Sport Fish Research Studies

Federal Aid Project F-394-R17

Eric R. Fetherman, Ph.D.
Aquatic Research Scientist

George J. Schisler, Ph.D.
Aquatic Research Leader

and

Brian W. Avila

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Job Progress Report

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STATE OF COLORADO

John W. Hickenlooper, Governor

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AQUATIC RESEARCH STAFF

George J. Schisler, Aquatic Research Leader
Kelly Carlson, Aquatic Research Program Assistant
Pete Cadmus, Aquatic Research Scientist/Toxicologist, Water Pollution Studies
Eric R. Fetherman, Aquatic Research Scientist, Salmonid Disease Studies
Ryan Fitzpatrick, Aquatic Research Scientist, Eastern Plains Native Fishes
Eric E. Richer, Aquatic Research Scientist/Hydrologist, Stream Habitat Restoration
Matthew C. Kondratieff, Aquatic Research Scientist, Stream Habitat Restoration
Dan Kowalski, Aquatic Research Scientist, Stream & River Ecology
Adam G. Hansen, Aquatic Research Scientist, Coldwater Lakes and Reservoirs
Kevin B. Rogers, Aquatic Research Scientist, Colorado Cutthroat Studies
Kevin G. Thompson, Aquatic Research Scientist, 3-Species and Boreal Toad Studies
Andrew J. Treble, Aquatic Research Scientist, Aquatic Data Management and Analysis
Brad Neuschwanger, Hatchery Manager, Fish Research Hatchery
Tracy Davis, Hatchery Technician, Fish Research Hatchery
David Karr, Hatchery Technician, Fish Research Hatchery

Jim Guthrie, Federal Aid Coordinator
Alexandria Austermann, Librarian

Prepared by: ______________________________
Eric R. Fetherman, Aquatic Research Scientist

Approved by: ______________________________
George J. Schisler, Aquatic Wildlife Research Chief

Date: 10/12/18

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State: Colorado **Project No. F-394-R16**

Project Title: Sport Fish Research Studies

Period Covered: July 1, 2016 – June 30, 2017

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, 2018.
2. Maintain genetic and disease integrity of brood stocks housed at the Bellvue Fish Research Hatchery and Poudre Rearing Unit through June 30, 2018.

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 2017 through January 2018, and reared through June 30, 2018. 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 2017, BFRH personnel checked all of the Hofer (GR)[1] and Harrison Lake (HL) brood fish (2, 3, and 4 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, 2017, 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 Category: Data Collection and Analysis*
- *Level 2 Action Strategy: Techniques development*
- *Level 3 Action Activity: 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 can preserve both the genetic and disease integrity of fish produced to replace hatchery brood stocks and for stocking. Studies will be conducted at both the Bellvue Fish Research Hatchery and Poudre Rearing Unit to determine the best options for maintaining pathogen-free whirling disease-resistant Rainbow Trout brood stocks.

Action #2 Accomplishments

Bacterial Kidney Disease (BKD), caused by *Renibacterium salmoninarum,* is widespread in most areas of the world where wild or cultured salmonid fish are present, and chronic disease can cause significant mortality in salmonids at most life stages (Fryer and Sanders 1981). *Renibacterium salmoninarum* is an intracellular, gram-positive diplobacillus that can be transmitted both horizontally (Bell et al. 1984; Murray et al. 1992) and vertically (Evelyn et al. 1986a, 1986b). Although Iodophor disinfection successfully inactivates surface bacteria, it does not eliminate intraovum infections (Evelyn et al. 1986a, 1986b), making traditional egg disinfection techniques ineffective for preventing vertical transmission during hatchery spawning. Additionally, prophylactic antibiotic-medicated feed treatments are not entirely protective (Fryer and Sanders 1981; Groman and Klontz 1983; Austin 1985; Elliot et al. 1989), and a potential for development of reduced antibiotic susceptibility exists in *R. salmoninarum* (Bell et al. 1988; Rhodes et al. 2008).

Colorado's hatchery system has had infrequent outbreaks of BKD for decades. *Renibacterium salmoninarum* outbreaks were a major problem in Colorado hatcheries in the 1950s and 1960s, and were often related to culture conditions, including poor water temperatures and cleanliness, and high-density and high-stress rearing conditions. Although BKD outbreaks were reduced by changing the rearing standards, *R. salmoninarum* was recently detected in Colorado hatcheries. Due to the potential for vertical transmission routes, *R. salmoninarum* can inadvertently be introduced to the hatchery rearing environment via wild spawning operations. This is the suspected introduction route to the CPW Glenwood Springs Hatchery in 2015. Roan Creek Cutthroat Trout brought onto the unit from wild spawn operations were incorporated into the raceway system, moved to the upper most raceway on the unit, and then subsequently tested positive for *R. salmoninarum*. Given the potential that the fish downstream of Roan Creek Cutthroat Trout were also positive for *R. salmoninarum*, and the unknown population-level consequences of stocking infected fish, the CPW Glenwood Springs Hatchery was depopulated to eliminate the disease. Depopulation resulted in the loss of thousands of pounds of fish, including five unique year classes of Roan Creek Cutthroat Trout, and the entire Hofer by Colorado River (H×C) Rainbow Trout brood stock for the state.

In 2015 and 2016, fish on the CPW Poudre Rearing Unit and BFRH also tested positive for *R. salmoninarum*. These two units house the entire Hofer (GR) brood stock for the state. As such, depopulation and loss of this brood stock, along with other important salmonid brood stocks, was not an option. CPW implemented a lethal spawning procedure at both units in which all spawned adults were culled and tested for *R. salmoninarum*. Offspring from these culled spawns were maintained separately until the results from the adult fish were obtained, and all offspring originating from parents that tested positive for *R. salmoninarum* were also culled. Although these types of culling operations can continue in perpetuity to maintain low to non-existent levels of infection in the offspring (Munson et al. 2010), they can be costly, time consuming, difficult to maintain, and result in the loss of fish after a single spawn when they are typically spawned two to three times. As an alternative, an experiment was conducted at the BFRH to examine the use of erythromycin injections with Erymin 200 (INAD study #12-781-17-016) to control *R. salmoninarum* in the brood stock fish. The objective of this INAD study was to determine the efficacy and safety of Erymin 200 injection treatments to reduce or minimize *R. salmoninarum* levels in BKD-positive female brood stock fish in order to control and/or prevent the vertical transmission of *R. salmoninarum* to the eggs and progeny.

Two- and three-year-old GR brood stock fish, averaging 2.0 kg, were used in this experiment, all of which were housed at the BFRH. A total of 334 fish were maintained as non-injected controls, whereas 200 fish were treated with Erymin 200 (Figure 1.2.1). Brood stock fish within the treatment group received three injections with Erymin 200 prior to spawning, with 21 days between the first and second injections, and 22 days between the second and third injections. Prior to injection, the sex of the fish was determined, if possible. Both male and female brood stock fish were injected with Erymin 200 in this experiment despite the lack of evidence that males can vertically transmit *R. salmoninarum*. The fish were then weighed to allow for a standardized dose of 25 mg Erymin 200 per kg of body weight. Although the original intent was to administer an intraperitoneal (IP) injection, examination of a mortality that occurred during the third injection event revealed that the needle being used for the injections was not long enough to have entered the IP cavity in the three-year-old fish. As such, it is suspected that all three-year-old fish used in this study received intramuscular (IM) versus IP injections. Body wall thickness was not examined on the two-year-old fish since the needle was no longer present at the hatchery during lethal spawn operations, so it is unknown whether two-year-old fish received IP or IM injections, but it was likely a combination of both.

Treated and control fish were lethally spawned on November 14, November 28, December 5, and December 12, 2017. During the lethal spawn operations, kidney samples were taken from all fish spawned to test for the presence of *R. salmoninarum*. Although unique male-female pairs were created during the spawning operations, a lack of space to maintain these families separately within the hatchery resulted in the need to pool two to three families per tank. The presence of *R. salmoninarum* was confirmed in the adults via a single round polymerase chain reaction (PCR) implemented at the CPW Aquatic Animal Health Lab (Brush, Colorado). If an adult tested positive for the presence of *R. salmoninarum*, the eggs from that adult, and any other families that may have been pooled with those fish, were moved to an isolation unit to finish hatching. All progeny held in the hatchery or isolation facility were maintained through swim-up, at which point a portion of the progeny were also tested for the presence of *R. salmoninarum* using five-fish pooled samples.

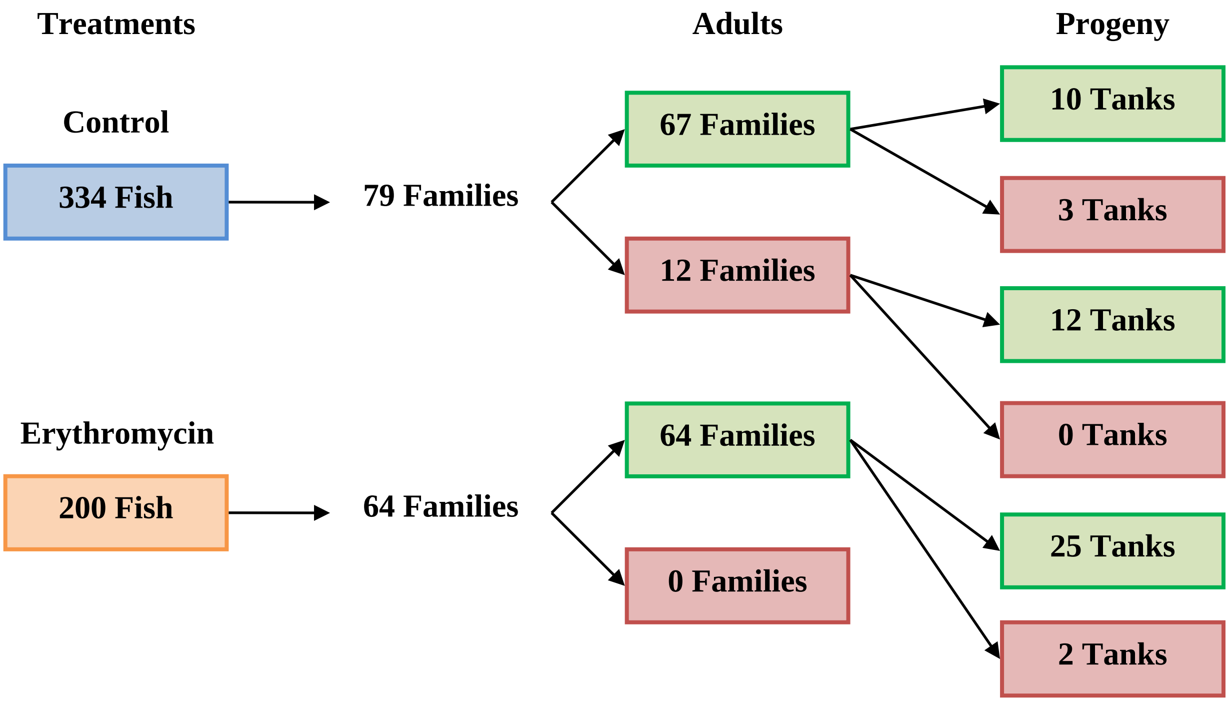

Figure 1.2.1. Number of fish spawned per treatment, number of families created from the spawns, number of families in which the adults tested positive (red) or negative (green) for *Renibacterium salmoninarum*, and the number of progeny tanks originating from those families that subsequently tested positive or negative for *R. salmoninarum*.

Seventy-nine families were created from the control fish, resulting in 25 pooled tanks of progeny. Sixty-four families were created from the Erymin 200-injected fish, resulting in 27 pooled tanks of progeny. Of the 13 pooled-family tanks originating from control adults that subsequently tested negative for *R. salmoninarum*, ten progeny tanks tested negative and three progeny tanks tested positive for the presence of *R. salmoninarum*. Similarly, of the 27 pooled-family tanks originating from treated adults that subsequently tested negative for *R. salmoninarum*, 25 progeny tanks tested negative and two progeny tanks tested positive for the presence of *R. salmoninarum* (Figure 1.2.1).

Fourteen adult brood stock fish, used to create 12 families, tested positive for *R. salmoninarum*, three 3-year-old females, three 3-year-old males, two 2-year-old females, and four 2-year-old males. All of the adult fish that tested positive for *R. salmoninarum* were in the control group. No adult fish treated with Erymin 200 tested positive for *R. salmoninarum*. All 12 families were moved to the isolation unit to finish hatching and swim-up. The progeny in all 12 tanks subsequently tested negative for the presence of *R. salmoninarum* (Figure 1.2.1). It was unknown whether the absence of *R. salmoninarum* in the progeny was a result of a lack of vertical transmission from the adult fish, or if the fish were actually positive but not harboring detectable concentrations of the bacteria. As a follow-up, these progeny fish were reared to fingerling size in low-flow, high-density rearing conditions to stress the fish and see if an outbreak of BKD would occur. Progeny from each tank were tested individually rather than in pooled five-fish groups, to obtain infection prevalence per tank. Although the tests are still being run, preliminary results suggest that only one fish tested positive for the presence of *R. salmoninarum* across all 12 tanks.

The results of this study highlight three major concerns for the management of BKD brood stocks in Colorado hatcheries. First, *R. salmoninarum*-positive offspring can originate from parents that test negative for the presence of *R. salmoninarum*. This result suggests that lethal spawn and testing operations may not be one hundred percent effective for eliminating *R. salmoninarum* in Colorado hatcheries unless maintaining separate families and subsequent testing of post-swim-up offspring continues as well. Second, *R. salmoninarum*-negative offspring can originate from parents that tested positive for the presence of *R. salmoninarum*, even when reared under stressful, crowded conditions. This result again highlights the importance of testing the offspring as well as the adults, as these negative offspring could potentially still be used for stocking rather than being culled.

Finally, injections with Erymin 200 were not completely successful in preventing vertical transmission of *R. salmoninarum*. The fact that the intended IP injections actually occurred as IM injections may have affected uptake of the antibiotic. Although erythromycin can be administered as an IM injection, IM injections are usually located in the upper rear quadrant of the fish to facilitate absorption, not in the muscle of the IP cavity. Additionally, it is possible that the injections reduced bacterial concentrations in the adults below a level where they could not be detected using PCR, but were still vertically transmitted at low concentrations to the progeny. A number of detection methods can be used for *R. salmoninarum*, each with its strengths and weaknesses, but so far none have demonstrated high analytical and diagnostic performance characteristics over the others (Elliot et al. 2013). Which diagnostic methods should be used to make management decisions, both within rearing facilities and wild fish populations is an ongoing topic of discussion and research for CPW.

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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. Conduct one hatchery feed study examining the growth and overall health of pure Hofer Rainbow Trout reared on four basic commercial diets by December 31, 2017.

2. Initiate one experiment to examine the effects of rearing density and feed on post-stocking survival of Rainbow Trout by June 30, 2017.
3. Conduct three experiments to determine differences in post-stocking survival rates based on hatchery diet and age-at-stocking by December 31, 2017.

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 regard to protein, lipids, vitamins, and additives such as astaxanthins, which can affect the shape, coloration, and, ultimately, angler satisfaction of the final product. The hatchery feed study will examine the growth and overall health of pure Hofer Rainbow Trout reared at the same feeding rate (% body weight per day) on the basic diet of four 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, and histological analysis of various tissue cells to determine fish health status.

Action #1 Accomplishments

This experiment expands on the hatchery feed experiment conducted at the BFRH in 2016, the motivation and feed descriptions for which can be found in Fetherman and Schisler (2017). The 2016 hatchery feed experiment examined the growth rate and health of fish fed on the basic feeds from four commercial feed manufacturers, Feed Company A, Feed Company B, Feed Company C, and Feed Company D. The manufacturer's recommended feeding rate (percent body weight per day [%BW/d]) for their feeds was followed in the 2016 experiment, with rates ranging from 5.4 to 0.72 %. Fish not only grew at different rates, but growth rate was not commensurate with feeding rate, with fish fed on Feed Company C reaching the goal weight of 200 g per fish three weeks sooner than fish fed on Feed Company D, despite Feed Company D having a significantly higher feeding rate, especially for smaller fish. These results suggested that in addition to feeding rate, feed quality also likely affected growth rate (Fetherman and Schisler 2017).

To examine the effects of feed quality on growth rate, feeds from the same four commercial feed manufacturers were evaluated in the 2017 hatchery feed experiment. However for this experiment, the feeding rate was standardized such that all fish switched to a lower feeding rate when they reached the same size, despite which feed company was being used. Feed Company B and C have similar recommended feeding rates, ranging from 3.3 to 1.0% BW/d, and fell between the wider range of feeding rates recommended by Feed Company D (5.4 to 1.4% BW/d) and the narrower range of feeding rates recommended by Feed Company A (2.41 to 0.72% BW/d). As such, the feeding rate for all feed companies was standardized to those recommended by Feed Company B (Tables 2.1.1, 2.1.2, 2.1.3, and 2.1.4).

Table 2.1.1. Feed Company A standardized feeding rate (percent body weight per day [% BW/d]) by feed size, fish size, and at a temperature of 52-54°F.

Feed Size	Weight (g)	Feeding Rate
#0	Hatch-0.30	3.3
#1	0.30-0.76	3.3
#2	0.76-0.8	3.3
#2	0.8-1.5	3.1
1.2 mm	1.5-3.0	3.0
1.2 mm	3.0-4.5	2.9
1.5 mm	4.5-8.0	2.9
1.5 mm	8.0-9.1	2.4
2.0 mm	9.1-22.7	2.4
3.0 mm	22.7-40	2.4
3.0 mm	40-45.4	1.4
4.0 mm	45.4-80	1.4
4.0 mm	80-91	1.0
5.0 mm	91-908	1.0

Table 2.1.2. Feed Company B standardized feeding rate (percent body weight per day [% BW/d]) by feed size, fish size, and at a temperature of 54°F.

Feed Size	Weight (g)	Feeding Rate
#0	Hatch-0.8	3.3
#1	0.8-1.5	3.1
#2	1.5-3.0	3.0
1.0 mm	3-8	2.9
2.0 mm	8-40	2.4
3.0 mm	40-80	1.4
4.0 mm	80-300	1.0

Table 2.1.3. Feed Company C standardized feeding rate (percent body weight per day [% BW/d]) by feed size, fish size, and at a temperature of 54°F.

Feed Size	Weight (g)	Feeding Rate
#0	Hatch-0.8	3.3
#1	0.8-1.5	3.1
#2	1.5-3.0	3.0
1.2 mm	3.0-5.0	2.9
1.5 mm	5.0-8.0	2.9
2 mm	8.0-18	2.4
2.5 mm	18-40	2.4
3 mm	40-75	1.4
4 mm	75-80	1.4
4 mm	>80	1.0

Table 2.1.4. Feed Company D standardized feeding rate (percent body weight per day [% BW/d]) by feed size, fish size, and at a temperature of 53°F.

Feed Size	Weight (g)	Feeding Rate
#0	Hatch-0.4	3.3
#1	0.4-0.8	3.3
#2	0.8-1.5	3.1
#2	1.5-2.3	3.0
#3	2.3-3.0	3.0
#3	3.0-6.0	2.9
#4	6.0-8.0	2.9
#4	8.0-11.0	2.4
3/32”	11.0-30.0	2.4
1/8”	30.0-40.0	2.4
1/8”	40.0-50.0	1.4
5/32”	50.0-80.0	1.4
5/32”	80.0-114.0	1.0
3/16”	114.0-151.0	1.0

Hofer Rainbow Trout used for this experiment were spawned at the BFRH in December 2016. A single male-female pair was used to create all of the eggs needed for this experiment as relationships among feed intake, growth, and feed efficiency are easier to determine using full-sib families (Silverstein 2006). Eggs were distributed to egg cups contained within four, 76-L (20-gallon) experimental tanks. Eggs were sized using a von Bayer trough (Piper et al. 1982), and initially counted by hand to determine the volume of eggs (mL) needed for each egg cup. This known volume was used to distribute eggs to each of the four egg cups. Egg mortality was monitored and recorded throughout the egg rearing process. After hatching, dead eggs and cripples were removed from the egg cups and recorded. Upon 50% swim-up, which occurred on January 31, 2017, fish were released into their tanks to begin feeding. Each tank initially contained between 1,175 and 1,190 swim-up fry.

Fish may take to feed better on different diets depending on attraction and palatability of the feed. Therefore, fish were fed the starter diet for the feed company to which each tank had been assigned. Feed companies were assigned to starter tank using a random number generator. Prior to feeding, a subset of 20 fish was removed from the tank and individually measured (total length; TL) and weighed to provide a baseline for estimation of feed conversion and growth in the first week post-swim-up. The average weight per fish and the number of fish per tank were used to set the daily feed amounts based on the standardized rate (%BW/d; Tables 2.1.1, 2.1.2, 2.1.3, and 2.1.4). Fish were fed eight times daily. Twenty fish were similarly measured and weighed to adjust feed amounts after the first week. Mortality was monitored and recorded to determine the percentage of fish that did not take to feed in each tank. At the end of the second week, another 20 fish were measured and weighed to estimate feed conversion and growth in the second week post-swim-up. Feeding fish for two weeks post-swim-up helped ensure that all fish included in the 2017 hatchery feed experiment were actively feeding prior to the start of the experiment. Data from the first two weeks was used to compare initial growth rates and feed conversion rates among the feed companies.

The hatchery feed experiment was started at two weeks post-swim-up, at which time 180 fish each were counted out of the starter tank and distributed into three replicate, 38-L (10-gallon) glass tanks for each feed company in FR1 (see Table 2.1.5 for tank assignments). More fish were used in this experiment compared to the 2016 experiment because extra fish were needed to conduct the feed and size-at-stocking experiments (Job No. 2, Action #3). All remaining fish in the starter tank were counted and euthanized. Counts and mortality records were used to determine the starting number of fish per tank at swim-up, and to back-calculate the mortality rate of fish that did not take to feed. An initial sample weight was taken for each tank by placing all 180 fish for a given replicate tank 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 calculate total amount of feed per day (g) for each tank using the standardized feeding rates. In addition, a subset of 20 fish were individually measured and weighed to calculate a Fulton's condition factor (K; Ney 1999) at the onset of the experiment.

Table 2.1.5. Assignment of feed company to tank, assigned using a random number generator.

Feed Company	Tank
D	1
C	2
A	3
B	4
D	5
C	6
B	7
A	8
B	9
C	10
D	11
A	12

Feeding occurred six times daily while fish remained in FR1, with one sixth of the day's total ration delivered to the tank at each feeding. It was assumed that all feed provided to the fish was consumed for the purpose of calculating feed conversion ratios. Given the GR's voracious appetite and ability to consume a large portion of the food presented to them, this assumption was likely met during this experiment. Throughout the entirety of the experiment, tanks and raceways were fed in a clockwise or counterclockwise direction, alternating rotations between the two directions, and the tank with which feeding began advanced by one tank daily. For example, on day one, tank 1 was fed first, and feeding occurred in a clockwise direction. On day two, tank 2 was fed first, and feeding occurred in a counterclockwise direction. This prevented an anticipated feeding response resulting from feeding in the same order every day that could have increased pre-feeding energy use and affected consumption efficiency.

Two to three batch weights of 20 fish each were obtained from each tank on a weekly basis and amount of feed fed per day was adjusted based on these weights. Daily feed amounts were adjusted for mortalities by subtracting the average weight of an individual fish from the previous weekly batch weight and recalculating the total feed per tank. Once a given tank reached the maximum average individual weight of the range for a given feed size, the tank was switched to the next size of feed and/or to a different feeding rate. A subset of 20 fish were individually measured and weighed on the day that feed rate was changed, which allowed for comparisons both across feeding rates within a feed company, and across feed companies at a given fish size. Fifteen of the 20 fish were returned to the tank after being processed. The remaining five fish were euthanized and dissected to obtain liver and viscera weights. Fin condition was also accessed on all 20 fish. Fin condition can be assessed to determine differences in fish appearance when using different feeds and feed delivery methods utilizing the Health/Condition Profile system (HCP; Goede and Barton 1990), which uses a rating scale between 0 and 3 and is based on the degree of hemorrhaging. Wagner et al. (1996) modified the HCP fin index to base scores on fin length, with 0 = perfect fin, 1 = slight erosion, and 2 = severe erosion. Fins were visually assessed for fin length using the scale developed by Wagner et al. (1996), but allowing for 0.5 scores between whole numbers. Each fin or fin pair (i.e., dorsal, caudal, anal, pelvic and pectoral) was assessed separately, and an average score for the fish was obtained since visual assessments of fish during the 2016 experiment showed that all fins did not exhibit the same amount of wear at the time of assessment, making it difficult to assign an average fin score.

To maintain suggested density indices of pounds per cubic foot less than or equal to half of the fish length in inches (Piper et al. 1982), fish started in the 38 L (10 gallon) glass tanks in FR1. Upon reaching an average of 3 grams per fish, fish were moved to 76 L (20 gallon) aluminum tanks within FR1, and the number of fish was counted and confirmed. Once fish reached an average of 15 grams per fish, they were moved from the tanks in FR1 to the BFRH fiberglass hatchery troughs. Again, the number of fish was counted and confirmed upon moving fish to the hatchery. Twelve hatchery troughs were used to rear the fish inside the hatchery to maintain replication. Fish in the hatchery were fed four times daily. Fish were held in one half of the trough until they reached an average of 65 grams per fish, at which point the divider was removed and the fish were allowed to use the entire trough for the remainder of the growth experiment. The experiment was concluded once fish reached an average of ≥ 210 grams of fish. At the end of the experiment, all fish remaining in a hatchery trough were euthanized, measured, and weighed, and 20 fish were dissected to obtain liver and viscera weights. Fifteen fish from each tank were kept alive and moved to round tanks where they continued to be fed on the same size and ration of feed until all fish from all feed companies had reached the final goal weight. Round tanks (four) contained 45 fish at the end of the experiment, 15 from each of three replicate troughs per feed company.

There are a number of standard metrics used to evaluate growth performance in feed comparison experiments, including weight gain (%), feed conversion ratio (FCR; grams of feed per gram of fish [g feed/g fish]), specific growth rate (SGR; % BW/d), feed intake (% BW/d), hepatosomatic index (HSI), and viscerosomatic index (VSI; Trushenski et al. 2011; Gause and Trushenski 2013), calculated using the following formulas:

$$Weight\ gain\ = 100 \times \frac{average\ \ final\ \ weight - average\ \ initial\ \ weight}{average\ \ initial\ \ weight}$$

$$Feed\ conversion\ ratio = 100 \times \frac{average\ \ initial\ \ feed\ \ consumption\ \ \ (dry\ \ matter\ )}{average\ \ individual\ \ \ weight\ gain}$$

$$Specific\ growth\ rate = 100\ \times \frac{log_e(average\ \ final\ \ weight) - log_e(average\ \ initial\ \ weight)}{days\ \ of\ \ feeding}$$

$$Feed\ intake = 100 \times \frac{total\ \ dry\ \ matter\ \ intake\ /(initial\ \ individual\ \ \ weight \times final\ \ individual\ \ \ weight)^{0.5}}{days\ \ of\ \ feeding}$$

$$HSI = 100 \times \frac{liver\ \ weight}{BW}$$

$$VSI = 100 \times \frac{total\ \ viscera\ \ weight}{BW}$$

Average individual values were calculated by dividing tank values by the number of fish in the tank at the time the data were collected. Parameters associated with feed consumption were based on average individual values calculated on a daily basis (i.e., average consumption values were calculated daily and summed over the course of the trial; Gause and Trushenski 2013). Weight gain, FCR, SGR, and feed intake were also calculated between each feeding rate change. HSI and VSI were computed for fish that were ≥ 2 grams; HSI and VSI were not calculated for feeding rate changes at which the average weight per fish was < 2 grams due to difficulty of dissection. The HSI and VSI indicate the amount of energy reserves stored in the liver and as fat in the viscera, excess energy that could be used during periods of low food availability after being stocked. The higher the HSI and VSI, the higher the amount of stored energy that can be utilized at a later date.

In addition to the growth metrics listed above, the coefficient of variation in length and weight was used to determine if certain feeds produce a wider range of variation in size than others (Wagner et al. 1996). The coefficient of variation (CV) is calculated as $CV = \frac{s}{\bar{y}}$, where s is the standard deviation in length or weight, and $\bar{y}$ is the mean. The CV was calculated when feeding rate changed for each feed company and used to determine when size variation began to occur during the experiment, if at all. Mortality, an important metric for assessing feed quality, especially at smaller sizes while fish are taking to feed (Kientz et al. 2012), was calculated between each feeding rate change, as well as for the entire growth period from hatch to the end of the experiment.

For each feeding rate change at which fish were dissected to obtain estimates of HSI and VSI, the intestine was preserved in 10% neutral buffered formalin and kept for later histological analysis performed by the FishVet Group (Portland, Maine) and the Aquatic Animal Medicine Research Laboratory (Grenada, West Indies). Sections were taken from the distal portion of the large intestine and examined for density of supranuclear vacuoles, goblet cell density, infiltration of eosinophilic granulocytes, and infiltration of mononuclear cells, all of which were scored on a semi-quantitative scale of one to five, and mucosal length, lamina propria width, and submucosal width, which were performed via digital measurements (µm; Table 2.1.6). Supranuclear vacuoles are clear glycogen deposits within the epithelium of the large intestine secondary to dietary pinocytosis and endocytosis, and are usually reduced during inflammation. Goblet cells are mucous secreting cells that increase in number secondary to chronic inflammation. Eosinophilic granulocytes and mononuclear cells (e.g., lymphocytes, plasma cells, and macrophages) infiltrate the submucosa and lamina propria secondary to antigenic stimulation. Although it is not uncommon to find small numbers of infiltrates within the intestine, increased numbers occur with inflammation. Mucosal length is the length of the mucosal villi beginning at the muscularis mucosa and ending at the distal tip of the epithelium. Mucosal villi decrease in length secondary to severe chronic inflammation. The width of the lamina propria can increase secondary to edema fluid inflammatory cell infiltrates, and other space-occupying lesions. Finally, the width of the submucosa changes similarly to that of the lamina propria with increased size due to edema fluid, inflammatory cell infiltrates, and other space-occupying lesions. Intestines were collected from fish reared on all four feed companies at five sizes: 1) extra small (70.7 mm TL, 3.8 g; following a feeding rate of 3.0% BW/d), 2) small (91.7 mm TL, 8.5 g; following a feeding rate of 2.9% BW/d), 3) medium (151.8 mm TL, 42.3 g; following a feeding rate of 2.4% BW/d), 4) large (192.2 mm TL, 85.4 g; following a feeding rate of 1.4% BW/d), and 5) extra large (252.6 mm TL, 195.3 g; following a feeding rate of 1.0% BW/d). All histological analyses were performed blind to feed company to prevent bias.

Table 2.1.6. Parameters of interest for the histological assessment of the intestine, and the semi-quantitative scoring system used to assess each parameter and to compare levels of inflammation across feed companies in the 2017 hatchery feed experiment.

