Sport Fish Research Studies

Federal Aid Project F-394-R16

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Federal Aid in Fish and Wildlife Restoration

Job Progress Report

Colorado Parks & Wildlife

Aquatic Research Section

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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 (*Oncorhynchus mykiss*) 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, 2017.
2. Maintain genetic and disease integrity of brood stocks housed at the Bellvue Fish Research Hatchery and Poudre Rearing Unit through June 30, 2017.

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 2016 through January 2017, and reared through June 30, 2017. Brood stocks will be marked, identified, and maintained by strain or cross and year class.

Action #1 Accomplishments

The whirling disease 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 different 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 2016, 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 17, 2016, 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 5 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 other 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.

[1] Hofer (H) is used interchangeably with 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.

The Bellvue Fish Research Hatchery was discovered to be positive for *Renibacterium salmoninarum* during their annual inspection on March 28, 2016. The only fish that were found to be positive at that time were the two- (16 month) and three- (28 month) year-old Hofer rainbow trout. Fish from these two positive lots were used in an erythromycin study conducted at the hatchery in fall 2016. The brood stock fish from the lots of two- and three-year-old fish found to be positive earlier in the year were treated with a single intraperitoneal injection of erythromycin, at a dose of 25 mg/kg, almost seven months later on October 20, 2016. Half of the fish in these lots remained untreated as a control. Feeding behavior was normal during the trial, and no other abnormal behaviors were observed following the injections.

Lethal spawning of the three year old treated and control fish commenced on November 15, 2016, and adults were subsequently spawned on November 29, December 6, and December 14, 2016. Adult fish were euthanized after spawning and tested for the presence of *R. salmoninarum* using single round polymerase chain reaction (PCR). Mortalities from these two groups were also tested via single round PCR when they occurred. Two-year-old treated and control fish were not lethally spawned so that they could be used to create future brood stocks again in 2017. Only one adult brood stock fish tested positive for *R. salmoninarum*, a three year old female Harrison Lake rainbow trout that had not been involved in the injection study, but was found as mortality on November 27, 2016.

Progeny were created from single male-female pairs out of either the treated or control lots. Eggs from each pair were held in separate egg cups until eye up to monitor egg mortality. Upon hatch, families were pooled since tank availability precluded rearing individual families until fry sampling could take place. Fry were sampled for testing using single round PCR on January 9, January 17, January 23, and January 31, 2017. Thirteen pooled family groups of progeny were found to be positive for *R. salmoninarum*, two treatment and four control families of Hofers, and six families of Harrison Lake rainbow trout that had not been a part of the injection experiments. All progeny in pooled families that tested positive for *R. salmoninarum* were euthanized; all negative pooled families were used for future brood stocks (see Job No.1, Action #1).

One potential reason that treated fish still became infected with *R. salmoninarum* is that we were only able to do one injection of erythromycin. Ideally, we would have done a three injection treatment. For CPW purposes, we considered the fall 2016 injection study to be a pilot study to determine how much effort would be needed to perform a larger study in the future. Results suggested that a single injection was not successful in preventing the presence of *R. salmoninarum* in treated progeny, although fewer treated families were found to be positive than were control families. A larger experiment utilizing a three injection treatment is planned for 2017, and results should be available in the next reporting cycle.

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

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, 2016.
2. Initiate one experiment to examining the efficacy of general and strain-specific vaccines for bacterial coldwater disease for Colorado hatcheries by June 30, 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 regards 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 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, fish protein and lipid content, fin wear rating, hepatosomatic index, viscerosomatic index, and histological analysis of various tissue cells to determine fish health status. In addition, angler satisfaction of the final product will be evaluated, including satisfaction with fish shape and coloration, flesh color, and taste.

Action #1 Accomplishments

The following describes the motivation, experimental design, methods, and results of a hatchery feed experiment conducted at the Bellvue Fish Research Hatchery (BFRH) in 2016. Reference to specific feeds and feed manufacturers has been intentionally omitted until a second experiment being conducted in 2017 can be completed. Results from the 2017 experiment will be available in the next reporting cycle.

Motivation

The purchase of commercial feeds for large scale hatchery production often dominates the operating budget for many state-run hatchery facilities. In general, feed costs are the largest expenditure for finfish producers (Trushenski et al. 2006), with feed costs covering up to 60-70% of the total expense of trout farming (Kim 1997). As such, hatcheries strive to minimize feed loss to improve profitability and reduce environmental impacts through changing portion sizes (Bailey and Alanärä 2006), assessing delivery methods (Wagner et al. 1996; Noble et al. 2007), and improving feed efficiency (Silverstein 2006). Selecting diets formulated and well suited for a target species can also help overcome financial challenges (Trushenski et al. 2006). Growing larger fish from the same amount of feed (i.e., improving feed efficiency) could not only reduce production costs, but also the environmental impacts of fish farming (Silverstein 2006).

Costs for diet formulations vary widely depending on ingredients, with protein representing the largest single and most costly component of finfish diets. Although fish meal is the "ideal protein" because the amino acid profile of the feed mimics the whole-body amino acid profile of the animal being fed, alternative sources of protein have been sought to reduce cost (Trushenki et al. 2006). The use of alternative protein sources, as well as variety in other ingredients such as lipids/fats, micronutrients, and fillers, may result in reduced cost, but also reduced feed intake and increased feed conversion ratios. Therefore, lower cost feeds may not always produce the best growth, potentially resulting in more food waste and higher long-term costs than more expensive feeds when evaluated on a cost-per-fish basis. The objective of the 2016 hatchery feed experiment was to assess differences in growth, condition, appearance, taste, and production cost per rainbow trout using four commercial trout feeds to determine if statewide annual and long-term production costs could be reduced using different commercial diets.

Feeds Evaluated

Feeds from four commercial trout feed companies were evaluated in this experiment, hereafter referred to as Feed Companies A, B, C, and D. To maintain low cost and consistency among the four 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 (Table 2.1.1). Though proportions are similar among diets produced by the four companies, the type and source of ingredients (often proprietary) used to produce the diets result in differences in cost and proposed feed conversion ratios. Each company also has their own recommendations for feeding rates (Tables 2.1.2, 2.1.3, 2.1.4, and 2.1.5), and these recommendations were followed to ensure that estimates of feed conversion and growth were obtained in a manner consistent with the expectations developed by each company for their feeds.

Table 2.1.1. Feed size and corresponding percent (%) crude protein, % crude fat, and description of the feed from the catalogs provided by Feed Companies A, B, C, and D.

Feed Size	% Crude Protein	% Crude Fat	Description
			FEED COMPANY A
#0 and #1 #2 and 1.2 mm	54 53	16 20	Premium fish oil and low temperature fishmeal give fry the best start in order to maximize survival and growth throughout the production cycle; premium ingredients and a high quality final product ensure high digestibility and excellent water quality. Particle size has been designed to match the ability of fish to consume feed.
1.5, 2, 3, 4, 5, 7, and 9 mm	43	14	Proven formulation with moderately high protein and low fat levels that delivers good growth potential while minimizing cost. Premium fishmeal and a blend of highly digestible alternative raw materials ensure good feed conversion.
			FEED COMPANY B
#0, #1, and #2	52	16	Nutrient-rich, crumbled starter feed suitable for trout, steelhead, and a range of other cold and warm water species, produced from a highly digestible extruded pellet.
1 and 2 mm	45	16	Medium-energy, extruded sinking or floating fry diet, specifically formulated for good growth and healthy fry.
3, 4, 6, and 8 mm	40	12	Proven low-cost, low-energy trout feed.
			FEED COMPANY C
#0 #1 and #2	53 52	18 20	Combines traditional dietary values with an increased level of alternative ingredients to reduce cost; contains an enhanced vitamin pack and natural pigment to promote healthy fish and natural coloration; natural palatability enhancers ensure an active first feeding response.
1.2, 1.5, 2, 2.5, and 3 mm	47	18	Mid-level energy fish feed for moderate or controlled growth; includes alternative ingredients to reduce cost; contains enhanced vitamin pack and pigment to promote healthy fish and natural coloration.
4 mm 6 mm 9 mm	45 43 40	24 24 24	High-energy trout and steelhead diet designed to give maximum growth and the lowest feed conversion rates. Pigment is included in sizes 6 and 9 mm to promote natural coloration.
			FEED COMPANY D
#0, #1, and #2	52	16	Especially formulated for first feeding fry and fingerling; nearly free of dust and fines; high levels of quality fish and animal protein; utilizes marine oils, beta glucans, pigments, and high vitamin levels, including stabilized vitamin C.
#3 and #4	45	15	Nutritionally complete and balanced; main components are high quality fish and animal proteins; finely ground to ensure superior digestibility and that fish receive full complement of nutrients.
3/32”, 1/8”, and 5/32”	40	12	Sinking pellets manufactured by steam pelleting; suitable for fish from 15 grams to 900 grams or more.
3/16” and 1/4”	40	10	Includes high levels of vitamins, minerals, and antioxidants; unique ingredients and balanced formulation proven to aid in the well being of brood fish and viable egg production; extrusion process allows slow sinking or floating pellets.

Table 2.1.2. Feed Company A suggested feeding rate (percent body weight per day [% BW/d]) by feed size, fish size, and at a temperature of 52-54°F. Note that Feed Company A's guidelines are for a moderate growth rate and about 5-6 feedings per day for starter sizes #0 and #1.

Feed Size	Count per Pound	Weight (g)	Feeding Rate
#0	5000-1500	0.09-0.30	2.41
#1	1500-1000	0.30-0.45	2.40
#1	1000-800	0.45-0.57	1.94
#1	800-600	0.57-0.76	1.73
#2	600-500	0.76-0.91	1.62
#2	500-300	0.91-1.5	1.5
1.2 mm	300-175	1.5-2.6	1.44
1.2 mm	175-100	2.6-4.5	1.41
1.5 mm	100-50	4.5-9.1	1.28
2.0 mm	50-20	9.1-22.7	1.14
3.0 mm	20-10	22.7-45	0.88
4.0 mm	10-5	45.4-91	0.78
5.0 mm	5-0.5	90.8-908	0.72

Table 2.1.3. Feed Company B 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
1.0 mm	150-60	2.6-3.1	3-8	2.9
2.0 mm	60-11	3.1-4.6	8-40	2.4
3.0 mm	11-6	4.6-7.4	40-80	1.4
4.0 mm	6-1.5	7.4-12.5	80-300	1.0

Table 2.1.4. 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
1.2 mm	150-90	2.6-3.1	3.0-5.0	2.9
1.5 mm	90-60	3.1-3.5	5.0-8.0	2.7
2 mm	60-25	3.5-4.6	8.0-18	2.4
2.5 mm	25-11	4.6-6.0	18-40	1.9
3 mm	11-6	6.0-7.4	40-75	1.4
4 mm	> 6	> 7.4	> 75	1.0

Table 2.1.5. 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
#3	100	2.8	4.5-6.0	2.9
#4	80	3.0	6.0-8.0	2.7
#4	60	3.5	8.0-11.0	2.5
3/32”	40	4.0	11.0-15.0	2.3
3/32”	30	4.5	15.0-21.0	2.2
3/32”	22	4.8	21.0-30.0	2.0
1/8”	15	5.5	30.0-38.0	1.8
1/8”	12	6.0	38.0-50.0	1.7
5/32”	9	6.5	50.0-76.0	1.6
5/32”	6	7.5	76.0-114.0	1.4
3/16”	4	8.5	114.0-151.0	1.3

Two samples of 125 grams each were retained from each feed size from each feed company. An analysis is being conducted by the U.S. Fish and Wildlife Service Bozeman Fish Technology Center (Bozeman, Montana) to ensure that the protein and lipid ratios are the same as those stated on the label for each feed. In addition, samples were dried and weighed to calculate amount of dry matter in each feed size and diet. The feed conversion ratio (see below) is dependent upon the weight of dry matter consumed in relation to the average individual weight gained, not total weight of the feed provided.

Hatchery Feed Experiment Methods

Rainbow trout (pure Hofer [GR]) used for this experiment were spawned at the BFRH in December 2015. 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, 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 29, 2016, fish were released into their tanks to begin feeding. Each tank initially contained between 812 and 817 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 recommended rate (percent body weight per day [%BW/d]) for each feed company (Tables 2.1.2, 2.1.3, 2.1.4, and 2.1.5). 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 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.

Table 2.1.6. Assignment of feed company to tank, assigned using a random number generator.

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

The hatchery feed experiment was started at two weeks post-swim-up, at which time 150 fish each were counted out of the starter tank and distributed into three replicate, 10-gallon glass tanks for each feed company in FR1 (see Table 2.1.6 for tank assignments). Any remaining fish in the starter tank were counted, euthanized, and retained for proximate analyses. 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 150 fish for a given replicate tank in a tarred 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) for each tank. 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.

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 given to the fish was consumed for the purpose of calculating the 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. This sampling schedule was similar to that used by other state hatcheries which sample smaller fish once a week, with time between samples increasing as fish get larger (Table 2.1.7). Daily feed amounts were adjusted for mortalities based on the average weight of an individual fish from the previous weekly sampling event. 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 (e.g., Feed Company D suggests multiple changes in feeding rate within each feed size [Table 2.1.5]). A subset of 20 fish were individually measured and weighed on the day that feed size was changed (note that a subset of fish were not processed when feeding rate changed within a feed size, as with Feed Company D). Fifteen of the 20 fish were returned to the tank after being processed. The remaining five fish were euthanized, dissected to obtain liver and viscera weights, and retained for proximate analysis. 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 using 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).

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 10 gallon glass tanks in FR1. Upon reaching an average of 5 grams per fish, fish were moved to 20 gallon aluminum tanks within FR1, and the number of fish was counted and confirmed. Once fish reached an average of 18 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 75 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 200 grams of fish, or on November 1, 2016, whichever came first. At the end of the experiment, all fish remaining in a hatchery trough tank were measured and weighed, and 20 fish were euthanized, dissected to obtain liver and viscera weights, and retained for proximate analyses. Fifteen fish from each tank were then moved to round tanks where they continued to be fed on the same size and ration of feed until they were used in the fish preference experiment (described below).

Table 2.1.7. Summary of sampling times and feed delivery methods by fish size and Colorado State Fish Hatchery.

Hatchery	Sample	Fish Size	Feeding Method
Pueblo (PUE)	Once a week	Less than 5”	Hand
	Once a month	Greater than 5”	Hand
Monte Vista (MVU)	Once a month	Less than 3”	Automatic Feeders
	Once a month	Greater than 3”	Hand
Spicer Facility (SLS)	Once a month	All	Hand
Durango (DUR)	Once a week	Subcatchables	Hand
Pitkin (PKN)	2-4 times per month	Less than 3”	Hand
	1-2 times per month	3-5”	Hand
	Once a month	Greater than 5”	Blower
Finger Rock (FRO)	Bi-weekly	All	Hand Blower in raceways
Roaring Judy (ROJ)	Once a week	Subcatchables	Hand Belt Feeder
	Once a month	Catchables	Hand up to 15/lb Blower
Chalk Cliffs (CCL)	Once a month	Subcatchables	Hand
		Larger than 20/lb	Truck
Glenwood Springs (GSU)	Once a month	Subcatchables	Hand Automatic Feeders
Bellvue-Watson (BWT)	Once a week	Less than 3”	Hand
	Bi-weekly	Greater than 3”	Hand
		Greater than 5”	Demand Feeders (supplemental)
Mount Shavano (MSO)	Biweekly	Less than 5”	Sweeny Vibratory Feeders Hand (3-8 times/day) Belt Feeders
		Greater than 5”	Hand (2 times/day)
Rifle (RIF)	Every 2-3 months (Rely on growth charts)	Subcatchables	Hand
		Catchables	Blowers

There are a number of standard metrics used to evaluate growth performance in feed comparison experiments, including weight gain (%), feed conversion ratio (FCR), specific growth rate (SGR; percent body weight per day [% 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. 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 for each size of feed for each company. HSI and VSI were computed for fish that were ≥ 2 grams; HSI and VSI were not calculated for feed sizes in 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 in 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 for each size of feed from each 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 for each size of feed for each company, as well as for the entire growth period from hatch to the end of the experiment.

An analysis of variance (ANOVA) implemented in SAS PROC GLM (SAS Institute 2016) was used to determine if there were differences in mortality, length, and weight between the feed companies following the first two weeks of feeding. Similarly, an ANOVA was used to determine if there were differences in overall feed conversion ratios, HSI, VSI, fin condition, CV length, CV weight, and mortality among the feed companies at the end of the experiment. Overall growth differences were not comparable since all feed companies did not reach the target weight of 200 grams per fish by the end of the experiment.

