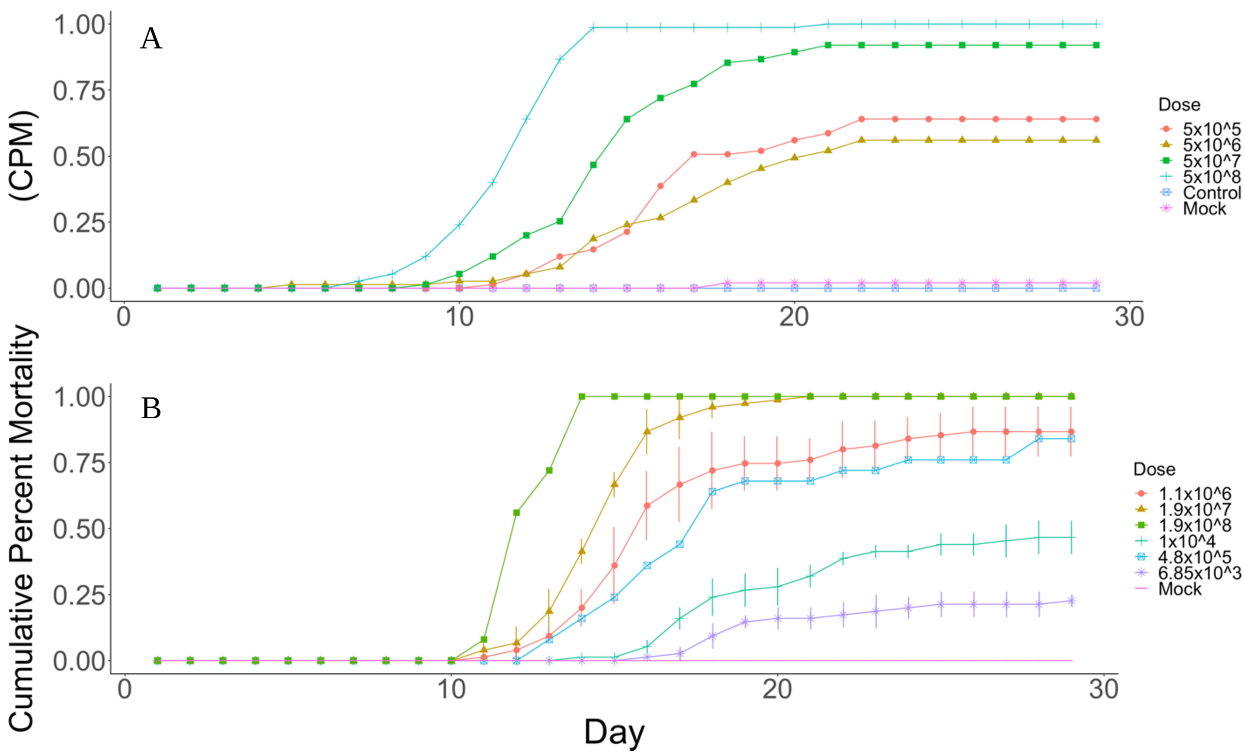

Figure 2.3.2. Cumulative Percent Mortality (CPM) curves constructed for challenge experiment two (A) and three (B). Vertical lines indicated standard deviations.

Table 2.3.2. Model selection results arranged by DIC (lowest to highest), with the lowest DIC value indicating the model most supported by the data.

Model	**DIC**	**Bayesian R-squared**
Intercept + Dose + Weight + Dose*Weight	44.67	0.48
Intercept + Dose + Weight	53.64	0.48
Intercept + Dose	63.68	0.44

$$p = \frac{e^{-1.231+1.354\text{x}10^{-7}(\text{Dose})+0.0347(\text{Weight})+2.387\text{x}10^{-9}(\text{Dose*Weight})}}{1+e^{-1.231+1.354\text{x}10^{-7}(\text{Dose})+0.0347(\text{Weight})+2.387\text{x}10^{-9}(\text{Dose*Weight})}}$$

Equation 2.3.1. Equation representing the probability of morality, with untransformed β coefficients, derived from the top predictive model and allowing future predictive abilities when conducting *F. psychrophilum* challenge experiments.