Parameter	**Scoring system**				
	1	**2**	**3**	**4**	**5**
Density of supranuclear vacuoles	Occupy almost entire apical area of enterocytes	Medium-sized vacuoles occupying less than half of the enterocytes	Small-sized near the apical membrane in many enterocytes	Scattered and small vacuoles in few enterocytes	No vacuoles observed
Goblet cell density	Scattered goblet cells observed	Increased number but sparsely distributed	Diffuse and widely spread	Densely grouped in some mucosal folds	Highly abundant and tightly packed
Infiltration of eosinophilic granulocytes	Scattered granulocytes observed in submucosa and lamina propria	Increased number but sparsely distributed	Diffuse and widely spread	Densely grouped in some mucosal folds	Highly abundant and tightly packed
Infiltration of mononuclear cells	Scattered lymphocytes and plasma cells in submucosa and lamina propria	Increased number but sparsely distributed	Diffuse and widely spread	Densely grouped in some mucosal folds	Highly abundant and tightly packed
Mucosal length	Performed via digital measurements. Average of three areas.				
Lamina propria width	Performed via digital measurements. Average of three areas.				
Submucosal width	Performed via digital measurements. Average of three areas.				

An analysis of variance (ANOVA) implemented in SAS PROC GLM (SAS Institute 2017) was used to determine if there were differences in survival, length, and weight among the feed companies following the first two weeks of feeding. Similarly, an ANOVA was used to determine if there were differences in overall FCR, CV length, CV weight, HSI, VSI, fin condition, and survival among the feed companies at the end of the experiment. Unlike the 2016 experiment, survival, FCR, fin condition, HSI, VSI, CV length, CV weight, and K were comparable across feed rate changes both within and across the feeds, and were compared using a two-factor ANOVA, with feed company and feeding rate change as the factors. Summary statistics are provided for SGR, weight gain, feed intake, length, and weight for each feeding rate within each feed company. Parameters of interest from the histological analysis of the intestines were also compared using a two-factor ANOVA, with feed company and fish size as the factors. Note that all results for which the feeding rate is shown, fish were collected when changing to a lower feeding rate. For example, results for 3.3% BW/d are from fish that had been fed at 3.3% BW/d prior to collection, and were being switched to 3.1% BW/d at the time of collection.

Table 2.1.7. Comparisons of overall survival (%), weekly survival (%), feed conversion ratios (FCR; g feed/g fish), weight gain (%), specific growth rate (SGR; % BW/d), feed intake (% BW/d), weekly length (mm; CV length in parentheses), weekly weight (g; CV weight in parentheses), and average fin rating among the four feed companies (A, B, C, and D) within the first two weeks post-swim-up. Different letters within the same row for a given metric represent significant differences among the feed companies.

Metric	**A**	**B**	**C**	**D**
Overall Survival	99.74^{a}	99.75^{a}	99.83^{a}	99.58^{a}
Week 1	99.83	99.75	99.83	99.66
Week 2	99.91	100.00	100.00	99.91
FCR	0.39	0.41	0.31	0.62
Weight Gain	165.85	155.00	214.87	96.39
SGR	5.75	5.51	9.56	3.97
Feed Intake	2.34	2.34	3.09	2.52
Length (CV)				
Start	24.25^{a} (0.04)	24.20^{a} (0.03)	24.15^{a} (0.03)	24.00^{a} (0.04)
Week1	28.80^{a} (0.03)	27.65^{bc} (0.05)	28.25^{ab} (0.03)	26.95^{c} (0.04)
Week2	31.85^{ab} (0.04)	31.30^{b} (0.05)	32.80^{a} (0.03)	27.80^{c} (0.04)
Weight (CV)				
Start	0.10^{a} (0.09)	0.10^{a} (0.07)	0.10^{a} (0.11)	0.10^{a} (0.13)
Week1	0.19^{a} (0.12)	0.18^{ab} (0.19)	0.18^{ab} (0.12)	0.16^{b} (0.09)
Week2	0.27^{b} (0.14)	0.26^{b} (0.17)	0.31^{a} (0.10)	0.19^{c} (0.15)
Average Fin Rating	0.00	0.00	0.00	0.00

There were no significant differences in overall survival in the first two weeks among the feed companies (Table 2.1.7), indicating that fish took to feed equally on all four feed companies. Feed conversion ratios (grams of feed needed to produce one gram of mass) varied among the feed companies, with Feed Company C having the lowest FCR and Feed Company D having the highest FCR. The lower the FCR, the more efficiently fish were able to convert feed to mass. Feed conversion ratios for Feed Companies B and C were fairly similar to the 2016 experiment

(Fetherman and Schisler 2017), likely a result of the similar feeding rates used in 2016 and 2017, whereas the FCR for Feed Company A was slightly lower, likely due to the higher feeding rate in 2017. The FCR for Feed Company D was higher than in the 2016 experiment (Fetherman and Schisler 2017), likely a result of the much lower feeding rate used in 2017 (5.4% BW/d in 2016 compared to 3.3% BW/d in 2017). Weight gain, SGR, and feed intake also varied among the feed companies, and only Feed Company C had a feed intake that approached the actual feeding rate compared to the other feed companies, which may suggest a difference in palatability among starter feeds. Length and weight did not differ among feed companies at the start of the experiment. However, both differed by the end of weeks one and two, with fish fed Feed Company D significantly smaller than fish fed Feed Companies A, B, or C at the end of the two week pre-feeding period. No fin wear was observed in the first two weeks, with fish from all feed companies having a fin rating of 0 (Table 2.1.7).

Table 2.1.8. Comparisons of survival (%), feed conversion ratios (FCR; g feed/g fish), weight gain (%), specific growth rate (SGR; % BW/d), and feed intake (% BW/d) among the four feed companies (A, B, C, D) and at standardized feeding rates (%BW/d) in the 2017 hatchery feed experiment. Different letters on the left side of survival and FCR values indicate significant differences among feeds within a feeding rate (columns), and different letters on the right side of these values indicate significant differences among feeding rates within a feed company (rows).

Parameter	**Feeding Rates**						
	3.3%	**3.1%**	**3.0%**	**2.9%**	**2.4%**	**1.4%**	**1.0%**
				Feed Company A			
Survival	^{a} 99.81 ^{z}	^{a} 100 ^{z}	^{a} 99.81 ^{z}	^{a} 100 ^{z}	^{a} 99.16 ^{z}	^{ab} 99.13 ^{z}	^{b} 99.54 ^{z}
FCR	^{a} 0.44 ^{z}	^{a} 0.47 ^{z}	^{a} 0.49 ^{z}	^{b} 0.68 ^{y}	^{a} 0.69 ^{y}	^{ab} 0.79 ^{y}	^{a} 0.77 ^{y}
Weight gain	225.54	99.82	98.42	158.68	408.75	72.44	155.78
SGR	5.62	4.94	4.89	3.39	2.90	1.56	1.18
Feed intake	2.65	2.41	2.46	2.41	2.22	1.25	0.95
				Feed Company B			
Survival	^{a} 99.81 ^{z}	^{a} 100 ^{z}	^{a} 100 ^{z}	^{a} 99.81 ^{z}	^{a} 99.78 ^{z}	^{ab} 99.11 ^{z}	^{b} 100 ^{z}
FCR	^{a} 0.49 ^{z}	^{a} 0.45 ^{z}	^{ab} 0.53 ^{z}	^{b} 0.65 ^{y}	^{a} 0.71 ^{y}	^{bc} 0.86 ^{x}	^{b} 0.90 ^{x}
Weight gain	252.97	88.06	108.717	159.02	399.26	94.73	152.14
SGR	5.40	5.31	4.49	4.07	2.87	1.43	0.99
Feed intake	2.83	2.43	2.47	2.77	2.25	1.25	0.93
				Feed Company C			
Survival	^{a} 100 ^{z}	^{a} 100 ^{z}	^{a} 100 ^{z}	^{a} 100 ^{z}	^{a} 99.79 ^{z}	^{a} 98.06 ^{y}	^{a} 97.74 ^{y}
FCR	^{a} 0.44 ^{z}	^{a} 0.48 ^{z}	^{a} 0.50 ^{z}	^{a} 0.53 ^{z}	^{a} 0.67 ^{y}	^{a} 0.69 ^{y}	^{a} 0.72 ^{y}
Weight gain	240.68	105.72	96.27	151.56	347.89	94.36	159.08
SGR	5.82	5.15	4.81	4.39	3.06	1.77	1.28
Feed intake	2.7	2.51	2.45	2.41	2.26	1.24	0.95
				Feed Company D			
Survival	^{a} 100 ^{z}	^{a} 100 ^{z}	^{a} 99.81 ^{z}	^{a} 100 ^{z}	^{a} 100 ^{z}	^{b} 100 ^{z}	^{b} 99.73 ^{z}
FCR	^{a} 0.50 ^{z}	^{a} 0.50 ^{z}	^{b} 0.62 ^{y}	^{b} 0.76 ^{x}	^{b} 0.99 ^{w}	^{c} 0.96 ^{w}	^{b} 0.93 ^{w}
Weight gain	324.98	99.83	99.16	164.42	398.33	89.00	160.11
SGR	4.78	4.93	4.03	3.20	2.03	1.30	0.97
Feed intake	2.60	2.51	2.52	2.51	2.22	1.26	0.94

Table 2.1.9. Comparisons of length (mm), coefficient of variation in length (CV length), weight (g), CV weight, Fulton's condition factor (K), hepatosomatic index (HSI), viscerosomatic index (VSI), and fin rating among the four feed companies (A, B, C, D) and at standardized feeding rates (% BW/d) in the 2017 hatchery feed experiment. Different letters on the left side of CV length, CV weight, K, HSI, VSI and fin rating values indicate significant differences among feeds within a feeding rate (columns), and different letters on the right side of these values indicate significant differences among feeding rates within a feed company (rows).

Parameter	Feeding Rates						
	3.3%	**3.1%**	**3.0%**	**2.9%**	**2.4%**	**1.4%**	**1.0%**
			Feed Company A				
Length	44.67	55.03	69.68	95.62	157.32	189.93	256.37
CV Length	^{a} 0.05 ^{z}	^{a} 0.07 ^{z}	^{a} 0.09 ^{z}	^{a} 0.05 ^{z}	^{a} 0.05 ^{z}	^{a} 0.07 ^{z}	^{a} 0.07 ^{z}
Weight	0.94	1.83	3.51	9.17	48.08	86.05	207.91
CV Weight	^{a} 0.16 ^{z}	^{a} 0.22 ^{z}	^{ab} 0.28 ^{z}	^{a} 0.14 ^{z}	^{a} 0.17 ^{z}	^{a} 0.21 ^{z}	^{a} 0.21 ^{z}
K	^{a} 1.05 ^{zy}	^{a} 1.08 ^{z}	^{ab} 1.01 ^{y}	^{b} 1.04 ^{zy}	^{ab} 1.22 ^{x}	^{a} 1.24 ^{x}	^{a} 1.22 ^{x}
HSI	N/A	N/A	^{a} 1.91 ^{z}	^{b} 2.00 ^{z}	^{b} 1.97 ^{z}	^{ab} 1.65 ^{zy}	^{a} 1.58 ^{y}
VSI	N/A	N/A	^{a} 14.38 ^{z}	^{b} 14.02 ^{zy}	^{a} 14.59 ^{z}	^{a} 12.60 ^{y}	^{a} 11.24 ^{x}
Fin rating	^{a} 0.01 ^{z}	^{a} 0.11 ^{z}	^{a} 0.31 ^{y}	^{b} 0.64 ^{x}	^{c} 0.79 ^{x}	^{a} 0.80 ^{x}	^{b} 1.16 ^{w}
			Feed Company B				
Length	44.9	53.45	69.63	94.08	153.52	194.17	260.30
CV Length	^{a} 0.05 ^{z}	^{a} 0.04 ^{z}	^{a} 0.06 ^{z}	^{a} 0.05 ^{z}	^{ab} 0.06 ^{z}	^{a} 0.06 ^{z}	^{a} 0.07 ^{z}
Weight	0.94	1.63	3.39	9.15	43.58	85.31	207.97
CV Weight	^{a} 0.18 ^{z}	^{a} 0.15 ^{z}	^{ab} 0.20 ^{z}	^{ab} 0.17 ^{z}	^{a} 0.21 ^{z}	^{a} 0.17 ^{z}	^{a} 0.20 ^{z}
K	^{a} 1.01 ^{zy}	^{a} 1.06 ^{yx}	^{b} 0.98 ^{z}	^{b} 1.08 ^{x}	^{b} 1.19 ^{w}	^{b} 1.16 ^{w}	^{c} 1.17 ^{w}
HSI	N/A	N/A	^{b} 1.49 ^{z}	^{c} 1.55 ^{z}	^{ab} 1.59 ^{z}	^{b} 1.71 ^{z}	^{a} 1.46 ^{z}
VSI	N/A	N/A	^{b} 12.24 ^{zy}	^{c} 11.44 ^{y}	^{b} 13.09 ^{z}	^{a} 11.68 ^{zy}	^{b} 9.99 ^{x}
Fin rating	^{b} 0.26 ^{z}	^{ab} 0.23 ^{z}	^{a} 0.27 ^{zy}	^{b} 0.49 ^{yx}	^{b} 0.59 ^{x}	^{b} 1.12 ^{w}	^{c} 1.27 ^{w}
			Feed Company C				
Length	46.11	55.67	71.32	92.57	147.72	189	259.51
CV Length	^{a} 0.03 ^{z}	^{a} 0.06 ^{z}	^{a} 0.05 ^{z}	^{a} 0.05 ^{z}	^{ab} 0.08 ^{z}	^{a} 0.07 ^{z}	^{a} 0.07 ^{z}
Weight	1.00	1.87	3.91	9.46	41.60	85.66	212.97
CV Weight	^{a} 0.13 ^{z}	^{a} 0.18 ^{zy}	^{a} 0.16 ^{zy}	^{ab} 0.17 ^{zy}	^{a} 0.26 ^{y}	^{a} 0.19 ^{zy}	^{a} 0.21 ^{zy}
K	^{a} 1.01 ^{z}	^{a} 1.07 ^{z}	^{a} 1.07 ^{z}	^{a} 1.18 ^{y}	^{a} 1.26 ^{x}	^{a} 1.26 ^{x}	^{ab} 1.20 ^{y}
HSI	N/A	N/A	^{ab} 1.63 ^{z}	^{a} 3.77 ^{y}	^{ab} 1.67 ^{z}	^{ab} 1.51 ^{z}	^{a} 1.48 ^{z}
VSI	N/A	N/A	^{b} 12.35 ^{y}	^{a} 16.22 ^{z}	^{b} 12.47 ^{y}	^{a} 11.21 ^{yx}	^{a} 11.05 ^{x}
Fin rating	^{a} 0.00 ^{z}	^{a} 0.10 ^{zy}	^{a} 0.23 ^{yx}	^{a} 0.26 ^{yx}	^{a} 0.30 ^{x}	^{a} 0.89 ^{w}	^{a} 0.96 ^{w}
			Feed Company D				
Length	43.30	53.70	68.33	94.00	152.38	193.8	262.53
CV Length	^{a} 0.05 ^{z}	^{a} 0.07 ^{zy}	^{a} 0.09 ^{zy}	^{a} 0.09 ^{zy}	^{b} 0.10 ^{y}	^{a} 0.09 ^{zy}	^{a} 0.07 ^{zy}
Weight	0.86	1.72	3.35	9.04	40.88	85.20	217.60
CV Weight	^{a} 0.16 ^{z}	^{a} 0.23 ^{zy}	^{b} 0.32 ^{y}	^{b} 0.30 ^{y}	^{a} 0.29 ^{zy}	^{a} 0.27 ^{zy}	^{a} 0.24 ^{zy}
K	^{a} 1.04 ^{zy}	^{a} 1.09 ^{yx}	^{ab} 1.01 ^{z}	^{b} 1.05 ^{zy}	^{c} 1.11 ^{x}	^{b} 1.14 ^{xw}	^{bc} 1.18 ^{w}
HSI	N/A	N/A	^{c} 1.08 ^{z}	^{c} 1.41 ^{z}	^{a} 1.31 ^{z}	^{a} 1.26 ^{z}	^{b} 1.87 ^{y}
VSI	N/A	N/A	^{ab} 13.48 ^{z}	^{b} 13.73 ^{z}	^{b} 12.71 ^{zy}	^{a} 11.62 ^{y}	^{ab} 10.36 ^{x}
Fin rating	^{b} 0.33 ^{z}	^{b} 0.37 ^{z}	^{a} 0.32 ^{z}	^{b} 0.68 ^{y}	^{bc} 0.76 ^{y}	^{c} 1.49 ^{x}	^{d} 1.60 ^{x}

Metrics associated with feed varied both among the feed companies and across the feeding rates within a feed company (Table 2.1.8). Survival was greater than 97.5% for all feeding rates and feed companies, and in general, varied little among the feed companies with the exception of lower survival rates exhibited by fish fed Feed Company C compared to Feed Company D at the 1.4 and 1.0% feeding rates. Similarly, within Feed Companies A, B, and C, there were no differences in survival across the feeding rates, but fish fed Feed Company C exhibited significantly lower survival at feeding rates of 1.4 and 1.0% (Table 2.1.8). Feed conversion ratios increased with an increase in fish size and decrease in feeding rate, as has been observed in previous years (Fetherman and Schisler 2017). Feed conversion ratios were similar among the feed companies at the higher feeding rates, but diverged as feeding rate decreased. Overall, Feed Companies A and C had similarly lower feed conversion ratios compared to feed Companies B and D, the feed conversion ratios of which were similar. Feed conversion ratios approached 1.0 for Feed Company D at the lower feeding rates. In general, feed conversion ratios were similar within a feed company between the 2016 and 2017 experiments, suggesting that feed quality had a larger effect on feed conversion ratios than did feeding rate. Weight gain, SGR, and feed intake varied similarly among the feeds across the feeding rates, as would be expected with fish that were of similar average size due to the standardized feeding rates (Table 2.1.8).

Individual growth, health, and appearance metrics also varied both among the feed companies and across the feeding rates within a feed company (Table 2.1.9). Coefficients of variation (CV) in both length and weight were generally lowest at a feeding rate of 3.3%, increasing with a decrease in feeding rate. Although there were no differences in CV length or CV weight among the feeding rates for Feed Companies A or B, fish in Feed Companies C and D both exhibited greater variability in both length and weight at lower feeding rates. CV weight for Feed Company D was especially high at feeding rates of 3.0 and 2.9%, and exceeded those exhibited by Feed Company D in the 2016 hatchery feed experiment (Fetherman and Schisler 2017). Fulton’s condition factor (K) was lowest at the higher feeding rates and increased as fish grew and feeding rates decreased, especially at feeding rates of 2.4, 1.4, and 1.0%. Although K differed among the feeds, the differences are likely not biologically relevant.

Hepatosomatic index (HSI) and viscerosomatic index (VSI) differed greatly among feeds and feeding rates (Table 2.1.9). For Feed Companies A and C, HSI and VSI were highest at feeding rates between 3.0% and 2.4%, and Feed Company C exhibited a significantly higher HSI and VSI at a feeding rate of 2.9%. This feeding rate corresponds to feed sizes between 1.2 and 2.0 mm, in which higher HSI and VSI values were observed in the 2016 hatchery feed experiment (Fetherman and Schisler 2017). Feed Company B showed an increasing trend in HSI to a feeding rate of 1.4%, decreasing at a feeding rate of 1.0%. Feed Companies A, B, and C had lower HSI and VSI values at a feeding rate of 1.0%, suggesting that larger feeds fed at lower feeding rates contained less storable energy than smaller, higher energy feeds fed at higher feeding rates. Feed Company D exhibited a trend of increasing HSI values through a feeding rate of 1.0%, although the VSI values obtained from fish reared at a feeding rate of 1.0% were similarly low relative to the other feed companies. Fin wear increased with an increase in fish size and decrease in feeding rate, and similar to the 2016 hatchery feed experiment (Fetherman and Schisler 2017), fin wear was lowest in Feed Company C, highest in Feed Company D, and fell between these two in Feed Companies A and B (Table 2.1.9).

Table 2.1.10. Semi-quantitative scores for density of supranuclear vacuoles, goblet cell density, infiltration of eosinophilic granulocytes, and infiltration of mononuclear cells, and measurements of mucosal length, lamina propria width, and submucosal width (μm) by feed company and fish size. Different letters on the left side of mucosal length values indicate significant differences among feeds within a fish size (columns), and different letters on the right side of these values indicate significant differences among fish sizes within a feed company (rows). Different letters in the overall column represent significant average differences among feed companies, and in the overall rows represent significant average differences among fish sizes.

Parameter	Extra Small	Small	Medium	Large	Extra Large	Overall
Feed Company A						
Vacuoles	2.7	2.8	3.3	3.5	3.5	3.2 ^{ab}
Goblet Cell Density	2.5	2.7	2.5	2.3	2.3	2.5 ^{a}
Granulocytes	1.3	2.5	3.0	3.2	2.8	2.6 ^{a}
Mononuclear Cells	1.0	1.0	1.2	1.2	1.0	1.1 ^{a}
Mucosal Length	^{a} 300.1 ^{z}	^{a} 296.7 ^{z}	^{a} 424.3 ^{z}	^{a} 365.3 ^{z}	^{b} 410.7 ^{z}	356.9 ^{b}
Lamina Propria Width	6.1	6.7	7.5	9.8	14.0	8.8 ^{a}
Submucosal Width	6.5	6.4	6.4	8.4	9.0	7.3 ^{a}
Feed Company B						
Vacuoles	2.7	2.5	3.3	4.0	2.7	3.0 ^{ab}
Goblet Cells	2.7	2.3	2.3	2.7	2.2	2.4 ^{a}
Granulocytes	1.5	2.3	3.0	3.0	2.5	2.5 ^{a}
Mononuclear Cells	1.0	1.0	1.3	1.0	1.0	1.1 ^{a}
Mucosal Length	^{a} 291.7 ^{z}	^{a} 280.9 ^{z}	^{a} 443.5 ^{yz}	^{a} 362.7 ^{z}	^{ab} 576.1 ^{y}	398.5 ^{ab}
Lamina Propria Width	6.1	7.1	9.0	10.3	13.4	9.2 ^{a}
Submucosal Width	6.5	6.2	7.7	8.9	8.8	7.6 ^{a}
Feed Company C						
Vacuoles	2.0	2.0	3.3	3.5	2.5	2.7 ^{b}
Goblet Cells	2.2	2.2	2.5	2.7	1.7	2.2 ^{a}
Granulocytes	1.3	2.5	3.0	2.8	2.5	2.4 ^{a}
Mononuclear Cells	1.0	1.0	1.2	1.2	1.0	1.1 ^{a}
Mucosal Length	^{a} 287.8 ^{z}	^{a} 364.1 ^{z}	^{a} 382.3 ^{z}	^{a} 374.6 ^{z}	^{a} 642.1 ^{y}	411.8 ^{a}
Lamina Propria Width	5.8	6.9	7.7	8.7	12.1	8.2 ^{a}
Submucosal Width	6.1	6.1	6.8	9.2	9.1	7.4 ^{a}
Feed Company D						
Vacuoles	4.0	2.0	4.0	3.8	3.5	3.5 ^{a}
Goblet Cells	3.0	2.0	2.3	2.7	2.0	2.4 ^{a}
Granulocytes	2.2	2.5	3.0	3.0	2.2	2.6 ^{a}
Mononuclear Cells	1.0	1.0	1.0	1.2	1.0	1.0 ^{a}
Mucosal Length	^{a} 255.0 ^{z}	^{a} 317.4 ^{yz}	^{a} 325.0 ^{yz}	^{a} 342.3 ^{yz}	^{b} 455.0 ^{y}	338.9 ^{b}
Lamina Propria Width	6.2	7.3	7.9	10.3	10.2	8.4 ^{a}
Submucosal Width	5.9	6.7	7.8	8.9	12.1	8.3 ^{a}
Overall						
Vacuoles	2.8 ^{ab}	2.3 ^{a}	3.5 ^{b}	3.7 ^{b}	3.0 ^{ab}	
Goblet Cells	2.6 ^{a}	2.3 ^{a}	2.4 ^{a}	2.6 ^{a}	2.0 ^{a}	
Granulocytes	1.6 ^{a}	2.5 ^{b}	3.0 ^{c}	3.0 ^{c}	2.5 ^{b}	
Mononuclear Cells	1.0 ^{a}	1.0 ^{a}	1.1 ^{a}	1.2 ^{a}	1.0 ^{a}	
Mucosal Length	351.7 ^{a}	415.1 ^{ab}	337.1 ^{c}	356.2 ^{bc}	421.7 ^{d}	
Lamina Propria Width	6.1 ^{a}	7.0 ^{ab}	8.0 ^{b}	9.8 ^{c}	12.5 ^{d}	
Submucosal Width	6.3 ^{a}	6.3 ^{a}	7.2 ^{a}	8.8 ^{b}	9.7 ^{b}	

There was a significant feed and size effect for the average density of supranuclear vacuoles in the intestines of fish reared on feeds from the four feed companies (Table 2.1.10). A size effect is common in these types of analyses, with increasing semi-quantitative scores from smaller to larger fish sizes due to longer antigen exposure. In this experiment, the semi-quantitative score for the density of supranuclear vacuoles, as well as the infiltration of eosinophilic granulocytes, dropped in the extra large fish, which is not typical. It is suspected that this is a result of sample location along the intestine in the extra large fish rather than a physiological effect of feed. With regard to the feed effect, Feed Company C had a significantly lower semi-quantitative score for density of supranuclear vacuoles than did Feed Company D, meaning that the density of the supranuclear vacuoles in Feed Company C were higher than for Feed Company D. A higher density of supranuclear vacuoles is considered an indication that more of the nutrients in the feed are being absorbed, which could be one reason why fish reared on Feed Company C and Feed Company D show a significant difference in growth rate (Figure 2.1.1). Overall, there was not a feed effect for goblet cell density, infiltration of eosinophilic granulocytes, or infiltration of mononuclear cells, suggesting that none of the feeds cause an increase in intestinal inflammation relative to the others. The lack of differences in the infiltration of mononuclear cells suggests that fish are not reacting to proteins in the feed and that, antigenically, the feeds are all well tolerated. This is supported by the lack of feed differences in the lamina propria and submucosal widths, which suggest that none of the feeds induced increased inflammation. Although there was a feed, size, and interactive effect for mucosal length, the lack of evidence of inflammation from the other parameters of interest suggests that mucosal length differences among the feeds was more likely a result of growth performance differences than feed effects on the intestines.

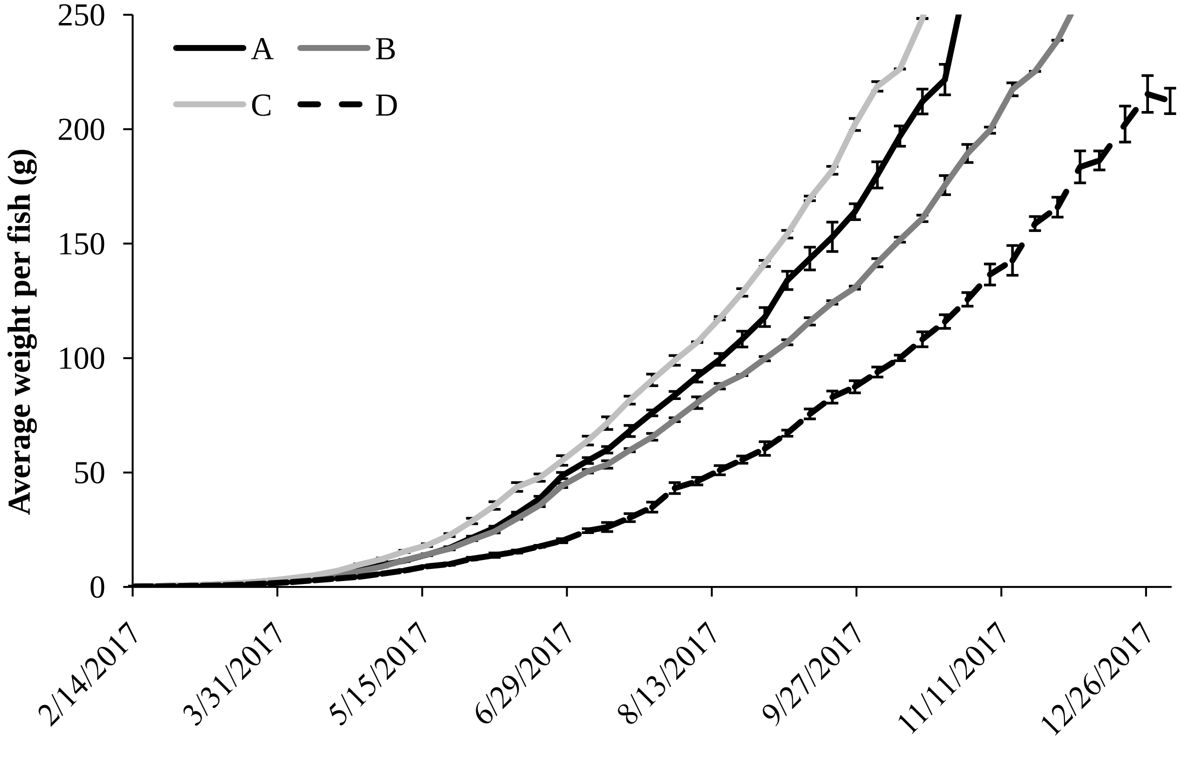

Figure 2.1.1. Average weekly weights of fish fed Feed Companies A, B, C, and D. Error bars represent differences among replicates (3) within a feed company.

Fish fed Feed Company C grew faster throughout the majority of the experiment than fish fed Feed Companies A, B, and D (Figure 2.1.1). Fish fed Feed Company A reached the goal weight of ≥ 201 g only two weeks after Feed Company C and exhibited a similar growth curve throughout the experiment, suggesting that feed quality was similar between the two companies. These results also suggest that the feeding rate recommended by Feed Company A and used in the 2016 experiment was too low for the GR reared at the BFRH, and that the poor growth performance of these fish in 2016 was entirely a result of the feeding rate (Fetherman and Schisler 2017). Fish fed Feed Company B grew slower than those fed Feed Companies A and C, reaching the goal weight six weeks later than Feed Company C and four weeks later than Feed Company A. Finally, fish fed Feed Company D exhibited much lower growth rates than those fed on the other three feeds, and did not reach the goal weight until 13 weeks after Feed Company C (Figure 2.1.1), suggesting that this feed was of lower quality than the other feeds used in this experiment. After reaching the goal weight, fifteen fish from each replicate from Feed Companies A, B and C were moved to round tanks and maintained at a feeding rate of 1.0% until the goal weight was reached by Feed Company D. By the time fish reared on Feed Company D reached the goal weight of ≥ 210 g on January 2, 2018, fish fed Feed Company D weighed 479 (± 23)g, fish fed Feed Company B weighed 347 (± 12.6) g, and fish fed Feed Company C weighed 577 (± 23.8) g.