Because feed size changes occurred at different fish sizes within each of the feed companies, growth and health metrics were not comparable among the feed companies throughout the majority of the experiment. Within a feed company, an ANOVA ($n = 4$) was used to compare differences in CV length, CV weight, HSI, VSI, and fin condition among the feed sizes. Summary statistics are provided for mortality, FCR, SGR, weight gain, feed intake, and K for each feed size within each feed company. Colorado hatcheries stock fish at various sizes including *Myxobolus cerebralis*-negative subcatchables at 75 mm, *M. cerebralis*-positive subcatchables at 150 mm, and *M. cerebralis*-negative and –positive catchables at 250 mm. Results from the various feed sizes are discussed in relation to these fish sizes at stocking.

Fish Preference Methods

Colorado stocks over 2.5 million catchable rainbow trout annually. Often, these fish are caught by anglers and taken for consumption shortly after being stocked. As a continuation of the hatchery feed experiment, thirty people, consisting of CPW employees and the general public participated in two preference tests pertaining to the appearance and taste of fish reared on the feeds from three feed companies, Feed Company B, Feed Company C, Feed Company D. Feed Company A was not included in this portion of the experiment because fish had not attained a large enough size to be comparable to the other three feed companies (see results section below). Preference tests were conducted at the Salud! Cooking School attached to the Whole Foods Market in Fort Collins, Colorado. The objective of these preference tests was to determine if there were differences in appearance and taste based on the feed company used to rear catchable rainbow trout in Colorado hatcheries.

For the appearance test, two fish from each of the three feed companies were randomly placed in clear, 20 gallon tanks. Feed company was not known to those participating in the test to reduce bias. Participants were asked to rate a number of metrics for the fish in each tank including fish color, fin quality, total length, body depth, head shape, body shape, and overall satisfaction. The rating scale ranged from 1 to 10, with 1 being completely unsatisfied with the appearance and 10 being completely satisfied with the appearance for each metric.

Two volunteer chefs prepared fish for the taste test. Chefs were asked to choose their favorite preparation style for the test. One chef pan seared the fish, and the fish were served with green beans and a white wine cream sauce. The other chef brined and smoked the fish prior to the event, and served the fish over a corn cake with cilantro lime sour cream. Because the chefs were the only impartial observers to work with the fish in their raw form, they were asked to rate several variables relating to fish appearance, workability, and overall satisfaction. Ratings were based on the chef's previous experience working with and preparing fish. The rating scale ranged from 1 to 10 and varied based on the characteristics being rated. Fillet color rating scale ranged from light (1) to dark (10). Aroma ranged from not fishy (1) to fishy (10). Texture ranged from not firm (1) to firm (10). Moisture content ranged from dry (1) to juicy (10).

Tenderness ranged from tough (1) to tender (10). Workability ranged from falling apart and hard to work with (1) to staying together and easy to work with (10). Lastly, chef's were asked to rate the overall acceptability of the fish ranging from not acceptable (1) to acceptable (10).

All three feed companies included in the test were prepared in the same manner by each chef. In addition, Whole Foods Market (WFM) rainbow trout were included as a store-purchased control to see how rainbow trout reared by Colorado hatcheries compared to those sold by Whole Foods. Participants were provided fish from each of the four groups, prepared in the two different styles (eight total fish to rate), throughout the evening. The order in which fish were presented to the participants was randomized to prevent association with a feed group between preparation styles, and participants were asked to independently rate each fish rather than try to do a comparison among the fish. Similar to the rating scale for appearance, the taste test rating scale ranged from 1 to 10, with 1 being completely unsatisfied with a given quality and 10 being completely satisfied with a given quality. Participants rated the fillet color, fishiness, fish texture, palatability, overall flavor (of the fish, not the cooking style) and overall satisfaction with taste. Participants were also asked to rate satisfaction with style of preparation. An ANOVA was used to compare differences in satisfaction for each quality included for appearance, chef preparation, and taste tests among the three feed companies (and the WFM fish included in the chef preparation and taste test).

Hatchery Feed Experiment Results

Table 2.1.8. Comparisons of overall survival (%), feed conversion (grams of feed per gram of fish [g feed/g fish]), weight gain (%), specific growth rate (SGR; % body weight per day [% BW/d]), feed intake (% BW/d), length (mm; CV length in parentheses), 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	99.27^{z}	99.88^{z}	99.75^{z}	99.51^{z}
Week 1	99.39	99.88	99.88	99.63
Week 2	99.87	99.87	99.87	99.87
Feed Conversion	0.41	0.42	0.33	0.56
Weight Gain	131.06	176.14	128.96	227.74
SGR	4.93	5.97	6.90	6.98
Feed Intake	2.08	2.64	2.31	4.17
Length (CV)				
Start	22.10^{z} (0.03)	22.55^{z} (0.04)	22.50^{z} (0.04)	22.05^{z} (0.05)
Week1	27.40^{z} (0.04)	27.35^{z} (0.05)	27.75^{z} (0.04)	27.55^{z} (0.05)
Week2	28.65^{z} (0.08)	28.95^{z} (0.09)	29.80^{z} (0.07)	31.90^{y} (0.04)
Weight (CV)				
Start	0.09^{z} (0.15)	0.09^{z} (0.10)	0.09^{z} (0.12)	0.08^{z} (0.12)
Week1	0.18^{z} (0.21)	0.20^{z} (0.16)	0.21^{z} (0.12)	0.20^{z} (0.17)
Week2	0.21^{x} (0.25)	0.24^{xy} (0.28)	0.27^{yz} (0.20)	0.30^{z} (0.12)
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 2.1.8), indicating that fish took to feed equally on all four feed types. Feed conversion (grams of feed needed to produce one gram of mass) varied among the feed companies, with Feed Company C having the lowest feed conversion rate and Feed Company D having the highest feed conversion rate. The lower the feed conversion rate, the more efficiently fish were able to convert feed to mass. Weight gain, specific growth rate, and feed intake also varied among the feed companies, and all three metrics were highly dependent upon the feeding rate used for each feed company (Tables 2.1.2, 2.1.3, 2.1.4, and 2.1.5). Length and weight did not differ at the start of the experiment, nor after one week of feeding. However, by the end of week two fish fed Feed Company D were significantly longer than fish fed the other three feed companies, and significantly heavier than fish fed Feed Companies A or B. Although fish from Feed Companies A, B, and C did not differ in length at the end of the first two weeks, fish fed Feed Company A were significantly smaller than fish fed Feed Companies C; the weight of fish fed Feed Companies B and C did not differ. No fin wear was observed in the first two weeks, with fish from all the feed companies having a fin rating of 0 (Table 2.1.8).

Table 2.1.9. Comparison of survival (%), feed conversion (g feed/g fish), weight gain (%), specific growth rate (SGR; % BW/d), feed intake (% BW/d), length (mm), CV length, weight (g), CV weight, Fulton's condition factor (K), hepatosomatic index (HSI), viscerosomatic index (VSI), and fin rating among the seven feed sizes used from Feed Company A. Different letters within the same row for a given metric represent significant differences among the feed sizes.

Metric	**Size 0**	**Size 1**	**Size 2**	**1.2 mm**	**1.5 mm**	**2.0 mm**	**3.0 mm**
Survival	100	98.83	100	99.75	99.19	98.05	99.39
Feed Conversion	0.48	0.48	0.45	0.55	0.62	0.67	0.70
Weight Gain	69.18	142.00	108.54	188.60	96.92	139.86	73.07
SGR	4.04	3.48	2.87	2.29	1.71	1.49	1.14
Feed Intake	1.97	1.73	1.29	1.31	1.07	1.03	0.81
Length	32.25	41.75	53.15	73.33	92.95	123.15	151.48
CV Length	0.08^{b}	0.08^{bc}	0.12^{a}	0.11^{ac}	0.11^{ac}	0.11^{abc}	0.08^{bc}
Weight	0.32	0.80	1.85	4.35	9.18	22.54	40.44
CV Weight	0.21^{b}	0.26^{abc}	0.36^{a}	0.33^{ac}	0.32^{abc}	0.31^{abc}	0.25^{bc}
K	0.95	1.08	1.18	1.07	1.10	1.16	1.14
HSI	N/A	N/A	1.00^{a}	1.23^{b}	1.43^{c}	1.53^{c}	1.14^{b}
VSI	N/A	N/A	9.23^{a}	9.60^{a}	10.87^{b}	10.79^{b}	8.16^{c}
Fin Rating	0.41^{a}	0.85^{b}	1.67^{c}	1.95^{de}	1.93^{de}	1.80^{d}	1.99^{de}

Feed Company A had the lowest suggested feeding rates of the four feed companies, starting at only 2.41% and dropping to 0.88% of the fish body weight per day by the end of the experiment (Table 2.1.2). Despite these low feeding rates, survival remained greater than 98% for each of the seven feed sizes, and did not differ among feed sizes (Table 2.1.9). Feed conversion increased with an increase in feed size, as is expected with larger fish and larger feed sizes. Weight gain was variable among the feed sizes and was dependent upon both the length of time fish were on a given size and the amount of feed provided of that size. Specific growth rate and feed intake both generally decreased with an increase in feed size, and were dependent upon feeding rate. This is the case for all four feed companies. Coefficients of variation in length and weight were smaller at the beginning of the experiment when fish were on size 0 and size 1 feed, increased when fish were on size 2, 1.2 mm, 1.5 mm, and 2.0 mm, and dropped towards the end of the experiment when fish were on 3.0 mm. Condition factor (K) was lowest when fish were on size 0 feed. Though variable, K was fairly similar across the remainder of the feed sizes. HSI and VSI were highest in fish on sizes 1.5 and 2.0 mm, suggesting that these feeds contained ingredients that allowed an increase in stored energy despite the low feeding rate. However, both metrics were reduced by the end of the experiment, suggesting that energy reserves were being used to compensate for the low feeding rates. Fin wear increased quickly at the beginning of the experiment, with fin ratings greater than 1.5 (up to 50% of the fin missing) observed in fish fed on the five largest feed sizes (Table 2.1.9). Fin wear likely occurred due to competitive interactions for the limited food availability as a result of the low feeding rates.

Myxobolus cerebralis-negative subcatchables would be stocked shortly after being on size 2 feed. Although K was highest for fish fed size 2 feed, HSI and VSI were low, suggesting that these fish would have very little stored energy and should be stocked in locations or at a time of year when food availability is high. Fin wear was evident in these fish, but given that they would not be immediately caught by anglers, it is likely the fins would regenerate prior to being caught. *Myxobolus cerebralis*-positive subcatchables would be stocked after being on 3.0 mm feed. Fish K was still relatively high in these fish, but HSI and VSI values suggest that these fish should be stocked when food availability is high. Fin wear was evident in these fish, and would be more noticeable if caught shortly after being stocked. Fish fed Feed Company A did not reach catchable size by the end of the experiment.

Table 2.1.10. Comparison of survival (%), feed conversion (g feed/g fish), weight gain (%), specific growth rate (SGR; % BW/d), feed intake (% BW/d), length (mm), CV length, weight (g), CV weight, Fulton's condition factor (K), hepatosomatic index (HSI), viscerosomatic index (VSI), and fin rating among the seven feed sizes used from Feed Company B. Different letters within the same row for a given metric represent significant differences among the feed sizes.

Metric	Size 0	Size 1	Size 2	1.0 mm	2.0 mm	3.0 mm	4.0 mm
Survival	99.78	99.77	100	100	100	98.12	98.86
Feed Conversion	0.47	0.48	0.53	0.56	0.75	0.90	1.00
Weight Gain	328.33	60.08	108.81	171.90	364.62	88.23	107.61
SGR	5.39	5.17	4.52	4.26	2.74	1.35	0.92
Feed Intake	2.77	2.45	2.43	2.49	2.25	1.24	0.94
Length	45.60	52.87	67.45	93.98	151.62	184.12	241.93
CV Length	0.05^{a}	0.06^{a}	0.08^{ab}	0.07^{a}	0.08^{a}	0.14^{b}	0.10^{ab}
Weight	1.06	1.74	3.72	9.45	43.92	76.70	174.56
CV Weight	0.17^{a}	0.20^{a}	0.24^{ab}	0.21^{ab}	0.23^{ab}	0.32^{b}	0.23^{ab}
K	1.11	1.15	1.19	1.13	1.23	1.17	1.20
HSI	N/A	1.75^{a}	1.61^{a}	2.47^{b}	1.66^{a}	1.77^{a}	2.11^{c}
VSI	N/A	12.75^{bcd}	11.89^{bde}	13.73^{c}	11.89^{de}	11.33^{ae}	10.37^{a}
Fin Rating	0.25^{de}	0.15^{e}	0.15^{e}	0.47^{dc}	0.43^{dc}	1.02^{b}	1.55^{a}

Suggested feeding rates for Feed Company B fell between those of Feed Companies A and D, and were similar to those of Feed Company C (Table 2.1.3). Survival was greater than 98% for each of the seven feed sizes, and did not differ among feed sizes (Table 2.1.10). Feed conversion increased with an increase in feed size, approaching 1.0 for fish on size 4.0 mm. Coefficients of variation in length and weight were smaller at the beginning of the experiment when fish were on size 0 and size 1 feed, increased when fish were on size 2, 1.0 mm, and 2.0 mm, were highest on size 3.0 mm, but dropped towards the end of the experiment when fish were on 4.0 mm. Though variable, K was fairly similar across the feed sizes, with the exception of size 0 when K was lowest. HSI and VSI were highest in fish on size 1.0 mm, suggesting that this size of feed had ingredients that allowed an increase in stored energy. Both metrics were variable but relatively high compared to other feed companies (Table 2.1.9 and Table 2.1.12) at all feed sizes. Fin wear remained fairly minimal until fish were on the last two feed sizes. Increased fin wear in fish fed 3.0 and 4.0 mm was likely due to the size of the fish relative to the size of the holding troughs and increased abrasion on the sides and bottom of the tank.

Myxobolus cerebralis-negative subcatchables would be stocked shortly after switching to a feed size of 1.0 mm. HSI and VSI were highest in fish on 1.0 mm feed suggesting that these fish would have a lot of stored energy and could be stocked in locations or at a time of year when food availability is low since the fish could depend upon these reserves until food availability increased. *Myxobolus cerebralis*-positive subcatchables would be stocked after being on 2.0 mm feed. Fish K was highest in these fish, and HSI and VSI values were still relatively high suggesting that these fish could also be stocked when food availability was low. Fin wear was not very evident in these fish. Catchable size fish stocked after being on size 4.0 mm had high stored energy in the liver, but less visceral fat than in previous sizes as indicated by the HSI and VSI values. However, stored energy is less important in these fish relative to smaller sizes since they are generally stocked in put-and-take fisheries and often caught and removed shortly after being stocked. Fin wear was evident in these fish, and would be noticeable when caught.

Suggested feeding rates for Feed Company C fell between those of Feed Companies A and D, and were similar to those of Feed Company B (Table 2.1.3). Survival was greater than 98.5% for each of the ten feed sizes, and did not differ among feed sizes (Table 2.1.11). Feed Company C was the only feed company to have a size of feed smaller than size 0, and feed conversion on mash was lower than any other size or type of feed used. Feed conversion increased with an increase in feed size. However, at the end of the experiment, feed conversion was still lower (0.73) than that of similar sized fish fed Feed Company B (Table 2.1.10) or Feed Company D (Table 2.1.12). Coefficients of variation in length and weight were variable throughout the experiment, but did not differ among the feed sizes, suggesting that fish size was fairly consistent and uniform no matter which size of feed the fish were being fed. Condition was lowest when fish were on mash and size 0 feeds, but fairly similar across the remainder of the feed sizes. HSI and VSI were highest in fish on sizes 1.2 mm, 1.5 mm, and 2.0 mm suggesting that these feed sizes had ingredients that allowed an increase in stored energy. The HSI and VSI values obtained on these three feeds were also twice as high as values obtained on any other size or type of feed, suggesting that fish on these feed sizes were likely quite a bit healthier than similar sized fish fed on the other feed companies. HSI and VSI values dropped to a more normal range exhibited by the other feed companies by the end of the experiment. Fin wear remained low to non-existent throughout the majority of the experiment. Increased fin wear in fish fed 4.0 mm was likely due to the size of the fish relative to the size of the holding troughs and increased abrasion on the sides and bottom of the tank.

Table 2.1.11. Comparison of survival (%), feed conversion (g feed/g fish), weight gain (%), specific growth rate (SGR; % BW/d), feed intake (% BW/d), length (mm), CV length, weight (g), CV weight, Fulton's condition factor (K), hepatosomatic index (HSI), viscerosomatic index (VSI), and fin rating among the ten feed sizes used from Feed Company C. Different letters within the same row for a given metric represent significant differences among the feed sizes.