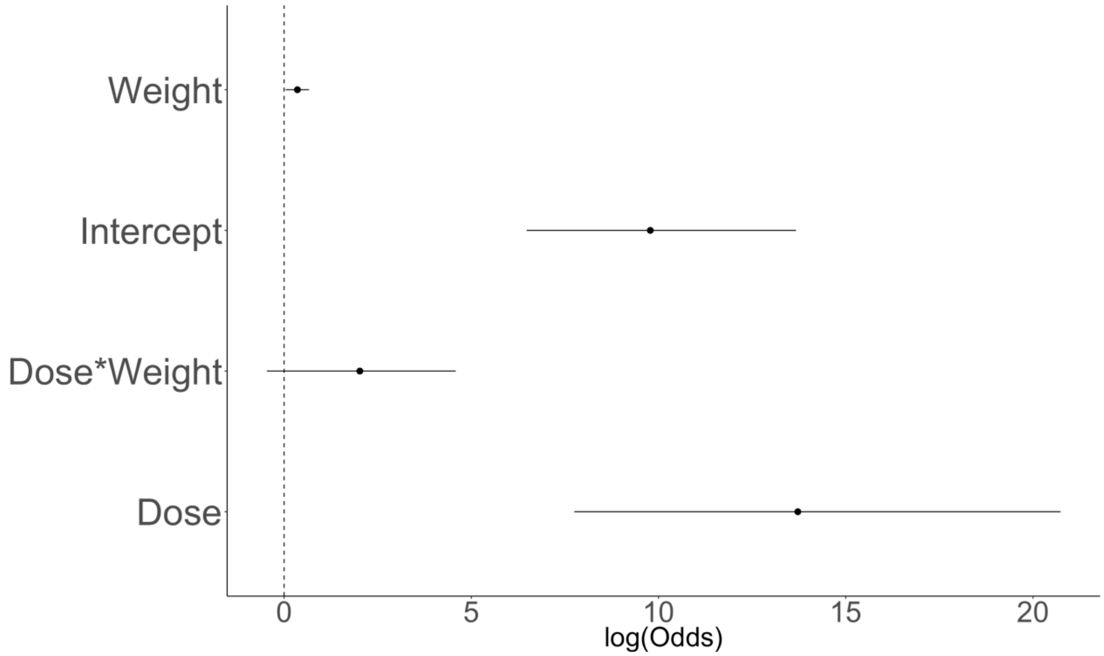

Figure 2.3.3. Top model log(Odds) posterior distributions for standardized β coefficients associated with each covariate (mean [black dot] and 95% credible intervals [black lines]).

The methods of *F. psychrophilum* culture and harvest presented here represent a deviation from previous methods where bacteria were harvested in the stationary phase. By harvesting bacteria in the log phase, bacteria are still actively replicating and the number of live bacteria concentrated for challenge exposure is maximized. Results suggest that there is both a dose and weight mortality response in challenged fish when using bacteria harvested in the log phase. Unique to this study is the inclusion of the dose-weight interaction term in the predictive equation, which has not been presented in the previous literature. The predictive equation can be used to standardize challenge experiment dose based on fish weight which will make future challenge experiment results more comparable when using similar harvest methods and the CSF259-93 isolate of *F. psychrophilum*. The same culture and harvest methods, as well as the predictive equation, will be used in future *F. psychrophilum* challenge experiments conducted by CPW and Colorado State University examining the incorporation of *F. psychrophilum* resistance into *Myxobolus cerebralis*-resistant Rainbow Trout strains, and the effects of *F. psychrophilum* and *M. cerebralis* co-infections in Rainbow Trout.

Job No. 3. Whirling Disease Resistant Domestic Brood Stock Development and Evaluation

Job Objective: These experiments are focused on the performance of the Hofer and Hofer × Harrison Lake strain as domestic production fish compared with other commonly used production fish.

Need

Whirling disease has a complex, two-host life cycle, with salmonids being the primary host of the disease. *M. cerebralis*-positive fish develop myxospores that are released upon death. The addition of these myxospores to a system perpetuates the disease; however, resistant fish contribute fewer myxospores than do susceptible fish. Evaluations are needed to determine which fish contribute more myxospores to a system, resistant fish reared in a *M. cerebralis*-positive hatchery environment, or susceptible fish reared in a *M.* cerebralis-negative hatchery environment. *Myxobolus cerebralis*-resistant and -susceptible strains can exhibit differences in survival and severity of infection when stocked into positive systems. Evaluations of survival and infection severity of the various strains stocked as fingerlings into lakes and reservoirs is needed to determine which strains are best suited for use in put-grow-and-take fisheries.

Objectives

1. Conduct up to four electrofishing surveys in Parvin Lake to evaluate survival and infection severity of various strains of rainbow trout stocked as fingerlings by November 30, 2018.

Approach

Action #1:

- *Level 1 Action Category: Direct Management of Natural Resources*
- *Level 2 Action Strategy: Wildlife disease management*
- *Level 3 Action Activity: N/A*

Samples of up to 60 fish will be collected from Parvin Lake during each survey via boat electrofishing conducted at night to increase capture probability. Up to four surveys will be conducted in fall of 2018, and summer of 2019. Coded wire tags will be recovered from each individual, and the batch code will associate that individual to a strain or cross and the year stocked. Survival will be assessed and compared among the strains and crosses using cumulative catch curves. Infection severity will be assessed through myxospore enumeration which will be conducted by the staff at the CPW Aquatic Animal Health Laboratory.

Action #1 Accomplishments

Sampling in Parvin Lake in 2018 and 2019 was focused on the field component of the hatchery rearing density experiment described in Job No. 2, Action #2. Details of the sampling and results for that project are reported therein. No additional tagged fish releases or sampling occurred as part of the rainbow trout fingerling survival experiment in 2018 or 2019.

Job No. 4 Whirling Disease Resistant Wild Strain Establishment, Brood Stock Development and Evaluation

Job Objective: These experiments are designed to establish, develop, and evaluate “wild” strain whirling disease-resistant Rainbow Trout for reintroduction into areas where self-sustaining populations have been lost due to whirling disease.

Need

Whirling disease caused significant declines in Rainbow Trout populations throughout Colorado following its accidental introduction and establishment in the late 1980s. *Myxobolus 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, dependant 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.

Objectives

1. Conduct one experiment designed to assess the post-stocking survival of sub-catchable and catchable Hofer × Harrison Lake (H×H) rainbow trout in the Yampa River by June 30, 2019.

Approach

Action #1:

- *Level 1 Action Category: Data Collection and Analysis*
- *Level 2 Action Strategy: Research, survey or monitoring – fish and wildlife populations*
- *Level 3 Action Activity: Abundance determination*

The Yampa River, along with Catamount Lake, in Steamboat, CO has become an important location for the establishment of a wild Hofer × Harrison Lake (H×H) brood stock. Habitat restoration activities and private land turnover has resulted in the need for multiple stocking strategies to maintain the integrity of the brood stock in the Yampa River. This study, initiated in 2017, compares the post-stocking survival rates of large, catchable H×Hs stocked on private land and subcatchable H×Hs stocked in public stretches of the Yampa River. Fish will be tagged with coded-wire and PIT tags, and multiple mark-recapture events will be used to assess survival.