Table 2.1.11. Comparison of overall survival (%), feed conversion ratios (FCR; g feed/g fish), coefficient of variation in length (CV length), CV weight, hepatosomatic index (HSI), viscerosomatic index (VSI), and fin rating (± SE) among the four feed companies (A, B, C, and D). Different letters within the same row for a given metric represent significant differences among the feed companies.

Metric	**A**	**B**	**C**	**D**
Survival	97.78^{ab} (± 0.81)	98.70^{ab} (± 0.74)	96.30^{b} (± 0.81)	99.63^{a} (± 0.37)
Feed Conversion	0.75^{b} (± 0.01)	0.85^{c} (± 0.01)	0.70^{a} (± 0.01)	0.93^{d} (± 0.01)
CV Length	0.06^{a} (± 0.004)	0.06^{a} (± 0.002)	0.06^{a} (± 0.003)	0.08^{b} (± 0.005)
CV Weight	0.20^{a} (± 0.012)	0.18^{a} (± 0.007)	0.18^{a} (± 0.010)	0.25^{b} (± 0.015)
HSI	1.73^{ab} (± 0.04)	1.53^{c} (± 0.02)	1.81^{a} (± 0.08)	1.56^{bc} (± 0.04)
VSI	12.57^{a} (± 0.18)	11.06^{c} (± 0.14)	12.06^{ab} (± 0.19)	11.63^{bc} (± 0.16)
Fin Rating	0.81^{b} (± 0.02)	0.88^{b} (± 0.02)	0.64^{a} (± 0.02)	1.11^{c} (± 0.02)

Over the course of the experiment, growth and health metrics varied among the four feed companies (Table 2.1.11). Overall feed conversion ratio (averaged from the start to end of the experiment) was lowest in fish fed Feed Company C. Feed Company A had a lower feed conversion ratio than did the other two feed companies, though it was higher than in the 2016 hatchery feed experiment (Fetherman and Schisler 2017), likely a result of the larger sizes attained by these fish in the 2017 experiment. Feed Companies B and D had the highest feed conversion ratios, with Feed Company D having a significantly higher feed conversion ratio than Feed Company B. Length and weight were significantly more variable in fish fed Feed Company D than Feed Companies A, B, or C. Fish fed Feed Company C exhibited higher HSI values than those fed Feed Companies B and D, which had the lowest overall HSI values. VSI values differed from HSI values in that Feed Company A had the highest overall VSI value, and was significantly higher than Feed Companies B and D, though did not differ from Feed

Company C. Overall fin condition was lowest (better) in fish fed Feed Company C, and highest in fish fed Feed Company A (Table 2.1.11). Fin condition differed by fin type, with the anal and pelvic fins showing significantly less wear than the dorsal, caudal, and pectoral fins. In general, fish fed Feed Companies A and C showed significantly less fin wear across all fin types than did fish fed Feed Companies B and D, with the exception of the pectoral fins, of which fish fed Feed Companies A, B, and D all showed severe erosion, and fish fed Feed Company C showed very slight erosion (Figure 2.1.2). Survival was one of the only metrics in which Feed Company D had an advantage over Feed Company C, with a significantly higher survival in fish reared on Feed Company D (Table 2.1.11).

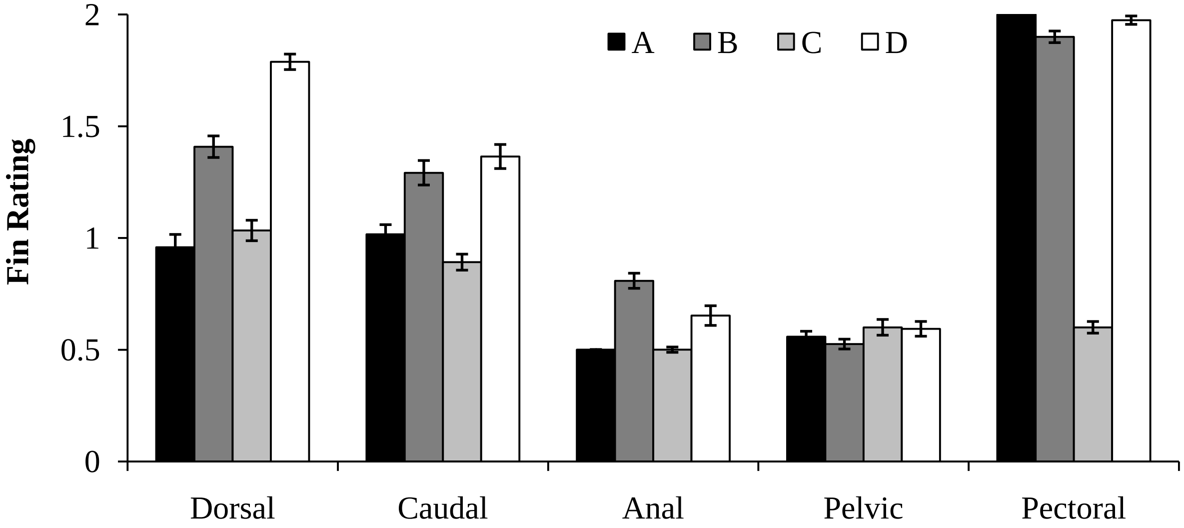

Figure 2.1.2. Average fin rating by fin type (dorsal, caudal, anal, pelvic, and pectoral) for fish fed Feed Companies A, B, C, and D that reached the goal weight of ≥ 210 g.

All feed companies produced catchable-size fish by the end of the experiment. On average, it took 0.38 lbs of feed to produce a catchable fish on Feed Company A, 0.44 lbs of feed to produce a catchable fish on Feed Company B, 0.35 lbs of feed to produce a catchable fish on Feed Company C, and 0.49 lbs of feed to produce a catchable fish on Feed Company D. Colorado produced and stocked 2,691,614 catchable Rainbow Trout in 2015. In order to produce this many catchable Rainbow Trout, 570 tons of feed would be needed of Feed Company A, compared to 657 tons of Feed Company B, 532 tons of Feed Company C, and 716 tons of Feed Company D. With natural protein sources, such as fish meal, becoming scarcer, it is important to reduce the amount of feed used to sustainably rear fish in aquaculture. Based on these results, Feed Company C is the most sustainable for producing Colorado's catchable size Rainbow Trout.

Colorado stocks millions of Rainbow Trout annually. In 2015, Colorado hatcheries stocked 12,447,260 *M. cerebralis*-negative subcatchable Rainbow Trout, averaging 2.53 in TL and 0.01 lbs, 58,604 *M. cerebralis*-positive subcatchable Rainbow Trout, averaging 6.16 in TL and 0.16 lbs, 1,900,652 *M. cerebralis*-negative catchable Rainbow Trout, averaging 10.23 in TL and 0.43 lbs, and 790,962 *M. cerebralis*-positive catchable Rainbow Trout, averaging 10.02 in TL and 0.41 lbs. Using the total amount of feed fed per individual, as well as the cost per pound of feed for each of the feed sizes used in the experiment (Table 2.1.12), the cost per fish was calculated for each size and number of Rainbow Trout stocked by Colorado (using the 2015 data presented above). The average cost to produce a fish in the hatchery feed experiment was lowest for fish reared on Feed Company D, increasing four to five cents each for rearing fish on Feed Companies B, A, and C, respectively (Table 2.1.13). The cost per pound of Feed Company C is about $0.75 greater than for Feed Companies B or D (Table 2.1.12). One potential reason could be the protein sources included in these feeds. Fish meal is the only protein source listed for Feed Company C, a much more expensive protein source than some listed for Feed Companies B and D, including blood meal, feather meal, poultry by-product meal, and soybean meal, in addition to fish meal. Because the cost of the size 0 feed from Feed Company D is higher than Feed Company B (Table 2.1.12), it is fairly comparable to produce over 12,000,000 subcatchable Rainbow Trout on both of these feeds (Table 2.1.13). However, it would be less expensive to produce the other sizes of fish on Feed Company D than on Feed Company B.

Table 2.1.12. Cost breakdown, by feed size, for each of the four feed companies (A, B, C, and D) used in the 2017 hatchery feed experiment.

Feed Company A		**Feed Company B**		**Feed Company C**		**Feed Company D**	
Size	**Cost/lb**	**Size**	**Cost/lb**	**Size**	**Cost/lb**	**Size**	**Cost/lb**
Size 0	$1.98	Size 0	$0.98	Mash	$2.05	Size 0	$2.55
Size 1	$1.98	Size 1	$0.98	Size 0	$2.05	Size 1	$0.99
Size 2	$1.98	Size 2	$0.98	Size 1	$2.05	Size 2	$0.99
1.2 mm	$1.54	1.0 mm	$0.65	Size 2	$2.05	Size 3	$0.55
1.5 mm	$1.18	2.0 mm	$0.60	1.2 mm	$1.50	Size 4	$0.55
2.0 mm	$1.05	3.0 mm	$0.65	1.5 mm	$1.19	3/32”	$0.501
3.0 mm	$0.99	4.0 mm	$0.60	2.0 mm	$1.14	1/8”	$0.431
4.0 mm	$0.90			2.5 mm	$1.10	5/32”	$0.431
5.0 mm	$0.77			3.0 mm	$1.06	3/16”	$0.431
				4.0 mm	$0.94		
Average	**$1.374**	***Average***	**$0.777**	***Average***	**$1.513**	***Average***	**$0.825**

Table 2.1.13. Cost per fish estimates based on feed cost per size (Table 2.1.12) and amount of feed used per fish. The cost to produce the number of Rainbow Trout of each size (negative subcatchables [Neg Sub], positive subcatchables [Pos Sub], negative catchables [Neg Catch], and positive catchables [Pos Catch]) reared and stocked by the state of Colorado in 2015 are also shown, as is the total cost of feed to produce 15,197,480 Rainbow Trout.

Feed Company	**Cost per fish**	**Neg Sub**	**Pos Sub**	**Neg Catch**	**Pos Catch**	**Total**
A	$0.33	$177,727	$7,772	$618,548	$257,411	$1,061,457
B	$0.27	$104,084	$5,455	$508,019	$211,414	$828,972
C	$0.36	$178,054	$7,921	$675,730	$281,207	$1,142,912
D	$0.22	$90,432	$4,957	$414,455	$172,477	$682,321

Similar to the results of the 2016 experiment (Fetherman and Schisler 2017), Feed Company C was the best performing feed of the four feeds tested. Fish fed Feed Company C reached the goal weight of ≥ 210 g two weeks, six weeks, and 13 weeks sooner than fish fed Feed Companies A, B, and D. Additionally, fish fed Feed Company C generally had more stored energy reserves, were less variable in size, and exhibited less fin wear than those fed Feed Company D. However, it costs 1.6 times more to produce a catchable size fish on Feed Company C than on Feed Company D. The second best alternative to Feed Company C appears to be Feed Company A, however, it still costs 1.5 times more to produce a catchable size fish on Feed Company A than Feed Company D. In general, there were not many differences among Feed Companies A and B, so Feed Company B may be a valid alternative for balancing cost and quality of fish produced by the state of Colorado.

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Action #2:

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

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. 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. This experiment will 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.

Action #2 Accomplishments

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 was designed 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.

Feeds from two commercial feed companies were evaluated, Feed Company C and D (using the same letter designations used in the hatchery feed experiment; Job No. 2, Action #1). 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 by Harrison Lake (H×H) Rainbow Trout were used for this experiment because they are one of the most common strains affected by BCWD outbreaks in Colorado hatcheries. 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 specific type of 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 monitored and 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.

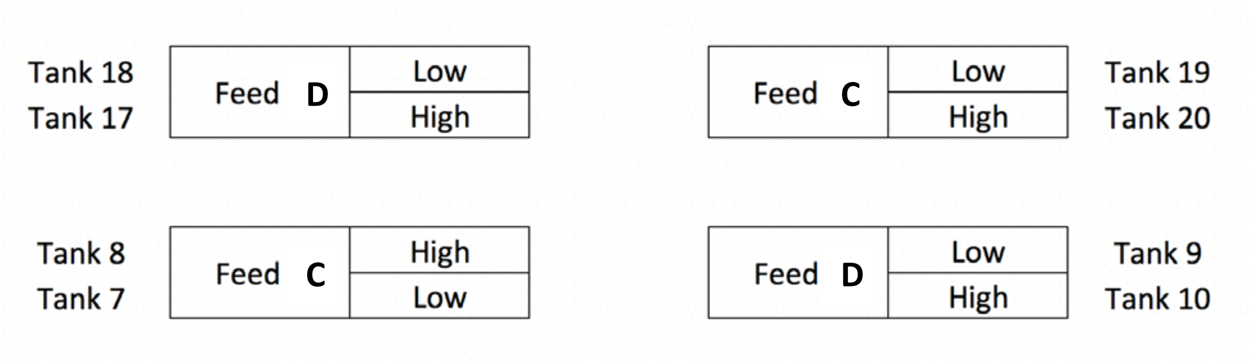

Figure 2.2.1. Paired experimental hatchery troughs used to rear the H×H at two densities (low and high) and on two feeds (C and D) at the BRFH.

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 and stocked into Parvin Lake (Red Feather Lakes, Colorado) in August 2017 so that rearing density and feed, not stocking density, were the only factors affecting post-stocking survival. Recaptures were conducted using a boat-mounted electrofishing unit every two weeks between September and October 2017, and one time per month in April and June 2018. Only the survival results for the two month post-stocking period between September and October 2017, and the seven month post-stocking period between August 2017 and April 2018 are shown here.

Survival analyses were conducted using a capture-recapture Cormack Jolly-Seber hierarchical Bayesian model. 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 detection probability and rearing density.

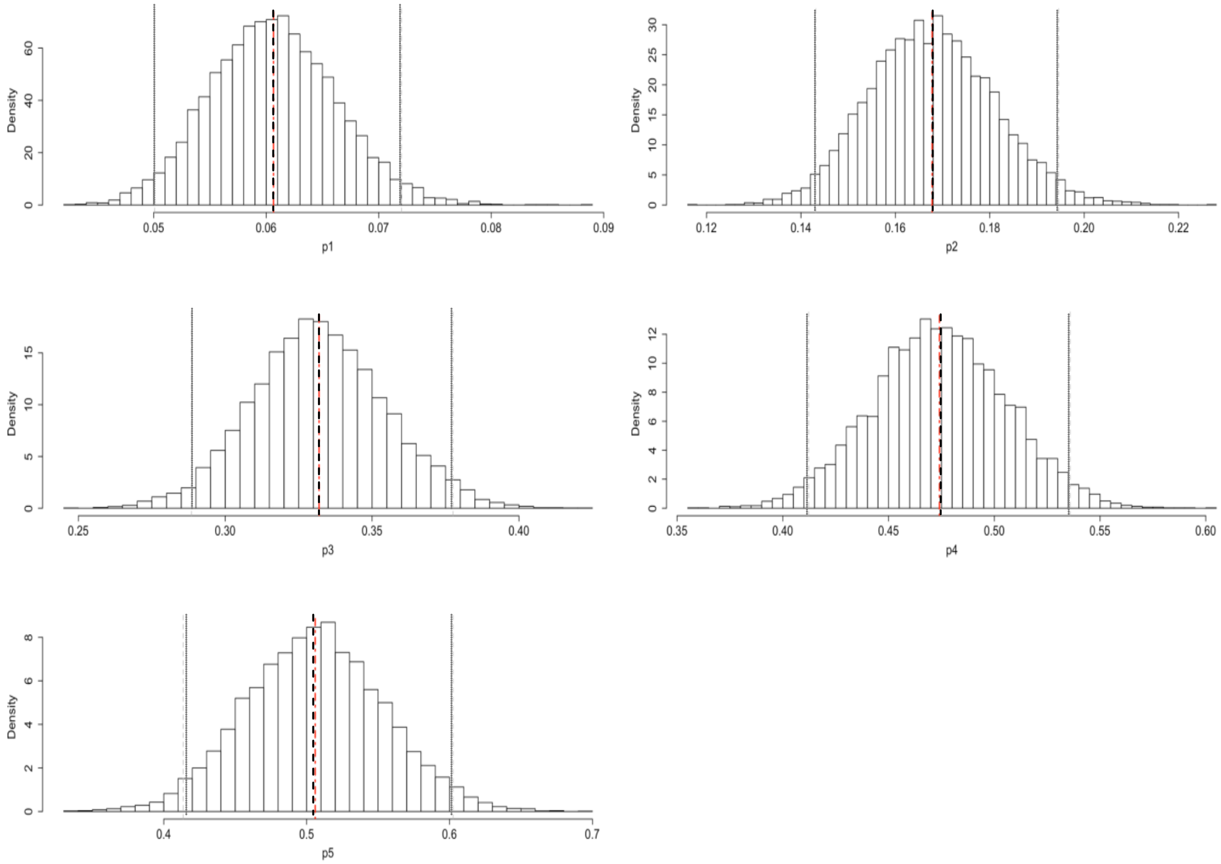

Figure 2.2.2. Posterior distributions for detection probabilities (ρ) associated with each recapture event, with ρ1-ρ4 representing biweekly sampling events occurring in September and October 2017, and ρ5 representing the sampling event occurring in April 2018.

Table 2.2.1. Mean survival [95% credible interval bounds] of Rainbow Trout reared at low and high densities, and on Feeds C and D, at two and seven months post-stocking into Parvin Lake.

Treatment		**Months Post-Stocking**	**Mean Survival**
Density	**Feed Company**		
Low	C	2	0.35 [0.34, 0.37]
High	C	2	0.34 [0.33, 0.35]
Low	D	2	0.35 [0.34, 0.36]
High	D	2	0.34 [0.33, 0.35]
Low	C	7	0.29 [0.28, 0.30]
High	C	7	0.27 [0.26, 0.28]
Low	D	7	0.28 [0.27, 0.29]
High	D	7	0.26 [0.26, 0.27]

Average detection probability (ρ) ranged between 0.05 and 0.50 between the five sampling events (Figure 2.2.2). Feed did not appear to affect post-stocking survival (Table 2.2.1), and as such, the posterior distributions for feed at the two time periods 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 2.2.3). Similarly, 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 2.2.3).

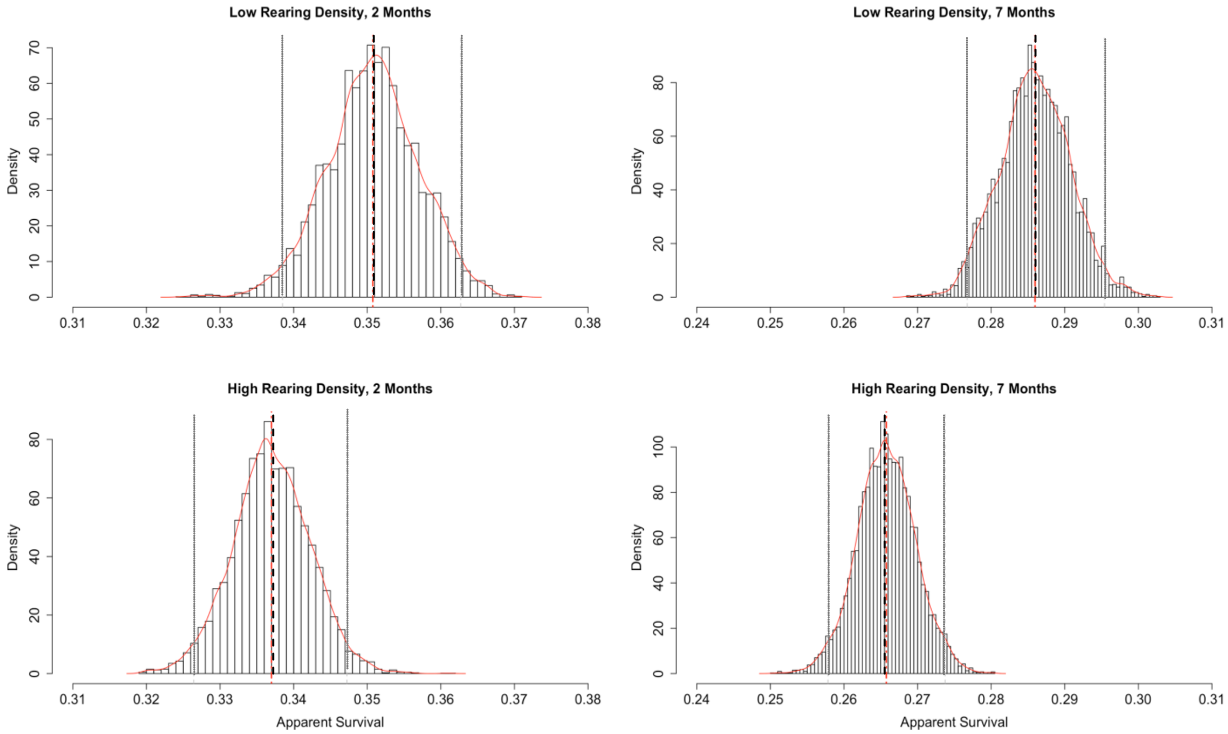

Figure 2.2.3. Posterior distributions for the average apparent survival for fish reared at low and high densities at two and seven months post-stocking.

Recapture events will continue through September 2018 to estimate an annual survival rate per treatment. In addition, throughout both the hatchery and lake portions of the experiment, subsets of fish were sacrificed to calculate the hepatosomatic index (HSI) and viscerosomatic index (VSI) for fish reared in and surviving from each treatment. These indices will be used to determine what factors may have contributed to post-stocking survival differences among the rearing densities. These additional analyses will be available in the next reporting cycle.

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: Artificial propagation studies*

Differences in feed quantity and quality of ingredients can affect health indices of hatchery-reared Rainbow Trout. Specifically, hepatosomatic indices (HSI; stored energy in liver) and viscerosomatic indices (VSI; fat content in viscera), among other qualities are known to differ among fish fed on different feeds and at various sizes or life stages. Higher HSI and VSI levels could result in increased health benefits and lead to higher post-stocking survival rates. Experiments will be conducted to examine the post-stocking survival rates of fish reared on the basic feeds from four major commercial fish feed manufacturers. One experiment will be conducted at each of three life stages, corresponding to the typical sizes of fish stocked by Colorado's hatchery system: 1) fingerlings, Rainbow Trout 3" in length; 2) subcatchables, Rainbow Trout 6" in length; and, 3) catchables, Rainbow Trout 10" in length.

Action #3 Accomplishments

Fish used in the feed and size-at-stocking survival experiments originated from the hatchery feed experiment (Job No. 2, Action #1). To determine potential differences in post-stocking survival and health of fish reared on the four feeds, A, B, C, and D (same feed and letter designations used in Job No. 2, Action #1), fish were transported to and held in tanks at the Parvin Lake Research Station (Red Feather Lakes, Colorado). Experimental tanks were supplied with unfiltered lake water so that water quality, food availability, etc. was the same in the tanks as it would have been had fish been stocked into the lake itself. However, conducting this experiment in tanks allowed detection of mortality, if it occurred, and prevented detection probability, which would have been much lower if fish were released into the lake, from affecting inferences regarding survival and health metrics at the conclusion of the trial period.

The first experiment with fingerling Rainbow Trout, averaging 77.9 (± 8.5) mm total length (TL) and 4.6 (± 1.6) g, began on April 19, 2017. All fish used in the experiment were measured, weighed, and Visual Implant Elastomer (VIE) tagged at the BFRH prior to transport up to the Parvin Lake Research Station. VIE tags were used to maintain replication within a tank, as ten fish per each of three replicates for each feed company were included in each experimental tank, a total of 30 fish per tank. Four, 76-L (20-gallon) experimental tanks were used for the experiment, one for each feed company. Fish remained undisturbed in the tanks for 50 days, with the experiment concluding on June 8, 2017. Fish were not fed during this time period, although food particles from the lake made it into the tanks through the water line, and the tanks were not cleaned to simulate being held in the lake environment. Upon completion of the experiment, fish were identified using VIE tag color, measured and weighed. Additionally, all fish were dissected to obtain liver and viscera weights.

Fulton's condition factor (K) was calculated for all individuals prior to and after the experiment using individual fish length and weight and the equation for K presented in Ney (1999). Hepatosomatic index (HSI) and viscerosomatic index (VSI) were calculated using the ratio of the liver or viscera weight to total weight of the fish (Trushenski et al. 2011; Gause and Trushenski 2013) at the conclusion of the experiment. The HSI and VSI indicate the amount of energy reserves stored in the liver and as fat in the viscera, excess energy that could be used during periods of low food availability after being stocked. The higher the HSI and VSI values, the higher the amount of stored energy that can be utilized at a later date. Measured HSI and VSI values were not available for fish immediately prior to the start of the experiment since the experiment began at a fish size that fell between feeding rate changes at which these values were assessed in the hatchery feed experiment. To obtain HSI and VSI values for fish at the start of the experiment, HSI and VSI of fish from the feeding rate prior to and after the feed and size-at-stocking experiment began were plotted. A regression line was fit to the data and used to calculate the values at the start of the experiment based on the daily change in HSI and VSI and the day on which the experiment started. A repeated measure analysis of variance (RM ANOVA) implemented in SAS Proc GLM (SAS Institute 2017), was used to determine if there were differences in pre-and post-experiment length, weight, condition factor, HSI, and VSI within and among fish reared on the feeds from the four feed companies.

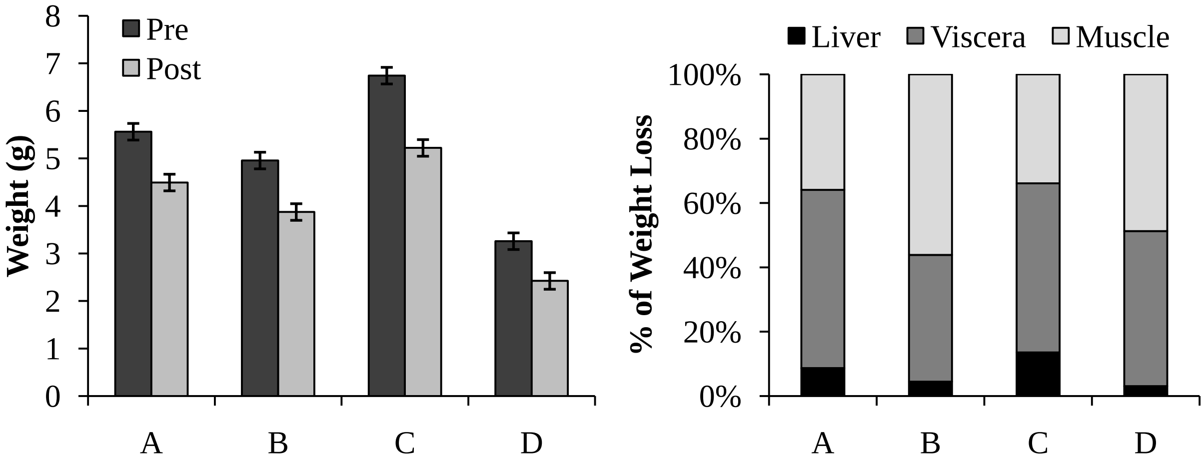

Figure 2.3.1. *Left panel:* Fingerling Rainbow Trout weight (g; SE bars) at the start (pre) and end (post) of the 50 day feed and size-at-stocking experiment by feed company (A, B, C, and D). *Right panel:* Location from which weight loss in the fingerling Rainbow Trout occurred for each feed company given the change in weight of the liver, viscera, and overall weight of the fish from the beginning to the end of the 50 day feed and size-at-stocking experiment.

No mortality occurred over the course of the experiment. As such, results are focused on the differences in fish condition and health from the beginning and end of the experiment. Overall, fish length did not change over the course of the 50 day experiment ($p = 0.68$) indicating that fish did not grow while in the experimental tanks. There was a significant decrease in weight from the beginning to end of the experiment ($p < 0.01$; Figure 2.3.1), although the interaction between weight at the beginning and end of the experiment and feed company was not significant ($p = 0.26$). The location in which weight loss occurred varied by feed group, with a larger percentage of the total weight loss occurring from absorption of the liver and viscera fat for fish fed Feed Companies A and C, and occurring as muscle loss for fish fed Feed Companies B and D. As a result of the weight loss exhibited by all fish in the experiment, there was also a significant reduction in K from the beginning to the end of the experiment ($p < 0.01$), with K averaging 1.07 (± 0.03) at the beginning of the experiment, and 0.78 (± 0.01) at the end of the experiment.

Differences in weight loss location were likely a result of the differences in primarily HSI among the feed companies at the beginning of the experiment ($p < 0.04$; Figure 2.3.2). Fish reared on all four feed companies experienced a significant decrease in HSI over the course of the experiment ($p < 0.01$). Decreases in HSI were larger for Feed Companies A and C, which is reflected in the location in which weight loss occurred in these fish, the liver, and is likely a result of having more available energy to draw from the liver at the beginning of the experiment compared to fish reared on Feed Companies B and D who relied on muscle absorption to account for smaller HSI at the beginning of the experiment. Additionally, fish reared on Feed Companies A and C also had significantly larger VSI at the beginning of the experiment compared to fish reared on Feed Companies B and D ($p < 0.01$; Figure 2.3.2). Significant reductions in VSI occurred for fish reared on all four feed companies, and VSI was similar among the feed companies at the end of the experiment, suggesting that fish in all four feed groups had absorbed all available visceral fat and energy in the viscera, and the final VSI reflected the minimum weight of the organs in all fish. Similar to the HSI, the larger reductions in VSI in fish reared on Feed Companies A and C suggest that there were more reserves available in the viscera in these fish compared to fish reared on Feed Companies B and D, resulting in less muscle absorption by the end of the experiment.

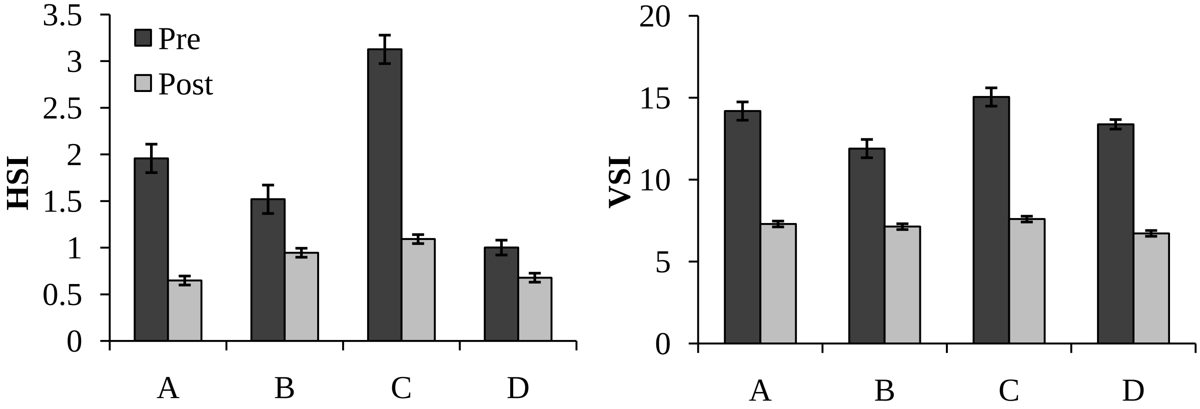

Figure 2.3.2. Hepatosomatic index (HIS; SE bars; left panel) and viscerosomatic index (VSI; SE bars; right panel) at the start (pre) and end (post) of the 50 day feed and size-at-stocking experiment for fingerling Rainbow Trout reared on the four feed companies (A, B, C, and D).