Metric	Mash	Size 0	Size 1	Size 2	1.2 mm	1.5 mm	2.0 mm	2.5 mm	3.0 mm	4.0 mm
Survival	99.88	99.78	99.77	100	99.50	100	99.19	99.72	99.40	98.77
Feed Conversion	0.33	0.41	0.45	0.45	0.51	0.72	0.60	0.70	0.63	0.73
Weight Gain	128.96	232.78	113.46	84.50	53.17	57.94	133.36	127.74	78.94	145.49
SGR	6.90	6.01	5.42	5.22	4.56	3.26	3.31	2.35	1.91	1.28
Feed Intake	2.31	2.63	2.51	2.40	2.33	2.36	2.04	1.69	1.23	0.97
Length	27.75	43.72	55.22	66.67	74.32	89.13	118.80	154.47	185.32	249.20
CV Length	0.04	0.05^{a}	0.06^{a}	0.06^{a}	0.08^{a}	0.07^{a}	0.08^{a}	0.09^{a}	0.09^{a}	0.10^{a}
Weight	0.21	0.93	2.07	3.65	5.25	8.52	21.14	46.92	84.20	199.56
CV Weight	0.12	0.14^{a}	0.17^{a}	0.20^{a}	0.25^{a}	0.21^{a}	0.23^{a}	0.24^{a}	0.21^{a}	0.23^{a}
K	0.98	1.10	1.22	1.21	1.25	1.19	1.24	1.23	1.27	1.25
HSI	N/A	N/A	1.66^{d}	1.41^{d}	3.56^{a}	4.14^{c}	4.16^{c}	2.25^{b}	1.59^{d}	1.70^{d}
VSI	N/A	N/A	13.38^{cd}	13.01^{c}	15.95^{f}	14.51^{de}	15.49^{ef}	12.48^{bc}	11.18^{ab}	10.40^{a}
Fin Rating	0.00	0.08^{d}	0.03^{d}	0.02^{d}	0.08^{d}	0.00^{d}	0.18^{cd}	0.33^{bc}	0.43^{b}	1.72^{a}

Myxobolus cerebralis-negative subcatchables would be stocked after being fed 1.2 mm feed. HSI and VSI values were high in these fish suggesting that these fish would have a lot of stored energy and could be stocked in locations or at a time of year when food availability is low. *Myxobolus cerebralis*-positive subcatchables would be stocked after being on 2.5 mm feed. HSI and VSI values were still relatively high in these fish suggesting that these fish could also be stocked when food availability was low. Fin wear was not evident in these fish. Catchable size fish stocked after being on size 4.0 mm had lower energy reserves, but reserves are not as necessary in these fish when stocked into put-and-take fisheries. Fin wear was evident in these fish, and would be noticeable when caught shortly after being stocked.

Feed Company D had the highest suggested feeding rates of the four feed companies (Table 2.1.5). Survival was greater than 97.5% for each of the nine feed sizes, and did not differ among feed sizes (Table 2.1.12). Survival was lowest at a feed size of 3/32", lower than the survival exhibited by fish on any other feed size within the other feed companies. Feed conversion increased with an increase in feed size, approaching 1.0 towards the end of the experiment. Coefficients of variation in length and weight did not differ among any of the feed sizes, suggesting that fish size was fairly consistent and uniform no matter which size of feed the fish were being fed. Condition was lowest when fish were on size 0 feed, but fairly similar across the remainder of the feed sizes. HSI and VSI were highest in fish fed on the smaller feed sizes, and lower by the end of the experiment. HSI and VSI values were some of the lowest seen in any of the feed sizes from any feed company. Overall, HSI and VSI values suggest that none of the sizes of fish typically stocked by the state of Colorado should be stocked when food availability is low because fish fed Feed Company D have low stored energy reserves relative to fish on other feeds, and could starve shortly after being stocked. Fin wear remained low throughout the majority of the experiment. Increased fin wear in fish fed 5/32” and 3/16” was likely due to the size of the fish relative to the size of the holding troughs and increased abrasion on the sides and bottom of the tank. Fin wear would be noticeable in catchable-size fish caught shortly after being stocked.

Table 2.1.12. Comparison of survival (%), feed conversion (g feed/g fish), weight gain (%), specific growth rate (SGR; % BW/d), feed intake (% BW/d), length (mm), CV length, weight (g), CV weight, Fulton’s condition factor (K), hepatosomatic index (HSI), viscerosomatic index (VSI), and fin rating among the nine feed sizes used from Feed Company D. Different letters within the same row for a given metric represent significant differences among the feed sizes.

Metric	**Size 0**	**Size 1**	**Size 2**	**Size 3**	**Size 4**	**3/32"**	**1/8"**	**5/32"**	**3/16"**
Survival	99.56	100	99.76	99.26	99.74	97.54	100	100	99.36
Feed Conversion	0.48	0.54	0.54	0.67	0.70	0.84	0.84	0.95	0.97
Weight Gain	59.87	171.24	132.52	149.61	81.49	152.55	69.60	120.45	58.37
SGR	7.82	7.13	6.02	3.92	3.14	2.20	1.89	1.47	1.20
Feed Intake	3.74	4.00	3.35	2.73	2.23	1.91	1.60	1.44	1.17
Length	34.55	45.95	60.55	82.57	102.02	137.52	164.42	210.98	248.59
CV Length	0.06^{a}	0.08^{a}	0.06^{a}	0.08^{a}	0.09^{a}	0.10^{a}	0.08^{a}	0.08^{a}	0.07^{a}
Weight	0.42	1.15	2.80	7.05	12.25	30.87	54.13	121.18	206.23
CV Weight	0.19^{a}	0.20^{a}	0.15^{a}	0.22^{a}	0.27^{a}	0.27^{a}	0.22^{a}	0.20^{a}	0.20^{a}
K	1.00	1.16	1.26	1.22	1.12	1.15	1.19	1.26	1.31
HSI	N/A	N/A	1.46^{bc}	1.53^{b}	1.31^{ab}	1.43^{bd}	1.26^{acd}	1.11^{a}	1.27^{acd}
VSI	N/A	N/A	13.24^{d}	12.94^{d}	12.25^{cd}	11.48^{bc}	11.01^{b}	10.46^{ab}	9.67^{a}
Fin Rating	0.00^{f}	0.08^{f}	0.03^{f}	0.37^{de}	0.55^{e}	0.77^{a}	0.30^{d}	1.58^{b}	1.95^{c}

Fish fed on Feed Company C grew faster throughout the majority of the experiment than fish fed on Feed Companies A, B, and D (Figure 2.1.1). However, on average, fish were fed a smaller percentage of their body weight per day on Feed Company C than on Feed Company D. In addition, the fish on Feed Company C reached the goal weight of 200 grams per fish three weeks sooner than fish fed on Feed Company D. Feed Companies B and D performed similarly through the first half of the experiment. After mid-July, fish on Feed Company D grew faster and reached larger body sizes than fish on Feed Company B. This is likely because fish on Feed Company B were fed at a lower percentage of their body weight per day than fish on Feed Company D throughout the second half of the experiment. Fish on Feed Company B did not reach a goal weight of 200 g per fish by the end of the experiment, averaging 165 g per fish at the last sample on October 25, 2016. Fish fed on Feed Company A did not perform well compared to the other feeds. This is likely due to the recommended feeding rates provided by the company being 0.22-3.0% lower than the other companies. Fish fed on Feed Company A averaged only 38 g per fish on October 25, 2016 (Figure 2.1.1).

Over the course of the experiment, growth and health metrics varied among the four feed companies (Table 2.1.13). Overall feed conversion (averaged from the start to end of the experiment) was lowest in fish fed on Feed Companies A and C. However, the fish on Feed Company A were still relatively small at the conclusion of the experiment when feed conversion rates are often lower. Feed conversion would likely have been higher at larger sizes for this feed company. As such, feed conversion rates for fish on Feed Company C were the lowest for fish reaching catchable size. Feed Companies B and D had the highest feed conversion rates, with Feed Company D having significantly higher feed conversion rates than Feed Company B. There was not a significant difference in CV length among the feed companies suggesting that fish were consistently variable in length on all four feeds. However, weight was significantly more variable in fish fed on Feed Company A than in fish fed on Feed Companies B, C, or D. Fish on Feed Company C exhibited higher HSI and VSI values than the other three feed companies. Although HSI was higher for fish fed on Feed Company B, VSI values did not differ between Feed Companies B and D. Feed Company A had the lowest HSI and VSI values of the feed companies. Overall fin condition was lowest (better) in fish fed on Feed Company C, and highest in fish fed on Feed Company A. Survival did not differ among the four feed companies (Table 2.1.13). Across all growth and health metrics, Feed Company C produced the best fish overall, followed by Feed Company B, D, and lastly, Feed Company A.

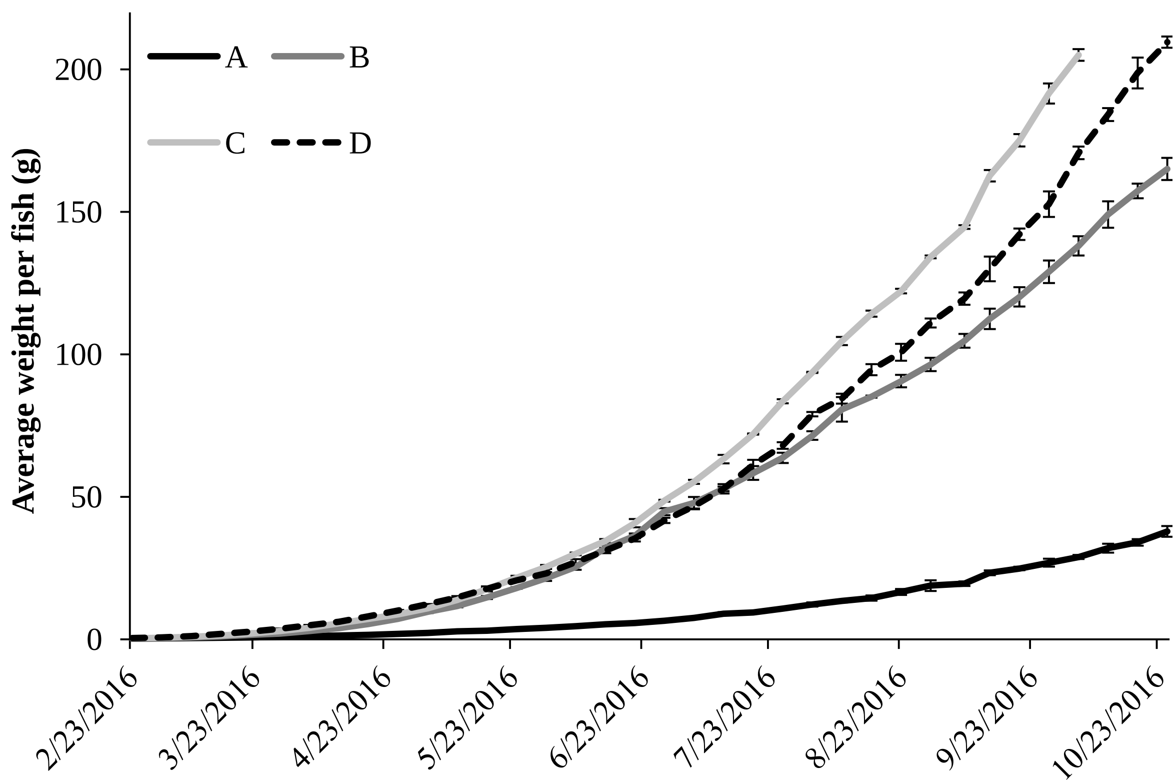

Figure 2.1.1. Average weekly weights of fish fed on Feed Companies A, B, C, and D. Error bars represent differences among replicates (3) within a feed company.

Feed Companies B, C, and D, produced catchable-size fish by the end of the experiment. On average, it took 0.37 lbs of feed to produce a catchable fish on Feed Company B, 0.33 lbs of feed to produce a catchable fish on Feed Company C, and 0.46 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, 556 tons of feed would be needed of Feed Company B, compared to 490 tons of Feed Company C, and 673 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.

Table 2.1.13. Comparison of overall survival (%), feed conversion (g feed/g fish), CV length, CV weight, hepatosomatic index (HSI), viscerosomatic index (VSI), and fin rating (± SE) among the four feed companies. Different letters within the same row for a given metric represent significant differences among the feed companies.

Metric	A	B	C	D
Survival	95.98^{a} (± 1.03)	97.11^{a} (± 1.18)	96.89^{a} (± 0.59)	95.56^{a} (± 0.59)
Feed Conversion	0.66^{a} (± 0.01)	0.90^{c} (± 0.01)	0.71^{b} (± 0.01)	0.94^{d} (± 0.01)
CV Length	0.10^{a} (± 0.01)	0.08^{a} (± 0.01)	0.08^{a} (± 0.01)	0.08^{a} (± 0.01)
CV Weight	0.29^{b} (± 0.02)	0.23^{a} (± 0.01)	0.21^{a} (± 0.01)	0.21^{a} (± 0.01)
HSI	1.22^{c} (± 0.02)	1.97^{b} (± 0.04)	2.32^{a} (± 0.09)	1.32^{c} (± 0.02)
VSI	9.14^{c} (± 0.14)	11.45^{b} (± 0.15)	12.51^{a} (± 0.18)	11.01^{b} (± 0.14)
Fin Rating	1.66^{c} (± 0.02)	0.97^{b} (± 0.03)	0.77^{a} (± 0.03)	1.04^{b} (± 0.03)

Fish Preference Results

Testers preferred the appearance of fish from Feed Company C over those from Feed Companies B and D, and fish from Feed Company B were preferred over those from Feed Company D (Table 2.1.14). With regard to fish color, fish from Feed Company C were preferred over those from Feed Company D. Fish from Feed Company B did not differ from either Feed Company C or D. Preference for fin quality did not differ among the feed companies. The total length, body depth, and body shape of fish from Feed Company C were preferred over Feed Companies B and D, which did not differ. Testers similarly preferred the head shape of fish from Feed Company C over Feed Company D, although the head shape of fish from Feed Company B did not differ from either Feed Company C or D. When it came to overall satisfaction, testers preferred the appearance of fish from Feed Company C over Feed Company D, but preference did not differ between Feed Company B and Feed Companies C and D (Table 2.1.14).

Table 2.1.14. Opinion-based ratings for appearance of fish reared on three of the four feed companies included in the hatchery feed experiment. Different letters within the same column for a given metric represent significant differences among the feed companies.

Feed Company	Average Rating	Fish Color	Fin Quality	Total Length	Body Depth	Head Shape	Body Shape	Overall Satisfaction
B	7.02^{c}	7.23^{ab}	6.97^{a}	6.91^{b}	6.68^{b}	6.88^{ab}	7.25^{b}	7.24^{ab}
C	7.8^{a}	7.59^{a}	7.27^{a}	8.01^{a}	8.15^{a}	7.41^{a}	8.13^{a}	8.04^{a}
D	6.43^{b}	6.53^{b}	6.14^{a}	6.24^{b}	6.81^{b}	6.24^{b}	6.47^{b}	6.57^{b}

Due to a small sample size (two chefs) there were few significant differences in the raw fish among the four groups included in the taste test, three groups reared at the BFRH and the Whole

Foods Market (WFM) fish. Fillet color was the only category in which differences were seen, with the WFM fish being darker than the fish reared at the BFRH, with the exception of fish from Feed Company C. There was no difference in fillet color among the fish reared at the BFRH (Table 2.1.15).

Table 2.1.15. Chef ratings for the four groups of fish included in the rainbow trout taste test, three from feed companies reared at the BFRH and the Whole Foods Market (WFM) fish. Different letters within the same column for a given metric represent significant differences among the feed companies/Whole Foods Market fish.

Feed/ Supplier	Color	Aroma	Texture	Moisture	Tenderness	Workability	Acceptability
B	2[b]	2[a]	7.5[a]	6.5[a]	8[a]	5[a]	6[a]
C	3[ab]	1.5[a]	4[a]	3.5[a]	7[a]	3[a]	4[a]
D	2[b]	2.5[a]	5.5[a]	5[a]	7.5[a]	5.5[a]	6[a]
WFM	6.5[a]	2[a]	8[a]	7.5[a]	6.5[a]	8.5[a]	9.5[a]

Table 2.1.16. Opinion-based ratings for the taste of fish reared on three of the four feed companies included in the hatchery feed experiment and the Whole Foods Market (WFM) fish provided as controls. Different letters within the same column for a given metric represent significant differences among the feed companies/WFM fish.