Action #1 Accomplishments

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 second 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 2,000 Rainbow Trout, averaging 337.8 (± 32.7) mm total length (TL) and 431.1 (± 126) g, were tagged with 32 mm PIT tags on May 30-31, 2018. 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, although 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 (707 fish), and 4) Green Creek/Kuntz ranches (668 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 right pelvic clip used for fish stocked into the Stagecoach Tailwater, a right pectoral clip used for fish stocked on the Wellar Ranch, a left pectoral clip used for fish stocked into the Service State Wildlife Area (SWA), BLM, and Foster's Ranch properties, and a left pelvic clip for fish stocked into the Green Creek and Kuntz ranches. PIT-tagged Rainbow Trout were stocked into the Yampa River on June 28, 2018. Known tag number groups 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 12,312 Rainbow Trout, averaging 68.8 (± 7.2) mm TL and 3.9 (± 1.3) g, were tagged with coded wire tags (CWT) on June 6-7, 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 1,101 fish were stocked into the Stagecoach Tailwater, 2,899 fish were stocked into the Wellar Ranch, 4,267 fish were stocked in the Service/BLM/Foster's Ranch section, and 4,045 fish were stocked in the Green Creek and Kuntz ranches. CWT Rainbow Trout were stocked into the Yampa River study section on June 8, 2018, 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 2018. The Foster Ranch, BLM property, Service Creek SWA, Wellar Ranch, and the Stagecoach Tailwater were sampled using a continuous single pass removal September 10-14, 2018. 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 2018 were adipose fin clipped prior to returning them to the river so that a unique encounter history could be created following the next recapture event in 2019. Additionally, CWT fish stocked in 2017 and recaptured in 2018 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 after being measured and weighed, and transported and released into the Chuck Lewis SWA downstream of Lake Catamount to prevent return to the study section. 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. The Green Creek and Kuntz ranches were not sampled in 2018 as low flow conditions precluded the use of raft electrofishing equipment needed to sample the deep holes formed by habitat restoration activities on the ranches.

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, transported downstream, and released into the Chuck Lewis SWA. 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.

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 only three occasions, a release occasion ("1" for all fish released in spring 2017 or 2018) and two encounter occasions ("1" for all fish encountered and "0" for all fish not encountered in fall 2017 and 2018). 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 survival and detection probability was estimated for each location.

Overall, 134 PIT-tagged Rainbow Trout were captured in the Stagecoach Tailwater, 451 were captured on the Wellar Ranch, and 571 were captured in the Service Creek SWA/BLM/Foster's Ranch section in 2018. Both PIT-tagged Rainbow Trout stocked in 2017 and 2018 contributed to the number of fish captured in each section, although fish stocked in 2018 outnumbered fish stocked in 2017 about 2:1. On average, and regardless of stocking location, PIT-tagged Rainbow Trout survival estimates were nearly equivalent between 2017 and 2018 across the entire Yampa River study section and higher than CWT Rainbow Trout survival estimates in both years (Figure 4.1.1). Two hundred eleven CWT Rainbow Trout were captured in the Stagecoach Tailwater, 167 were captured on the Wellar Ranch, and 603 were captured in the Service Creek SWA/BLM/Foster's Ranch section in 2018. A large majority of the CWT fish captured were stocked in 2018, with only 6, 2, and 4 CWT Rainbow Trout stocked in 2017 captured in the Stagecoach Tailwater, Wellar Ranch, and Service/BLM/Foster's Ranch sections, respectively. On average, CWT Rainbow Trout stocked in 2018 had higher survival estimates than did CWT

Rainbow Trout stocked in 2017 (Figure 4.1.1). Wild Rainbow Trout juveniles, PIT-tagged in 2017 at ≤ 150 mm total length (TL), exhibited similar survival estimates to the stocked CWT Rainbow Trout, whereas wild Rainbow Trout adults, PIT-tagged in 2017 at > 150 mm TL exhibited similar survival estimates to stocked PIT-tagged Rainbow Trout (Figure 4.1.1).

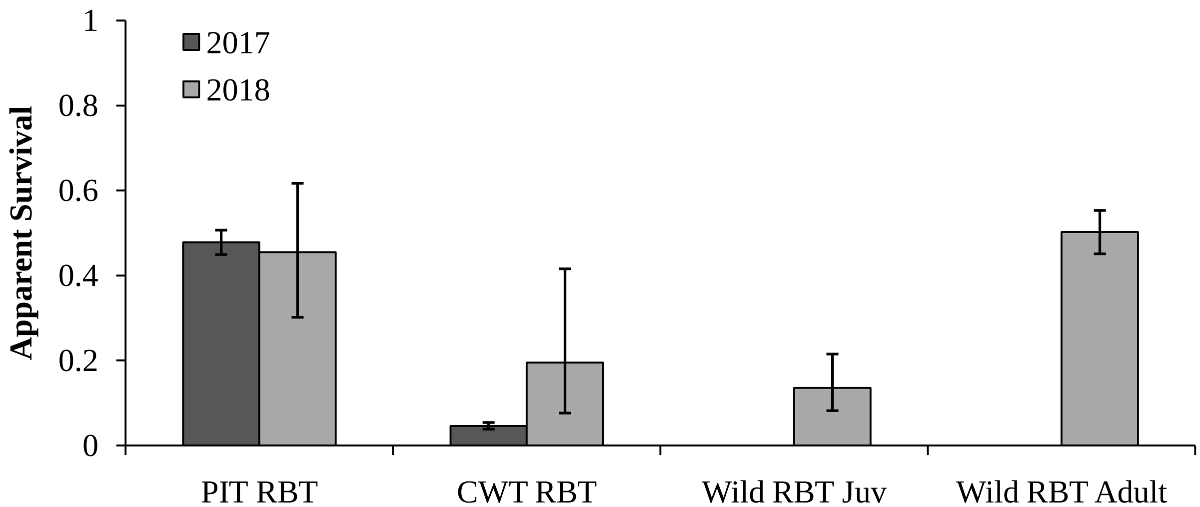

Figure 4.1.1. Apparent survival estimates for PIT- and coded wire-tagged (CWT) Rainbow Trout (RBT) stocked in the Yampa River in 2017 and 2018, and for wild Rainbow Trout PIT tagged during population estimates conducted in 2017 and recaptured in 2018.