The second experiment with subcatchable Rainbow Trout, averaging 201.2 (± 21.2) mm TL and 98.3 (± 35.3) g, began on August 23, 2017. All fish used in the experiment were measured, weighed, and VIE tagged at the BFRH prior to transport up to the Parvin Lake Research Station. VIE tags were used to maintain replication within a tank, as ten fish per each of three replicates for each feed company were included in each experimental tank, a total of 30 fish per tank. Four, 946-L (250-gallon) experimental tanks were used for the experiment, one for each feed company. Fish remained undisturbed in the tanks for 50 days, with the experiment concluding on October 12, 2017. Fish were not fed during this time period, although food particles from the lake made it into the tanks through the water line, and the tanks were not cleaned to simulate being held in the lake environment. Unfortunately, as a result of density and temperature issues within the experimental tanks, high mortality occurred. Because mortality was not suspected to be a result of nutrition, since all tanks experienced similarly high mortality rates, and because there were very few fish remaining at the end of experiment from which to compare size, condition, and health metrics, this experiment was dropped from the post-stocking survival and feed comparisons.

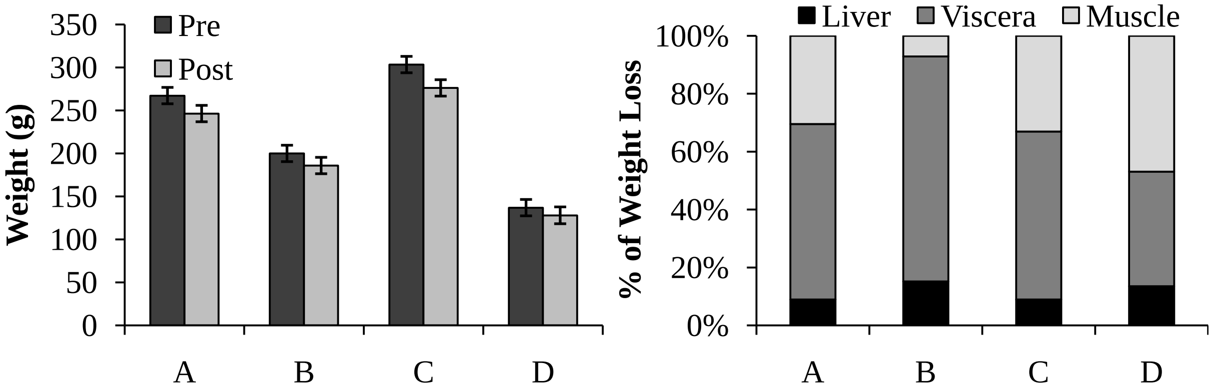

Figure 2.3.3. *Left panel:* Catchable Rainbow Trout weight (g; SE bars) at the start (pre) and end (post) of the 50 day feed and size-at-stocking experiment by feed company (A, B, C, and D). *Right panel:* Location from which weight loss in the catchable Rainbow Trout occurred for each feed company given the change in weight of the liver, viscera, and overall weight of the fish from the beginning to the end of the 50 day feed and size-at-stocking experiment.

The third and final experiment with catchable Rainbow Trout, averaging 263.2 (± 25.9) mm TL and 218.5 (± 74) g, began on November 8, 2017. All fish used in the experiment were measured, weighed, and VIE tagged at the BFRH prior to transport up to the Parvin Lake Research Station. A subset of five fish from each feed company were dissected to obtain initial estimates of HIS and VSI. This experiment was modified from the first two to reduce densities and to prevent excess mortality due to crowding and poor water quality which occurred in the subcatchable experiment. As such, VIE tags were used to identify fish by feed company. Four, 946-L (250-gallon) experimental tanks were used for the experiment, each containing five fish from each feed company, a total of 20 fish per tank. Fish remained undisturbed in the tanks for 50 days, with the experiment concluding on December 27, 2017. Fish were not fed during this time period, although food particles from the lake made it into the tanks through the water line, and the tanks were not cleaned to simulate being held in the lake environment. Upon completion of the experiment, fish were identified using VIE tag color, measured and weighed. Additionally, all fish were dissected to obtain liver and viscera weights. An RM ANOVA implemented in SAS Proc GLM (SAS Institute 2017), was used to determine if there were differences in pre-and post-experiment length, weight, condition factor, HSI, and VSI within and among fish reared on the feeds from the four feed companies.

Overall, fish length did not change over the course of the 50 day experiment (p = 0.84) indicating that fish did not grow while in the experimental tanks. There was a significant decrease in weight from the beginning to end of the experiment (p = 0.01; Figure 2.3.3), although the interaction between weight at the beginning and end of the experiment and feed company was not significant (p = 0.79), suggesting that feed quality did not affect the ability to maintain weight in catchable Rainbow Trout. Patterns of weight loss differed from the fingerling Rainbow Trout. Fish reared on Feed Company B experienced very little muscle loss in comparison to the fingerling fish reared on the same feed, and muscle loss was much lower in fish reared on Feed Company B than in the other three feed companies in the catchable Rainbow Trout experiment. Fish reared on Feed Company D were the only fish to experience up to 50% muscle loss, with fish reared on Feed Companies A and C losing only about 25% muscle mass (Figure 2.3.3). Similar to the weight data, although there was significant reduction in K in all fish between the beginning and end of the experiment (p < 0.01), decreasing from 1.20 (± 0.04) to 1.10 (± 0.03) over the course of the 50 day experiment, there was not a significant reduction in K within a feed company between the beginning and end of the experiment (p = 0.12).

HSI and VSI both decreased significantly over the course of the experiment (p < 0.01 for both metrics; Figure 2.3.4), suggesting that the liver and viscera fat were both absorbed to keep fish alive over the course of the experiment, and the weight loss location results suggest that for all fish except those fed on Feed Company D, the liver and viscera were primarily source of energy consumption over the course of the experiment. HSI decreased significantly within feeds from the beginning to the end of the experiment, but VSI did not (p = 0.62), suggesting that the liver was the primary source of energy consumption during the experiment, and that this source was likely drawn from prior to switching to viscera fat for energy.

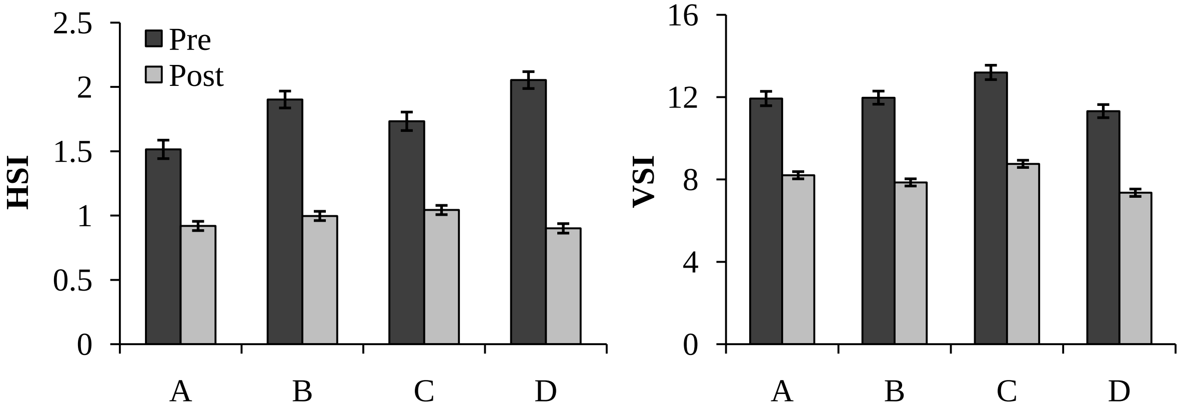

Figure 2.3.4. Hepatosomatic index (HIS; SE bars; left panel) and viscerosomatic index (VSI; SE bars; right panel) at the start (pre) and end (post) of the 50 day feed and size-at-stocking experiment for catchable Rainbow Trout reared on the four feed companies (A, B, C, and D).

The results of the feed and size-at-stocking experiments show that feed quality is more of factor affecting the health and condition of fish when they are smaller than when they are larger. Fingerling Rainbow Trout experienced much larger declines in weight and condition compared to catchable rainbow trout reared on the same feeds. Additionally, weight loss location differs in food-deprived fingerling and catchable Rainbow Trout, with fingerling Rainbow Trout losing proportionally more muscle mass overall than catchable Rainbow Trout. It is expected that if these fingerling Rainbow Trout were to be released into a body of water in which food availability was low upon release, fish reared on Feed Companies A and C would survive better than those reared on Feed Companies B and D because of the energy reserves the fish were able to access in the liver and viscera, rather than the muscle. Muscle absorption and loss of muscle mass is expected to affect swimming ability and predator avoidance in these fish, decreasing their survival in the wild. Fingerling Rainbow Trout reared on Feed Companies A and C exhibited higher HSI and VSI than did the catchable Rainbow Trout, a pattern that has been observed in the hatchery feed experiments (Fetherman and Schisler 2017; Job No. 2, Action #1), likely a result of the higher energy content in the smaller feed sizes from these feed companies compared to Feed Companies B and D.

Overall, the results of these experiments support the conclusions of the hatchery feed experiment that Feed Companies A and C should be considered for use on a larger scale within Colorado's hatcheries. Both feeds produce healthier, higher quality Rainbow Trout that would be expected to exhibit higher post-stocking survival rates. Additionally, both feeds produce catchable Rainbow Trout in a shorter amount of time than do Feed Companies B and D, with Feed Company C producing catchable fish two weeks sooner than Feed Company A, and both producing catchable fish at least 1 to 3 months sooner than Feed Companies B and D (Job No. 2, Action #1).

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.

Gause, B. R., and J. T. Trushenski. 2013. Sparing fish oil with beef tallow in feeds for Rainbow Trout: effects of inclusion rates and finishing on production performance and tissue fatty acid composition. North American Journal of Aquaculture 75:495-511.

Ney, J. J. 1999. Practical use of biological statistics. Pages 167-191 *in* C. C. Kohler and W. A. Hubert, editors. Inland fisheries management in North America, 2nd edition. American Fisheries Society, Bethesda, Maryland.

SAS Institute. 2017. SAS system software, release 9.4. SAS Institute, Cary, North Carolina.

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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 four electrofishing surveys in Parvin Lake to evaluate survival and infection severity of various strains of Rainbow Trout stocked as fingerlings by November 30, 2017.
2. Initiate an experiment to determine differences in susceptibility to *M. cerebralis* infection among relatively new strains of Rainbow Trout to Colorado's hatchery program by June 30, 2018.

Approach

Action #1:

- *Level 1 Action* *<u>Category</u>: Direct Management of Natural Resources*
- *Level 2 Action* *<u>Strategy</u>: Wildlife disease management*
- *Level 3 Action* *<u>Activity</u>: 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 2017, and summer of 2018. 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.

<u>Action #1 Accomplishments</u>

Rainbow Trout *Oncorhynchus mykiss* post-stocking survival and infection severity studies were conducted in Parvin Lake (Red Feather Lakes, Colorado), located 45 miles northwest of Fort Collins, Colorado. In addition to the Rainbow Trout stocked for these evaluations, the reservoir is stocked annually with fingerling Brown Trout *Salmo trutta* and splake *Salvelinus namaycush x S. fontinalis*. The reservoir was also stocked with tiger muskies *Esox masquinongy x E. lucius* ostensibly between 2000 and 2003 to control the abundant White Sucker *Catostomus commersoni* population. An inlet trap that was historically used for wild Rainbow Trout spawning operations has also been operated more recently to remove White Suckers from the reservoir in the months of May through July during their annual spawning run up the inlet stream. Numbers of White Suckers and salmonids captured in the trap vary from year to year, but White Sucker numbers appear to have been greatly reduced in recent years (Figure 3.1.1).

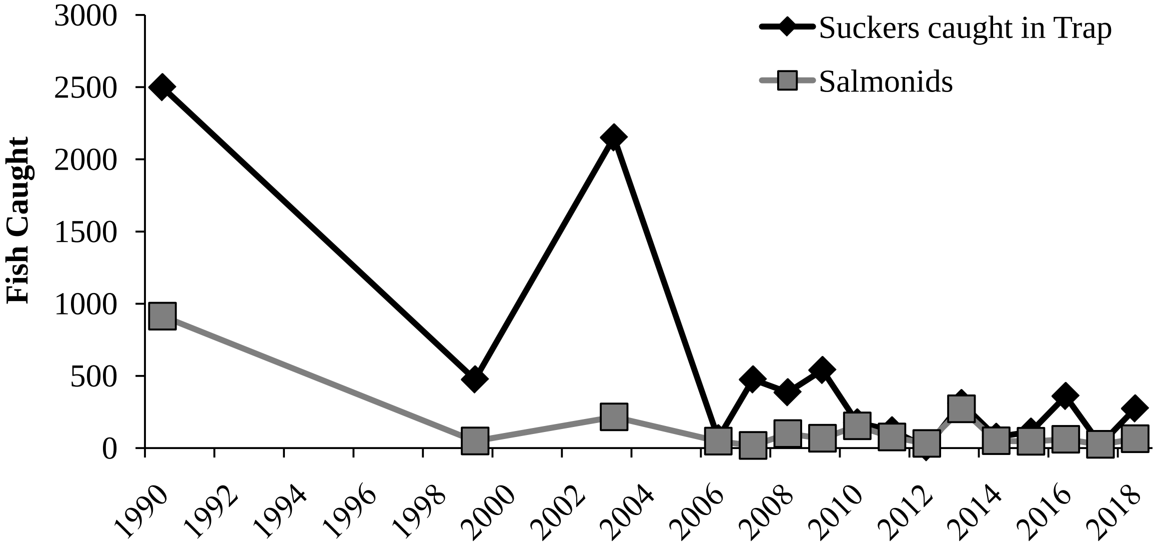

Figure 3.1.1. Number of White Suckers and salmonids captured in the Parvin Lake inlet trap (May-July) in years where data are available.

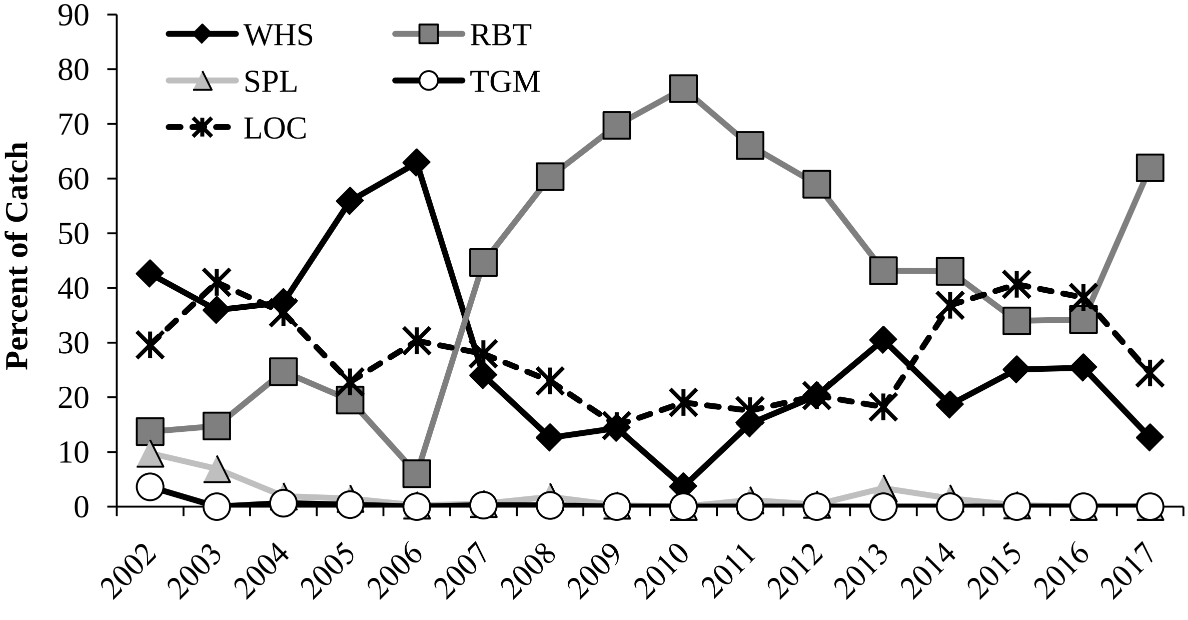

Figure 3.1.2. Percent of catch for White Suckers (WHS), Rainbow Trout (RBT), slake (SPL), tiger muskie (TGM), and Brown trout (LOC) during fall electroshocking surveys (2002 – 2017).

Table 3.1.1. Acronyms for various Rainbow Trout strains used in these experiments.

Acronym	Description
HAR	Pure Harrison Lake Rainbow Trout, also described as HL in this document.
GR	Pure Hofer Rainbow Trout, also described as HOF or Hofer in this document.
HXN	Hofer Rainbow Trout (described above) crossed with fall spawning Snake River Cutthroat Trout (see SRN below).
HXH	Hofer Rainbow Trout crossed with Harrison Lake Rainbow Trout. Proportion of Hofer to Harrison is typically provided with parentheses. For example, (75:25) would be 75% Hofer and 25% Harrison Lake. If no parentheses or other designation are provided, as with the Colorado Parks and Wildlife species codes, these fish are typically from Crystal River Hatchery brood stock. That stock originated from a mixture of HXH year classes consisting of (75:25) and (87.5:12.5) crosses.
HHN	Crystal River Hatchery HXH brood stock (as described above) crossed with fall spawning Snake River Cutthroat Trout (see SRN below).
HH1	Crystal River Hatchery HXH brood stock (as described above) crossed with SR1.
HH2	Crystal River Hatchery HXH brood stock (as described above) crossed with SR2.
HN1	Hofer Rainbow Trout crossed with SR1.
HN2	Hofer Rainbow Trout crossed with SR2.
SRN	Fall spawning Auburn strain Snake River Cutthroat Trout housed at Crystal River Hatchery.
SR1	Pure Wyoming spring spawning Snake River Cutthroat Trout, brought to Crystal River Hatchery to increase genetic diversity of SRN.
SR2	Cross of any form of SRN with SR1, including 50:50, 75:25, and other back-crosses. This is the lot created to increase Snake River Cutthroat Trout brood stock diversity, but intended to be as close to old fall spawn timing as possible.
RXN	Standard hatchery Rainbow Trout (usually Bellaire or Tasmanian strains) crossed with Snake River Cutthroat Trout (SRN, SR1 or SR2).
HXC	Hofer Rainbow Trout crossed with Colorado River Rainbow Trout. As with the HXH strains, parentheses or other designations are typically used to delineate the proportional crosses of these fish. Early crosses used in these experiments were 50:50 crosses from the Glenwood Springs Hatchery. After 2012 these fish are subsequent generational crosses (matings of the original HXC year classes with other HXC year classes).
GBN	Recreational Greenback Cutthroat Trout (not pure, used for recreational stocking opportunities).
HGBN	Hofer Rainbow Trout crossed with recreational Greenback Cutthroat Trout (not pure, used for recreational stocking opportunities).
GRR	Gunnison River Rainbow. This strain originated from wild egg takes at the East Portal of the Gunnison River, where a wide variety of wild and domestic Rainbow Trout strains, including HXC, had been stocked for many years.
HXG	Cross of Hofer Rainbow Trout with Gunnison River Rainbow Trout.
PRR	*Psychrophilum* Resistant Rainbow. This strain originated from USDA breeding efforts, and was imported to Colorado from Utah.

In the current reporting period, electrofishing events at Parvin Lake were conducted every two weeks between September and October 2017, and one time per month in April and June 2018, related to the experiment examining density and feed effects on post-stocking survival of

Rainbow Trout (Job No. 2, Action #2). The annual fall population sampling event was conducted on November 6, 2017. A fall survey has been conducted annually since 2002 to monitor species composition and growth in Parvin Lake. A shift from a population dominated by White Suckers to one dominated by Rainbow Trout has occurred since 2006 (Figure 3.1.2).

Past evaluations of Rainbow Trout strains stocked as fingerlings have been occurring in this location since 2009. A variety of strains, batch marked with coded wire tags, have been stocked over this time period (Table 3.1.1). Recaptures during each individual sampling event have been recorded, and recoveries compared between stocked groups in each individual year (Table 3.1.2). Advanced analysis of this large data set is possible to compare recoveries over the duration of the stocking period using recapture modeling in Program MARK. These analyses are in progress and will be provided in future reports.

Table 3.1.2. Annual stocking events, dates, strains, average length (L; mm), total pounds (Lbs), and number stocked (#), and recoveries of coded wire tagged fish by year at Parvin Lake.

Year/Tag #	Date/Strain	L	Lbs	#	Year/Number Recovered									
					07	**08**	**09**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
2007	**8/14/2007**													
623737	GR	147	225	2800	11	8	6	2	0	0	0	0	0	0
623838	RXN	122	125	2800	34	53	45	40	9	1	0	0	0	0
623939	HAR	97	64.2	2800	17	32	13	15	3	0	0	0	0	0
624040	HXH (50:50)	104	75.5	2800	23	15	17	15	4	0	0	0	0	0
624141	HXH (75:25)	104	76.6	2800	12	9	11	3	0	0	0	0	0	0
2008	**7/31/2008**													
623333	HAR	91	38.4	2050		7	21	11	6	1	0	0	0	0
624444	HXH (50:50)	117	78.2	2050		15	36	15	8	3	0	0	0	0
625555	HXH (75:25)	117	81.7	2050		11	11	2	0	0	0	0	0	0
626666	GR	127	103	2050		10	0	1	0	0	0	0	0	0
627777	RXN	127	103	2050		17	34	27	19	2	0	0	0	0
2009	**8/12/2009**													
621212	GR	150	83.7	1005			11	14	1	0	0	0	0	0
621313	TAS	167	119.6	1005			13	18	8	3	0	0	0	0
621414	HXH (50:50)	150	83.7	1005			15	19	6	4	4	3	1	0
621515	HXH (75:25)	150	83.7	1005			13	20	7	2	0	0	0	0
621717	HXH (87.5:12.5)	150	83.7	1005			10	16	2	3	1	0	0	0
621818	HHN	132	55.8	1005			5	32	15	8	1	1	0	0
621919	RXN	127	50.3	1005			8	27	19	4	2	3	0	0
622020	HAR	117	42.2	1005			17	43	7	6	4	1	0	0
2010	**7/6/2010**													
622121	HHN	112.4	260	7511				17	97	92	38	19	5	1
629999	RXN	106.7	219	7380				17	127	93	40	10	2	1

Table 3.1.2 continued. Annual stocking events, dates, strains, average length (L; mm), total pounds (Lbs), and number stocked (#), and recoveries of coded wire tagged fish by year at Parvin Lake.

Year/Tag #	**Date/Strain**	**L**	**Lbs**	**#**	**Year/Number Recovered**									
					07	**08**	**09**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
2011	**11/3/2011**													
620260	GR	76.2	32.4	3000						1	0	0	0	0
620261	HXC	76.2	32.4	3000						5	0	2	2	1
620262	HN2	76.2	32.4	3000						17	16	16	3	2
620263	RXN	76.2	32.4	3000						27	27	17	3	1
2012	**10/29/2012**													
622323	SR2	92.5	40.3	2116							1	8	3	0
622424	HXC	110.1	68.9	2116							15	7	0	0
622525	HN2	100.3	52.1	2116							25	21	5	2
622626	GR	126.8	105.3	2116							5	0	0	0
2013	**No fish stocked**													
2014	**4/4/2014**													
622929	GR	216	426	1,734								9	4	0
622727	HXC	216	426	1,734								24	10	4
622828	HN2	216	426	1,734								32	3	2
2015	**4/1/2015**													
620283	GR	193	212	1,125									11	0
620284	HXC	169	141	1,125									30	2
620285	GBN	110	33	1,125									27	7
2016	**4/28/2016**													
620286	HGBN	239	264	740										32
620287	HXC	257	332	740										27
620292	GR	267	371	740										23

Action #2:

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

The Gunnison River Rainbow (GRR) is a relatively new strain of Rainbow Trout to Colorado's hatchery system. Originating from the East Portal of the Gunnison River, this strain appears to have developed a natural resistance to M. cerebralis. The GRR will be crossed with the resistant German Rainbow (GR) in this experiment to determine if resistance of the cross is increased over that of the pure GRR strain. The Psychrophilum Resistant Rainbow (PRR), a strain *resistant to Bacterial Coldwater Disease, is also a relatively new strain of Rainbow Trout to Colorado's hatchery system. Although the strain performs well when exposed Flavobacterium psychrophilum, the resistance of this strain to M. cerebralis is unknown. This experiment will compare the whirling disease resistance and post-stocking survival of the pure GRR, the pure GR, a cross of the GR and GRR, and the PRR.*

Action #2 Accomplishments

The East Portal of the Gunnison River is currently being managed as a wild Rainbow Trout brood stock location. H×C fingerlings were stocked in the East Portal of the Gunnison River every year from 2006 to 2012. Testing in 2011 identified an average of 21.3% of the genetic background of adult fish sampled from the population to be associated with the GR strain, which was the highest rate for that location observed in our testing. However, subsequent sampling in 2015 resulted in identification of only 3.7% GR in the adult population. Sampling of fry produced naturally in this location have exhibited even lower (< 1%) of GR genetics. Because this population is reproductively isolated and unique these fish became known as the Gunnison River Rainbow (GRR) strain.

Testing of whirling disease resistance in the GRR strain from wild egg collections at the East Portal of the Gunnison River were conducted in 2012 and 2014, with promising results. In these experiments (Fetherman et al. 2013, 2014, 2015, 2016), we found that the GRR strain appeared to demonstrate some resistance to the effects of *Myxobolus cerebralis*. This finding was fortuitous, because an outbreak of bacterial kidney disease at the CPW Glenwood Springs Hatchery in 2015 resulted in elimination of the H×C brood stock housed at that location. The GRR strain was a good candidate as a replacement brood stock with resistance to whirling disease which could be used for re-establishment of wild Rainbow Trout populations.

Replacement of the Glenwood springs brood stock with a wild, yet *M. cerebralis* resistant, strain was important. Some questions as to the level of resistance that should be used in a brood stock and stocking efforts to re-establish wild Rainbow Trout populations were raised, including the resistance that would be found in a cross of the GRR with the pure GR strain. Because this cross had never been created artificially or tested for resistance, a pilot experiment was designed and implemented to address the issue.

Spawning and Rearing

In 2017, milt was collected from the Rainbow Trout in the East Portal during the spring spawning season and transported to the CPW Poudre Rearing Unit, where it was used to fertilize eggs of pure GR females to make several lots of H×G strain eggs. A group of pure GRR fish was also created with eggs and milt collected from the Rainbow Trout in the East Portal. Both sets of eggs were later transported to the CPW Bellvue Fish Research Hatchery where they were reared to swim-up stage. Once past the swim-up stage, the fish were brought to the CPW Parvin Lake Research Station for exposure to *M. cerebralis*.

Three groups of GRR and three groups of H×G were maintained in separate 76-L flow through tanks within the Parvin Lake Research Station. Each group contained 25 fish, which were exposed to an average of 2,000 triactinomyxons (TAMs) per fish, obtained from worm cultures maintained at the Parvin Lake Research Station, on July 5, 2017. Fish were reared until April 18, 2018, for a total of 2,042 degree-days (ºC). At the end of the experiment, all remaining fish were sacrificed using an overdose of MS-222. Lengths, weights, and signs of infection (cranial, spinal, lower jaw, and opercular deformities, and blacktail) were recorded from each individual. Heads were removed, placed in individually labeled bags, and sent to the CPW Aquatic Animal Health Lab for myxospore enumeration (O'Grodnick 1975) using the Pepsin-Trypsin Digest (PTD) method (Markiw and Wolf 1974). Fin clips were also taken from each individual to determine genetic background.

Results and Discussion

Overall, the myxospore counts in the GRR fish averaged 17,301 myxospores per fish (ranging from 0 to 125,422 myxospore per fish), and in the H×G averaged 3,572 myxospores per fish (ranging from 0 to 38,072 myxospores per fish (Figure 3.2.1). The myxospore counts for the pure GRR were very similar to those found in the 2012 and 2014 experiments. In the 2012 experiment, in which exposures were conducted in ambient hatchery water at the CPW Poudre Rearing Unit, the GRR fish that were exposed had an overall average of 19,461 myxospores per fish (ranging from 0 to 324,561 myxospores per fish; note this value was erroneously reported as 17,028 myxospores per fish in previous reports [Fetherman et al. 2014, 2015] due to a calculation error). In the experiment conducted in 2014, the GRRs developed an average of 38,063 myxospores when measured as the average of all fish, and 40,159 myxospores as measured by an average of groups (differences due to unequal sample sizes per group), ranging from 0 to 814,867 myxospores per fish (Schisler 2015; Fetherman et al. 2016).

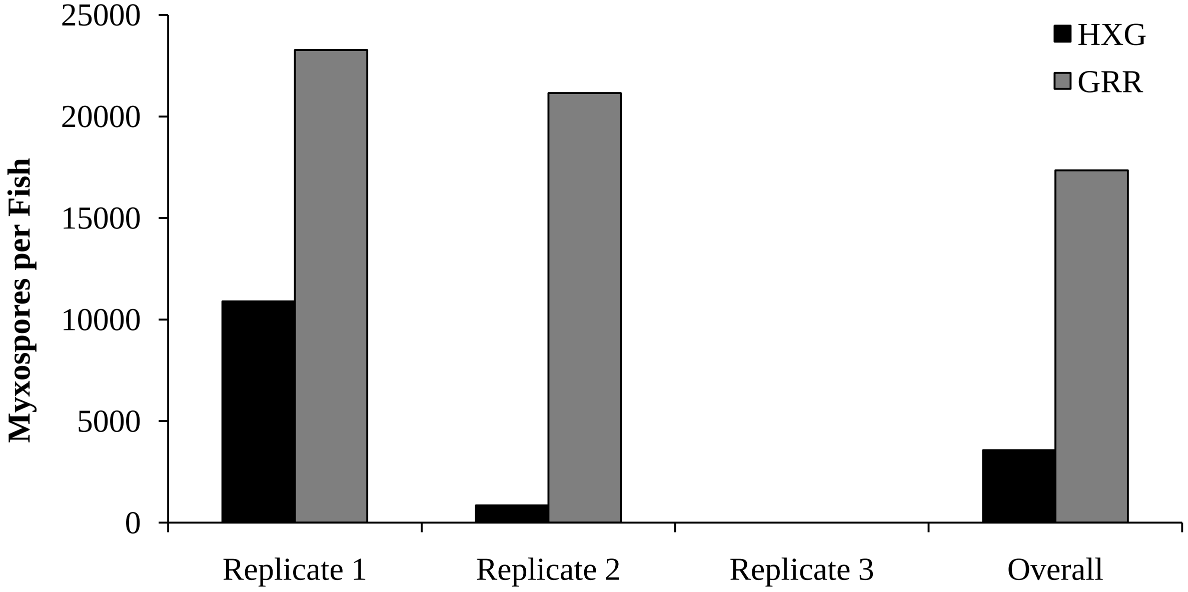

Figure 3.2.1. Average number of myxospores per fish, in three replicate groups and overall, for the H×G and GRR fish exposed to 2,000 triactinomyxons per fish in 2017.