Feed/ Supplier	Average Rating	Fillet Color	Fishiness	Fish Texture	Palatability	Overall Flavor	Overall Satisfaction
B	5.85[bc]	5.51[a]	5.33[a]	5.79[a]	6.15[ab]	6.12[ab]	6.13[ab]
C	6.3[a]	6.27[a]	5.46[a]	6.04[a]	6.52[a]	6.78[a]	6.78[a]
D	5.64[c]	5.56[a]	5.21[a]	5.69[a]	5.68[b]	5.85[b]	5.85[b]
WFM	6.18[ab]	6.21[a]	5.46[a]	6.12[a]	6.28[ab]	6.57[ab]	6.52[ab]

On average, fish from Feed Company C had the best taste, although they did not differ from the fish provided by WFM. The taste of the fish from Feed Company C was rated higher than fish from either Feed Company B or D, with participants being least satisfied with fish from Feed Company D. The four groups did not differ with regards to fillet color, fishiness, or fish texture. However, fish from Feed Company C were more palatable and had a better overall flavor than fish from Feed Company D, and people were more satisfied overall with the fish from Feed Company C than Feed Company D (Table 2.1.16). Overall, participants preferred the pan-seared preparation (8.2 out of 10) over the smoked preparation (5.1 out of 10). Both recipes were made available for use by Colorado's anglers by putting them in the Colorado Parks and Wildlife blog (https://coloradooutdoorsmag.com/2016/11/21/taste-tested-recipes-for-your-next-trout-cookout/).

Feed Cost Comparisons

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 total length (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 2.1.17), the cost per fish was calculated for each size and number of rainbow trout stocked by Colorado (using the 2015 data presented above) for Feed Companies B, C, and D, the three feed companies that produced a catchable-size rainbow trout by the end of the experiment.

Table 2.1.17. Cost breakdown, by feed size, for each of the four feed companies used in the 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	$2.43	Size 0	$2.55
Size 1	$1.98	Size 1	$0.98	Size 0	$2.43	Size 1	$0.99
Size 2	$1.98	Size 2	$0.98	Size 1	$2.43	Size 2	$0.99
1.2 mm	$1.54	1.0 mm	$0.65	Size 2	$2.43	Size 3	$0.55
1.5 mm	$1.18	2.0 mm	$0.60	1.2 mm	$1.88	Size 4	$0.55
2.0 mm	$1.05	3.0 mm	$0.65	1.5 mm	$1.58	3/32”	$0.501
3.0 mm	$0.99	4.0 mm	$0.60	2.0 mm	$1.55	1/8”	$0.431
				2.5 mm	$1.51	5/32”	$0.431
				3.0 mm	$1.47	3/16”	$0.431
				4.0 mm	$1.36		
Average	**$1.529**	***Average***	**$0.777**	***Average***	**$1.907**	***Average***	**$0.825**

Table 2.1.18. Cost per fish estimates based on feed cost per size (Table 2.1.17) and amount of feed used per fish for Feed Companies B, C, and D. The cost to produce the number of fish of each size reared and stocked by the state of Colorado in 2015 are also shown, as is the total cost of feed to meet the numbers of fish produced.

Feed Company	Cost per fish	Neg Sub	Pos Sub	Neg Catch	Pos Catch	Total
B	$0.23	$80,871	$5,514	$435,432	$181,206	$703,024
C	$0.47	$180,930	$11,104	$894,971	$372,445	$1,459,451
D	$0.20	$79,064	$4,797	$386,558	$160,867	$631,286

The average cost to produce a fish in the hatchery feed experiment was lowest for fish reared on Feed Company D, increased slightly for fish reared on Feed Company B, and was greatly increased for fish reared on Feed Company C (Table 2.1.18). The cost per pound of Feed Company C is $1.00-$1.50 greater than for Feed Companies B or D (Table 2.1.17). 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 2.1.17), it is fairly comparable to produce over 12,000,000 rainbow trout on both of these feeds (Table 2.1.18). However, it would be less expensive to produce the other sizes of fish on Feed Company D than on Feed Company B.

Discussion

Fish fed Feed Company C grew faster, had a better feed conversion rate, had more stored energy reserves for use if stocked when food availability is low, and generally had less fin wear than the other feed companies. Less feed was used to produce a catchable fish on Feed Company C, making it more sustainable than either Feed Company B or D. Participants also preferred the appearance and taste of fish from Feed Company C. However, it costs at least twice as much to raise a fish on Feed Company C than on the other feed companies. Although fish grew slower on Feed Company B than on Feed Company D, fish tended to exhibit lower feed conversions and have more stored energy reserves than fish reared on Feed Company D. In addition, less feed was used with Feed Company B than with Feed Company D, and fish from Feed Company B were preferred for appearance over Feed Company D. It is slightly more expensive to produce a fish on Feed Company B than Feed Company D, but the increased cost may be worth the benefits gained in fish health and angler satisfaction with the final product.

Currently, cost calculations are based on feed costs alone, and do not incorporate other costs incurred by the hatchery system such as transportation, equipment use and maintenance, and employee pay. In addition, other factors identified by this experiment, such as fish in Feed Company C reaching a goal weight of 200 g three weeks sooner than Feed Company D, have not been included in the cost calculations. It is expected that there would be a significant savings in equipment maintenance, water use, and personnel costs if fish could be stocked out sooner on one feed over another. Stocking fish sooner also opens up space for slower-growing strains or species, and could allow more, larger fish of these slower-growing species to be produced annually. Finally, growth differences could allow more biologist stocking requests to be met on time with the target size fish.

The difference in time to reach the target weight at the end of the experiment between Feed Companies C and D, despite the fact that Feed Company C was fed at a lower rate than Feed Company D suggests that there is a large difference in quality between these two feeds. The way that this experiment was designed, feed rate had more of an influence on growth than did feed quality, in general. To determine if there is a difference in feed quality that results in differences in growth rate between the feed companies, this experiment is being conducted again in 2017. In general, the design of the experiment is largely the same as the one run in 2016. However, feed rates have been standardized across the feed companies, ranging from 3.3% to 1.0%. Results for this experiment will be available in the next reporting cycle.

Bailey, J., and A. Alanärä. 2006. Effect of feed portion size on growth of rainbow trout, *Oncorhynchus mykiss* (Walbaum), reared at different temperatures. Aquaculture 253:728-730.

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.

Goede, R. W., and B. A. Barton. 1990. Organismic indices and an autopsy-based assessment as indicators of health and condition of fish. Pages 93-108 *in* S. M. Adams, editor. Biological indicators of stress in fish. American Fisheries Society Symposium 8. Bethesda, Maryland.

Kientz, J., M. E. Barnes, and D. J. Durben. 2012. Performance of commercial starter diets during first-feeding of landlocked fall Chinook salmon. Proceedings of the South Dakota Academy of Science 91:101-106.

Kim, K. I. 1997. Re-evaluation of protein and amino acid requirements of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 151:3-7.

Nay, 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.

Noble, C., K. Mizusawa, K. Suzuki, and M. Tabata. 2007. The effect of differing self-feeding regimes on the growth, behaviour and fin damage of rainbow trout held in groups. Aquaculture 264:214-222.

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.

SAS Institute. 2016. SAS system software, release 9.4. SAS Institute, Cary, North Carolina.

Silverstein, J. T. 2006. Relationships among feed intake, feed efficiency, and growth in juvenile rainbow trout. North American Journal of Aquaculture 68:168-175.

Trushenski, J. T., C. S. Kasper, and C. C. Kohler. 2006. Challenges and opportunities in finfish nutrition. North American Journal of Aquaculture 68:122-140.

Trushenski, J., J. Rosenquist, and B. Gause. 2011. Growth performance, tissue fatty acid composition, and consumer appeal of rainbow trout reared on feeds containing terrestrially derived rendered fats. North American Journal of Aquaculture 73:468-478.

Wagner, E. J., M. D. Routledge, and S. S. Intelman. 1996. Assessmnet of demand feeder spacing on hatchery performance, fin condition, and size variation of rainbow trout *Oncorhynchus mykiss*. Journal of the World Aquaculture Society 27:130-136.

Action #2:

- *Level 1 Action Category: Coordination and Administration*
- *Level 2 Action Strategy: Coordination and Administration*
- *Level 3 Action Activity: Program/project administrative support*

Bacterial coldwater disease is a major disease of concern in Colorado, sometimes causing high losses of salmonid species in many state hatcheries. Although there are treatment options for the disease, treatment can be costly and time consuming, and effectiveness can vary. Disease prevention through vaccination or genetic resistance could help prevent future losses. A Ph.D.-level experiment will be proposed and developed in conjunction with Colorado State University by June 30, 2017. This project will be designed to investigate several aspects of bacterial coldwater disease, including: 1) determining the susceptibility of various strains and species of *salmonids reared by Colorado hatcheries to bacterial coldwater disease, 2) determining of the types or strains of bacterial coldwater disease in Colorado hatcheries, 3) evaluating whether the current general vaccine is effective against Colorado's strains of bacterial coldwater disease, and 4) if the general vaccine is not effective, developing strain-specific vaccines for use in Colorado hatcheries. The project may also evaluate the plausibility of incorporating newly-developed bacterial coldwater disease resistant rainbow trout into the Colorado hatchery system.*

Action #2 Accomplishments
A contract has been established to fund Ph.D. student Brian Avila through the Colorado Cooperative Fish and Wildlife Research Unit (Major Advisor, Dana L. Winkelman) in the Department of Fish, Wildlife and Conservation Biology at Colorado State University. The Ph.D. project will focus on issues relating to Bacterial Coldwater Disease (BCWD), caused by the bacterium *Flavobacterium psychrophilum*, in Colorado hatcheries. The study design will incorporate a number of field and laboratory experiments to evaluate the health and post-stocking survival of fish being reared in Colorado hatcheries at various densities and on different feeds, the post-stocking survival of healthy fish versus those that were exposed to BCWD and treated or not treated for the infection in the hatchery prior to stocking, and the effects of stocking density on post-stocking survival to determine if stocking densities, and by extension, rearing densities, could be decreased to reduce BCWD infection and mortality in the hatchery. Recently, a strain of *F. psychrophilum*-resistant rainbow trout (PRR) has been incorporated into Colorado's hatchery system. Additional laboratory experiments will be conducted to evaluate the susceptibility of the PRR, and crosses of the PRR with the Hofer and/or Hofer by Harrison Lake (H×H) rainbow trout strains, to both *Myxobolus cerebralis* and *F. psychrophilum*.

The first experiment was initiated in Spring 2017 at the BFRH. In this experiment, H×H are being reared at two different densities within hatchery troughs. One density represents the density used to avoid crowding by holding trout at densities in pounds per cubic foot less than or equal to half of their length in inches (Piper et al. 1982). The other density represents rearing densities often encountered to meet production goals in the Colorado hatchery system of pounds per cubic foot 1.5-2 times the fish length in inches. In addition, H×H are being reared on the basic feeds from Feed Companies C and D (see Job No. 2, Action #1). Mortality and basic health indices will be measured for fish reared on the various combinations of rearing density and feed. After three months, fish will be passive integrated transponder (PIT) tagged using 12 mm tags and stocked into Parvin Lake (Red Feather Lakes, Colorado) to evaluate the effects of rearing density and feed on post-stocking survival. Results of this experiment should be available in the next reporting cycle.

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.

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, although 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, 2016.

Approach

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

Samples of up to 60 fish will be collected from Parvin Lake during each survey via boat electrofishing conducted at night to increase capture probability. Up to four surveys will be conducted in fall of 2016, and summer of 2017. 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 Brush Fish Health Laboratory.

Action #1 Accomplishments
Three varieties of rainbow trout (740 fish of each variety) that had been grown to catchable size at the BFRH were stocked into Parvin Lake (Red Feather Lakes, Colorado) on April 28, 2016. These consisted of Hofer by cutthroat trout (HGBN; tag # 620286), Hofer by Colorado River rainbow trout (H×C; tag # 620287), and pure Hofer rainbow trout (HOF; tag # 620292), stocked at 2.80/lb (162 g each), 2.23/lb (203 g each), and 1.99/lb (228 g each), respectively. Samples were collected by night electrofishing events in June and October 2016. Each fish was weighed and measured, and tags were extracted for strain identification. Heads were submitted to the Aquatic Animal Health Laboratory (Brush, Colorado) for myxospore enumeration. June samples consisted of 32.6% HGBN, 15.2% HXC, 21.7% HOF, and 30.4% from previous years of stocking. HGBN averaged 244 mm total length (TL) and 146 g weight, H×C averaged 263 mm TL and 179 g, and HOF averaged 273 mm TL and 212 g. October sampling consisted of 28.8% HGBN, 33.9% HXC, 22.0% HOF, and 15.3% from previous years of stocking. HGBN averaged

297 mm TL and 264 g, H×C averaged 323 mm TL and 344 g, and HOF averaged 308 mm TL and 294 g in weight. No *Myxobolus cerebralis* myxospores were found in any of the fish collected in June or October 2016.

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 rainbows 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, 2016.
2. Conduct one fry abundance estimate in the Gunnison River by November 30, 2016.
3. Conduct one adult abundance estimate in the upper Colorado River by June 30, 2017.
4. Conduct five fry abundance estimates in the upper Colorado River by November 30, 2016.
5. Complete genetic sampling 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 by to determine genetic background by June 30, 2017.
6. 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, 2017.

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 2016. 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.

<u>Action #1 Accomplishments</u>
Adult population estimates were conducted in the Ute Park section of the Gunnison River (Figure 4.1.1) October 3-6 2016. 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 caudal fin punches, measured to the nearest millimeter, and weighed to the nearest gram. In addition, 60 genetic samples were collected from rainbow trout (see Job No. 4, Action #5) encountered throughout the reach to determine the proportion of GR genes present in the adult spawning population. Population estimates were calculated using the Lincoln-Peterson estimator (Van Den Avyle and Hayward 1999).

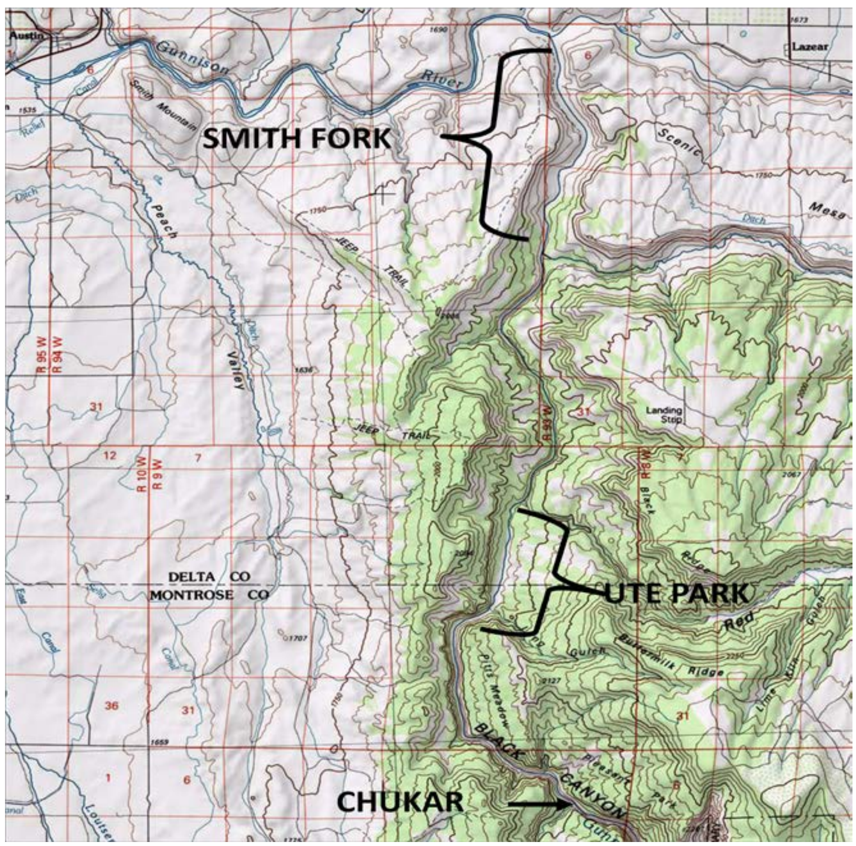

Figure 4.1.1. Map of the Gunnison River showing the location of Ute Park where fry and adult population estimates were conducted in October 2016.