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 survival rates were similar for fish stocked above and below the Service Creek confluence with the Yampa River (Figure 4.1.2). Short-term survival estimates for CWT Rainbow Trout stocked in 2017 were significantly higher than annual survival estimates, and in general, survival was higher for fish stocked above the Service Creek confluence than below (Figure 4.1.2). 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). Recapture, or lack thereof, in future sampling occasions in 2019 and 2020 will help determine the fate of these individuals.

Short-term (June-September 2018) survival estimates were higher for PIT-tagged Rainbow Trout compared to CWT Rainbow Trout in all sampled sections of the Yampa River, and in general, did not differ among the sampled sections for either the PIT-tagged or CWT Rainbow Trout (Figure 4.1.3). Both PIT-tagged and CWT Rainbow Trout stocked into the Green Creek and Kuntz ranches in 2018 were recovered in other sections of the river during sampling in September 2018. However, no sampling occurred in these two sections in 2018. Therefore, although survival estimates appear lower in the Green Creek and Kuntz ranches since recoveries were based on detecting fish that moved into other sections within the Yampa River (Figure 4.1.3), it is probable that survival and retention in these ranches were higher than estimated, and future sampling occasions conducted in these locations in 2019 and 2020 will be used to adjust these estimates.

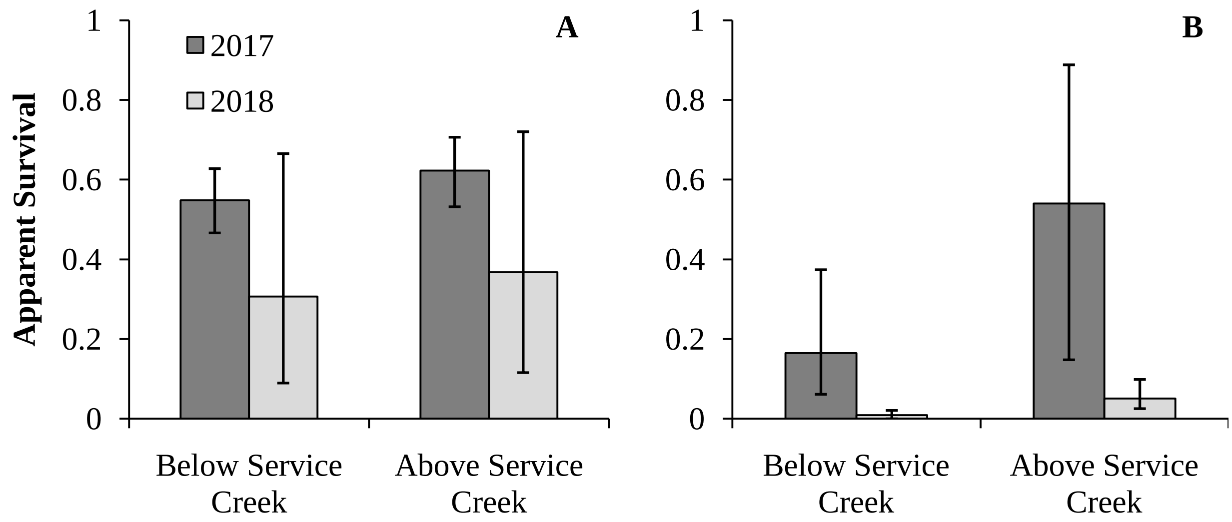

Figure 4.1.2. Short-term (May-September 2017) and annual (September 2017-September 2018) apparent survival estimates for PIT-tagged (panel A) and CWT Rainbow Trout (panel B) stocked above or below the Service Creek confluence with the Yampa River in spring 2017.