Concurrent with these experiments, replacement brood fish collection efforts were conducted. In the spring of 2016, GRR milt was collected during wild spawning operations in the East Portal of the Gunnison River. Milt was transported to the CPW Poudre Rearing Unit and mixed with eggs from GR females. Eggs from several families were sent to the CPW Glenwood Springs Hatchery to start the new H×G brood stock. However, due to constraints on space and the need to have flexibility in producing pure GRR or H×G fish, the H×G fish created in 2016 were stocked out in favor of retaining both the pure GR and pure GRR parental strains at the facility. Pure GRR fish were created from spawns in the East Portal in 2016, 2017, and 2018. Pure GR brood stock were brought to the CPW Glenwood Springs Hatchery as eggs from the CPW Poudre Rearing Unit in 2017 and 2018. This new brood strategy will allow the CPW Glenwood Springs Hatchery to produce eggs of either parent strain, or H×G, as demand requires.

The results of this study further validate the theory that the GRR fish have some resistance to *M. cerebralis* compared to many other varieties of Rainbow Trout. However, there is a wide range of susceptibility within these fish, so some caution should be used when implementing stocking efforts, recognizing that some proportion of the fish produced from this location will produce very high levels of infection. The myxospore counts among the H×G fish were quite low, as might be expected from a cross of the highly resistant GR strain with a strain exhibiting resistance characteristics such as the GRR. In situations where a high level of *M. cerebralis* resistance is required for rearing or stocking, the H×G may be preferred. Biologists should weigh the pros and cons of each variety when making stocking requests, using the faster growing and more resistant H×G where competition is low and food supplies are abundant, and the pure GRR in areas where competition or potential predation may be high and food supplies are limited, particularly in areas where *M. cerebralis* is less prevalent due to environmental conditions.

PRR Exposure Experiment

A pilot exposure experiment was initiated in 2016, and concluded in August of 2017, to test the relative susceptibility of the *Psychrophilum* Resistant Rainbow (PRR) to infection due to *M. cerebralis* (Schisler 2017). When exposed to 2,500 TAMs per fish under laboratory conditions, these fish produced average myxospore counts of 32,385 and 79,322 myxospores per fish when tested with PTD at 4 and 14 months, respectively. These myxospore loads are not unusually high compared to other domestic strains we have tested in the past at these levels of TAM exposure, but are severe enough to cause disease among exposed fish. It is possible that crossing of the coldwater disease resistant strain (PRR) with whirling disease resistant strains may be a useful variety to reduce infection severity from both pathogens in domestically-reared Rainbow Trout.

Fetherman, E. R., and G. J. Schisler. 2013. Sport Fish Research Studies. Federal Aid Project F-394-R12. Federal Aid in Fish and Wildlife Restoration, Job Progress Report. Colorado Parks and Wildlife, Aquatic Wildlife Research Section. Fort Collins, Colorado.

Fetherman, E. R., and G. J. Schisler. 2014. Sport Fish Research Studies. Federal Aid Project F-394-R13. Federal Aid in Fish and Wildlife Restoration, Job Progress Report. Colorado Parks and Wildlife, Aquatic Wildlife Research Section. Fort Collins, Colorado.

Fetherman, E. R., and G. J. Schisler. 2015. Sport Fish Research Studies. Federal Aid Project F-394-R14. Federal Aid in Fish and Wildlife Restoration, Job Progress Report. Colorado Parks and Wildlife, Aquatic Wildlife Research Section. Fort Collins, Colorado.

Fetherman, E. R., and G. J. Schisler. 2016. Sport Fish Research Studies. Federal Aid Project F-394-R15. Federal Aid in Fish and Wildlife Restoration, Job Progress Report. Colorado Parks and Wildlife, Aquatic Wildlife Research Section. Fort Collins, Colorado.

Markiw, M. E., and K. Wolf. 1974. *Myxosoma cerebralis*: isolation and centrifugation from fish skeletal elements – sequential enzymatic digestions and purification by differential centrifugation. Journal of the Fisheries Research Board of Canada 31:15-20.

O'Grodnick, J. J. 1975. Whirling disease *Myxosoma cerebralis* spore concentration using the continuous plankton centrifuge. Journal of Wildlife Diseases 11:54-57.

Schisler, G. J. 2015. WD positive aquaculture facility exemption annual report. October 21, 2015. Colorado Parks and Wildlife, Fort Collins, CO. 3 pp.

Schisler, G. J. 2017. WD positive aquaculture facility exemption annual report. October 16, 2017. Colorado Parks and Wildlife, Fort Collins, CO. 8 pp.

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 adult abundance estimate in the Gunnison River by November 30, 2017.
2. Conduct one adult abundance estimate in the upper Colorado River by June 30, 2018.
3. Conduct five fry abundance estimates in the upper Colorado River by November 30, 2017.
4. Complete genetic analyses for one study designed to determine genetic background of naturally produced Rainbow Trout fry and recruits from previous stockings in the Gunnison and Colorado Rivers to determine genetic background by June 30, 2018.

5. Complete sampling for one study designed to examine the long-term side-by-side survival of pure Hofer and Hofer by Colorado River Rainbow (H×C) stocked as fry in the Cache la Poudre, Colorado, and South Platte drainages by June 30, 2018.
6. Initiate one experiment designed to assess the post-stocking survival of subcatchable and catchable Hofer × Harrison Lake (H×H) Rainbow Trout in the Yampa River by June 30, 2018.

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 adult abundance estimate in the Gunnison River will occur in fall 2017. Two-pass mark-recapture estimates will be obtained using a boat-mounted electrofishing unit. All fish captured will be measured, and fish captured on the second pass will be weighed. Adult abundance in the Gunnison River is being estimated as part of a study monitoring long-term trends in abundance and survival in, and recruitment to, the adult wild Rainbow Trout population.

Action #1 Accomplishments

Adult salmonid population estimates were conducted in the Ute Park section of the Gunnison River (Figure 4.1.1) October 3-6, 2017. A boat-mounted electrofishing unit was used to complete the population estimates. All fish captured on the mark run were given a caudal fin punch, measured to the nearest millimeter, and returned to the river. On the recapture run, fish were examined for the presence of a caudal fin punch, measured to the nearest millimeter, and weighed to the nearest gram. Population estimates were calculated using the Lincoln-Peterson estimator (Van Den Avyle and Hayward 1999).

Typically in high flow years in the Gunnison River, the Brown Trout population declines, with particularly large declines for the age-1 cohort. However, that was not the case in 2017 despite record high flows. An estimated 9,631 (± 1,729 [95% CI]) Brown Trout were present per mile in the Ute Park section of the Gunnison River in 2017, which was the second largest population estimate recorded for Brown Trout since 1981. The Rainbow Trout population also increased, reaching the highest estimate obtained since 2003 at 522 (± 232) Rainbow Trout per mile, likely a result of the previous and continued fry stocking of the Gunnison River Rainbow (GRR) throughout the Gunnison River. All age classes of Brown Trout were present, and the age-1 cohort was large, which is more typical of low- to moderate-flow years like 2016 (Figure 4.1.2). The age-1 Brown Trout population was the largest observed in the Gunnison River for any year in which the average flow was greater than 2,000 cfs. All age classes of Rainbow Trout were also present, and the age-1 cohort was similarly large compared to a low- to moderate-flow year (Figure 4.1.3). Both Brown Trout and Rainbow Trout exhibited an average body condition that was lower than that observed in 2016, likely due to effects of competition as a result of the overall high number of fish in the river.

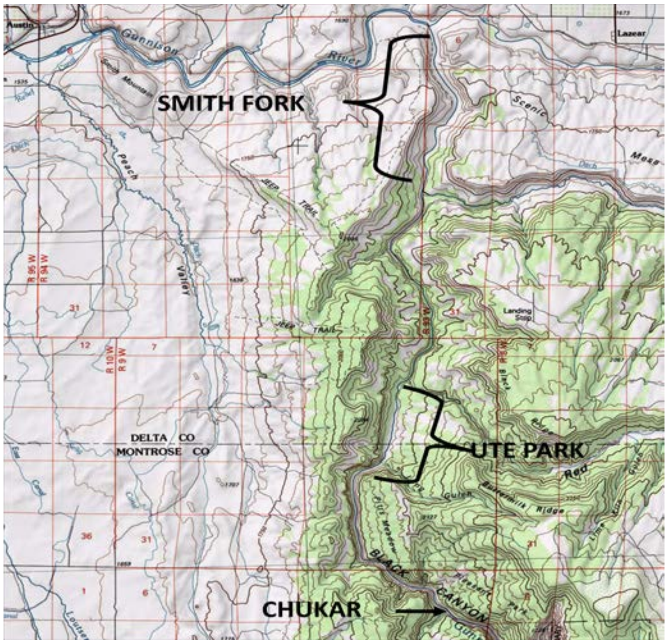

Figure 4.1.1. Map of the Gunnison River showing the location of Ute Park where fry and adult population estimates were conducted in October 2017.

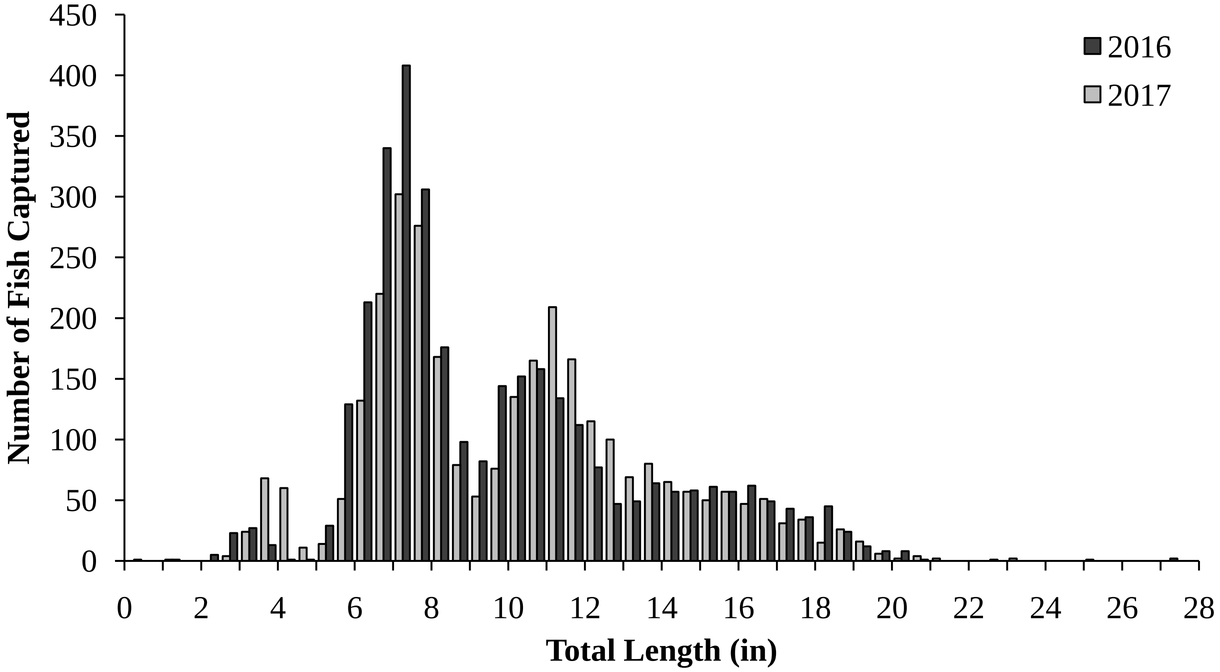

Figure 4.1.2. Number of Brown Trout captured by total length (in) during the 2016 and 2017 adult salmonid population estimates in the Ute Park section of the Gunnison River. Data was obtained from Eric Gardunio, CPW Aquatic Biologist, Montrose, Colorado.

It appears that high flows did not have a negative impact on the Brown Trout population. Flows peaked at 12,700 cfs, and were over 10,000 cfs for six days. It is possible that the magnitude and duration of these flows allowed more shoreline refuge habitat to be available for the age-1 Brown Trout than what is typically available in years where high flows peak and recede more quickly, increasing their survival. Despite two consecutive years of strong age-1 Brown Trout cohorts, the adult Rainbow Trout population continued to increase, and wild Rainbow Trout fry were found at all fry sites prior to stocking the GRR fry, an encouraging result for the future recruitment of this species. Continued stocking of GRR from the East Portal of the Gunnison River should help the Rainbow Trout population continue on its current upward trend.

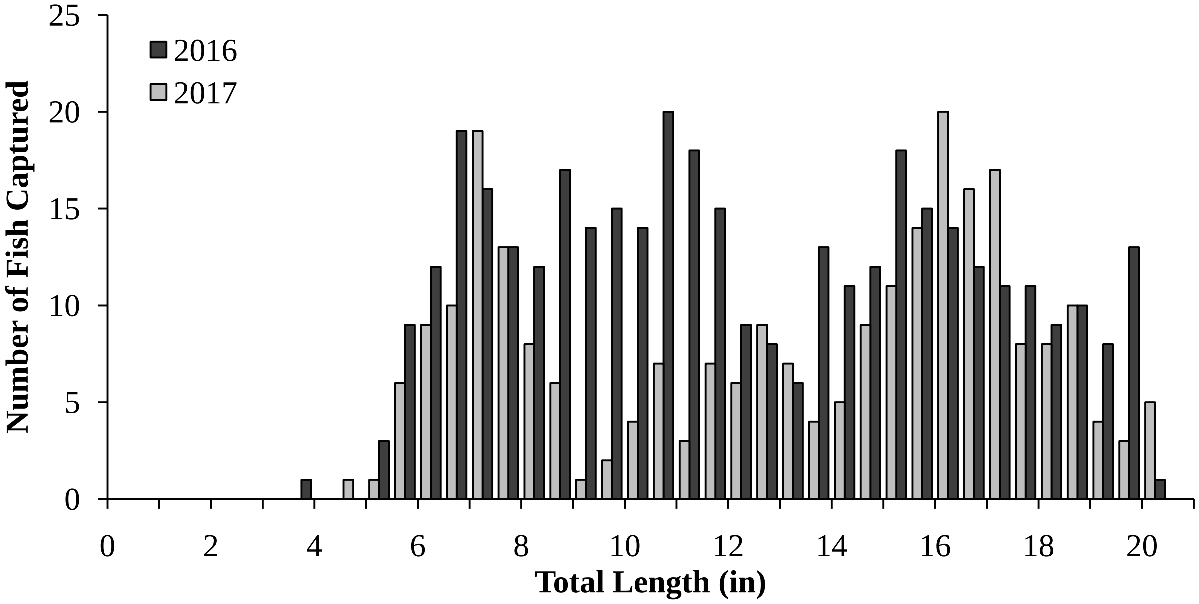

Figure 4.1.3. Number of Rainbow Trout captured by total length (in) during the 2016 and 2017 adult salmonid population estimates in the Ute Park section of the Gunnison River. Data was obtained from Eric Gardunio, CPW Aquatic Biologist, Montrose, Colorado.

Van Den Avyle, M. J., and R. S. Hayward. 1999. Dynamics of exploited fish populations. Pages 127-166 *in* C. C. Kohler and W. A. Hubert, editors. Inland fisheries management in North America, 2nd edition. American Fisheries Society, Bethesda, Maryland.

Action #2:

- *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 adult abundance estimate in the upper Colorado River will occur in spring 2018. Two-pass mark-recapture estimates will be obtained using two raft-mounted electrofishing units. All fish captured will be measured and weighed. Adult abundance in the upper Colorado River is being estimated as part of a study designed to determine if stocking large numbers of Rainbow Trout fry is an effective management strategy for increasing the adult Rainbow Trout population through recruitment.

<u>Action #2 Accomplishments</u>

An adult salmonid population estimate was conducted in the 3.9 mile Chimney Rock/Sheriff Ranch study section of the upper Colorado River in May 2018, with the mark run occurring on May 7, 2018, and the recapture run occurring on May 9, 2018. Two raft-mounted, fixed-boom electrofishing units were used to conduct the population estimates. All fish captured on the mark run were given a caudal fin punch for identification on the recapture run, measured to the nearest millimeter, and returned to the river. On the recapture run, fish were examined for the presence of a caudal fin punch, measured to the nearest millimeter, and weighed to the nearest gram. Population estimates were calculated using the Lincoln-Peterson estimator with a Bailey (1951) modification, which accounted for fish being returned to the population following examination of marks on the recapture run, making them potentially available for subsequent recapture.

An estimated 8,157 (± 407) adult Brown Trout were present in the Chimney Rock/Sheriff Ranch study section in 2018, nearly 2,100 less than 2017 (Fetherman and Schisler 2017). Overall, 2,184 (± 104) Brown Trout were present per mile in the study section, averaging 310 (± 52) mm total length (TL) and 299 (± 122) g. All age classes of Brown Trout were represented in the sample, including several juvenile (≤ 150 mm TL) Brown Trout, but the majority of the Brown Trout captured were age 3+ (Figure 4.2.1).

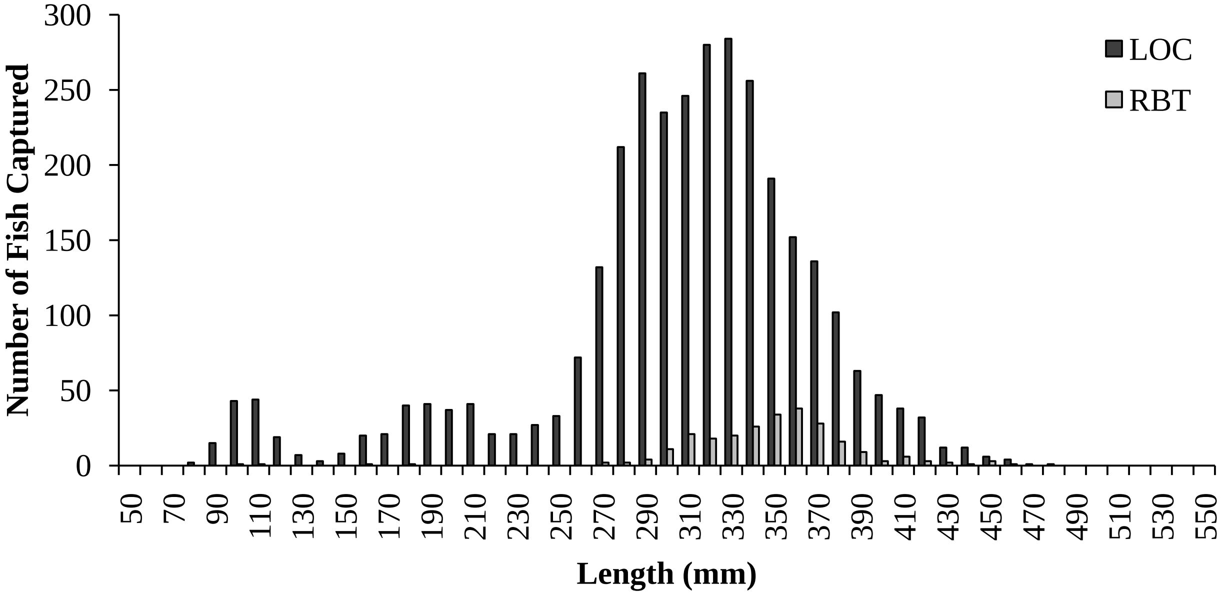

Figure 4.2.1. Number of Brown Trout (LOC) and Rainbow Trout (RBT) captured by total length (mm) during the 2018 adult salmonid population estimates in the Chimney Rock/Sheriff Ranch study section of the upper Colorado River.

Rainbow Trout densities decreased between 2017 and 2018, with an estimated 827 (± 138) adult Rainbow Trout present in the study section in 2017 (Fetherman and Schisler 2017), and 513 (± 65) present in 2018. Although the Rainbow Trout population in the upper Colorado River had exhibited an exponential increase in abundance since 2013, the lower survival rates exhibited by the Hofer (GR) fry (see Job No. 4, Action #3) resulted in fewer adult Rainbow Trout present in the study section, with an estimated 132 (± 17) present per mile in 2017 (Figure 4.2.2). Adult Rainbow Trout averaged 341 (± 43) mm TL and 404 (± 107) g, larger than the average size

Rainbow Trout encountered in 2017, likely a result of the high number of age 3+ Rainbow Trout captured during the 2018 population estimates, relative to the other age classes (Figure 4.2.3). Very few fish were captured less than 270 mm, suggesting that in addition to low fry survival, GR fish were not recruiting well to the adult population. To support this, age 2 fish (150-300 mm TL) were much less prevalent in the population than in previous years. The age 3+ Rainbow Trout population increased in 2018, and was larger than in previous years, suggesting that once fish reach age 2, survival and recruitment to age 3+ is high, although the majority of the fish in this age class are likely still H×C from previous fry stocking events in 2013-2015 (Figure 4.2.4).

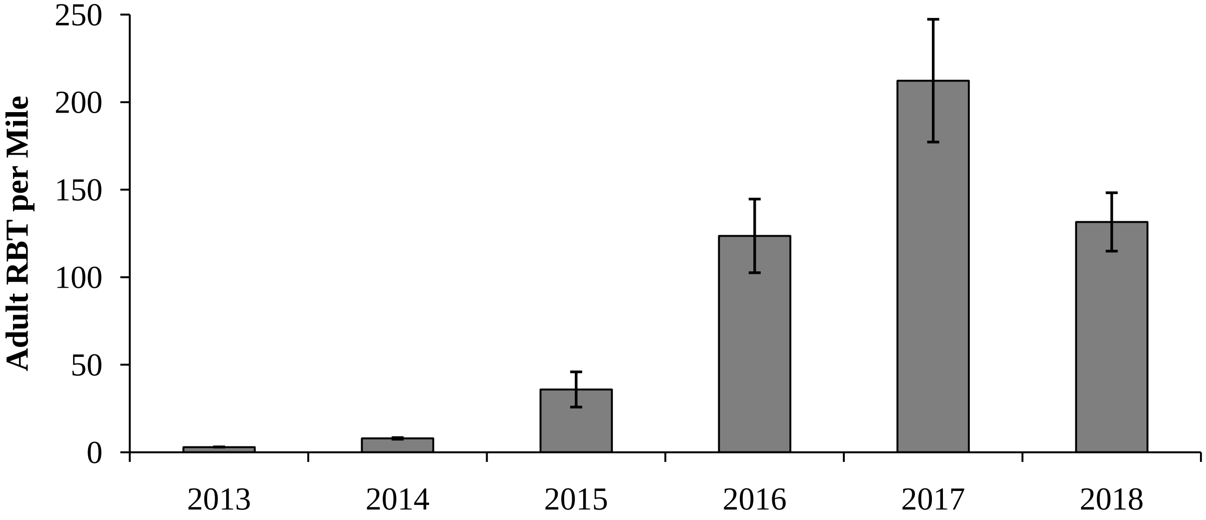

Figure 4.2.2. Estimated number of adult Rainbow Trout (RBT) per mile in the Chimney Rock/Sheriff Ranch study section of the upper Colorado River between 2013 and 2018.

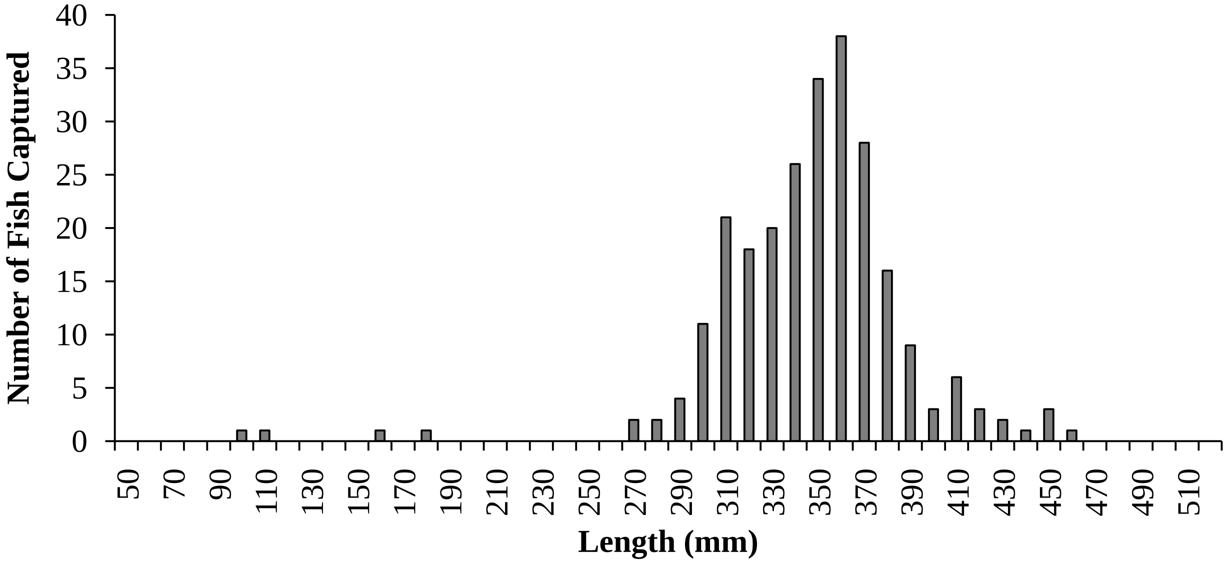

Figure 4.2.3. Number of Rainbow Trout (RBT) captured by total length (mm) during the 2018 adult salmonid population estimates in the Chimney Rock/Sheriff Ranch study section of the upper Colorado River.

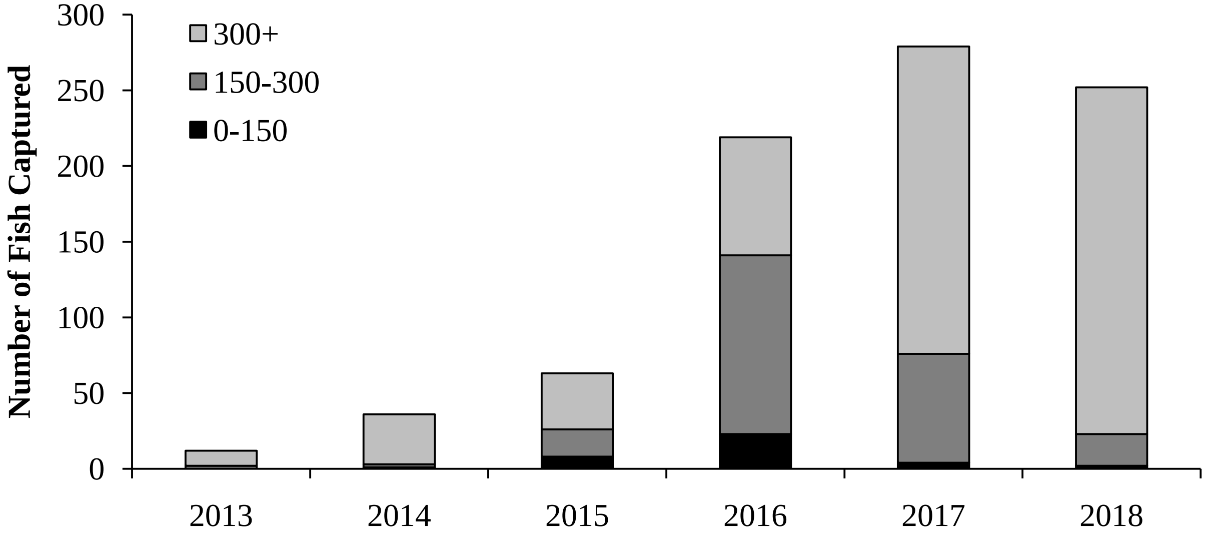

Figure 4.2.4. Number of age 1 (≤ 150 mm TL), age 2 (150-300 mm TL) and age 3+ (> 300 mm TL) Rainbow Trout (RBT) captured in the Chimney Rock/Sheriff Ranch study section of the upper Colorado River between 2013 and 2018.

The adult Rainbow Trout population in the upper Colorado River exhibited its first decline in abundance since Rainbow Trout fry stocking began in the river in 2013. The smaller age class of age 2 fish, along with the lower observed fry abundances following GR fry stocking (Job No. 4, Action #3), suggest that the GR fry do not survive as well as the H×C fry in the upper Colorado River. This is in contrast to results from Avila et al. (*In press*) suggesting similar survival rates between GR and H×C fry, however, that study was conducted in smaller streams with less competition and predation. Although the Brown Trout population declined in 2018, the 2017 abundance estimates for Brown Trout were higher than they had been in nearly two decades, which may have contributed to the lower survival rates in the GR fry stocked in 2017. GR fry will be stocked for a third and final year in 2018. The lower adult Brown Trout abundances may contribute to a higher survival rate in these fish, although the forecasted lower water year could increase predatory interactions between the two species. Additional fry sampling and N-mixture models will be used to determine the post-stocking fate of the GR fry in 2018, and determine if lower post-stocking survival rates, lower recruitment to consecutive age classes, or both are contributing to the decline in the adult Rainbow Trout population in the upper Colorado River.

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

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

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.

Action #3:

- *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*

Three-pass removal estimates for Rainbow Trout fry abundance, accomplished using two Smith-Root LR-24 backpack electrofishing units, will be conducted in the upper Colorado River in June, July, August, September, and October of 2017. Seven sites will be sampled, three on State Wildlife Areas below Byers Canyon, and four on the Chimney Rock/Sheriff Ranches upstream of Byers Canyon. All fry encountered will be measured and checked for signs of M. cerebralis infection. Fry abundance in the upper Colorado River is being estimated as part of a study designed to determine if stocking large numbers of Rainbow Trout fry is an effective management strategy for increasing the adult Rainbow Trout population through recruitment. Fry abundance estimates conducted in 2017 will be used to determine if fish from previous fry stocking events have recruited to the adult spawning population, are reproducing, and contributing offspring to the population.