An estimated 7,262 (± 996) brown trout were present per mile in the Ute Park section of the Gunnison River in 2016, and represented 89.74% of the total catch. Brown trout averaged 260 (± 93) mm total length (TL). All age classes were represented in the sample, including several juvenile (≤ 150 mm TL) brown trout, but a large proportion of the fish caught fell into the age 2 size class (150-250 mm; Figure 4.1.2). Brown trout density estimates were on the lower end of density estimates obtained in Ute Park in the mid to late 2000s (Schisler et al. 2011).

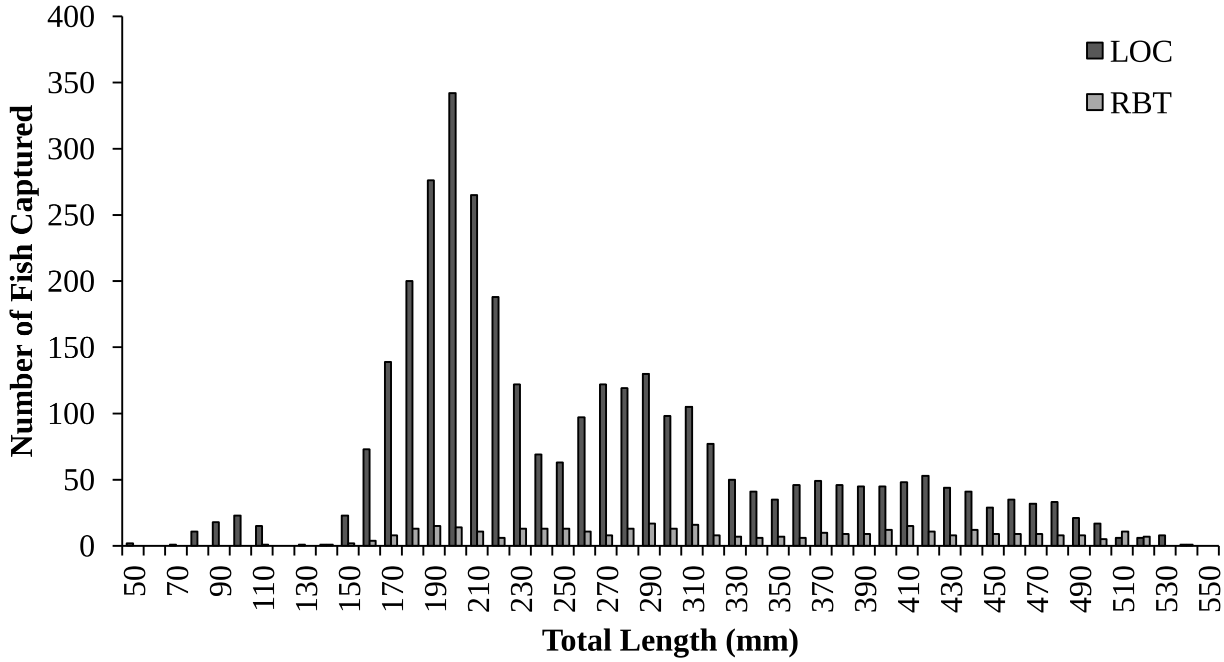

Figure 4.1.2. Number of brown trout (LOC) and rainbow trout (RBT) captured by total length (mm) during the 2016 adult population estimates in the Ute Park section of the Gunnison River.

An estimated 756 (± 93) rainbow trout were present per mile in Ute Park in 2016, making up the other 10.26% of the total catch. Rainbow trout averaged 325 (± 105) mm TL. Several age classes of rainbow trout were present, but were not easily identifiable using the length-frequency histogram (Figure 4.1.3). Several small peaks between 150 and 330 mm could represent multiple age classes, or differences in emergence, growth, and size-at-stocking for wild and stocked fish within the same age class. All size classes of adult rainbow trout (> 150 mm TL) were represented in the rainbow trout population in 2016 (Figure 4.1.3).

Rainbow trout densities in 2016 were approximately four times higher than they were in 2010 (Schisler et al. 2011). This can partially be attributed to the recent stocking and use of the Gunnison River Rainbow (GRR) fry in the Ute Park section of the Gunnison River. The GRR originates from the East Portal of the Gunnison River in the Black Canyon of the Gunnison National Park. Recent laboratory evaluations showed that the GRR had begun to develop resistance to *Myxobolus cerebralis* through continued natural reproduction and low-level exposure to the parasite (Fetherman and Schisler 2016). The combination of resistance and origin from the Gunnison River makes this strain well suited for use in the Ute Park section of the Gunnison River. Data from the 2016 population estimates suggest that the GRR are surviving and recruiting to the adult rainbow trout population in Ute Park. Biologists will continue to stock the GRR in the Gunnison Gorge for the foreseeable future. The GRR is also currently being developed as a hatchery brood stock for use in other Colorado rivers.

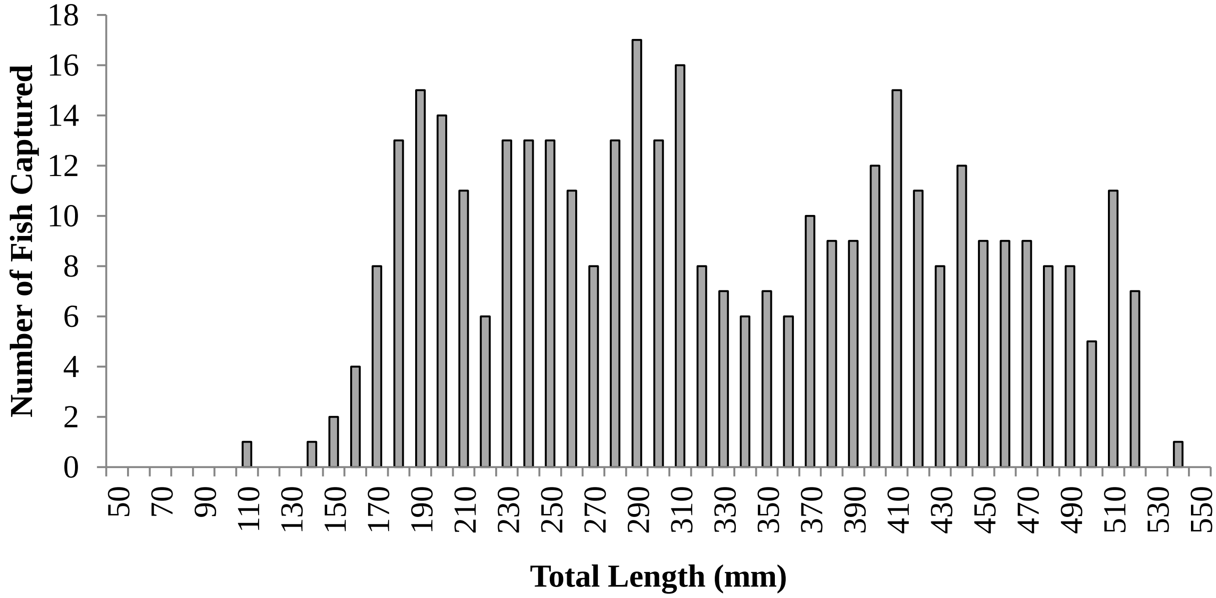

Figure 4.1.3. Number of rainbow trout (RBT) captured by total length (mm) during the 2016 adult population estimates in the Ute Park section of the Gunnison River.

Fetherman, E. R., and G. J. Schisler. 2016. Sport Fish Research Studies. Federal Aid in Fish and Wildlife Restoration Project F-394-R-15, Job Progress Report. Colorado Parks and Wildlife, Aquatic Wildlife Research Section. Fort Collins, Colorado.

Schisler, G. J., E. R. Fetherman, and B. Neuschwanger. 2011. Salmonid Disease Studies. Federal Aid in Fish and Wildlife Restoration, FINAL Progress Report F-394-R10. Colorado Division of Wildlife, Aquatic Wildlife Research Section. Fort Collins, 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*

Three-pass removal estimates for rainbow trout fry abundance, accomplished using a three electrode bank shocking unit, will be conducted in the Gunnison River in August 2016. Eight sites will be sampled, two above the Ute Park section of the Gunnison Gorge, three within Ute Park, one within the interior of the Gunnison Gorge downstream of Ute Park, and two below the confluence of the Smith Fork and Gunnison Rivers. All fry encountered will be measured and checked for signs of M. cerebralis infection. Fry 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 wild rainbow trout population, as well as the ability of the rainbow trout population to become self-sustaining.

Action #2 Accomplishments
Fry estimates were not conducted in August 2016 due to logistical constraints. Three pass removal estimates were conducted at three sites in the Ute Park section of the Gunnison River (see Figure 4.1.1) in conjunction with the adult population estimates conducted in October 2016. 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. Genetic samples were collected from all rainbow trout encountered in each site (see Job No. 4, Action #5). 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).

An estimated 8,884 (± 2,371) brown trout fry, averaging 75.9 (± 2.2) mm total length (TL), and 671 (± 360) rainbow trout fry, averaging 56.3 (± 3.9) mm TL, were present per mile in the Gunnison River in October 2016. Brown trout did not exhibit any signs of infection, but averaged 17,196 (± 9,242) myxospores per fish. The majority of the rainbow trout encountered were Gunnison River Rainbow fry that had been stocked by raft throughout the Gunnison River in August 2016. As such, none of the rainbow trout fry exhibited signs of disease, nor had any myxospores, likely because they had not been in the river long enough to develop myxospores. Rainbow trout genetic samples were sent to the University of California Davis to be included as part of a project to evaluate the genetics of wild rainbow trout populations established using *Myxobolus cerebralis*-resistant rainbow trout (see Job No. 4, Action #5).

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

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*

The adult abundance estimate in the upper Colorado River will occur in spring 2017. 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.

Action #3 Accomplishments
An adult population estimate was conducted in the 3.9 mile Chimney Rock/Sheriff Ranch study section of the upper Colorado River in April 2017, with the mark run occurring on April 17, 2017, and the recapture run occurring on April 19, 2017. 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 caudal fin punches, 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.

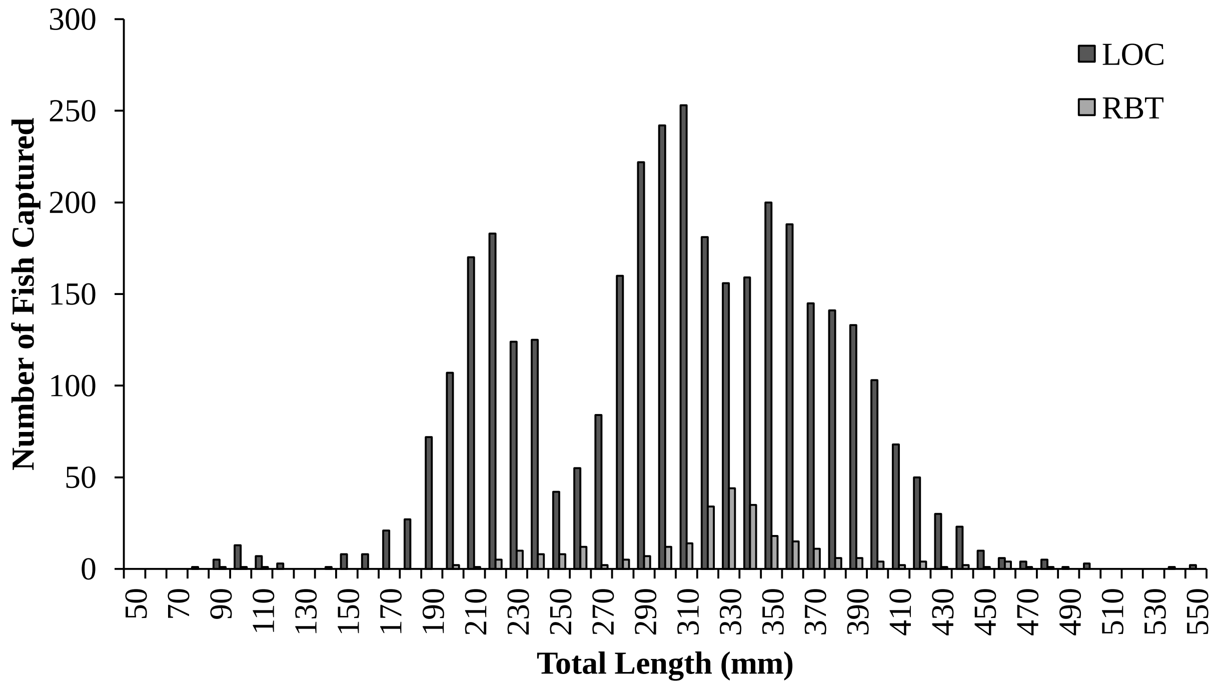

Figure 4.3.1. Number of brown trout (LOC) and rainbow trout (RBT) captured by total length (mm) during the 2017 adult population estimates in the Chimney Rock/Sheriff Ranch study section of the upper Colorado River.

An estimated 10,253 (± 550) adult brown trout were present in the Chimney Rock/Sheriff Ranch study section in 2017, nearly 3,500 more than 2016 (Fetherman and Schisler 2016). Overall, 2,629 (± 141) brown trout were present per mile in the study section, averaging 305 (± 65) mm total length (TL) and 295 (± 160) 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+ (> 260 mm TL; Figure 4.3.1).

Rainbow trout densities doubled between 2016 and 2017, with an estimated 335 (± 56) adult rainbow trout present in the study section in 2016 (Fetherman and Schisler 2016), and 643 (± 102) present in 2017. The rainbow trout population in the upper Colorado River has exhibited an exponential increase in abundance since 2013, with an estimated 165 (± 26) present per mile in the study section in 2017 (Figure 4.3.2). Adult rainbow trout averaged 320 (± 53) mm TL and 357 (± 176) g, larger than the average size rainbow trout encountered in 2016, likely a result of the higher number of age 3+ rainbow trout captured during the 2017 population estimates (Figure 4.3.3). Hofer (GR) fry stocked in 2016 (see Job No. 4, Action #4) were represented in the smaller length classes (90-140 mm TL), indicating that fry stocked in 2016 survived the winter and were recruiting. Very few fish were captured between 110 and 190 mm TL suggesting that the majority of the age 1 rainbow trout grew at least 100 mm TL in their second year in the river. Age 2 fish (150-300 mm TL) were less prevalent in the population than in previous years suggesting a weaker age class in 2018. The age 3+ rainbow trout population increased in 2017, and was much larger than in previous years, suggesting that survival of the age 2 fish from 2016 was high and that these fish had recruited to the adult spawning population (Figure 4.3.4).

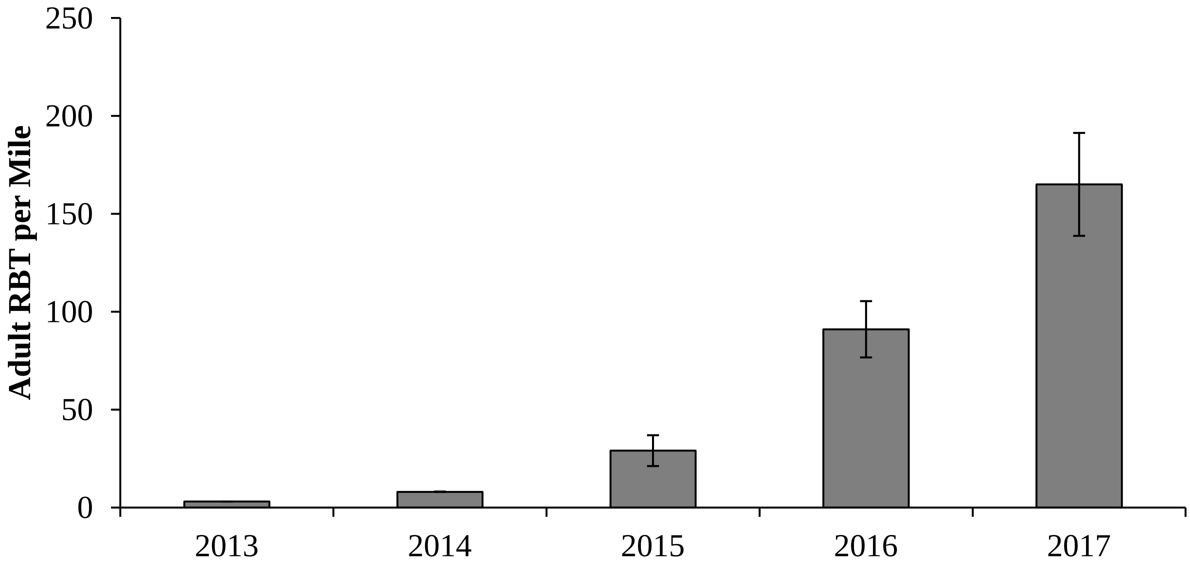

Figure 4.3.2. Estimated number of adult rainbow trout (RBT) per mile in the Chimney Rock/Sheriff Ranch study section of the upper Colorado River between 2013 and 2017.