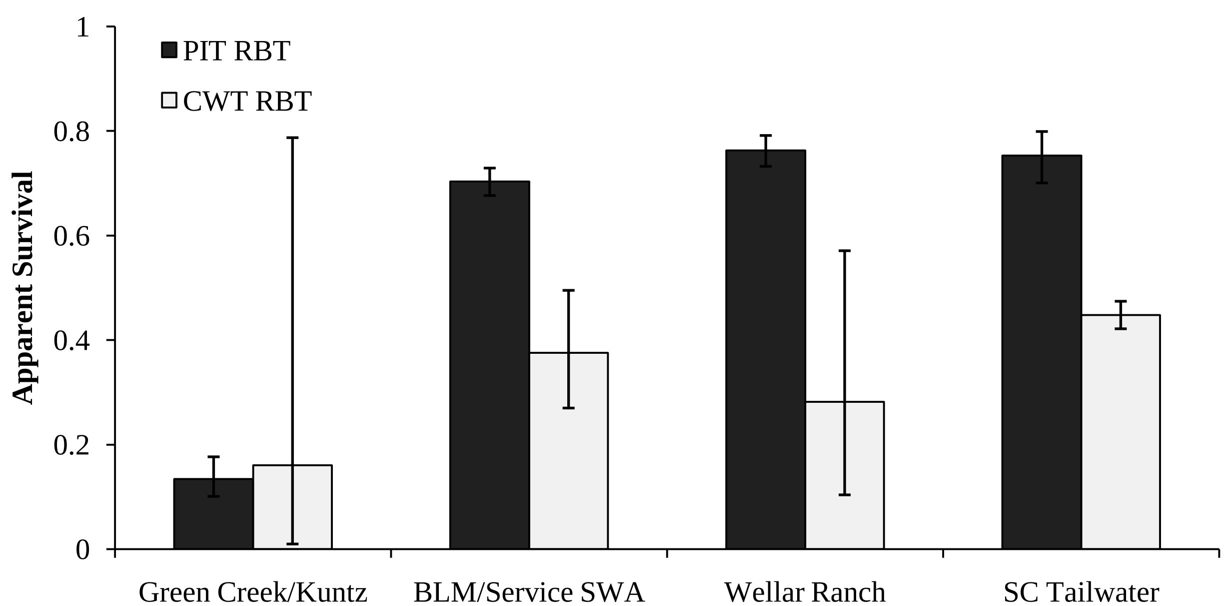

Figure 4.1.3. Short-term (June-September 2018) apparent survival estimates for PIT-tagged and CWT Rainbow Trout stocked in four sections of the Yampa River study section in spring 2018.

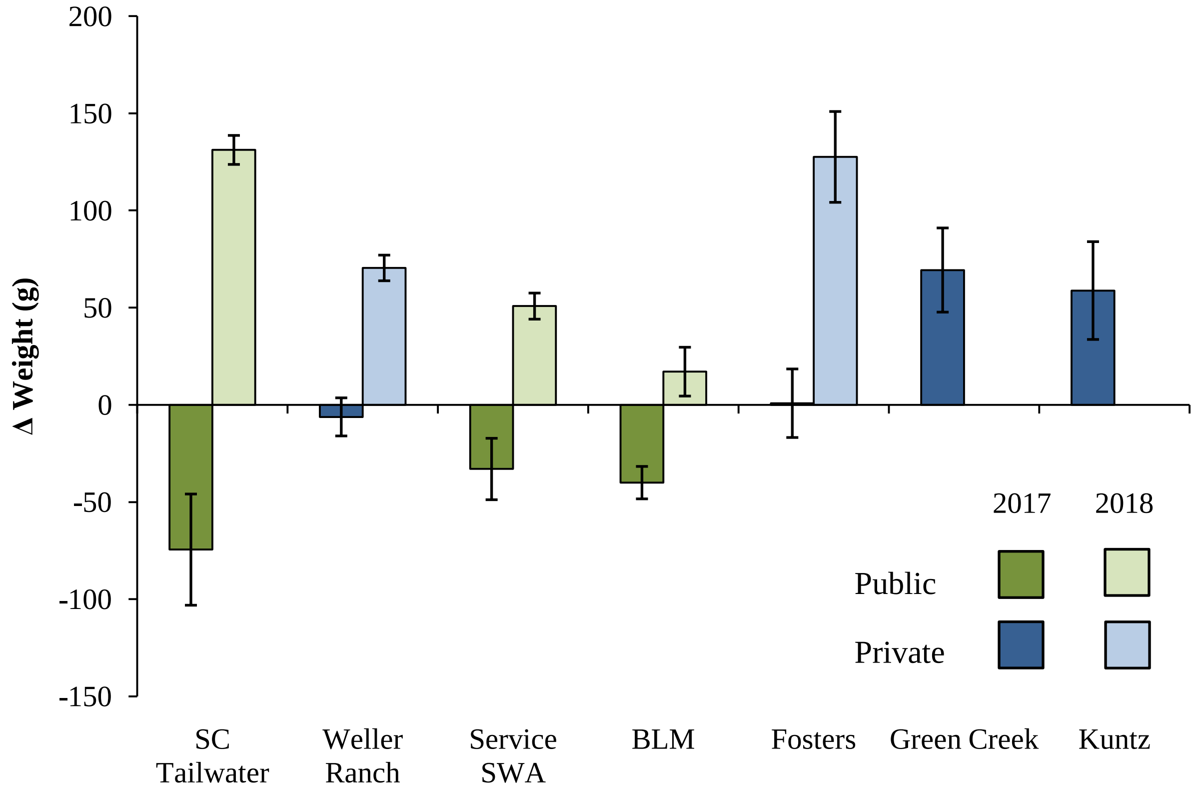

Figure 4.1.4. Short-term change in weight (g) of PIT-tagged Rainbow Trout stocked and recovered in either 2017 or 2018 in public and private land sections of the Yampa River.

Coded wire-tagged Rainbow Trout stocked in spring 2018 grew well, averaging 182.3 (± 17.6) mm TL and 70.5 (± 21.3) g upon recapture in fall 2018. Coded wire-tagged Rainbow Trout stocked in spring 2017 continued to grow and nearly doubled in size from that reported in 2018 (Fetherman et al. 2018), averaging 307.2 (± 26.7) mm TL and 287.9 (± 109.7) g upon recapture in fall 2018. 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 gained weight in all locations prior to recapture in fall 2018 (Figure 4.1.4), which could be a result of the smaller size of fish stocked in 2018 in comparison to those stocked in 2017 leading to a greater growth potential in the 2018 fish. 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 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 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.