Action #3 Accomplishments

The current phase of the Colorado River Rainbow Trout fry stocking evaluations began in 2013. In 2013, 2014, and 2015, the 3.9 mile stretch of the upper Colorado River between Hitching Post Bridge on the Chimney Rock Ranch and the Sheriff Ranch (Figure 4.3.1) was stocked with 100,000 to 250,000 Hofer by Colorado River (H×C) Rainbow Trout fry annually. Due to disease issues within Colorado hatcheries in late 2015, H×C Rainbow Trout fry were not available for stocking in 2016. Recent studies showed that the pure Hofer (GR) survives just as well as the H×C when stocked as fry into small streams (Avila et al. *In press*), but the survival of the GR had not been evaluated in a larger river. As such, approximately 60,000 GR fry were stocked by raft into this stretch of the upper Colorado River on July 13, 2016, and approximately 70,000 GR fry were stocked using the same methods on July 20, 2017. Two-thirds of the Rainbow Trout fry were loaded into large coolers supplied with a constant flow of oxygen on the stocking raft at the Hitching Post Bridge. Rainbow Trout were stocked in the margins on both sides of the river in the 0.8 mile stretch between Hitching Post Bridge and the upper extent of the Red Barn access road. The final third of the Rainbow Trout fry were loaded onto the raft from the Red Barn access road, and fry were similarly stocked on both sides of the river from this point to the irrigation diversion structure located at Red Barn (0.4 miles). No fish were stocked below the diversion structure as they had been in previous years (Fetherman and Schisler 2016) due to the lower number of fry available.

Pre-stocking fry population estimates were conducted at seven sites in the upper Colorado River two weeks prior to stocking the GR in July, and post-stocking fry population estimates were conducted at the end of July, August, September, and October 2017. Fry estimates completed prior to GR stocking provided information on the number of fry occurring from natural reproduction of both Rainbow Trout and Brown Trout, whereas the estimates completed at the end of July, August, September, and October provided information regarding the post-stocking survival of the GR fry and survival of wild Brown Trout fry. Although this current study is focused on the Chimney Rock/Sheriff Ranch study section, three reference sites below Byers Canyon were used to compare survival of wild fry to those of the stocked GR. Sampling sites (*n*

= 3) below Byers Canyon include the Kemp-Breeze, Lone Buck, and Paul Gilbert State Wildlife Areas. The Colorado River below Byers Canyon had been stocked with H×C fry between 2010 and 2015, but no fry were stocked in 2017 to allow evaluation of natural reproduction and determine if there was evidence for a self-sustaining Rainbow Trout population in this section of the river. Sampling sites (*n* = 4) in the Chimney Rock/Sheriff Ranch study section include the Sheriff Ranch, upper and lower Red Barn, and the Hitching Post Bridge (Figure 4.3.1), historical sites used to evaluate fry production and survival in this section.

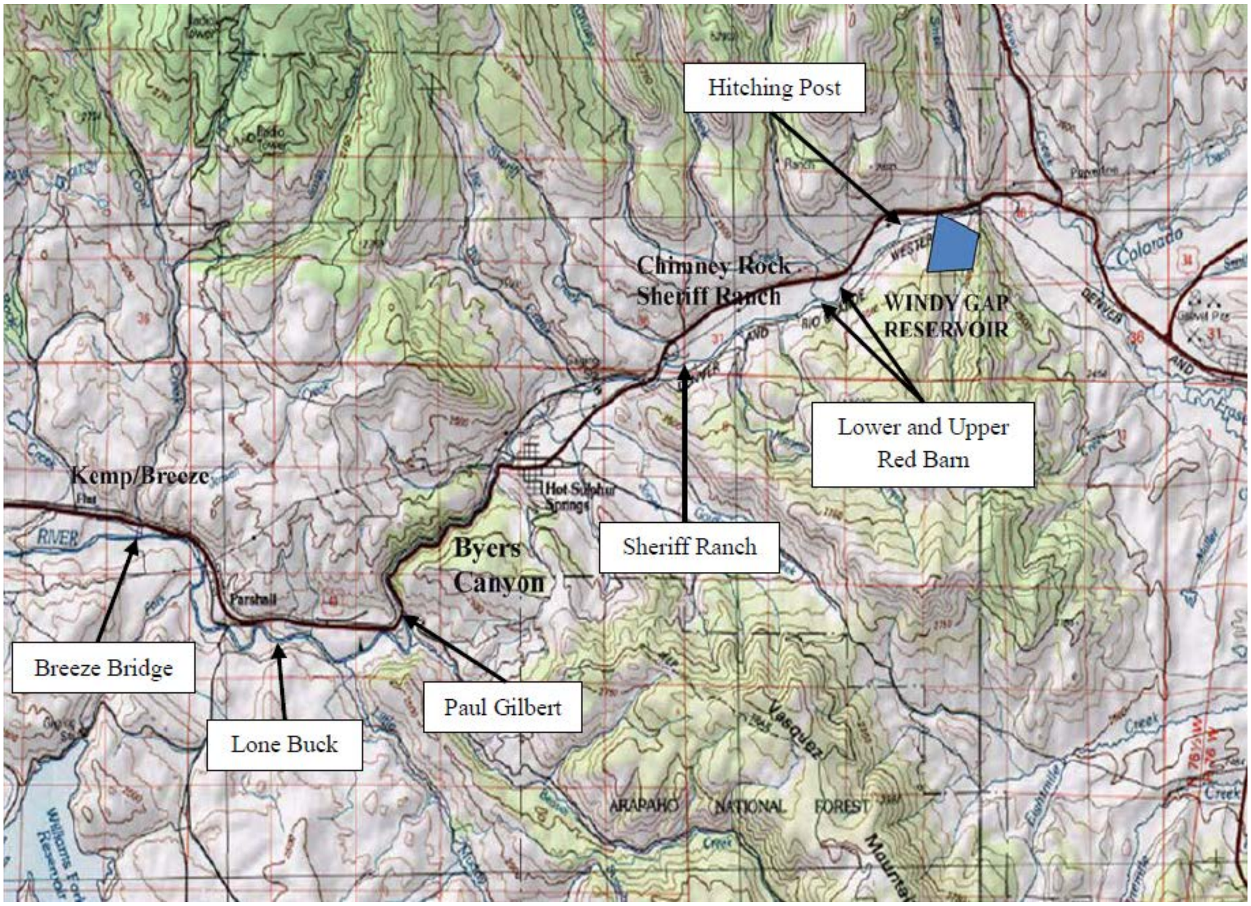

Figure 4.3.1. Map of the upper Colorado River study area showing the seven sites at which salmonid fry population estimates were conducted in July, August, September, and October 2017.

Salmonid fry estimates were accomplished using two Smith-Root LR-24 backpack electrofishing units running side-by-side to cover available fry habitat. Three passes were completed through each of the 50 foot long study sites, and fry were removed on each pass. All salmonid fry encountered were measured and returned to the site. In October 2017, genetic samples were taken from five Rainbow Trout fry at each site, and five Brown Trout and five Rainbow Trout were collected from each site to obtain myxospore counts. Myxospore enumeration was completed at the Aquatic Animal Health Laboratory (Brush, Colorado). Fry density estimates were calculated using the three-pass removal equations of Seber and Whale (1970).

Brown Trout fry densities were highest in early July, with densities reduced by half by the end of August, and an estimated 2,406 (± 354) Brown Trout fry per mile remaining in October (Figure 4.3.2). Wild Rainbow Trout fry densities below Byers Canyon were highest in late August (141 ± 77), but dropped to an estimated 70 (± 35) Rainbow Trout fry per mile in October. Pre-stocking, wild Rainbow Trout fry densities above Byers Canyon were similar to those below Byers Canyon (Figure 4.3.2). Rainbow Trout fry densities above Byers Canyon, which were composed mostly of stocked GR fry, peaked at the end of July. Densities dropped significantly between July and August, which is not unusual in the first month following fry stocking, although the decrease was larger than it had been in previous years when stocking H×C fry (Fetherman and Schisler 2015, 2016). Similar to Rainbow Trout fry density estimates in 2016 (Fetherman and Schisler 2017), Rainbow Trout fry densities were significantly lower than Brown Trout fry densities in August, September, and October. By the end of October, stocked Rainbow Trout fry densities above Byers Canyon did not differ from the wild fry densities Below Byers Canyon (Figure 4.3.2). Overall, Brown Trout and Rainbow Trout fry exhibited very similar patterns in density in 2016 and 2017 (Fetherman and Schisler 2017). However, when comparing GR and H×C fry abundances averaged over the first five years of fry stocking, the GR fry exhibited much lower survival rates than did the H×C fry (Figure 4.3.3).

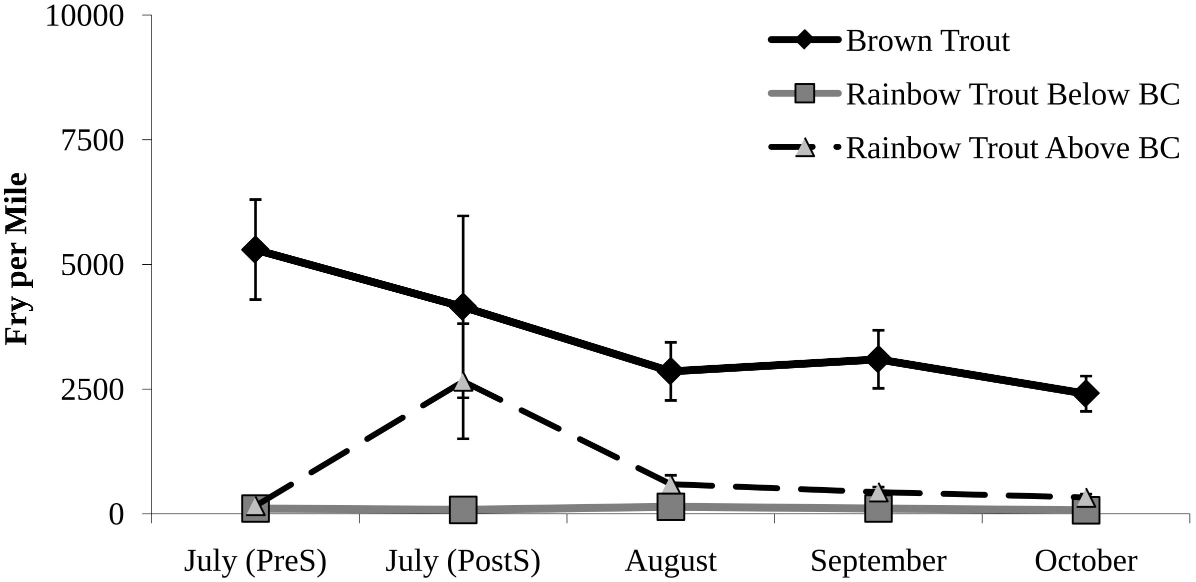

Figure 4.3.2. Upper Colorado River Brown Trout fry density estimates averaged across all seven sampling sites, and Rainbow Trout fry density estimates above and below Byers Canyon (BC; fry/mile; SE bars) for the July pre- and post-stocking (PreS and PostS) sampling occasions, as well as sampling occasions occurring at the end of August, September, and October 2017.

Myxospore counts for Brown Trout fry averaged 12,103 (± 4,436) myxospores per fish and was higher than myxospore counts observed in 2016 (Fetherman and Schisler 2017). Of the 35 Brown Trout collected, only one fish exhibited an opercular deformity, whereas no signs of disease were observed in the other 34 fish. Myxospore counts for Rainbow Trout fry averaged 2,471 (± 1,366) myxospores per fish, lower than in previous years (Fetherman and Schisler

2017). Disease signs were observed in 13% of the Rainbow Trout fry encountered in October 2017. Signs of disease in rainbow trout included opercular and spinal deformities, as well as exophthalmia.

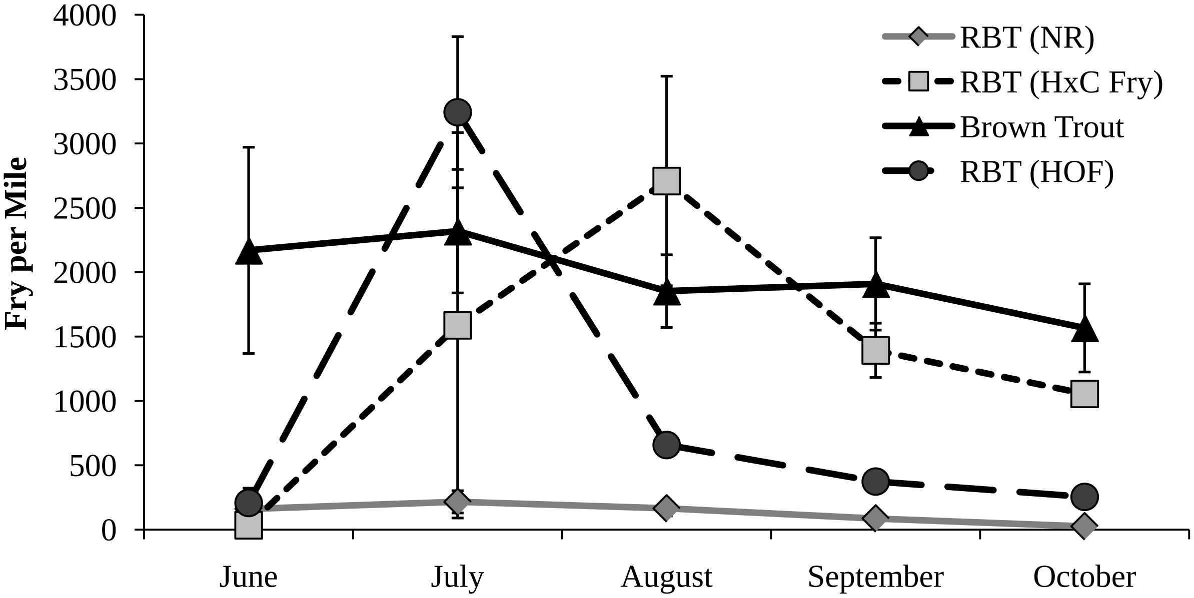

Figure 4.3.3. Upper Colorado River Brown Trout and wild Rainbow Trout (RBT [NR]) fry density estimates averaged between 2013 and 2017, H×C fry (RBT [H×C Fry]) density estimates averaged between 2013 and 2015, and GR fry (RBT [HOF]) density estimates averaged between 2016 and 2017 (fry/mile; SE bars).

The GR fry apparent survival in the upper Colorado River has been lower than expected given recent results suggesting that survival was similar between GR and H×C fry (Avila et al *In press*). Low water years in 2016 and 2017 and increased Brown Trout abundances in 2017 may have contributed to lower GR fry survival in the upper Colorado River. Alternatively, the GR fry may not survive as well in a large river situation compared to the small streams evaluated by Avila et al. (*In press*) for a number of reasons including reduced predator avoidance behaviors, selection of suboptimal habitats for feeding or growth, and reduced resilience to competitive interactions with Brown Trout fry. GR fry will be stocked and evaluated for a third and final year in 2018. Additional fry sampling and N-mixture models will be used to determine the post-stocking fate, as well as the distribution and habitat selection, of the GR fry in 2018.

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

Fetherman, E. R., and G. J. Schisler. 2015. Sport Fish Research Studies. Federal Aid Project F-394-R14. Federal Aid in Fish and Wildlife Restoration, Job Progress Report. Colorado Parks and Wildlife, Aquatic Wildlife Research Section. Fort Collins, Colorado.

Fetherman, E. R., and G. J. Schisler. 2016. Sport Fish Research Studies. Federal Aid Project F-394-R15. Federal Aid in Fish and Wildlife Restoration, Job Progress Report. Colorado Parks and Wildlife, Aquatic Wildlife Research Section. Fort Collins, Colorado.

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.

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

Action #4:

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

Genetic analyses will be completed by the Genomic Variation Lab at the University of California Davis using single nucleotide polymorphisms (SNPs) to determine the proportion of Hofer genetic markers in each individual. Data from these analyses will be used to assess long-term genetic trends in Rainbow Trout populations established using M. cerebralis-resistant Rainbow Trout.

Action #4 Accomplishments

Genetic sample processing and analysis was recently completed for two major projects examining the genetic contribution of *Myxobolus cerebralis*-resistant Rainbow Trout in wild Rainbow Trout populations. The first project focuses on the genetics of wild Rainbow Trout populations established using *M. cerebralis*-resistant Rainbow Trout strains and crosses. The objective of this project is to examine patterns in Rainbow Trout population genetics over time, looking specifically for changes in population genetic composition due to stocking and/or forms of selection. Genetic data will be compared with stocking records, known genetic composition of fish being stocked (baseline genetic data collections from Colorado hatcheries), and changes in genetic composition over time. The project focuses primarily on the Colorado River and Gunnison River, but also includes data from other rivers where similar patterns have been observed. Approximately 1,800 samples are included in this project.

Recently, genetic analyses have suggest that fish that express mainly Colorado River Rainbow (CRR) or other wild Rainbow Trout genes are surviving better than those that are high-proportion Hofer (GR). Due to repeated sampling, we can look at these changes not only over years, but also within a year for fry populations, and across different locations throughout the river system. In addition, genetic data will be paired with myxospore counts and other disease metrics to show how disease resistance has changed over time. Genetic data collected from the upper Colorado River fry populations (see Job No. 4, Action #3) in 2012 is used as an example of how we plan on using this data to explain changes in genetic composition over time.

H×Cs were first stocked into the upper Colorado River study section on the Chimney Rock and Sheriff Ranches beginning in 2006, with additional introductions occurring in 2009 and 2010.

Although survival of these stocked catchable-size H×Cs was low, they did reproduce, and an increasingly larger proportion of the fry population was classified as GR-cross fish naturally produced by the stocked H×Cs (Fetherman et al. 2014; Figure 4.4.1). Along with an increase in GR-cross fry, average myxospores per fish in the fry collected from the river in October decreased from 45,036 (± 8,650) in 2009 to 2,672 (± 4,379) in 2011 (Fetherman et al. 2014).

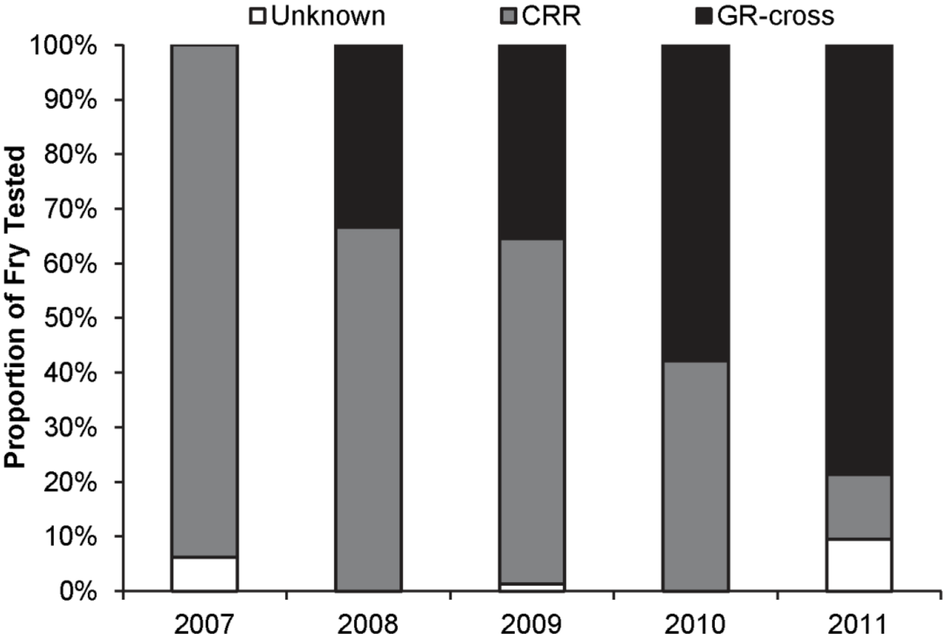

Figure 4.4.1. Proportion of the wild Rainbow Trout fry population assigned as Colorado River Rainbow (CRR), Hofer-cross (GR-cross) or unknown in the upper Colorado River between 2007 and 2011 (figure from Fetherman et al. 2014).

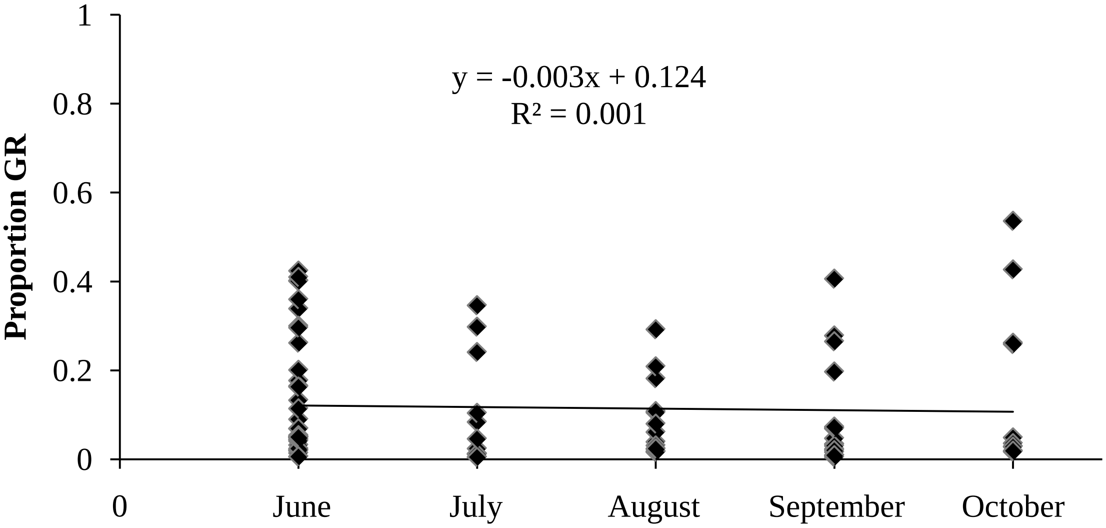

Figure 4.4.2. Proportion of GR genes in wild Rainbow Trout fry collected from the upper Colorado River on the Chimney Rock and Sheriff Ranches between June and October 2012.

Towards the end of this same time period, genetic data collected from wild Rainbow Trout fry in the Gunnison River suggested that within a year, a high proportion of the fry population was GR-cross shortly after emergence in July, but that the proportion shifted back towards the CRR by October, potentially a result of selection for a more wild-type fish that still had enough GR genetics to survive exposure to *M. cerebralis*. To determine if that same pattern was occurring in the upper Colorado River, samples collected from wild Rainbow Trout fry in June through October 2012 were plotted and a trend line applied to the data (Figure 4.4.2). Although the trend line has a rather poor fit due to the spread of the data ($R^2 = 0.001$), the slope of the line is slightly negative, suggesting that a similar trend was occurring in the upper Colorado River. Some outliers collected in September and October that exhibited a higher proportion GR than the majority of the other fry collected affected both the fit and slope of the trend line. Using data collected from wild Rainbow Trout throughout the upper Colorado River fry sites, and comparing the myxospore counts obtained from those fish, we found that as the proportion GR increases, average myxospores per fish decrease. Again, this trend holds despite a few outliers collected in the mid range of proportion GR that developed a high number of myxospores per fish (Figure 4.4.3). These types of trends and analyses will be conducted for all years of data from various rivers across the state to look at within year, across year, and statewide changes in wild Rainbow Trout genetics over time.

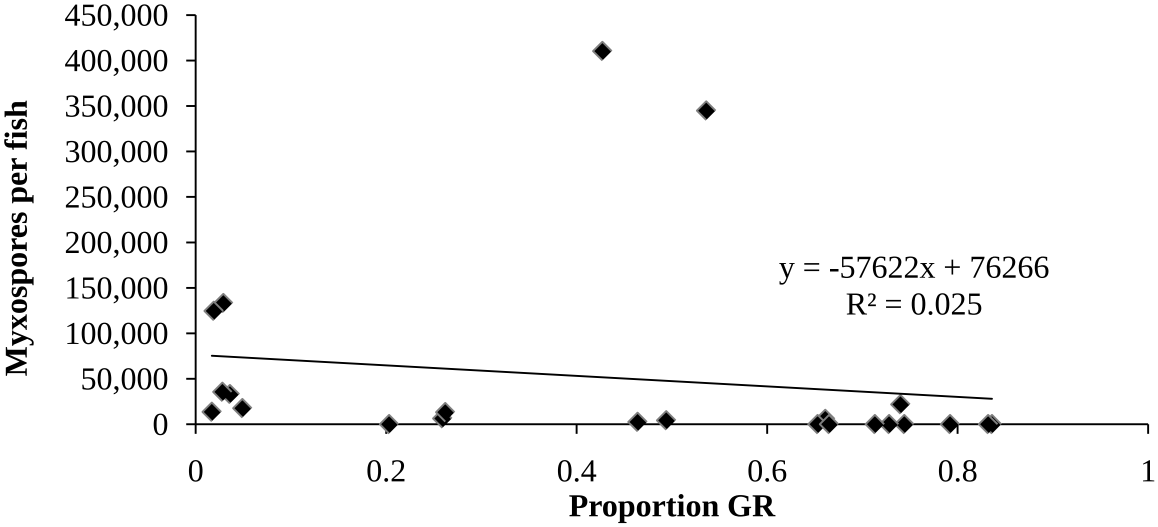

Figure 4.4.3. Average number of myxospores per fish as a function of proportion GR, by individual, for the wild Rainbow Trout fry collected from the upper Colorado River fry sites in October 2012.

The second project focuses on the development of wild, *M. cerebralis*-resistant Rainbow Trout brood stocks in Colorado, with a focus on two locations, the East Portal of the Gunnison River, and Harrison Creek and Catamount Lake near Steamboat Springs, Colorado. The Harrison Creek and Catamount Lake location is being used to develop a wild brood stock of H×Hs which can be used to supplement and/or recover hatchery populations of H×Hs, if needed. H×Hs have been stocked primarily into Catamount Lake since 2010, and genetic samples have been collected during the spawning period in April and May opportunistically since the start of this project (Figure 4.4.4).

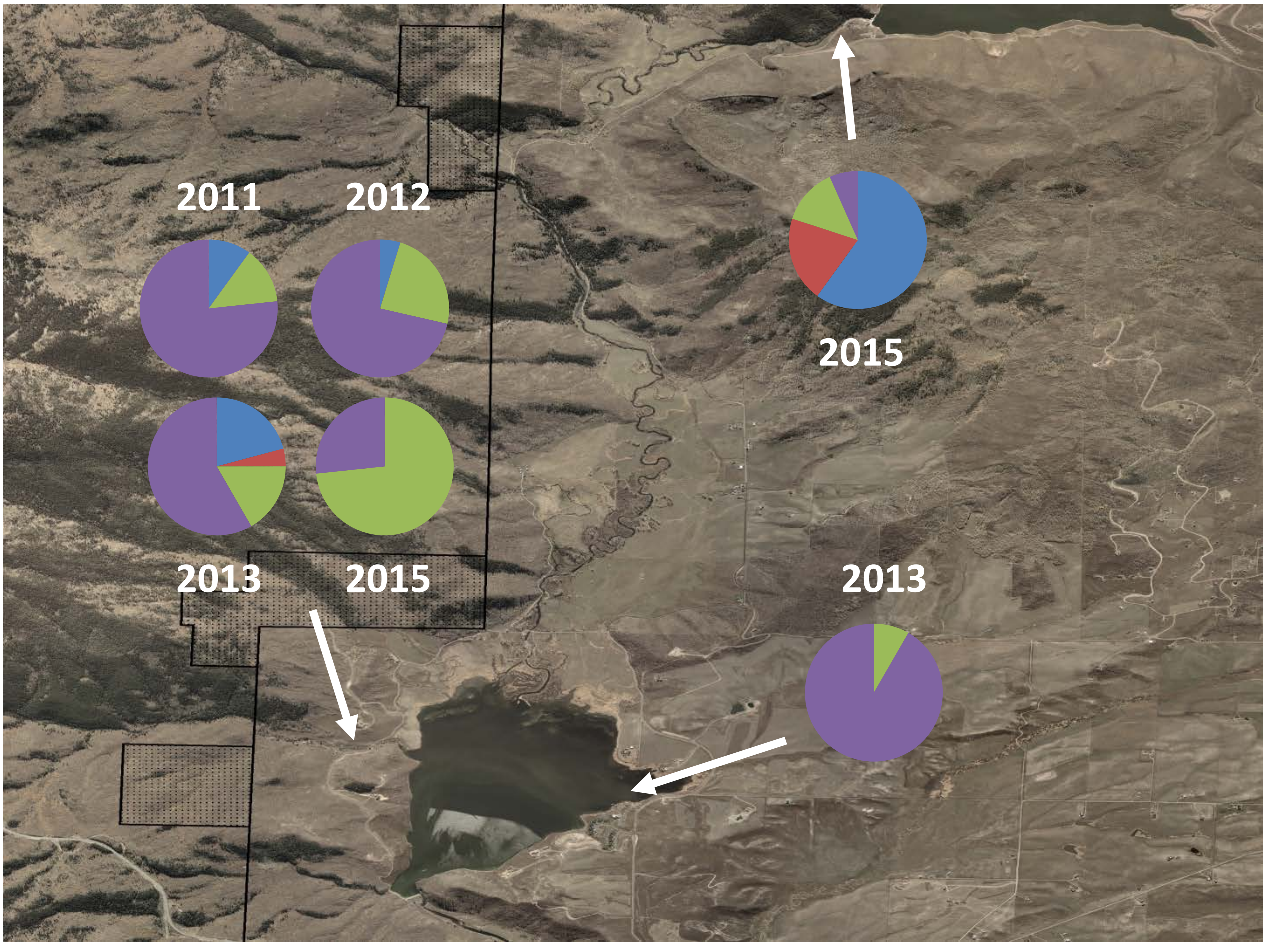

Figure 4.4.4. Map of the Yampa River, Catamount Lake, and Harrison Creek near Steamboat Springs, Colorado. Pie charts show the percentage of fish that contain a low-proportion GR genes (0-25; blue), a mid-range proportion of GR genes (25-50 [red] and 50-75 [green]), or a high-proportion GR genes (75-100; purple), by year and location.

Genetic results collected from fish captured in trap nets near the boat ramp (primary lake stocking location) suggest that the fish in Lake Catamount maintained a high proportion GR since they began being stocked in 2010. Although high proportion GR fish were collected from Harrison Creek in 2011, 2012, and 2013, lower proportion GR fish were collected from the creek in 2015 (Figure 4.4.4). This is likely a result of spawning between high-proportion GR fish in Harrison Creek resulting in a lower proportion of GR genes in offspring that started returning to the creek to spawn in 2015. Because Harrison Creek is intended to be the primary spawn take location for the brood stock, a high-proportion GR H×H population is desired. High-proportion GR fish are now stocked as fingerlings in Harrison Creek rather than Lake Catamount every year to increase the proportion GR in the population of fish returning to Harrison Creek to spawn. Genetic samples collected in 2015 from the Stagecoach Reservoir tailwater suggested that high-proportion GR fish stocked in Catamount Lake were not distributing throughout the entire river as previously thought, with much lower proportion GR fish found in the tailwater than what had been represented in Harrison Creek and Catamount Lake in that and previous years. These results partially led to the inception of the Yampa River comparative survival experiment (see

Job No. 4, Action #6) in which high-proportion GR fish were stocked throughout the entire Yampa River from the Stagecoach Reservoir tailwater to just upstream of Catamount Lake.