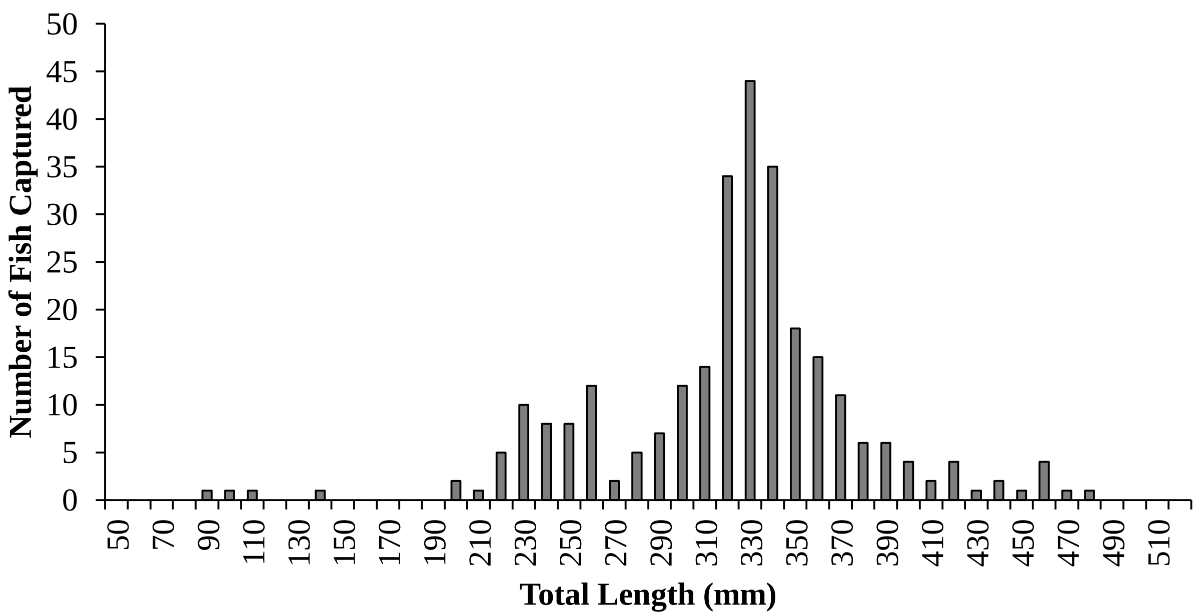

Figure 4.3.3. Number of rainbow trout (RBT) captures by total length (mm) during the 2017 adult population estimates in the Chimney Rock/Sheriff Ranch study section of the upper Colorado River.

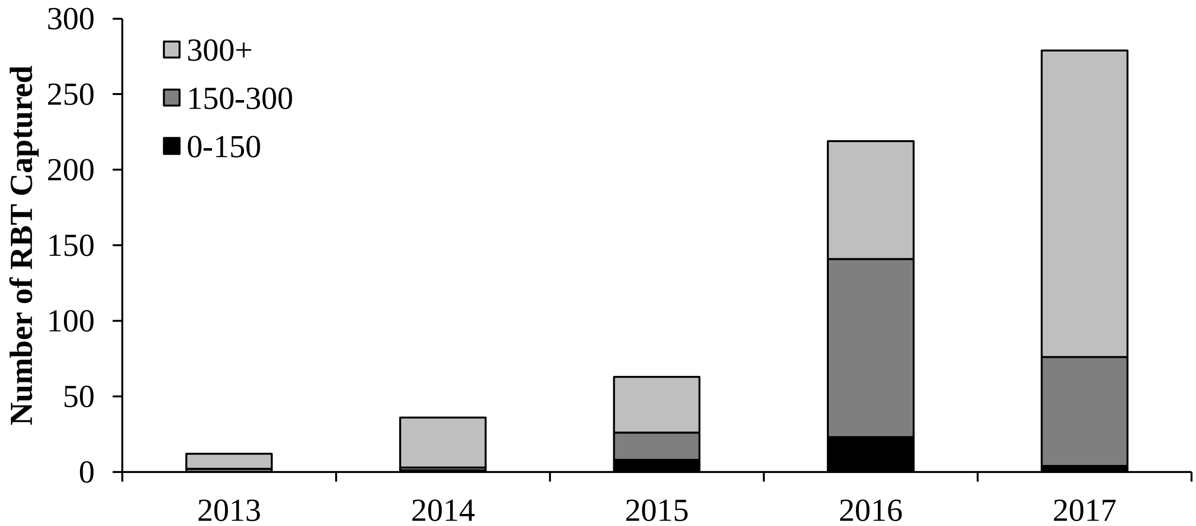

Figure 4.3.4. Number of age 1 (≤ 150 mm TL), age 2 (150-300 mm TL) and age 3+ (> 300 mm TL) rainbow trout (RBT) captured in the Chimney Rock/Sheriff Ranch study section of the upper Colorado River between 2013 and 2017.

The adult rainbow trout population in the upper Colorado River continues to grow at an exponential rate since fry stocking began in 2013. The strong age class of age 2 fish present in the population in 2016 appeared to survive well and has recruited to the adult spawning population, increasing the number of 300+ mm TL fish present in the population. Fry density estimates conducted in 2017 will be used to confirm that these fish are naturally reproducing in the upper Colorado River. The age 2 year class in 2017 was smaller than the previous year suggesting that not as many fish had recruited from the 2015 fry stocking. A number of factors could have contributed to this, including the increased adult brown trout population which could be competing with or predating upon smaller size classes, increased infection rates evident in the rainbow trout fry collected in October 2016 (see Job No. 4, Action #4), or low water events that could have increased both competitive and predatory interactions between rainbow trout and brown trout. The presence of GR juveniles in the adult population estimate is encouraging, and represents the first documentation of GR fry survival in a larger river system in Colorado. Future adult population estimates will be used to determine whether GR fry exhibit similar survival and recruitment rates to the H×C fry stocked between 2013 and 2015.

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. 2016. Sport Fish Research Studies. Federal Aid in Fish and Wildlife Restoration Project F-394-R-15, Job Progress Report. Colorado Parks and Wildlife, Aquatic Wildlife Research Section. Fort Collins, Colorado.

Action #4:

- *Level 1 Action <u>Category</u>: Data Collection and Analysis*
- *Level 2 Action <u>Strategy</u>: Research, survey or monitoring – fish and wildlife populations*
- *Level 3 Action <u>Activity</u>: 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 2016. 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 2016 will be used to determine if fish from previous fry stocking event have recruited to the adult spawning population, are reproducing, and contributing offspring to the population.

Action #4 Accomplishments

The current phase of the Colorado River 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 4.4.1) was stocked with 100,000 to 250,000 Hofer by Colorado River (H×C) 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 2016), but the survival of the GR had not been evaluated in a larger river. As such, approximately 80,000 GR fry were stocked by raft into this stretch of the upper Colorado River on July 13, 2016. 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 days prior to stocking the GR in July, and post-stocking fry population estimates were conducted at the end of July, August, September, and October 2016. 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 2016 to allow evaluation 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 4.4.1), historical sites used to evaluate fry production and survival in this section.

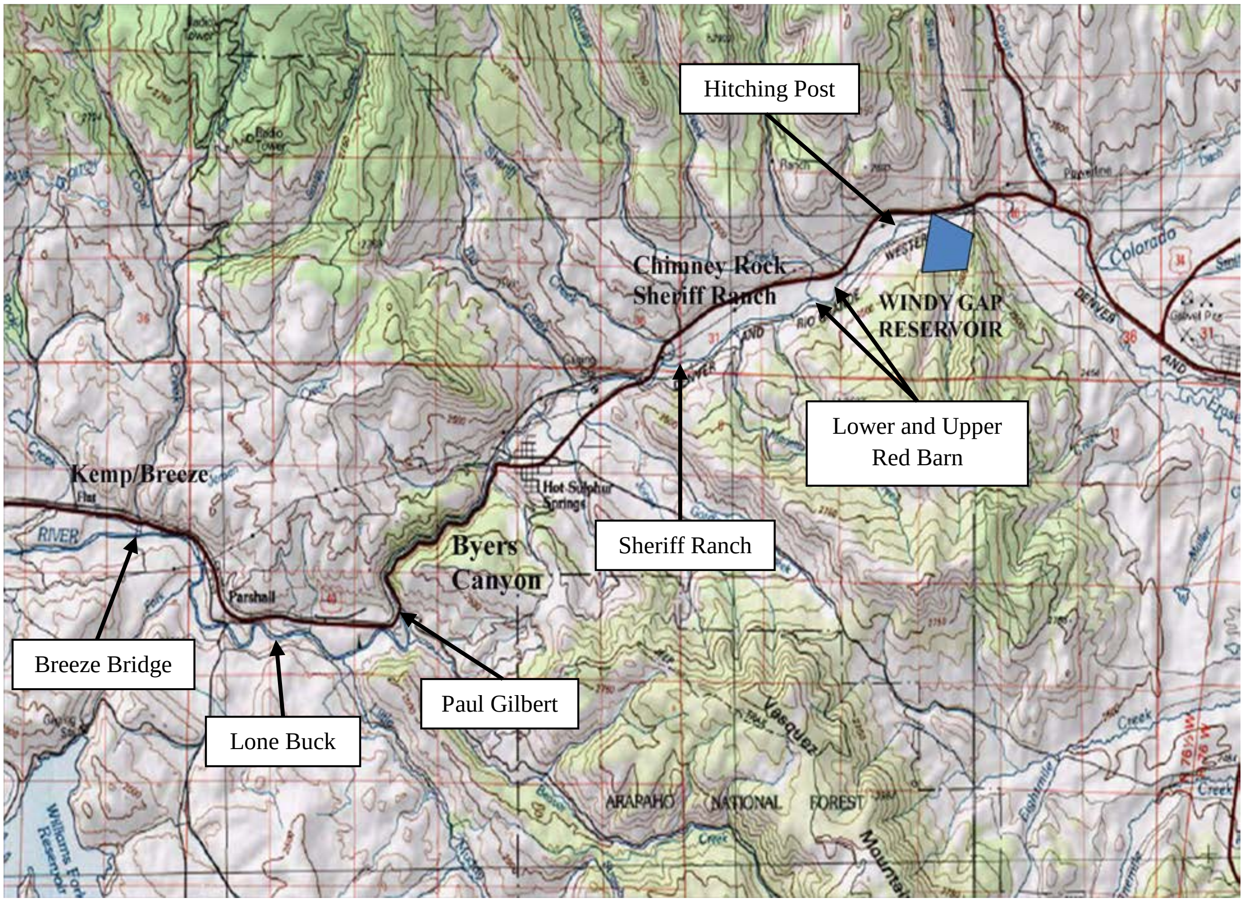

Figure 4.4.1. Map of the upper Colorado River study area showing the seven sites at which fry population estimates were conducted in July, August, September, and October 2016.

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 2016, genetic samples were taken from five rainbow trout fry at each site (see Job No. 4, Action #5), and five brown trout and five rainbow trout were collected from each site to obtain myxospore counts. Myxospore enumeration was completed 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 1,681 (± 303) brown trout fry per mile remaining in October (Figure 4.4.2). Wild rainbow trout fry densities below Byers Canyon were relatively constant between early July (214 ± 106) and late September (459 ± 274), but dropped to an estimated 106 (± 1) per mile in October. Pre-stocking, wild rainbow trout fry densities above Byers Canyon were similar to those below Byers Canyon (Figure 4.4.2). Rainbow trout fry densities, 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 (Fetherman and Schisler 2015, 2016). For the first time since the fry stocking experiments began in 2013, 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 4.4.2).

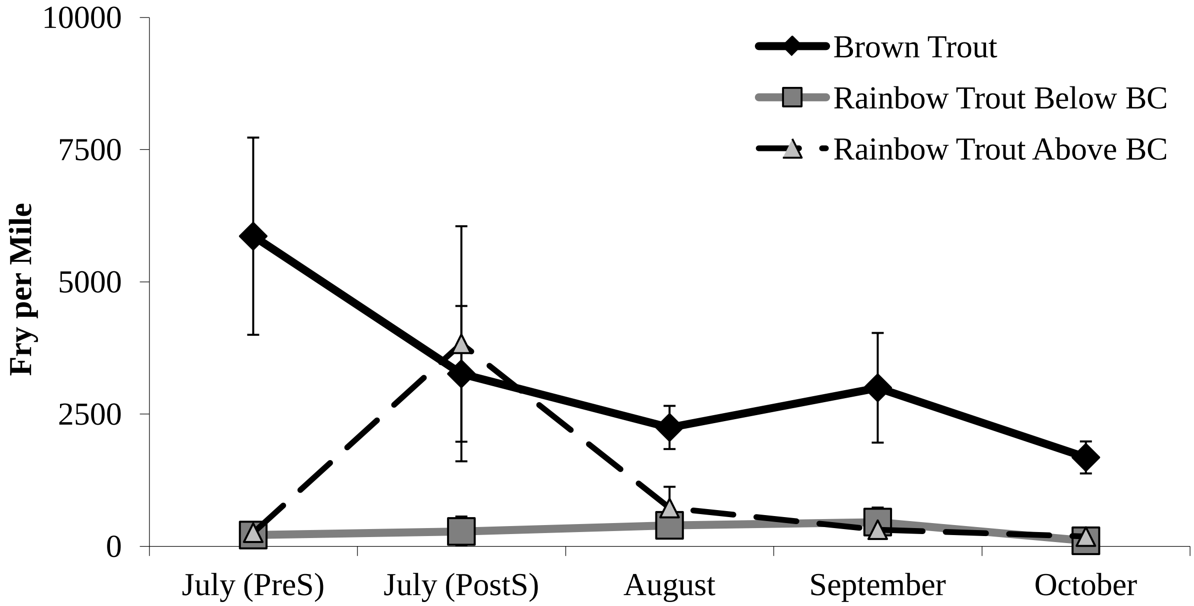

Figure 4.4.2. Upper Colorado River brown trout fry density estimates averaged across all seven sampling sites, and rainbow trout fry density estimates above and below Byers Canyon (BC; fry/mile; SE bars) for the July pre- and post-stocking sampling occasions, as well as sampling occasions occurring at the end of August, September, and October 2016.

Myxospore counts for brown trout fry averaged 3,630 (± 1,471) myxospores per fish and did not differ from myxospore counts observed in previous years (Fetherman and Schisler 2016). Disease signs were observed in only 9% of the brown trout fry encountered in October 2016, and most fry only exhibited a single sign of disease. Signs of disease in brown trout included cranial, spinal, opercular and caudal deformities. Myxospore counts for rainbow trout fry averaged 5,525 (± 1,908) myxospores per fish, higher than in previous years (Fetherman and Schisler 2016). Disease signs were observed in 20% of the rainbow trout fry encountered in October 2016. Signs of disease in rainbow trout included cranial, opercular, spinal, and lower jaw deformities, as well as exophthalmia, and several fish exhibited many of these signs simultaneously. Upon closer inspection of the myxospore counts from upstream to downstream, a spike in myxospore counts was observed at the Lower Red Barn fry site with a reduction in myxopores per fish moving downstream from this site (Figure 4.4.3). Low myxospore counts at the Hitching Post fry site suggested that Windy Gap Reservoir was not the potential source of infection for these higher myxospore counts as it had been in previous years. A water diversion structure for the Chimney Rock Ranch located just downstream of the Lower Red Barn fry site slows flows upstream of the structure. These slower flows may have created a depositional area providing habitat for the *Tubifex tubifex* worms, and perpetuating infection in both this section and for a ways downstream of the structure.