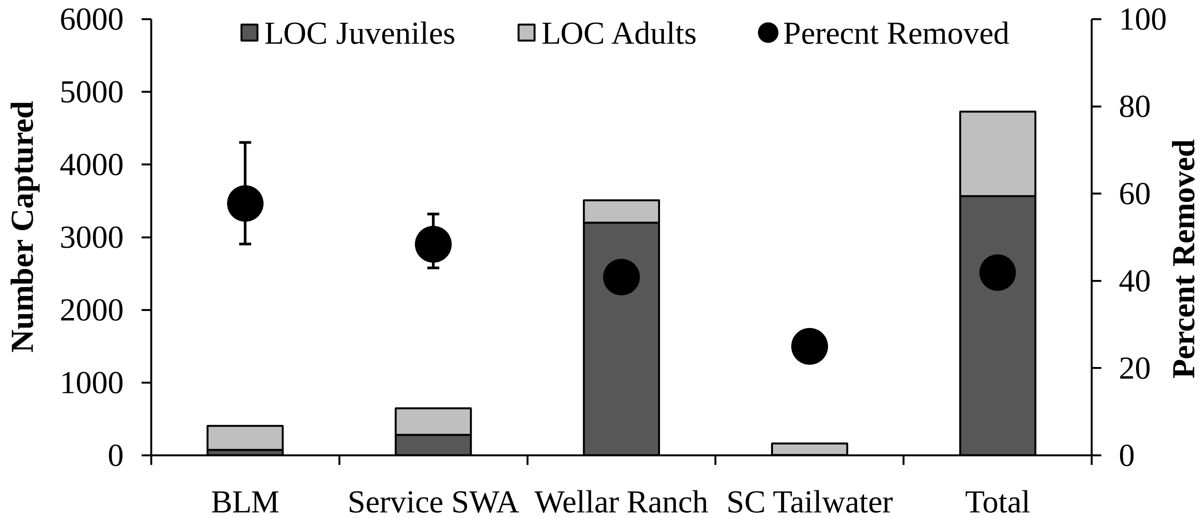

Figure 4.1.5. Number of juvenile and adult Brown Trout captured in the BLM, Service SWA, Wellar Ranch, and Stagecoach (SC) Tailwater sections of the Yampa River in fall 2018, and corresponding estimated percent Brown Trout removed based on two-pass removal abundance estimates conducted in each of the four sections.

A total of 4,727 Brown Trout, only 100 less than 2017 (Fetherman et al. 2018), were removed and relocated to the Chuck Lewis SWA, 42% of the estimated 11,276 Brown Trout present in the Yampa River in fall 2018. The percentage of Brown Trout removed from the BLM, Service SWA and Wellar Ranch ranged between 40 and 60%, whereas only 25% of the Brown Trout population present in the Stagecoach Tailwater was removed (Figure 4.1.5). The Stagecoach Tailwater 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. Additionally, Rainbow Trout greatly outnumber Brown Trout in the tailwater, which could result in human-induced sampling error when the crew’s search image is focused on the more abundant species. Three quarters of the Brown Trout removed in 2018 were juveniles, and the majority of those fish were captured and removed from the Wellar Ranch, a section of the river known for its juvenile rearing habitat. Despite similar numbers of Brown Trout being removed throughout the Yampa River study section in 2017 and 2018, fewer adults were removed in 2018, suggesting that the 2017 removals had an effect on both adult and recruiting juvenile Brown Trout populations throughout the section.

Habitat assessments were conducted in five sections of the Yampa River in 2018, corresponding to the two-pass abundance estimates sites in the Stagecoach Tailwater, Wellar Ranch, Service SWA, and BLM sections, and a representative section of similar length on the upper Green Creek Ranch. Site surveys were conducted to create longitudinal profiles which will be used to generate estimates of channel length, stream and valley slope, sinuosity, identify bedform features, and measure residual pool depths across the study reach. Additionally, cross section data collected during the site surveys will be used to compare average bankful widths, average bankful depths, average width to depth ratios, bankfull cross-sectional area, and average entrenchment ratios for all reaches. Pebble counts were conducted in each section, and will be used to characterize bed materials, especially the percentage of fines in each of the reaches. Brown Trout redd counts were conducted in each section to determine availability of spawning habitat, and pebble samples were collected from redds in each section to determine bed material type and size requirements for spawning. Bank vegetation ratings and estimates of large wood and cover were conducted to examine and compare the availability of additional habitat types and the potential contribution of terrestrial inputs in each section. Lastly, aquatic insect samples were collected from representative pool and riffle habitats to compare benthic macroinvertebrate composition and density, and potential fish prey availability, in each section.

Table 4.1.1. Abundance estimates for adult and juvenile Brown Trout (LOC), wild adult and juvenile Rainbow Trout (RBT), PIT- and coded wire-tagged (CWT) Rainbow Trout, Rainbow Trout that could not be identified (unknown), Mottled Sculpin, Speckled Dace, Mountain Whitefish, and Brook Trout [95% CIs], obtained from the two pass removals conducted in the BLM, Service Creek SWA, Wellar Ranch, and Stagecoach Tailwater sections of the Yampa River in fall 2018.

Species/Type	BLM	Service Creek SWA	Wellar Ranch	Stagecoach Tailwater
LOC (Adults)	543 [424, 663]	467 [443, 492]	207 [187, 229]	648 [620, 676]
LOC (Juv)	158 [142, 175]	874 [730, 1,018]	8,376 [8,006, 8,746]	----
RBT (Wild Adults)	558 [477, 639]	232 [219, 245]	82 [78, 87]	2,204 [2,159, 2,249]
RBT (Wild Juv)	425 [316, 535]	581 [434, 728]	2,688 [2,500, 2,878]	229 [200, 259]
RBT (Wild Recaps)	37 [34, 39]	26 [26, 26]	14 [13, 14]	529 [518, 539]
RBT (2018 CWT)	267 [250, 283]	810 [775, 845]	234 [202, 266]	1,049 [983, 1,115]
RBT (2017 CWT)	9 [8, 10]	18 [17, 18]	----	41 [36, 47]
RBT (PIT-tagged)	162 [137, 188]	247 [242, 253]	206 [199, 212]	815 [799, 830]
Mottled Sculpin	2,533 [2,230, 2,837]	4,517 [3,821, 5,212]	395 [334, 456]	57 [44, 70]
Mountain Whitefish	148 [0, 335]	886 [837, 935]	1,332 [1,183, 1,482]	42 [33, 52]
Brook Trout	110 [102, 118]	168 [57, 280]	22 [16, 28]	97 [85, 110]
Fathead Minnow	----	----	195 [161, 229]	29 [19, 39]
White Sucker	----	----	8 [3, 13]	----

Although the habitat data collected in 2018 is still being compiled and analyzed, 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 rearing habitat for Brown Trout and Rainbow Trout. In comparison, the deep pools in the Stagecoach Tailwater 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 and Specled Dace than do other sections of the river (Table 4.1.1). 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.