In addition to wild samples collected from both brood stock locations, which will be examined for patterns over time and space as part of this genetic analysis, exposure experiments were also conducted using eggs collected from both brood stock locations. Similar to the analysis described above looking at the effect of proportion GR on myxospore counts from the upper Colorado River fry population, data from these exposure experiments, along with the genetic data collected from the adults and offspring used in these experiments, will be used to examine how and when eggs should be collected from brood stock locations, how resistance is passed on and maintained in these populations, and to inform management decisions regarding the use of these brood stocks in the future.

Fetherman, E. R., D. L. Winkelman, M. R. Baerwald, and G. J. Schisler. 2014. Survival and reproduction of *Myxobolus cerebralis* resistant Rainbow Trout in the Colorado River and increased survival of age-0 progeny. PLoS ONE 9(5):e96954.

Action #5:

- *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*

Hofer by Colorado River Rainbow (H×C) fry have been stocked in the upper Colorado River, and survival and recruitment has resulted in increasing adult Rainbow Trout populations in several locations. Previous laboratory work suggested that there was little difference in physiological performance between H×C and pure Hofer Rainbow Trout, suggesting that stocking pure Hofer fry may be a viable management option. Recently, a graduate student (Brian Avila) from Colorado State University evaluated the survival and recruitment to age-1 of pure Hofer and H×C Rainbow Trout stocked as fry in three tributaries each of the Cache la Poudre, Colorado, and South Platte Rivers. H×C were coded wire tagged prior to stocking so that the two strains could be easily identified during field sampling. Results suggested that there was no difference in short-term (two month), overwinter, or annual survival rates between the Hofer and H×C, and indicated that both strains continued to persist in eight of the nine streams stocked one year post-stocking. Three pass removal estimates, accomplished using three Smith-Root LR-24 backpack electrofishing units, will be conducted in July/August 2017, and will be used to determine if both strains continue to persist in these streams two years after being stocked, as well as to evaluate growth differences between the strains. Additionally, if Rainbow Trout have spawned, estimates will be conducted to assess fry abundance, and genetic samples will be collected to determine which of the strains successfully spawned in each tributary.

Action #5 Accomplishments

Avila et al. (*In press*) showed that similar survival rates were obtained for the pure Hofer (GR) and Hofer by Colorado River Rainbow (H×C) when stocked as fry into nine streams throughout Colorado, and into laboratory mesocosms with a Brown Trout predator. However, the ability, especially of the GR, to survive, recruit, and potentially reproduce in these streams was unknown. To determine if the GR, H×C, or both continued to persist in the streams, two pass removal estimates were conducted in one site (220 ft, on average) within eight streams located in three major river drainages in July/August 2017: 1) Sheep Creek (Cache la Poudre), 2) North Fork of the Cache la Poudre River (Cache la Poudre), 3) Lone Pine Creek (Cache la Poudre), 4) Willow Creek (Colorado), 5) Spielberg Creek (Colorado), 6) Rock Creek (Colorado), 7) Tarryall Creek (South Platte), and 8) Michigan Creek (South Platte). One stream included in the original field experiment, Jefferson Creek (South Platte), was not sampled in 2017 because Rainbow Trout were not encountered in August 2015 and were thought to no longer be present in the stream. Population estimates were accomplished using two to three LR-24 backpack electrofishing units, depending on stream width. Fish from each pass were maintained in separate net pens until both passes were completed, at which time fish were measured, weighed, and returned to the creek within the site. Rainbow Trout encountered during the population estimates were scanned with a metal detector to determine presence (H×C) or absence (GR) of coded wire tags. Density estimates were calculated using a two pass removal estimator. Although several species of fish were encountered across the eight streams, density estimates were only calculated for salmonids (Table 4.5.1).

In addition to the population estimates obtained from the sampling sites, between 0.5 and 1 mile of each stream was shocked using a single pass to look for the presence of Rainbow Trout. When Rainbow Trout were encountered, the fish were scanned with a metal detector to determine presence (H×C) or absence (GR) of coded wire tags, measured, weighed, and returned to the river. Counts were maintained for other salmonids captured within the reach, but are not presented here. Given that Rainbow Trout were not previously present in any of these streams, if Rainbow Trout fry or juveniles were captured, it was considered evidence of reproduction from one of the two stocked strains. To verify which strain reproduced, genetic samples were taken from all fry and juvenile Rainbow Trout encountered.

Only one H×C was found within one of the population estimation sites in Michigan Creek, resulting in an estimate of 25 H×C per mile. During the single pass presence/absence shocking, Rainbow Trout stocked in 2014 were found in seven of the eight streams, with Sheep Creek being the only stream in which Rainbow Trout were not found. Overall, 12 GR and 12 H×C were found across the seven creeks, and both H×C and GR were found in six of the seven creeks. Rock Creek was the only creek in which both strains were not found, with two GR persisting in the creek. GR averaged 299 mm total length (TL) and 267.8 g, whereas the H×C averaged 309.5 mm TL and 301.9 g.

Evidence of reproduction was found in two creeks, Spielberg Creek and Lone Pine Creek. In Spielberg Creek, one Rainbow Trout fry (50 mm TL, 0.5 g) was found during the single pass presence/absence shocking. The proportion of GR in this fish was 0.275, suggesting it was a result of H×C reproduction. In Lone Pine Creek, three juvenile Rainbow Trout, averaging 233.3 mm TL and 127.6 g, and five Rainbow Trout fry, averaging 67.6 mm TL and 3.5 g, were found during the single pass presence/absence shocking. Genetic testing showed that the juvenile Rainbow Trout had an average proportion GR of 0.15 ± 0.09, and the Rainbow Trout fry had an average proportion GR of 0.19 ± 0.02. Given these proportions, it is most likely that all eight of these fish originated from H×C reproduction since the proportion GR was well outside of the range for known GR fish (Fetherman and Schisler 2017). Additionally, since the proportion GR was similar between the two age classes, it is likely that both age classes were first generation offspring from the H×C stocked during this experiment in 2014.

Table 4.5.1. Density estimates (fish per mile), length, and weight (± SE) for Brown Trout (LOC), Brook Trout (BRK), and the GR and H×C Rainbow Trout strains for each stream sampled in July/August 2017.

	LOC	**BRK**	**GR**	**HXC**
		Sheep Creek		
Density	2160 ± 7916	1764 ± 233	0	0
Length	131.2 ± 16.1	101. ± 6.6	NA	NA
Weight	39.3 ± 10.2	18.5 ± 3.1	NA	NA
		North Fork of the Cache la Poudre River		
Density	2432 ± 354	0	0	0
Length	99.7 ± 9.1	NA	NA	NA
Weight	36.6 ± 10.6	NA	NA	NA
		Lone Pine Creek		
Density	2352 ± 294	0	0	0
Length	119.8 ± 6.6	NA	NA	NA
Weight	31.8 ± 6.2	NA	NA	NA
		Willow Creek		
Density	Inestimable	388 ± 409	0	0
Length	106.5 ± 27.6	119.7 ± 17.8	NA	NA
Weight	28.1 ± 18.1	27.3 ± 9.0	NA	NA
		Spielberg Creek		
Density	563 ± 47	0	0	0
Length	135.8 ± 19.7	NA	NA	NA
Weight	80.8 ± 30.2	NA	NA	NA
		Rock Creek		
Density	1630 ± 93	517 ± 183	0	0
Length	109.5 ± 7.7	85.5 ± 10.8	NA	NA
Weight	46.6 ± 6.7	21.6 ± 7.0	NA	NA
		Tarryall Creek		
Density	2380 ± 115	24 ± 0	0	0
Length	140.9 ± 7.5	245 ± 0.0	NA	NA
Weight	52.9 ± 7.5	124 ± 0.0	NA	NA
		Michigan Creek		
Density	3878 ± 68	530 ± 27	0	25 ± 0
Length	155.1 ± 6.7	186.0 ± 12.7	NA	260 ± 0.0
Weight	76.5 ± 8.2	95.5 ± 11.3	NA	199 ± 0.0

Avila (2016) showed that average stream temperature had an effect on Rainbow Trout survival. The results of these continued shocking events suggest that average stream temperature can also affect sexual maturity and reproduction in the H×Cs. Lone Pine Creek is the lowest elevation and warmest stream, on average, included in this experiment. Water temperatures in Lone Pine

Creek likely accelerated sexual maturity in the H×Cs allowing them to spawn not only once, but twice, since being stocked in 2014. Spielberg Creek is a mid-elevation stream relative to the others included in the experiment, and the discovery of a single Rainbow Trout fry in this creek suggests that this was the first year the H×Cs spawned in this creek. If reproduction were to occur in higher elevation, cooler streams in which Rainbow Trout were still present, such as Tarryall and Michigan Creek, it is likely that this would not occur for the first time until 2018. Unfortunately, the ability of the GR strain to reproduce in the wild remains largely unknown. Although Nehring (2014) showed that the GR can survive and reproduce in a pond setting, there is still no evidence that the same can occur in a stream environment. However, continued collection of genetic samples from wild Rainbow Trout fry in the upper Colorado River, where the GR have been introduced as fry for the last two years (see Job No. 4, Action #2, #3), could show that the GR are capable of reproducing in a riverine environment.

Avila, B. W. 2016. Survival of Rainbow Trout fry in the wild: A comparison of two whirling disease resistant strains. Master's Thesis, Colorado State University, Fort Collins, Colorado.

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

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.

Nehring, R. B. 2014. Fishery management interventions to reduce *Myxobolus cerebralis* infections in fish ponds on the Cap K Ranch (2000-2013): A final report. Colorado Parks and Wildlife, Aquatic Research Section. Montrose, Colorado.

Action #6:

- *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 Springs, 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 will compare 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 #6 Accomplishments

The following describes the motivation, experimental design, methods, and first year results of a four year comparative survival experiment being conducted in the Yampa River between Stagecoach Reservoir and Lake Catamount.

Motivation

Hofer (GR) by Harrison Lake (HL) Rainbow Trout *Oncorhynchus mykiss* crosses (i.e., H×H) have been stocked into Lake Catamount, Harrison Creek (tributary to Lake Catamount), and the Yampa River and tributaries between Lake Catamount and Stagecoach Reservoir (Steamboat Springs, CO) since 2007, with the objectives of reducing *Myxobolus cerebralis* infection levels within the Yampa River and establishing a wild H×H Rainbow Trout brood stock in Lake Catamount. Reintroduction of H×H Rainbow Trout to the system followed a population-level collapse of the Rainbow Trout fishery, evident by 2006, caused by the introduction and establishment of *M. cerebralis*. Annual electrofishing surveys conducted in the fall of 2006 revealed four missing age classes within the Rainbow Trout population. The remaining low numbers of senescent fish would have been lost by the following spring, and the population would have crashed without additional supplementation. Maintaining wild brood stocks of whirling disease-resistant Rainbow Trout in whirling disease-endemic locations is important for several reasons. First, fish are reared in wild environments while simultaneously experiencing disease pressure. As such, fish that survive long-term exposure to whirling disease and continue to maintain resistant genotypes, while exhibiting characteristics necessary for continued survival in lake and stream environments, are retained in the system and contribute to future generations through natural and artificial spawning events. Periodically introducing offspring from wild brood stocks back into hatchery environments can prevent the loss, via artificial selection, of both disease resistance and wild characteristics in hatchery populations. Additionally, recent concerns with disease resulting in losses of year classes or entire brood stocks in Colorado hatcheries have elucidated the importance of maintaining sources of whirling disease-resistant Rainbow Trout that can be used to re-establish hatchery brood stocks, when necessary.

Laboratory exposure experiments conducted in 2013 demonstrated that many of the H×H families created using fish from Harrison Creek maintained their resistance to whirling disease. However, family groups were created using pooled egg groups from two unique male-female pairs for this experiment. As such, variability in the adult genetics resulted in variability in the fry genetics, and a wide range of myxospore counts among family groups. Genetic results suggested that high proportion GR family groups had low myxospore counts, whereas family groups in which the proportion GR was lower had higher and more variable myxospore counts. Pooling of the groups made it difficult to predict the genetic and resistance outcomes of the progeny (Fetherman and Schisler 2015). One option to ensure only resistant offspring are being incorporated into hatchery brood stocks during future egg takes is to maintain and rear unique male-female pairs until the genetic composition of the adults can be ascertained. However, this option is both costly and logistically challenging. Another option is to continue to stock high-proportion GR or pure GR fish to reduce the proportion of susceptible fish in the population. Sampling in 2015 showed that a large portion of the fish returning to spawn in Harrison Creek were high proportion GR fish, however, in other locations, such as in the Stagecoach Reservoir tailwater, lower proportion GR persisted in the population and had the potential to continue out-crossing with these fish (Job No. 4, Action #4). As such, only pure GR and/or high-proportion GR fish will be stocked during this experiment.

Stream restoration has become an increasingly popular management tool for improving fish habitat in order to increase fish biomass and density, fish size (quality), and improve overall habitat for all aquatic organisms. While there is evidence to suggest that restoration activities have helped increase fish biomass, density, and quality, as well as improve overall stream ecological function, there continues to be some debate over the efficacy of restoration to meet fisheries goals and controversy over the cost-effectiveness of restoration techniques. There also exists some doubt over the long-term sustainability of stream restoration treatments to function at a high performance level. Historically, many stream restoration activities have a relatively expensive price tag. There is a paucity of studies documenting the cost-benefits associated with stream restoration work compared to any gains in ecological function (particularly related to fisheries). This study is designed to investigate how Rainbow Trout survival changes in response to stocking size, habitat condition, and manipulation of competition and predation pressure through removal of Brown Trout *Salmo trutta*.

Although the effects of Brown Trout removal on Rainbow Trout survival have been found to be equivocal for locations in which Brown Trout were or were not removed from the Cache la Poudre River (Fetherman et al. 2015), Brown Trout were removed from a much shorter section of river than that in which the removal will occur in this study. Fetherman et al. (2015) suggested that given the differences in short-term survival and movement of Rainbow Trout in the Cache la Poudre River following Brown Trout removal, the effects may have been more apparent if the removal section had been longer and Brown Trout had not moved back into the section from other locations. As such, positive effects of Brown Trout removal on survival are expected for both sizes of Rainbow Trout stocked into the Yampa River given both the length and isolation, preventing recolonization, of the removal section.

Unique management issues continue to complicate our ability to maintain a resistant H×H brood stock in this location of the Yampa River. Portions of the river are in various states of degradation, ranging from degraded on the Service Creek State Wildlife Area and Bureau of Land Management (BLM) properties, and sections owned by the private sector, to recently restored reaches in the Stagecoach Reservoir tailwater (construction completed in 2013) and on a portion of the Yampa River through private land (construction completed in 2017). A variety of ownerships exist throughout this portion of the Yampa River, including private landowners, private fishing associations, and public land in the form of State Parks, State Wildlife Areas, and BLM (Figure 4.6.1). Different stocking strategies are needed to meet management goals and maintain stakeholder relations throughout the system. Currently, the two strategies being proposed for this study are the continued stocking of smaller H×H fingerlings and stocking larger (~ 14”) H×H catchables throughout the approximately seven miles of river, upstream of Catamount Reservoir, including portions of the private section. Initial landowner support and cooperation has been very positive.

Although not common, size-based comparative survival studies have been conducted for Lake Trout *Salvelinus namaycush* (Bronte et al. 2006; Gunn et al. 1987), Brown Trout (Hesthagen and Johnsen 1992), and a combination of Brook Trout *Salvelinus fontinalis*, Brown Trout, and Rainbow Trout (Mullan 1956). In general, these studies showed that larger fish exhibited higher survival rates than smaller fish. While initial survival rates of larger stocked fish may be higher, Godin et al. (1994) found low survival rates into the second year post-stocking in four British Columbia river systems. Some of these early studies relied on angler tag returns, with the possibility that anglers returned tags to a larger degree from larger trout than smaller trout (Mullan 1956).

This study focuses on generating estimates of survival using different techniques, such as electrofishing and Passive Integrated Transponder (PIT) tags, which will alleviate potential size bias from angler returns. Most of the aforementioned studies were conducted in lake or small stream environments, and the range in stocking sizes was smaller, and therefore not comparable to fish sizes we are using in this experiment. More commonly, the survival of these various sizes have been assessed independently for specific management situations. For example, Stuber et al. (1985) showed that stocked fingerling Rainbow Trout return-to-creel in Dillon Reservoir was only 4.8%, and survival was affected by both predation from resident Brown Trout and competition with Kokanee Salmon *Oncorhynchus nerka*. Conversely, stocked catchable Rainbow Trout have exhibited long-range movements and high levels of activity post-stocking, leading to low return rates and survival compared to resident fish (Bettinger and Bettoli 2002). More closely related to this study, Fetherman et al. (2014) showed that survival rates of whirling disease-resistant Rainbow Trout stocked as subcatchables in the Colorado River, over a five year period, were only 0.007. However, survival of stocked whirling disease-resistant Rainbow trout fry appears to increase over that of subcatchables, and fish stocked as fry in the Colorado River are recruiting to the adult population (Fetherman and Schisler 2017). In addition, pure GR fry have exhibited similar post-stocking survival rates as lower proportion GR fry when stocked into small streams (Avila et al. *In press*). Several prior comparative survival studies (Godin et al.1994; Meyer et al. 2012) have focused on impacts of stocking hatchery Rainbow Trout strains on top of existing wild populations. Given the persistent whirling disease infection within our study system, the existing population was founded on stocking GR genetics. As such, the current re-established wild population is made up of the same variety of fish being stocked in this study.

The primary goal of this study is to evaluate survival of H×H Rainbow Trout in the Yampa River through a range of habitat conditions, manipulations of a resident Brown Trout population, and stocking strategies. As such, this project has three major objectives. The first is to determine if there is a length-specific effect on survival due to river habitat condition (restored versus impaired reaches). To accomplish this objective, the annual apparent survival rates of catchable and fingerling-size *M. cerebralis*-resistant Rainbow Trout will be estimated for fish stocked into both restored and impaired reaches of the Yampa River. The second objective of this study is to determine if large-scale Brown Trout removal will affect annual apparent survival rates of both catchable (competition) and fingerling (competition and predation) Rainbow Trout. To accomplish this objective, Brown Trout will be removed from the Yampa River on an annual basis during the study period. The third objective of this study is to determine if a reduced stocking density results in similar annual survival rates in fingerling Rainbow Trout, with potential implications for hatchery management. To meet this objective, the fingerling Rainbow Trout stocking density will be reduced in the third year of the study to less than half of what had been stocked in the two years previous.

Site Description

The *M. cerebralis*-resistant Rainbow Trout comparative survival experiment will take place in the Yampa River between Stagecoach Reservoir dam and Lake Catamount. The total length of the riverine portion of the study section is approximately 7.7 miles. This section contains approximately 5 miles of private land, and approximately 2.7 miles of public land accessible via Stagecoach State Park, Service Creek State Wildlife Area, and BLM (Figure 4.6.1). Approximately 0.25 miles of the river has been restored in recent years within the State Park portion of the Stagecoach Reservoir tailwater. Additionally, in 2017, 2.0 miles of restoration work was completed on the main stem of the Yampa River, on the Green Creek Ranch located 0.5 to 2.5 miles upstream of Lake Catamount. Given the potential for downstream migration out of the river, current sampling events on Lake Catamount will continue throughout the study period and be used to update post-stocking survival estimates based on recovery location.

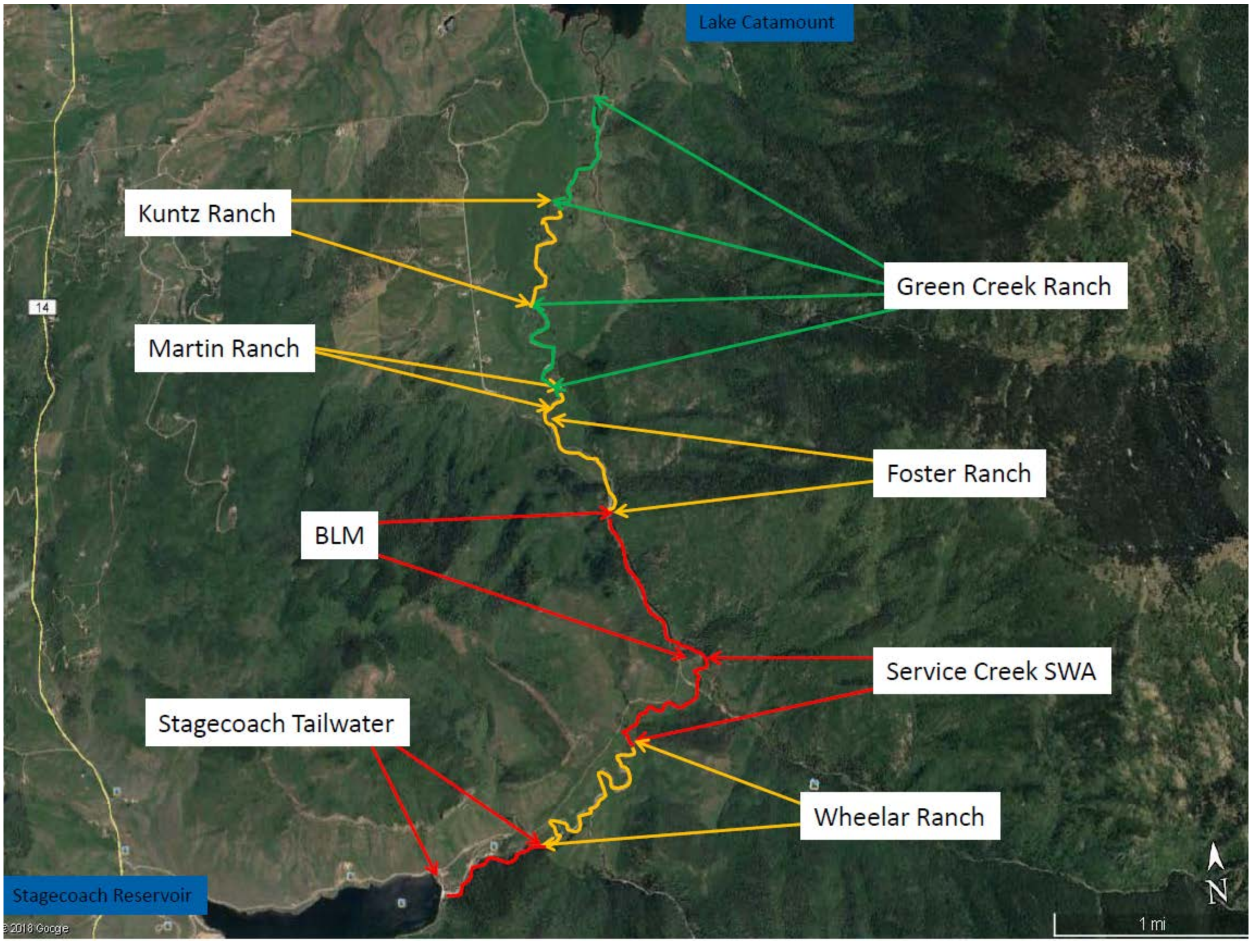

Figure 4.6.1. Map of the Yampa River (flowing north) between Stagecoach Reservoir on the upstream end of the study section, and Lake Catamount on the downstream end of the study section. Colors represent different landownership designations, with red indicating public land, and yellow and green indicating private land.

Experimental Methods for Year 1 of the Comparative Survival 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 381.6 (± 48.9) mm total length (TL) and 684.9 (± 180.7) g, were tagged with 32 mm PIT tags on May 11-12, 2017. All fish were anesthetized using CO_2, 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 two groups of 1,000 fish each during tagging, with known tag numbers in each group, for stocking above and below the Service Creek confluence with the Yampa River which roughly splits the study section in half. Both groups were secondarily fin clipped to identify stocking location in the event of tag loss, with a left pelvic clip for fish stocked above the Service Creek confluence, and a right pelvic clip for fish stocked below the confluence. PIT-tagged Rainbow Trout were stocked into the Yampa River on May 15, 2017. 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 12,093 Rainbow Trout, averaging 58.9 (± 6.1) mm TL and 2.3 (± 0.7) g, were tagged with coded wire tags (CWT) on May 17-18, 2017. All fish were anesthetized using tricaine methanesulfonate (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 six 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 5,548 fish were stocked above the Service Creek confluence, 1,102 in the Stagecoach Tailwater, 1,431 on the Wellar Ranch, and 3,015 on the Service Creek SWA between the Wellar Ranch and confluence with Service Creek, and 6,545 fish were stocked below the Service Creek confluence, 2,157 on the Service Creek SWA and BLM property, 1,732 on the Foster Ranch, and 2,656 on the Green Creek and Kuntz Ranches. CWT Rainbow Trout were stocked into the Yampa River study section on May 22, 2017, and evenly distributed throughout the study section in the same manor of the catchable Rainbow Trout stocked one week earlier.

Two five-electrode catrafts were used to complete the Rainbow Trout recapture events in fall 2017. The Foster Ranch, BLM property, Service Creek SWA, portions of the Wellar Ranch, and the Stagecoach Tailwater were sampled using a continuous single pass removal September 11-15, 2017. There was not enough time to sample the entire study section in a single week of sampling. As such, an additional continuous single pass removal was conducted on a 0.5 mile section of the Wellar Ranch on October 19, 2017. Additionally, the Green Creek Ranch was sampled using a continuous single pass removal using raft-mounted, throw-electrode electrofishing equipment to sample the deep holes formed by habitat restoration activities on November 2-3, 2017. All fish captured during the electrofishing efforts were removed from the river and held in net pens until all fish 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 fish with CWTs were additionally adipose fin clipped prior to release so that a unique encounter history could be created for all CWT fish on the next recapture event in 2018. Brown Trout from all sections with the exception of the Foster Ranch, Green Creek Ranch, and Kuntz 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, Green Creek Ranch, and Kuntz Ranch, as well as all other species encountered throughout the remainder of the study section, including Brook Trout, Mountain Whitefish *Prosopium williamsoni*, Mottled Sculpin *Cottus baridii*, and Speckled Dace *Rhinichthys osculus*, were measured, weighed, and returned to the section from which they were captured.

Two pass removal estimates were conducted in four standard sampling sites on the BLM property, Service Creek SWA, Wellar Ranch, and Stagecoach Tailwater to estimate the number of fish per mile in each section used to inform patterns of habitat use and estimate the percent of the Brown Trout population removed from each section. All fish captured were removed from the river and held in net pens by pass until they could be processed. PIT- or coded wire-tagged 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 standard sampling site, and standardized to number of fish per mile for comparison of abundance and habitat use. Additionally, the length of the entire study 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 two occasions, a release occasion ("1" for all fish released in spring 2017) and an encounter occasions ("1" for all fish encountered and "0" for all fish not encountered in fall 2017). 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 between the spring and fall 2017. Fish released above and below the Service Creek confluence with the Yampa River were treated as two separate groups in the analysis and survival and detection probability was estimated for each location. Because there was only one sampling occasion, detection and survival probabilities were confounded, and as such, are presented together as a single probability.

Results

Overall, 419 PIT-tagged rainbow trout were captured above the Service Creek confluence, and 391 PIT-tagged Rainbow Trout were captured below the Service Creek confluence. There was no difference in the survival/detection probability of the catchable Rainbow Trout between the two locations in which they were stocked in spring 2017, although catchable Rainbow Trout exhibited higher survival/detection probabilities than the fingerling Rainbow Trout stocked into the same locations (Figure 4.6.2). Eighty-nine CWT Rainbow Trout were captured below the Service Creek confluence, and 288 were captured above the Service Creek confluence. Fingerling Rainbow Trout survival/detection probability was higher above Service Creek than below. However, it has been documented that the GR-crosses move downstream shortly after being stocked (Fetherman and Schisler 2013), and it is possible that some of the fish stocked below the Service Creek confluence moved downstream into Lake Catamount where they could not be detected during the fall sampling efforts.

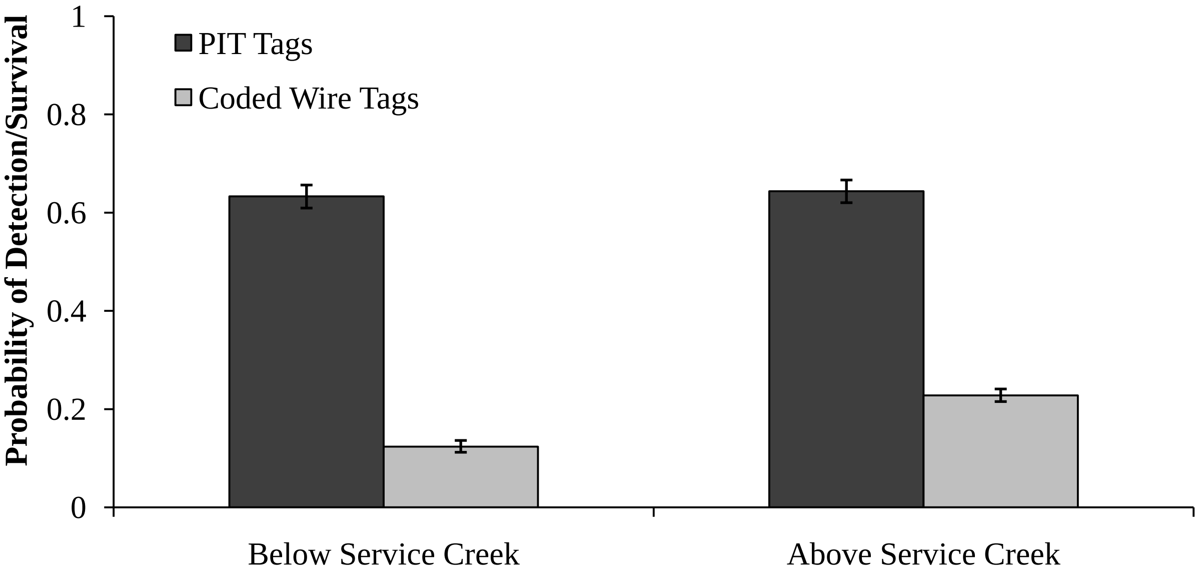

Figure 4.6.2. Survival/detection probabilities for PIT- and coded wire-tagged Rainbow Trout stocked in the Yampa River above and below the Service Creek confluence in spring 2017.

Figure 4.6.3. Change in weight of PIT-tagged Rainbow Trout stocked in public and private land sections of the Yampa River in spring 2017 and recaptured in fall 2017.

Coded wire-tagged Rainbow Trout grew well, averaging 186.1 (± 19.5) mm TL and 74.8 (± 22.8) g upon recapture in fall 2017. PIT-tagged fish growth appeared to be correlated with land use as well as fishing pressure, with fish stocked into publicly accessible locations losing weight between spring and fall 2017, and fish stocked on private land gaining weight over that same time period. In the Stagecoach Tailwater, which arguably receives the highest fishing pressure of the public use areas, PIT-tagged Rainbow trout lost twice as much weight as fish stocked into the Service Creek SWA and BLM sections (Figure 4.6.3).

Table 4.6.1. Estimated number of Brown Trout adults and juveniles [95% CIs] in the BLM, Service Creek SWA, Wellar Ranch, and Stagecoach Tailwater sections of the Yampa River given the estimated number of fish per mile (Table 4.6.2) and the length of the section, the number of adult and juvenile Brown Trout removed per section, and the percentage of the Brown Trout population that was removed and relocated in the Chuck Lewis SWA downstream of Lake Catamount.