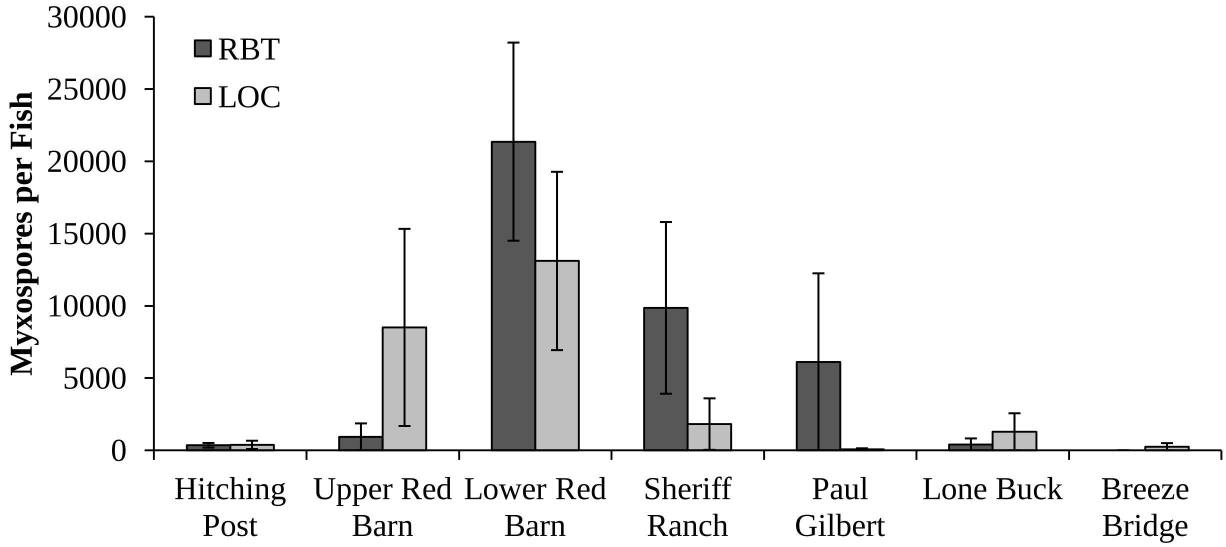

Figure 4.4.3. Average myxospores per fish (SE bars) for brown trout and rainbow trout fry collected from seven sites in the upper Colorado River in October 2016.

The GR fry apparent survival in the upper Colorado River was lower than expected given recent results suggesting that survival was similar between GR and H×C fry (Avila 2016). Several factors could have resulted in lower apparent survival rates. First, 2016 was a relatively low water year in the upper Colorado River. Low water levels were perpetuated in September 2016 when water through the bypass channel was turned off for a short period of time. This occurred concurrent with the monthly fry estimates. During this time, the river flowed only through the low flow channel, and the majority of the fry sites usually located immediately adjacent to the shoreline were located in the middle of the river. This low water event likely resulted in crowding of fish in the low flow channel, which could have increased brown trout predation rates on stocked rainbow trout fry. Additionally, rainbow trout fry could have moved to different locations in the river during this time, resulting in lower detection in September and October. Second, little is known about the post-stocking behavior of the GR fry in a larger system. It is possible that these fish continue to persist in the upper Colorado River, but have moved out of the sites where they would typically be detected. GR fry may also move away from the shoreline earlier or at a smaller size than the H×C, making them harder to detect. Future adult population estimates will help inform whether GR continue to persist in this section of the river. Several GR were captured during the spring 2017 population estimates, suggesting that these fish continue to persist and will recruit to the adult population. GR fry stocked in 2016 should be easier to detect during the 2018 adult population estimates.

Avila, B. W. 2016. Survival of rainbow trout fry in the wild: a comparison of two whirling disease resistant strains. M.S. thesis, Department of Fish, Wildlife and Conservation Biology, Colorado State University, Fort Collins, CO.

Fetherman, E. R., and G. J. Schisler. 2015. Sport Fish Research Studies. Federal Aid in Fish and Wildlife Restoration Project F-394-R-14, 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 in Fish and Wildlife Restoration Project F-394-R-15, 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 #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: Genetics*

Genetic samples will be collected from up to 30 rainbow trout fry and adults each during the abundance estimates described in Action #1-4. Non-lethal genetic samples consist of an l × 1 cm square of fin material (smaller from fry, if necessary) retrieved using scissors. Scissors will be burned off between each individual sample collection to prevent contamination among the samples. Samples will be maintained in individually labeled tubes filled with 95% ETOH and held at cold temperatures prior to analysis. 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.

Action #5 Accomplishments

Genetic samples were collected from rainbow trout fry and adults captured during fry and adult population estimates in the upper Colorado River (see Job No. 4, Action #3 and Action #4) and the Gunnison River (see Job No. 4, Action #1 and Action #2). Genetic samples consisted of fin clips taken from the upper caudal fin. Fins were preserved in 95% ethanol alcohol and stored in the freezer for later analysis.

In addition to genetic sample collection, samples collected and analyzed in previous years were organized by date and location from which the samples were collected. Since sample collection started in 2008, analysis methods have changed over time, with previous samples being analyzed using microsatellite markers (msats) or single nucleotide polymorphisms (SNPs) to determine cross between the Hofer (GR) and other wild strains, including the Colorado River (CRR) and Harrison Lake (HL) rainbow trout strains. However, this system of classification was becoming outdated since many rivers have been stocked with a number of different *Myxobolus cerebralis*-resistant rainbow trout crosses, and identification to strain or cross was becoming harder and less reliable as these fish began to spawn and intermix. More recently, samples have been analyzed using SNPs and the proportion of GR genes in any given individual was quantified for a metric that could be used to analyze data across stream locations and collection dates despite strain or cross stocked.

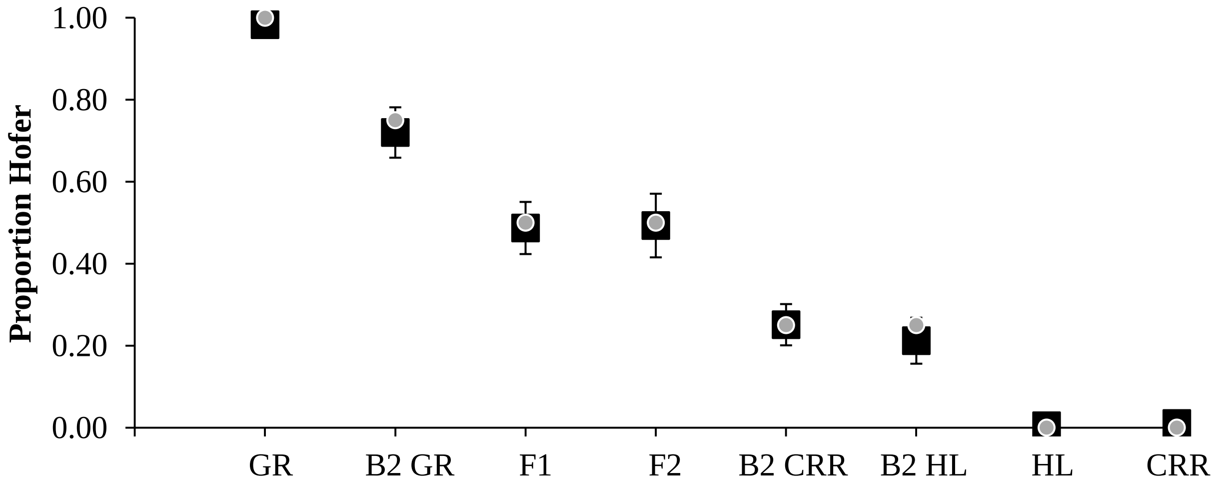

Figure 4.5.1. Median proportion GR genes identified using SNPs in fish known to be pure Hofer (GR), pure CRR, pure HL, or crosses therein (black boxes; SD bars). The proportion GR expected for each strain or cross is represented by the gray circle.

Although it would be most cost effective to reanalyze old samples for proportion GR using previous techniques, the marker sets developed for both the msats and SNPs differed from strain to strain, and as such, could have resulted in variable results. To determine if all previous samples run as msats needed to be rerun as SNPs prior to quantification of proportion GR, an analysis was conducted using blind samples to see how the results obtained using the two methods compared. First, known samples were used to determine if proportion GR in the various strains and crosses were similar to those expected. In general, results for proportion GR obtained from known samples did not deviate from expectations when using either SNPs (Figure 4.5.1) or msats (Figure 4.5.2), however, there was higher variability in proportion GR in the F1, F2, and B2 crosses using msats (Figure 4.5.2).

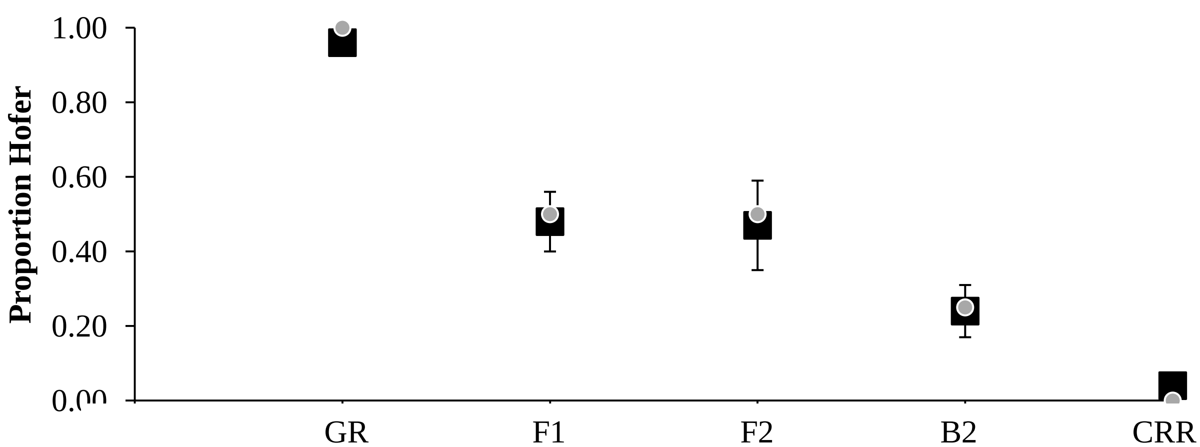

Figure 4.5.2. Median proportion GR genes identified using msats in fish known to be pure Hofer (GR), pure CRR, or crosses therein (black boxes; SD bars). The proportion GR expected for each strain or cross is represented by the gray circle.

Blind samples were then run to determine if the proportion GR results differed between msats and SNPs (Figure 4.5.3). A paired t-test was used to compare results obtained using msats or SNPs within a strain or cross. For the GR, F1, F2 and B2 (BC_CRR), proportion GR did not differ significantly between the msats and SNPs. For the CRR, the proportion GR obtained using msats (median = 0.055) was significantly higher than that obtained using SNPs (median = 0.019; $p = 0.01$). However, given that both of these values are much lower than those obtained for the first generation backcross between the F1 and pure CRR (B2 or BC_CRR), it was determined that samples previously run as msats did not need to be rerun as SNPs prior to analyzing these samples for proportion GR.

Expected	1.0	0.50	0.50	0.25	0
Msat Median	0.97	0.51	0.42	0.20	0.06
SNP Median	0.97	0.49	0.49	0.24	0.02

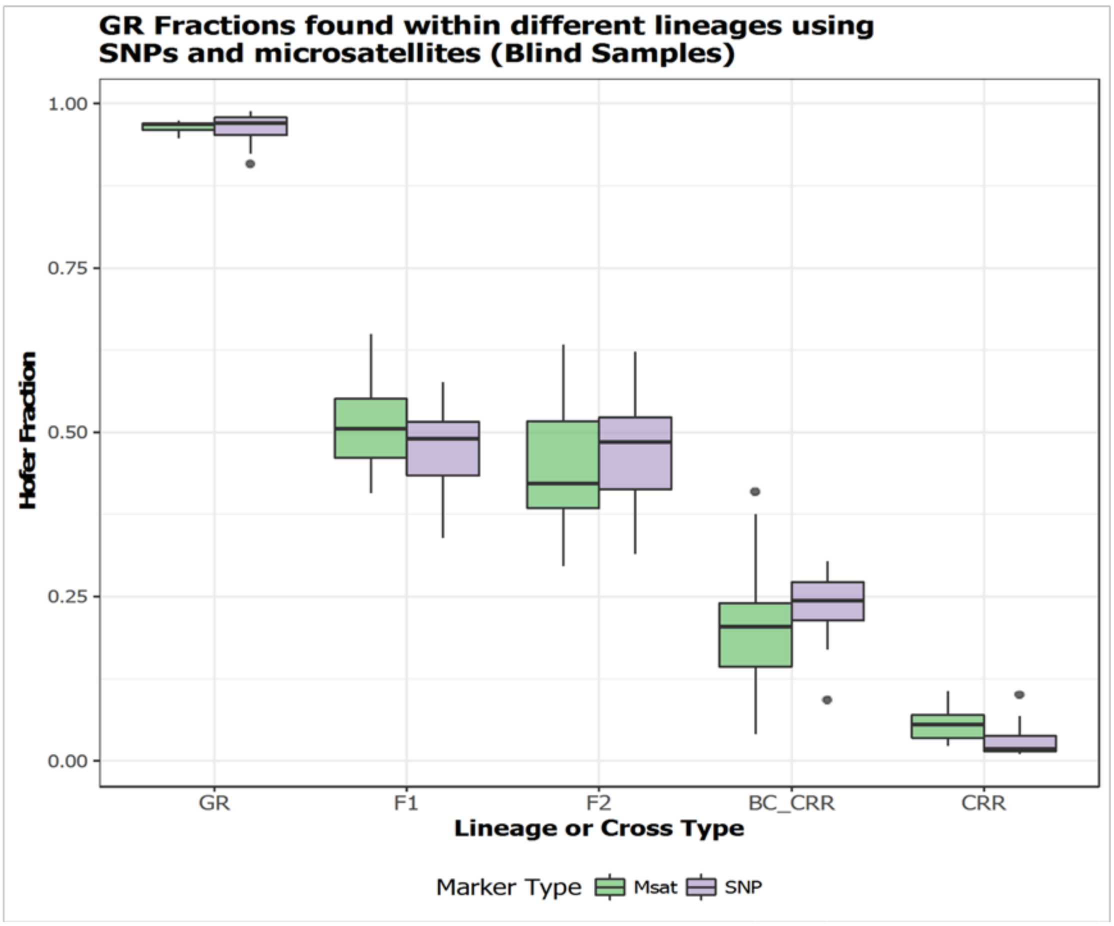

Figure 4.5.3. Comparison of proportion Hofer genes quantified for blind samples using either msats (green box plots) or SNPs (purple box plots) for the pure Hofer (GR), pure CRR, and crosses (F1, F2, and BC CRR [also known as B2]). Median values obtained from the msats and SNPs are shown above each strain or cross as are the expected values. Developed by Dr. Melinda Baerwald, University of California Davis.

Two projects are currently in the final stages of genetic sample processing and analysis, with synthesis of genetic results expected in the next reporting cycle. 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. Patterns have emerged recently that suggest that fish that express mainly CRR genes are surviving better than those that are high-proportion 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. The project focuses primarily on the Colorado and Gunnison Rivers, but also includes data from other rivers where different patterns have been observed. Approximately 1,800 samples are included in this project. Of those, 641 samples have already been run as SNPs and proportion GR quantified. Of the remaining samples, 685 have been run as msats, but proportion GR has not yet been quantified, and 472 samples remain to be processed using SNPs and analyzed for proportion GR. These 472 samples were recently sent to the Genomic Variation Laboratory at the University of California Davis for processing and analysis.

The second project focuses on the development of wild, *M. cerebralis*-resistant rainbow trout brood stocks in Colorado. One attempt at developing a wild Hofer by Colorado River (H×C) brood stock occurred in the two mile stretch of the Gunnison River in the East Portal. This two-mile section of river is located in the Black Canyon of the Gunnison National Park. It is bordered on the upstream end by Crystal Dam and on the downstream end by a water diversion for the Montrose Canal. Infection levels within the East Portal are relatively low compared to other rivers in the state. Due to its enclosed nature, H×Cs were stocked in the East Portal between 2006 and 2012 in an attempt to develop a wild H×C brood stock. However, a wild population of CRRs, stocked for decades prior to the establishment of *M. cerebralis*, continued to persist this section of river. Despite several attempts to establish the H×C, this strain performed relatively poorly compared to its wild counterparts. As such, only a small proportion of the rainbow trout in this location exhibited GR genetic characteristics after years of stocking. Exposure experiments were conducted to determine if the CRRs from the East Portal had developed resistance to *M. cerebralis* as a result of continued low-level exposure and natural reproduction. Resistance of the CRR was determined to be fairly high, with relatively low myxospore counts compared to CRRs in previous laboratory experiments. This has lead to this brood stock being known as the Gunnison River Rainbow (GRR). H×Cs are no longer stocked in this location, and GRRs have been adopted as a new strain in Colorado's hatchery system. Wild spawns are taken every spring to aid in the development of this new hatchery brood stock.