The results from the second 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 than 2017, suggesting that a reduction in the adult Brown Trout population may have reduced predation and increased the survival of these fish. 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 year of the study as well. Habitat data collected in 2018, and recapture data to be collected in 2019, 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 has had on survival of the stocked fingerling Rainbow Trout. In addition, the number of CWT Rainbow Trout stocked in Yampa River in 2019 was reduced by 50% to evaluate the potential effects of stocking density and Brown Trout population reduction on survival of the CWT 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 will be 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 in Colorado hatcheries.

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

Huggins, R. M. 1989. On the statistical analysis of capture experiments. Biometrika 76:133-140.

Huggins, R. M. 1991. Some practical aspects of a conditional likelihood approach to capture experiments. Biometrics 47:725-732.

White, G. C., and K. P. Burnham. 1999. Program MARK: survival estimation from populations of marked animals. Bird Study 46(Supplement):120-138.

Job No. 5. Technical Assistance

Job Objective: Provide information on impacts of fish disease on wild trout populations to the Management and Hatchery Sections of Colorado Parks and Wildlife and other resource agencies. Provide specialized information or assistance to the Hatchery Section. Contribute editorial assistance to various professional journals and other organizations upon request.

Need

Fishery managers and hatchery supervisors often request information regarding the impacts of fish disease on wild or hatchery trout populations. Effective communication between researchers, fishery managers and hatchery supervisors is essential to the management of Rainbow Trout populations in Colorado. In addition, the publication process requires a minimum of two peer reviews from other researchers in the same field, and CPW researchers are often chosen as peer reviewers for scientific journals. Technical assistance is often unplanned, and is addressed on an as-needed basis.

Objectives

1. Provide one fishery manager or hatchery supervisor with information regarding the impacts of disease on wild or hatchery trout populations by June 30, 2019.
2. Complete one peer review of a manuscript submitted to a scientific journal by June 30, 2019.

Approach

Action #1:

- *Level 1 Action <u>Category</u>: Technical Assistance*
- *Level 2 Action <u>Strategy</u>: Technical assistance*
- *Level 3 Action <u>Activity</u>: With individuals and groups involved in resource management decision making*

Provide technical assistance to fishery managers or hatchery supervisors upon request. Technical assistance may consist of providing information regarding fish disease, assisting with data analysis, or a presentation of projects to keep all interested parties informed of current results.

<u>Action #1 Accomplishments</u>
Internal presentations to CPW staff were used to update fishery managers on current research and to help inform management decisions regarding disinfectant use, predator training of hatchery populations of Arkansas darters, and the stocking and use of *Myxobolus cerebralis*-resistant Rainbow Trout in Colorado waters. Two presentations were given at the CPW statewide aquatic biologist meeting, one at the CPW Plains Fish Research Meeting, and one at an internal stakeholder meeting for the Colorado River Headwaters Project:

- Wells, C., and E. R. Fetherman. 2019. Disinfection of aquatic/fisheries field equipment. 2019 Colorado Parks and Wildlife Aquatic Biologist Meeting. Salida, Colorado. January 23, 2019.
- Fetherman, E. R., B. Atkinson, and M. Kondratieff. 2019. Comparative survival of catchable and fingerling rainbow trout stocked in the Yampa River. 2019 Colorado Parks and Wildlife Aquatic Biologist Meeting. Salida, Colorado. January 23, 2019.
- Kopack, C. J., E. R. Fetherman, R. M. Fitzpatrick, E. D. Broder, and L. M. Angeloni. 2019. Can training species of conservation concern prior to release increase survival. 2019 Colorado Parks and Wildlife Plains Fish Research Meeting. Fort Collins, Colorado. February 12, 2019.
- Fetherman, E. R., and J. Ewert. Colorado River 2018 salmonid research/population monitoring. Colorado River Headwaters Project Meeting. Frisco, Colorado. April 24, 2019.

External presentations provided an opportunity to give research updates to fishery managers both within and outside of the state of Colorado. In addition, these talks provided information regarding the history and importance of being a part of a professional society to the student subunits of the Colorado/Wyoming Chapter of the American Fisheries Society. One talk was given at the Colorado Aquaculture Association meeting, three at the Colorado/Wyoming Chapter of the American Fisheries Society Meeting, one at the 2019 AFS Fish Health Section Meeting, and three to the student subunits of the Colorado/Wyoming Chapter of the American Fisheries Society:

- Avila, B., T. Riepe, and E. Fetherman. 2019. Upcoming research projects. 2019 Annual Meeting of the Colorado Aquaculture Association. Mt. Princeton Hot Springs, Colorado. February 1, 2019.
- Kopack, C., E. Fetherman, R. Fitzpatrick, E. D. Broder, and L. Angeloni. 2019. Can training species of conservation concern prior to release increase survival? 2019 Annual Meeting of the Colorado/Wyoming Chapter of the American Fisheries Society. Fort Collins, Colorado. February 28, 2019.
- Riepe, T., D. L. Winkelman, and E. R. Fetherman. 2019. The past, present, and future of bacterial kidney disease. 2019 Annual Meeting of the Colorado/Wyoming Chapter of the American Fisheries Society. Fort Collins, Colorado. February 28, 2019.
- Avila, B. W., D. L. Winkelman, E. R. Fetherman, and J. Drennan. 2019. Culturing *Flavobacterium psychrophilum* and standardization of experimental exposures. 2019 Annual Meeting of the Colorado/Wyoming Chapter of the American Fisheries Society. Fort Collins, Colorado. February 28, 2019.
- Fetherman, E. R. 2019. Benefits of AFS membership and a career as a CPW Aquatic Research Scientist. Colorado State University Student Subunit of the Colorado/Wyoming Chapter of the American Fisheries Society. Fort Collins, Colorado. February 21, 2019.
- Fetherman, E. R. 2019. Benefits of AFS membership and a career as a CPW Aquatic Research Scientist. Colorado Mesa University Student Subunit of the Colorado/Wyoming Chapter of the American Fisheries Society. Grand Junction, Colorado. March 12, 2019.
- Fetherman, E. R. 2019. Benefits of AFS membership and a career as a CPW Aquatic Research Scientist. University of Wyoming Student Subunit of the Colorado/Wyoming Chapter of the American Fisheries Society. Laramie, Wyoming. May 7, 2019.

- 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.

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, CO. 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 2019:

- Fetherman, E. R. 2018. Salmonid disease research in Colorado. Guest lecture, Introduction to Fisheries. Front Range Community College. Fort Collins, Colorado. March 28, 2019.

A similar fish tagging lab was presented to the Colorado State University Wildlife Management Short Course on March 26, 2019. The Wildlife Management Short Course is a week long course that teaches non-field professionals that work for natural resources agencies about the research, data collection, and management that is conducted by their colleagues.

Guest lectures were also given at the University of Washington and Colorado State University:

- 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? University of Washington. Tacoma, Washington. March 4, 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? Colorado State University. Fort Collins, Colorado. April 2019.

Manuscripts published in peer-reviewed scientific journals help to inform fisheries management decisions locally, nationally, and internationally. Two manuscripts were published in peer-reviewed scientific journals:

- 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. DOI: 10.1002/aah.10040.
- Fetherman, E. R., P. Cadmus, A. L. Jefferson, and M. K. Hura. 2019. Increasing copper concentrations do not affect *Myxobolus cerebralis* triactinomyxon viability. Journal of Fish Diseases, Early View. DOI: 10.1111/jfd13048.

Two other manuscripts were prepared for publication, but have not yet been submitted:

- Kopack, C. J., E. D. Broder, E. R. Fetherman, R. M. Fitzpatrick, and L. M. Angeloni. *In preparation*. Behavior and survival of Arkansas darters (*Etheostoma caragini*) in response to abiotic enrichment and predator recognition training: Evidence of genotype by environment interactions. Intended for submission to Biological Conservation.
- Kopack, C. J., E. D. Broder, E. R. Fetherman, L. M. Angeloni, and C. K. Ghalambor. *In preparation*. Fisheries conservation makes sense in light of evolution. Intended for submission to Trends in Ecology and Evolution.

Social media has increasingly been used to inform the public about management and research activities being conducted by CPW. Two videos were submitted for posting to the CPW Facebook Page. The first video showed electrofishing crews in the Yampa River and explained why fish were being recaptured for the research project being conducted (see Job No. 4, Action #1). The second was a series of video clips 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.

Interviews were provided for two video production efforts. The first was an interview with Elliot Kennerson, a producer of the YouTube and Facebook series "Deep Look", during which New Zealand mudsnail disinfection experiments and protocols were discussed for an upcoming episode regarding the mudsnails. The second was an interview with Ben Bortner, Hog Leg Fly Fishing, who is putting together a video on whirling disease and its history in the Gunnison Gorge for a traveling fly fishing film festival.

Reviewed and helped correct a press release regarding the survival success of the Gunnison River Rainbow in the Arkansas River.

Technical assistance milestones included providing information and discussion for internal management decisions regarding bacterial kidney disease in Colorado's hatchery system, determining the cost of treating diseases in Colorado's hatchery system, and disinfectant alternatives to quaternary ammonium compounds for both internal and public use.

Technical assistance milestones also included assistance with data collection and analysis on two projects being conducted by CPW researchers and biologists:

- Analyzed movement and detection probabilities by species for an enclosure experiment and a long-term movement experiment conducted at the FCRID fish passage structure on the Cache la Poudre River in Fort Collins.
- 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.

Action #2:

- *Level 1 Action Category: Technical Assistance*
- *Level 2 Action Strategy: Technical assistance*
- *Level 3 Action Activity: N/A*

Provide review of manuscripts submitted to scientific journals upon request.

Action #2 Accomplishments

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

- Anonymous. Identification of parasites from farmed goldfish and effectiveness of two herbal extracts to control the parasitic infection. Submitted to the Proceedings of the National Academy of Sciences, India Section B: Biological Sciences.

- Peterson, D. P., and H. M. Neville. Comparison of methods to verify upstream passage by trout at remediated culverts in four Rocky Mountain streams. Submitted to the North American Journal of Fisheries Management.
- Saleh, M., R. Montero, B. Köllner, G. Kumar, A. Sudhagar, A. Friedl, and M. El-Matbouli. Kinetics of local and systemic immune cell responses in whirling disease infection and resistance in rainbow trout. Submitted to Parasites and Vectors.
- Polivka, C. M., and S. M. Claeson. 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.
- Anonymous. 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.