	BLM	**Service Creek SWA**	**Wellar Ranch**	**Stagecoach Tailwater**
Estimated LOC Adults	419 [390, 448]	277 [238, 316]	446 [429, 464]	209 [198, 220]
Number Removed	334	332	515	169
% Removed	80 [75, 86]	120 [105, 140]	116 [111, 120]	81 [77, 85]
Estimated LOC Juveniles	77 [65, 89]	332 [218, 445]	9,505 [9,217, 9,794]	----
Number Removed	29	248	3,160	10
% Removed	38 [33, 45]	75 [56, 113]	33 [32, 34]	----
Estimated Total LOC	*496* *[455, 537]*	*609* *[456, 761]*	*9,951* *[9,646, 10,213]*	*209* *[198, 220]*
Total Removed	*363*	*580*	*3,675*	*179*
% Removed	*73* *[68, 80]*	*95* *[76, 127]*	*37* *[36, 38]*	*86* *[81, 90]*

A total of 4,797 Brown Trout were removed from the Yampa River and relocated to the Chuck Lewis SWA in fall 2017, 42% of the estimated 11,267 Brown Trout in the Yampa River. The percentage of the Brown Trout population removed from the BLM, Service Creek SWA, and Stagecoach Tailwater was relatively high (> 79%; Table 4.6.1). This is likely a result of the higher numbers of adult versus juvenile Brown Trout in these sections compared to the Wellar Ranch where only 37% of the Brown Trout population was removed. Juvenile Brown Trout were present on the Wellar Ranch in much higher numbers than in the other three locations. In addition, the Yampa River through the Wellar Ranch is much wider and contains more rooted vegetation than the other sections, which likely affected capture probability of the juvenile Brown Trout in this section of the River.

Table 4.6.2. 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 2017.

Species/Type	BLM	Service Creek SWA	Wellar Ranch	Stagecoach Tailwater
LOC (Adults)	419 [390, 448]	369 [317, 421]	446 [429, 464]	835 [790, 880]
LOC (Juv)	77 [65, 89]	442 [291, 593]	9,505 [9,217, 9,794]	----
RBT (Wild Adults)	295 [271, 319]	271 [220, 322]	257 [245, 269]	2,127 [2,085, 2,168]
RBT (Wild Juv)	524 [401, 647]	432 [281, 583]	1,451 [1,376, 1,525]	327 [302, 353]
RBT (CWT)	58 [41, 76]	125 [76, 175]	89 [75, 103]	604 [528, 680]
RBT (PIT-tagged)	95 [82, 108]	143 [133, 154]	151 [145, 157]	560 [540, 581]
RBT (Unknown)	10 [6, 13]	82 [72, 91]	48 [45, 52]	----
Mottled Sculpin	2,523 [2,412, 2,653]	1,935 [1,378, 2,492]	158 [137, 179]	----
Speckled Dace	10 [6, 14]	----	----	----
Mountain Whitefish	86 [71, 102]	411 [330, 492]	271 [243, 300]	14 [11, 17]
Brook Trout	166 [104, 228]	170 [112, 227]	117 [102, 132]	68 [62, 74]

Habitat assessments have not yet been completed for the Yampa River, but are planned for fall 2018. Each segment will be mapped using GPS topographic survey gear. Surveys will consist of longitudinal profiles, cross sections, and pebble counts. Longitudinal profiles 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 reaches. Cross sections will be used to compare average bankfull widths, average bankfull depths, average width to depth ratios, bankfull cross-sectional area, and average entrenchment ratios across all reaches. Pebble counts will be used to characterize bed materials, especially the percentage of fines in each of the reaches. A stage-discharge relationship will be generated to monitor the hydrology within reaches for the extent of the study period. Additional habitat assessments may be done to monitor riparian vegetation condition, concentration of large wood, presence of various cover types, conduct stream classification (stream and valley types), monitor active bank erosion, compare baseflow to bankfull discharge ratios, and measure the degree of vertical and lateral connectivity (related to bed incision or aggradation respectively). Historical land use and practices within the study segment will be researched in order to understand underlying causes of stream impairment documented through various habitat assessments.

Although formal habitat assessments have not yet occurred, population abundance estimates and length-frequency histograms can be used to provide an initial look at how the fish are distributed and what habitats are used by which age classes. For example, the BLM section appears to be good habitat for adult Brown Trout and juvenile wild Rainbow Trout. The deep pools in the Stagecoach Tailwater appear to support large numbers of adult Brown Trout and Rainbow Trout, as well as stocked PIT- and coded wire-tagged Rainbow Trout, but lower numbers of Rainbow Trout juveniles and no Brown Trout juveniles (Table 4.6.2). Although the Wellar Ranch is considered impaired with regard to adult salmonid habitats, the wide riffle and highly vegetative conditions appear to be good rearing habitats for juvenile salmonids (Table 4.6.2; Figure 4.6.4). Conversely, the newly constructed deep pools on the Green Creek Ranch appear to hold larger numbers of adult salmonids, and fewer numbers of juvenile salmonids (Figure 4.6.4). Formal habitat assessments conducted in fall 2018 will be used to identify specific habitat characteristics and stream forms with land use and level of impairment, and associate these characteristics with retention and survival of the various age classes of salmonids throughout the reach.

Discussion

The results from the first year of this study suggest that catchable Rainbow Trout exhibited higher survival rates initially than fingerling Rainbow Trout stocked in the Yampa River. This result is similar to what was observed by Godin et al. (1994), although larger fish exhibited lower survival rates in the second year of that study. The survival rates observed in this study are short-term (4 month) survival rates. It is possible that overwinter or runoff conditions have since affected the survival of the stocked Rainbow Trout. The second year of estimates will be more informative relative to the annual survival rates and potential for recruitment of these stocked fish. It is likely that the Brown Trout removal will also affect the survival rates of catchable and fingerling Rainbow Trout stocked in 2017 and 2018.

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 the pressure of high-density culture, and potential issues with disease that come with high-density culture, in Colorado hatcheries.

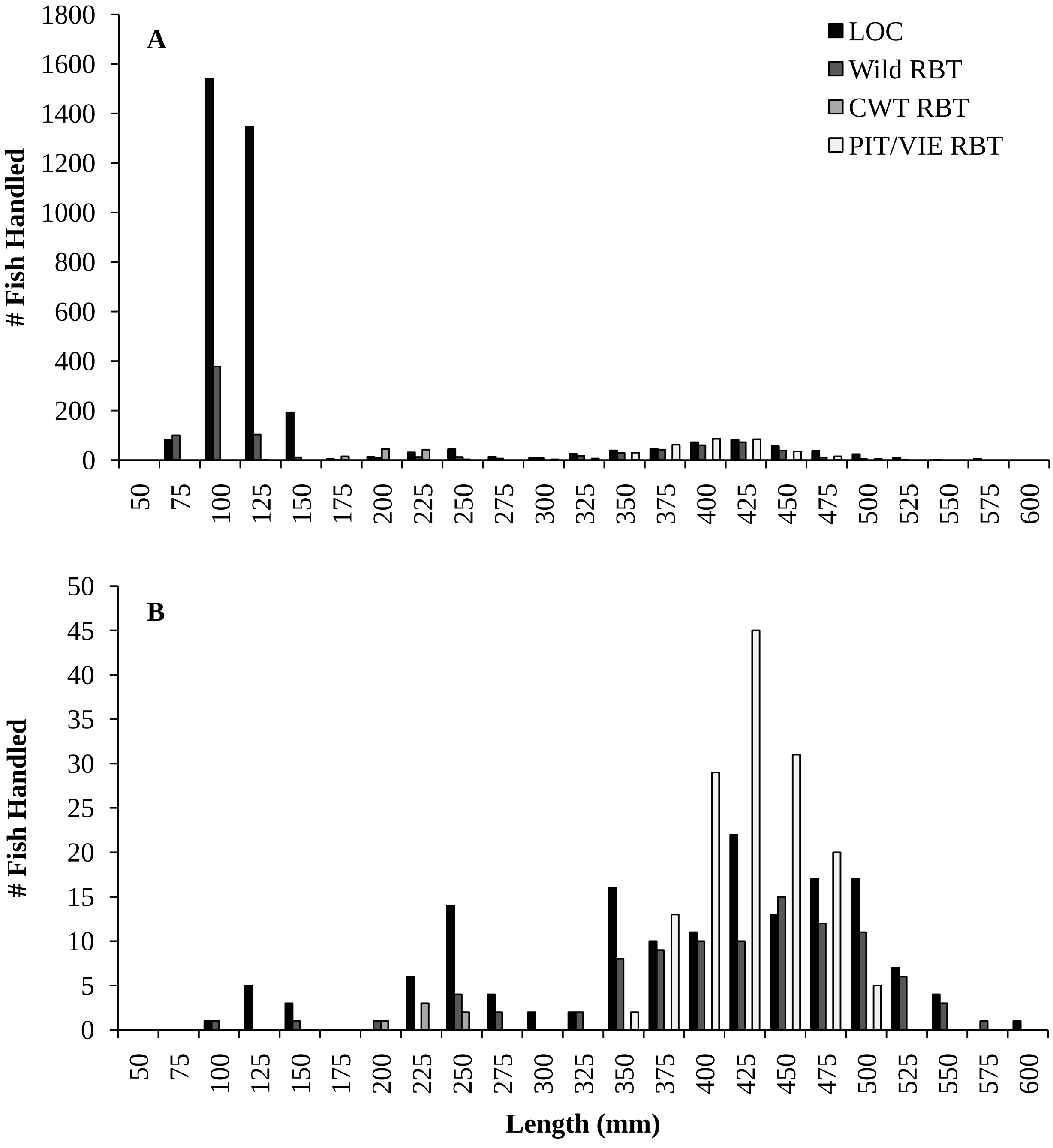

Figure 4.6.4. Length-frequency histograms for the Wellar Ranch (A) and Green Creek Ranch (B) showing the size class distribution of Brown Trout (LOC), and wild, PIT-tagged, VIE-tagged, and coded wire-tagged Rainbow Trout on the two ranches in fall 2017. Note the difference in scale on the y-axis.

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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, 2018.
2. Complete one peer review of a manuscript submitted to a scientific journal by June 30, 2018.

Approach

Action #1:

- *Level 1 Action Category: Technical Assistance*
- *Level 2 Action Strategy: Technical assistance*
- *Level 3 Action Activity: 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.

Action #1 Accomplishments

Internal presentations to CPW staff were used to update fishery managers on current research and to help inform management decisions regarding the stocking and use of *Myxobolus cerebralis*-resistant Rainbow Trout in Colorado waters. Two presentations were given at the CPW statewide aquatic biologist meeting:

- Atkinson, B., E. Fetherman, and M. Kondratieff. 2018. Comparative survival of whirling disease resistant catchable and fingerling Rainbow Trout stocked in restored versus impaired reaches of the Yampa River. Colorado Parks and Wildlife 2018 Aquatic Biologist Meeting. Gunnison, Colorado. January 16, 2018.
- Fetherman, E. R., and J. Ewert. 2018. Fry stocking in the upper Colorado River. Colorado Parks and Wildlife 2018 Aquatic Biologist Meeting. Gunnison, Colorado. January 17, 2018.

Stakeholders involved in CPW research projects appreciate being informed on current results and how everyone benefits from their continued involvement in a research project. A presentation regarding current results and research plans for 2018 was given to private landowners involved in the Rainbow Trout comparative survival experiment being conducted in the Yampa River (see Job No. 4, Action #6):

- Atkinson, B., E. Fetherman, and M. Kondratieff. 2018. Comparative survival of whirling disease resistant catchable and fingerling Rainbow Trout stocked in restored versus impaired reaches of the Yampa River. CPW meeting with Yampa River private landowners. Steamboat Springs, Colorado. May 14, 2018.

External presentations provided an opportunity to give research updates to fishery managers both within and outside of the state of Colorado. Four presentations were given at the Colorado Aquaculture Association meeting, a joint meeting of Colorado's public and private hatchery managers, the Colorado State University Cooperative Fish and Wildlife Research Unit Coordinating Committee Meeting, and chapter and division meetings of the American Fisheries Society:

- Fetherman, E. R., B. Neuschwanger, T. Davis, D. Karr, and C. Praamsma. 2018. Comparison of basic trout feeds for Rainbow Trout. 2018 Annual Meeting of the Colorado Aquaculture Association. Mt. Princeton Hot Springs Resort, Nathrop, Colorado. February 2, 2018.
- Avila, B. W., D. L. Winkelman, and E. R. Fetherman. 2018. Manipulating rearing density as a strategy for increasing survival of Rainbow Trout fry pre- and post-stocking. Colorado Cooperative Fish and Wildlife Research Unit Coordinating Committee Meeting. Fort Collins, Colorado. February 22, 2018.
- Avila, B. W., D. L. Winkelman, and E. R. Fetherman. 2018. Factors affecting survival of hatchery-reared Rainbow Trout fry in the wild. 2018 Annual Meeting of the Colorado/Wyoming Chapter of the American Fisheries Society. Laramie, Wyoming. March 1, 2018.

- Richer, E. E., E. R. Fetherman, and M. C. Kondratieff. 2018. Haunted rivers: Application of mobile RFID-GPS systems to evaluate the prevalence of ghost PIT tags. 43rd Annual Meeting of the Western Division of the American Fisheries Society. Anchorage, Alaska. May 24, 2018.

Symposia at professional meetings allow groups of professionals working on similar topics to come together and present and discuss their research to each other and other interested professionals. A symposium entitled "Fishing for solutions to economically and ecologically important salmonid diseases" was co-organized by CPW and Colorado State University at the Western Division of the American Fisheries Society annual meeting in Anchorage, Alaska. Five presentations were given as part of this symposium:

- Winkelman, D. L., and E. R. Fetherman. 2018. Introduction to the symposium on "Fishing for solutions to economically and ecologically important salmonid diseases". 43rd Annual Meeting of the Western Division of the American Fisheries Society. Anchorage, Alaska. May 24, 2018.
- Avila, B. W., D. L. Winkelman, and E. R. Fetherman. 2018. Whirling disease-resistant Rainbow Trout fry survival: A comparison of two strains. 43rd Annual Meeting of the Western Division of the American Fisheries Society. Anchorage, Alaska. May 24, 2018.
- Fetherman, E. R., B. W. Avila, and J. Ewert. 2018. Whirling disease-resistant Rainbow Trout fry stocking in the upper Colorado River. 43rd Annual Meeting of the Western Division of the American Fisheries Society. Anchorage, Alaska. May 24, 2018.
- Avila, B. W., D. L. Winkelman, and E. R. Fetherman. 2018. Quality vs. quantity: Manipulating rearing density to increase survival of Rainbow Trout fry pre- and post-stocking. 43rd Annual Meeting of the Western Division of the American Fisheries Society. Anchorage, Alaska. May 24, 2018.
- Fetherman, E. R., B. Neuschwanger, T. Davis, C. L. Wells, and A. Kraft. 2018. Erythromycin injections for controlling bacterial kidney disease in Colorado hatcheries. 43rd Annual Meeting of the Western Division of the American Fisheries Society. Anchorage, Alaska. May 24, 2018.

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 fish tagging methods used in research and management across Colorado, and were given a chance to try the various tagging methods on live fish. The second, a formal presentation, was given to the class in March 2018:

- Fetherman, E. R. 2018. Salmonid disease research in Colorado. Front Range Community College, Fisheries Management Class. Fort Collins, Colorado. March 21, 2018.

A guest lecture was also given at Colorado State University:

- Fetherman, E. R. 2017. Colorado Parks and Wildlife Sport Fish Research Studies. Guest lecture, FW260 – Principles of Wildlife Management. Department of Fish, Wildlife, and Conservation Biology, Colorado State University. Fort Collins, Colorado. November 9, 2017.

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:

- Richer, E. E., E. R. Fetherman, M. C. Kondratieff, and T. A. Barnes. 2017. Incorporating GPS and mobile radio frequency identification to detect PIT-tagged fish and evaluate habitat utilization in streams. North American Journal of Fisheries Management 37(6):1249-1264.
- Hodge, B. W., E. R. Fetherman, K. B. Rogers, and R. Henderson. 2017. Effectiveness of a fishway for restoring passage of Colorado River cutthroat trout. North American Journal of Fisheries Management 37(6):1332-1340.

In addition to those manuscripts published in peer-reviewed journals, one other manuscript was submitted for publication:

- Avila, B. W., D. L. Winkelman, and E. R. Fetherman. *In press*. Survival of whirling disease resistant Rainbow Trout fry: A comparison of two strains. Submitted to the Journal of Aquatic Animal Health.

Popular articles in magazines and newspapers inform anglers and other members of the public about our research results, how these results affect them, and the work that continues to occur to increase the quality and quantity of angling opportunities in Colorado. This year, we provided publications and other relevant information to Dan Omasta with Colorado Trout Unlimited who was writing an article for TU members focused on the basic, proper steps for decontaminating fishing gear in Colorado, as well as information on the brown trout removal project conducted in the Cache la Poudre River, which was incorporated into an article in Southwest Fly Fishing Magazine:

- Cache la Poudre River, CO: Wild Trout. Author: Ken Proper. Southwest Fly Fishing Magazine. October 2017.

Interviews were also given to Mary Taylor Young for an article in Colorado Outdoors, and to Bruce Finley with the Denver Post:

- Building a better Rainbow Trout: Colorado biologists on breeding blitz to revive species ravaged by whirling disease. Author: Bruce Finley. Denver Post. March 4, 2018.
- Whatever happened to whirling disease? Author: Mary Taylor Young. Colorado Outdoors 2018 Fishing Guide. A Colorado Department of Natural Resources and Colorado Parks and Wildlife publication. June 2018.

Reviewed talking points and provided additional background and history on the research conducted in the East Portal of the Gunnison River regarding the Gunnison River Rainbow Trout for an interview of the aquatic biologist and district wildlife manager in Montrose on CBS National News (aired June 2018).

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

- Examined differences in deformity development related to feed type and quality in Snake River Cutthroat Trout (Appendix A).
- Designed and collected data for an experiment being conducted at the Bellvue-Watson Fish Hatchery in which a split lot of Hofer x Harrison Lake x Snake River Cutthroat Trout (HHN)

are being reared on feeds from two different manufacturers from swim-up through stocking as catchable-sized fish. Endpoints for the study include fish length, weight, condition, CV length, CV weight, and mortalities due to disease.

- 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 three manuscripts submitted to scientific journals:

- Anonymous. Elimination of *Myxobolus cerebralis* in Placer Creek, a native Cutthroat Trout stream in Colorado. Submitted to the Journal of Aquatic Animal Health.
- Baker, C. F., H. Reeve, D. Baars, D. Jellyman, and P. Franklin. Efficacy of 12 mm half-duplex PIT tags in monitoring fish movements through stationary antenna systems. Submitted to the North American Journal of Fisheries Management.
- Bond, R. M., C. L. Nicol, J. D. Kiernan, and B. C. Spence. Occurrence, fate, and confounding influence of ghost PIT tags in an intensively monitored watershed. Submitted to the Canadian Journal of Fisheries and Aquatic Sciences.

Appendix A
Feed Quality and Deformity Formation in Snake River Cutthroat Trout

Feed quality can affect many processes in salmonid fishes including growth, health indices, appearance, and taste. The effects of basic feeds from four commercial feed manufacturers have been evaluated for Rainbow Trout reared in Colorado hatcheries (Fetherman and Schisler 2017; Job No. 2, Action #1), but have not been evaluated for other species of salmonids. Anecdotal evidence from the CPW Crystal River Hatchery suggested that deformity incidence in Snake River Cutthroat Trout may vary with feed. This was determined after switching feeds from Feed Company D to Feed Company C in subsequent years and following standard practice of removing deformed individuals from future brood stocks at the fingerling life stage. Fewer deformed individuals were removed after feeding Feed Company C than in previous years of feeding Feed Company D. The following describes an experiment conducted at the BFRH used to test if deformity incidence in Snake River Cutthroat Trout fingerlings differs by feed company.

Seven unique male-female families were spawned at the CPW Crystal River Hatchery in fall 2017 and shipped in individual egg crates to the BFRH for experimentation. Upon receiving the eggs, the three families that contained enough eggs for experimentation (≥ 2000; families 1, 4, and 5) were chosen based on egg counts performed at the CPW Crystal River Hatchery. Space constraints at the BFRH prevented all seven families from being used in the experiment. Eggs from each family were placed in floating egg boxes in the experimental raceways and counted out using egg siphons. Two thousand eggs from each family were split into two adjoining hatchery troughs, each containing one thousand eggs, and held until they hatched. During this time, dead eggs were removed from the trough to prevent fungal infections.

Fish were reared in the same troughs in which they had hatched. Crippled fish and mortalities were removed daily. Feeding began upon swim-up, which occurred on January 4, 2018. The two troughs for each family were each fed different feeds, Feed Company C or Feed Company D. Similar to the 2016 hatchery feed experiment (Fetherman and Schisler 2017), the manufacturer’s recommendations for feed size and feeding rate were followed in this experiment (Tables A.1 and A.2). Fish were fed six times daily through the conclusion of the experiment on March 26, 2018. Upon conclusion of the experiment, 50 fish were removed from the trough, measured and weighed, and checked for deformity type and number. Additionally, five fish from this group were dissected to obtain liver and viscera weights for comparisons of hepatosomatic index (HSI) and viscerosomatic index (VSI) between the feed companies.

Table A.1. Feed Company C suggested feeding rate (% BW/d) by feed size, fish size, and at a temperature of 54°F.

Feed Size	Count per Pound	Length (in)	Weight (g)	Feeding Rate
#0	3000-570	Hatch-1.7	0.15-0.8	3.3
#1	570-300	1.7-2.1	0.8-1.5	3.1
#2	300-150	2.1-2.6	1.5-3.0	3.0

Table A.2. Feed Company D suggested feeding rate (% BW/d) by feed size, fish size, and at a temperature of 53°F.

Feed Size	Count per Pound	Length (in)	Weight (g)	Feeding Rate
#0	< 1,200	< 1.3	< 0.4	5.4
#1	1,200	1.3	0.4-0.8	5.4
#2	600	1.5	0.8-1.5	4.5
#2	300	2.0	1.5-2.3	3.9
#3	200	2.3	2.3-4.5	3.5

The remaining fish were individually checked for deformities, and type of deformity or deformities were recorded for each fish. Nine deformities were identified in this experiment, 1) spinal deformities, bends or twists of the spine that caused a deviation from the typical fusiform shape, 2) lower jaw deformities, shortened lower jaws or lower jaws that were bent to one side, 3) upper jaw and cranial deformities, deformities causing shortening of the upper jaw or depressions in the skull, 4) caudal deformities, most often a nearly 90 degree dip in the spine posterior of the adipose fin, 5) missing eyes, where the eye did not appear to form correctly causing it to be small or nonexistent, 6) bulging eyes or exopthalmia, where the eye extended out of its socket, 7) caudal fin deformities, typically consisting of deformations where the caudal fin connected to the body along the top of the fish, 8) dorsal fin deformities, where the dorsal fin was not fully formed, containing only a couple of deformed rays, or nonexistent, and 9) opercular deformities, where the operculum was pulled back or eroded along the edge or in the center, exposing the gills. Although all deformities ranged in severity, a severity score was not associated with the deformities, and all deformity types were recorded as present or absent.

Since the effect of feed on growth and health indices had never been evaluated for Snake River Cutthroat Trout, an analysis of variance (ANOVA), implemented in SAS PROC GLM (SAS Institute 2017), was used to determine if there were differences in survival, fish weight, feed conversion ratios, HSI, and VSI among the families and feed companies. Additionally, an ANOVA was used to determine family and feed effects on deformity presence, number of deformities per fish, and deformity type and expression, as well as whether fish weight was affected by deformity presence or number.

There was not an effect of feed on survival, but survival did differ among families. Family 5 exhibited significantly higher survival (97%) than families 1 and 4 (84% and 86%, respectively). Feed conversion ratios differed by feed, with fish fed on Feed Company C having a significantly lower feed conversion ratio (0.70) than fish fed on Feed Company D (1.0). Although the feed conversion ratio was similar for Rainbow Trout and Snake River Cutthroat Trout fed on Feed Company C, Snake River Cutthroat Trout fed on Feed Company D had a higher feed conversion rate than did Rainbow Trout fed on Feed Company D (see Fetherman and Schisler 2017; Job No. 2, Action #1). Despite the lower feed conversion ratios, fish fed on Feed Company D weighed significantly more at the end of the experiment than fish fed on Feed Company C (4.41 and 4.07 g, respectively), likely a result of the higher feeding rate used for Feed Company D. Fish reared on Feed Company C had a significantly higher HSI and VSI (1.28 and 11.1, respectively) than did fish fed Feed Company D (1.09 and 9.8, respectively). Overall, HSI and VSI values for the

Snake River Cutthroat Trout were lower than for Rainbow Trout (Fetherman and Schisler 2017; Job No. 2, Action #1), suggesting that the two species differ in the way that the feeds are processed and excess energy stored in the liver and viscera.

Deformity incidence differed among the families, but was not affected by feed. A significantly larger percentage of the fish in family 1 developed a deformity in comparison to families 4 and 5 (21, 13 and 10%, respectively), and a larger percentage of fish expressed deformities in family 4 than family 5. On average, 14% and 15% of fish fed on Feed Company C and Feed Company D expressed deformities, respectively. Among deformed individuals, the number of deformities differed by both family and feed, with family 1 expressing a larger number of deformities (1.5) than family 4 (1.3) or family 5 (1.1), and fish fed Feed Company C expressing a higher number of deformities (1.4) than fish fed Feed Company D (1.2). The presence of a deformity affected fish weight, with fish that did not have a deformity weighing an average of 4.3 g, and fish with a deformity weighing an average of 3.7 g. Additionally, in the fish that expressed at least one deformity, the number of deformities also affected fish weight such that the more deformities a fish expressed, the smaller the fish (Figure A.1).

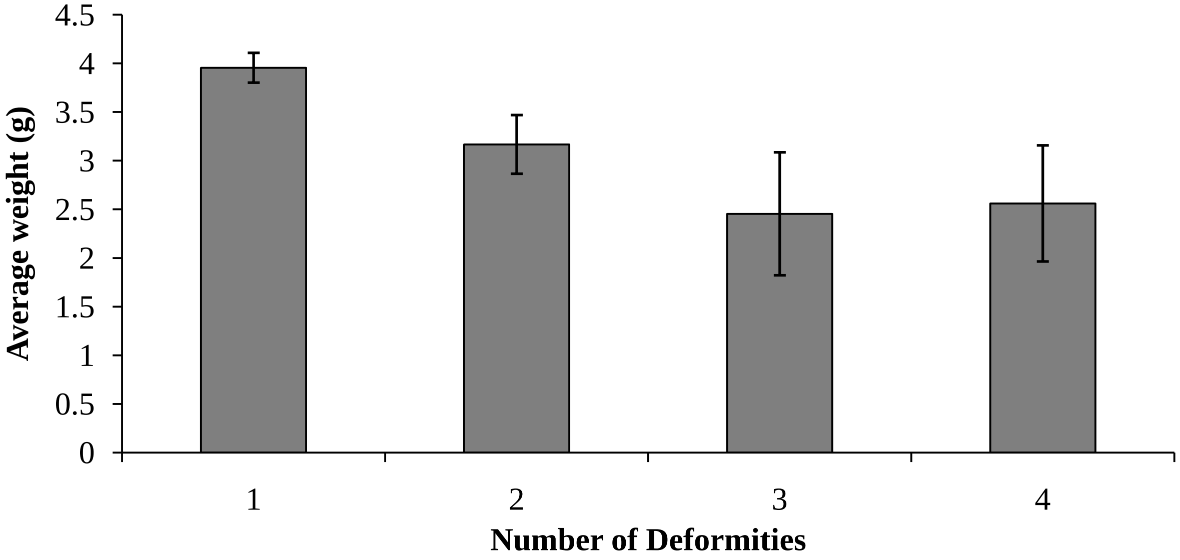

Figure A.1. Average weight (g; SE bars) of Snake River Cutthroat Trout expressing one, two, three, or four deformities.

Overall, opercular deformities were the most common deformity observed in the experiment, followed by upper jaw and cranial deformities, caudal deformities, spinal deformities, and lower jaw deformities (Table A.3). Bulging eyes were also fairly common, and were generally observed in conjunction with cranial deformities, explaining why more bulging eyes were observed in family 1 than the other two families. Missing eye, caudal fin and dorsal fin deformities were the least common deformities, and were generally observed in one family, suggesting these deformities were likely associated with family genetics rather than feed company. Certain deformities were differentially expressed by family or feed company (Table A.3). For example, the number of fish expressing opercular deformities was similar among the feed companies in families 1 and 4, but fish fed Feed Company C in family 5 expressed fewer opercular deformities than did fish fed on Feed Company D in that same family. Upper jaw and cranial deformities were more common in family 1 compared to families 4 and 5. A higher number of fish fed on Feed Company C exhibited caudal deformities than did fish fed on Feed Company D in all families.

Table A.3. Number of deformities observed by deformity type for all fish included in the experiment, and by family and feed company.

Deformity Type	All	Family 1		Family 4		Family 5	
		Feed C	Feed D	Feed C	Feed D	Feed C	Feed D
Spinal	92	19	12	14	17	26	4
Opercular	333	53	67	56	53	29	75
Lower Jaw	80	18	15	10	9	14	14
Upper Jaw/Cranial	164	51	59	18	21	9	6
Caudal	106	56	18	15	3	13	1
Missing Eye	4	3	0	0	1	0	0
Bulging Eye	66	26	26	6	6	2	0
Caudal Fin	8	5	2	1	0	0	0
Dorsal Fin	2	0	0	0	0	2	0

Although deformity type and deformity number were affected by feed company, overall deformity expression did not differ by feed. As such, switching feeds from Feed Company D to Feed Company C is not likely to result in lower deformity formation in Snake River Cutthroat Trout, especially if hatchery practice is to remove fish exhibiting any kind of deformity during the brood stock selection process. However, given the critical evaluation of even slight deformities made by the observers in this experiment, deformity severity may have more of an effect on deformity identification during hatchery sorting. Family had a large effect on incidence of deformities, with some families containing 11% more deformed fish than other families in this experiment. It is therefore likely that the observed reduction in deformity incidence in the CPW Crystal River Hatchery was a result of spawning fish that were less likely to have offspring that expressed deformities than families spawned in previous years rather than changing the feed company. There could be additional potential benefits, however, of switching feeds from Feed Company D to Feed Company C in Snake River Cutthroat Trout brood stocks, including lower (better) feed conversion ratios, which should result in higher growth rates when extending feeding beyond the fingerling life stage, and higher HSI and VSI values, which could result in healthier fish and potentially better egg quality if excess energy storage carries over into egg formation in future brood stock fish.

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.

SAS Institute. 2017. SAS system software, release 9.4. SAS Institute, Cary, North Carolina.