The second brood stock that this project focuses on is the development of a wild Hofer by Harrison Lake (H×H) brood stock in Lake Catamount and the Yampa River near Steamboat Springs. Similar to the East Portal, the Yampa River is enclosed between Lake Catamount and the Stagecoach Reservoir Dam. H×Hs were first stocked in the late 2000's to establish this wild brood stock. Two exposure experiments have been conducted to monitor the *M. cerebralis* resistance of this brood stock. One experiment used wild rainbow trout collected from various tributary streams in 2010. The second was conducted using fish created from parents returning to Harrison Creek (tributary to Lake Catamount) to spawn. Experiments showed that myxspore counts in the Yampa River had been reduced as a result of stocking H×Hs. However, resistance was variable among families created from fish spawning in Harrison Creek as a result of outcrossing with other strains of rainbow trout that continued to persist in this location. Finally, experiments showed that the higher the GR component in a given family, the higher the *M. cerebralis* resistance. Approximately 1,330 samples are included in this project. Of those, 298 samples have been run as SNPs and analyzed for proportion GR. Of the remaining samples, 140 have been run as SNPs and 550 have been run as msats, but proportion of GR has not yet been quantified, and 343 samples remain to be processed using SNPs and proportion GR quantified. These 343 samples were recently sent to the Genomic Variation Laboratory at the University of California Davis for processing and analysis.

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*

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 2016, 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.

Action #6 Accomplishements

Colorado State University masters degree student Brian Avila recently completed his thesis in which he describes the results of experiments designed to determine if there were differences in post-stocking survival between *M. cerebralis*-resistant rainbow trout strains when stocked as fry (Avila 2016). Brian conducted two experiments, one in the lab and one in the field, examining the post-stocking survival of the pure Hofer (GR) and Hofer by Colorado River (H×C) rainbow trout strains. For the field experiment conducted in 2014-2015, 5,000 rainbow trout fry from each strain were stocked into one mile study reaches of nine Colorado streams. Coded wire tags inserted in the nose of the H×C made differentiation between the two strains possible during recapture events, and allowed an estimation of survival and growth rate at two-months post-stocking, over winter, and annually. In the laboratory experiment, a 50:50 mix of the GR and

H×C was stocked into a large open mesocosm with a wild brown trout predator, and survival was estimated over a 24-hour time period where cover was or was not present in the mesocosm.

Table 4.6.1. Density estimates (fish per mile), length, and weight (± SE) for brown trout (LOC), and the GR and H×C rainbow trout strains for each stream sampled in July/August 2016.

	LOC	GR	HXC
	Sheep Creek		
Density	449 ± 18	0	0
Length	121.8 ± 5.8	NA	NA
Weight	28.6 ± 2.0	NA	NA
	North Fork of the Cache la Poudre River		
Density	3129 ± 396	0	14 ± 14
Length	103.8 ± 5.8	NA	245.0 ± 0
Weight	39.8 ± 4.2	NA	146.0 ± 0
	Lone Pine Creek		
Density	2822 ± 486	12 ± 12	0
Length	113.7 ± 4.9	143.0 ± 0	NA
Weight	32.4 ± 7.4	31.0 ± 0	NA
	Willow Creek		
Density	285 ± 98	0	0
Length	147.6 ± 31.5	NA	NA
Weight	97.9 ± 23.5	NA	NA
	Spielberg Creek		
Density	430 ± 227	0	28 ± 28
Length	164.0 ± 20.4	NA	285.0 ± 15.0
Weight	117.4 ± 14.6	NA	258.0 ± 35.0
	Rock Creek		
Density	1033 ± 388	0	8 ± 8
Length	113.9 ± 26.2	NA	220.0 ± 0
Weight	34.8 ± 21.6	NA	120.0 ± 0
	Tarryall Creek		
Density	1888 ± 208	16 ± 16	48 ± 48
Length	161.3 ± 9.7	245.0 ± 0	241.7 ± 14.4
Weight	73.9 ± 12.4	165.0 ± 0	159.7 ± 33.6
	Michigan Creek		
Density	3542 ± 333	12 ± 12	12 ± 12
Length	122.5 ± 17.2	210.0 ± 0	225.0 ± 0
Weight	42.2 ± 15.5	94.0 ± 0	113.0 ± 0

In summary, the field experiment revealed that apparent survival and growth rate was influenced by strain and stream characteristics, primarily average temperature, within the first year. After two months in the wild, the H×C exhibited a higher growth rate than the GR, opposite of what is typically seen in the hatchery. However, after 12 months there was no significant difference in apparent survival or growth rate between the GR and H×C. The laboratory experiment corroborated the findings from the field experiment, revealing that there were no differences in survival between the strains when confronted with brown trout predation, whether or not cover was present. Overall, the results from these experiments indicated that the GR may be a viable alternative for fry stocking purposes in streams that contain *M. cerebralis* (Avila 2016). However, when stocking rainbow trout as fry, the goal is not only survival through the first year, but continued persistence and recruitment to the adult spawning population.

To determine if the GR, H×C, or both continued to persist in the streams, three pass removal estimates were conducted in two sites (220 ft, on average) within eight streams located in three major river drainages in July/August 2016: 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 2016 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 all three 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 the three-pass removal equations of Seber and Whale (1970) for each site, and density estimates from the two sites were averaged to obtain an estimate of fish per mile for each stream.

Rainbow trout were encountered in six of eight streams in 2016 (Table 4.6.1), and a number of other species were encountered in the various streams during sampling, including brown trout (Table 4.6.1), brook trout, longnose dace, longnose sucker, mottled sculpin, speckled dace, white sucker, creek chub, Johnny darter, and brook stickleback (Table 4.6.2). Both GR and H×C were encountered in Michigan and Tarryall Creeks, both of which had shown higher annual GR and H×C apparent survival rates than many of the other streams in 2015 (Avila 2016). Of the remaining four streams, H×C continued to persist in three (North Fork of the Cache la Poudre River, Spielberg Creek, and Rock Creek), whereas the GR persisted in only one (Lone Pine Creek). The lack of rainbow trout in Willow Creek was not unexpected given the low annual apparent survival rates estimated in 2015. However, not encountering rainbow trout in Sheep Creek was unexpected since Sheep Creek had the highest annual apparent survival rate for H×Cs in 2015 (Avila 2016). It is possible that rainbow trout continue to persist in Sheep Creek, and were just not encountered in the study sites in 2016.

The sampling scheme for 2017 will be changed so that the entire one mile study reach is sampled during electrofishing efforts, which will help in determining if rainbow trout continue to persist in these streams outside of the shorter study sites. Additionally, longitudinal sampling will be used to detect the presence of rainbow trout fry. Because rainbow trout were not present in any of these streams prior to being stocked in 2014, the presence of rainbow trout fry would indicate natural reproduction by one of the two strains. To determine which strain spawned and contributed offspring to the population, genetic samples will be collected from all rainbow trout fry encountered in 2017 using the techniques described in Job No. 4, Action #5.

Table 4.6.2. Density estimates (fish per mile), length, and weight (± SE) for additional fish species encountered in each stream sampled in July/August 2016. Additional species encountered included brook trout (BRK), longnose dace (LND), longnose sucker (LGS), mottled sculpin (MTS), speckled dace (SPD), white sucker (WHS), creek chub (CRC), Johnny darter (JOD), and brook stickleback (BSB).

	BRK	LND	LGS	MTS	SPD	WHS	CRC	JOD	BSB
Sheep Creek									
Density	1243 ± 83	350 ± 118	26 ± 1	0	0	0	0	0	0
Length	108.2 ± 2.5	101.1 ± 1.3	125.5 ± 3.5	NA	NA	NA	NA	NA	NA
Weight	20.4 ± 2.5	10.2 ± 0.1	20.3 ± 0.7	NA	NA	NA	NA	NA	NA
North Fork of the Cache la Poudre River									
Density	14 ± 14	445 ± 17	106 ± 52	0	0	0	0	0	0
Length	56.0 ± 0	82.1 ± 2.1	167.3 ± 3.3	NA	NA	NA	NA	NA	NA
Weight	1.3 ± 0	5.5 ± 0.9	56.6 ± 3.1	NA	NA	NA	NA	NA	NA
Lone Pine Creek									
Density	0	4342 ± 1289	89 ± 37	0	0	260 ± 212	24 ± 24	12 ± 12	12 ± 12
Length	NA	64.2 ± 1.2	110.6 ± 16.4	NA	NA	193.8 ± 160.3	78.0 ± 1.0	49.0 ± 0	53.0 ± 0
Weight	NA	2.8 ± 0.1	20.3 ± 10.3	NA	NA	120.8 ± 120.4	5.3 ± 0.9	1.5 ± 0	0.8 ± 0
Willow Creek									
Density	22 ± 22	0	0	4732 ± 777	286 ± 145	0	0	0	0
Length	195.0 ± 17.0	NA	NA	55.4 ± 0.9	77.7 ± 9.7	NA	NA	NA	NA
Weight	83.5 ± 25.5	NA	NA	3.1 ± 0.1	6.1 ± 0.3	NA	NA	NA	NA
Spielberg Creek									
Density	0	0	412 ± 412	4842 ± 4842	2458 ± 2458	0	0	0	0
Length	NA	NA	143.2 ± 22.6	67.8 ± 14.1	66.3 ± 11.2	NA	NA	NA	NA
Weight	NA	NA	32.1 ± 16.5	4.3 ± 2.8	2.6 ± 1.5	NA	NA	NA	NA
Rock Creek									
Density	1891 ± 1132	0	44 ± 44	0	0	0	0	0	0
Length	71.5 ± 8.6	NA	107.8 ± 24.4	NA	NA	NA	NA	NA	NA
Weight	7.2 ± 3.6	NA	13.5 ± 10.5	NA	NA	NA	NA	NA	NA
Tarryall Creek									
Density	127 ± 95	0	0	0	0	0	0	0	0
Length	137.4 ± 3.4	NA	NA	NA	NA	NA	NA	NA	NA
Weight	35.4 ± 10.4	NA	NA	NA	NA	NA	NA	NA	NA
Michigan Creek									
Density	1131 ± 131	0	12 ± 12	0	0	0	0	0	0
Length	113.0 ± 14.8	NA	259.0 ± 0	NA	NA	NA	NA	NA	NA
Weight	34.1 ± 12.4	NA	175.0 ± 0	NA	NA	NA	NA	NA	NA

Avila, B. W. 2016. Survival of rainbow trout fry in the wild: a comparison of two whirling disease resistant strains. M.S. thesis, Department of Fish, Wildlife and Conservation Biology, Colorado State University, Fort Collins, CO.

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

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, 2017.
2. Complete one peer review of a manuscript submitted to a scientific journal by June 30, 2017.

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 use of *Myxobolus cerebralis*-resistant rainbow trout and rearing *M. cerebralis*-resistant rainbow trout in Colorado hatcheries. Three presentations were given at the CPW Southwest Biology Days and statewide hatchery managers meetings:

- Fetherman, E. R., and B. W. Avila. 2016. Overview of whirling disease-resistant rainbow trout success in Colorado. Colorado Parks and Wildlife Southwest Biology Days. Montrose, Colorado. May 19, 2016.
- Fetherman, E. R. 2016. Rainbow trout wild and hatchery research projects update. Colorado Parks and Wildlife Hatchery Managers Meeting. Wray, Colorado. September 27, 2016.
- Fetherman, E. R., B. Neuschwanger, C. Praamsma, and T. Davis. 2017. Comparison of basic trout feeds for rainbow trout. Colorado Parks and Wildlife Hatchery Managers Meeting. Frisco, Colorado. May 9, 2017.

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, chapter and division meetings of the American Fisheries Society, and to Colorado Trout Unlimited:

- Fetherman, E. R. 2017. Comparison of basic feeds for producing rainbow trout. 2017 Annual Meeting of the Colorado Aquaculture Association. Mt. Princeton Hot Springs, Colorado. February 3, 2017.
- Hodge, B. W., E. R. Fetherman, R. Henderson, and K. B. Rogers. 2017. PIT technology elucidates the biological effectiveness of a fishway. 2017 Joint Meeting of the Utah and Colorado/Wyoming Chapters of the American Fisheries Society. Grand Junction, Colorado. February 22, 2017. Received award for best professional presentation.
- Avila, B. W., D. L. Winkelman, and E. R. Fetherman. 2017. Survival of rainbow trout fry in the wild: a comparison of two whirling disease resistant strains. The Cutthroat Chapter of Colorado Trout Unlimited. Highlands Ranch, Colorado. March 21, 2017.
- Kondratieff, M., E. Fetherman, B. Fox, N. Kolden, and K. Kinzli. 2017. Whitewater parks: Implications for fish habitat and fish passage. Annual Meeting of the Western Division of the American Fisheries Society. Missoula, Montana. May 21-25, 2017.

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 2017:

- Fetherman, E. R. 2017. Salmonid disease research in Colorado. Front Range Community College, Fisheries Management class. Fort Collins, Colorado. March 22, 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:

- Kopack, C. J., E. D. Broder, E. R. Fetherman, J. M. Lepak, and L. M. Angeloni. 2016. The effect of a single prerelease exposure to conspecific alarm cue on poststocking survival in three strains of rainbow trout (*Oncorhynchus mykiss*). Canadian Journal of Zoology 94(9):661-664. DOI: 10.1139/cjz-2016-0086.

- Fetherman, E. R., J. A. Wardell, C. J. Praamsma, and M. K. Hura. 2016. Critical dissolved oxygen tolerances of whirling disease-resistant rainbow trout. North American Journal of Aquaculture 78:366-373.

In addition to those manuscripts published in peer-reviewed journals, two other manuscripts were submitted for publication:

- Richer, E. E., E. R. Fetherman, M. C. Kondratieff, and T. A. Barnes. *In review*. Incorporating GPS and mobile radio frequency identification to detect PIT-tagged fish and evaluate habitat utilization in streams. Submitted to the North American Journal of Fisheries Management.
- Hodge, B. W., E. R. Fetherman, R. Henderson, and K. B. Rogers. *In review*. Effectiveness of a fishway for restoring passage of Colorado River cutthroat trout. Submitted to North American Journal of Fisheries Management.

Newsletters produced by and for fisheries associations and societies provide an outlet for keeping other fisheries professionals informed regarding the research that the state of Colorado is conducting. Two articles were written for The Fishline, the quarterly newsletter of the Colorado Aquaculture Association, and The Tributray, the newsletter for the Western Division of the American Fisheries Society:

- Fetherman, E. R., and G. J. Schisler. 2016. Hofer rainbow trout research update. The Fishline, Official Publication: Colorado Aquaculture Association. Volume XVIII No. 1. Spring 2016.
- Fetherman, E. R. 2016. Whirling disease resistant rainbow trout fry stocking evaluations. Western Division of the American Fisheries Society Newsletter, The Tributary. Volume 40, Issue 2. Summer 2016.

Public involvement in the appearance and taste preference tests for the hatchery feed experiment (Job No. 2, Action #1) got people interested in the research being conducted regarding the quality of rainbow trout reared and stocked by the Colorado hatchery system. Working with CPW public relations specialist Alicia Cohn, a blog was written for the CPW website which helped inform the public about the research being conducted to produce a higher quality, better tasting rainbow trout using different feeds at the BFRH. Additionally, recipes were provided by local chefs for anglers to use the next time they are preparing rainbow trout they caught. The blog can be accessed at https://coloradooutdoorsmag.com/2016/11/21/taste-tested-recipes-for-your-next-trout-cookout/.

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

- Collected brown trout scales and genetic samples for a study examining movement rates between Spinney Reservoir and the Middle Fork of the South Platte River. Assisted with age determination and calculations of growth rate obtained from the scale samples, and worked with geneticists at the University of California Davis to complete an analysis regarding relatedness between brown trout with various spotting patterns.
- Consulted on study design and interpretation of data collected from a sentinel cage study used to determine if a surface water source near the CPW Roaring Judy Hatchery is pathogen free and appropriate to use for increasing hatchery flow rates during the summer months.

- Completed AIC analysis comparing factors affecting adult salmonid abundance and biomass in whitewater park and control pools in the St. Vrain River in Lyons, Colorado.

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:
- Gibson-Reinemer, D. K., J. H. Chick, T. D. VanMiddlesworth, M. VanMiddlesworth, and A. F. Casper. Widespread and enduring demographic collapse of invasive common carp (*Cyprinus carpio*) in the Upper Missouri River System. Submitted to Biological Invasions.
- Korman, J., and M. D. Yard. Trends in recruitment, abundance, survival, and growth over a boom-and-bust cycle of a Rainbow Trout tailwater population. Submitted to Transactions of the American Fisheries Society.
- Anonymous. Elimination of *Myxobolus cerebralis* in Placer Creek, a native cutthroat trout stream in Colorado. Submitted to Journal of Aquatic Animal Health.