Sport Fish Research Studies

Federal Aid Project F-394-R15

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

and

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

Federal Aid in Fish and Wildlife Restoration

Job Progress Report

Colorado Parks & Wildlife

Aquatic Research Section

Fort Collins, Colorado

August 2016

STATE OF COLORADO

John W. Hickenlooper, Governor

COLORADO DEPARTMENT OF NATURAL RESOURCES

Bob Randall, Executive Director

COLORADO PARKS & WILDLIFE

Bob Broscheid, Director

WILDLIFE COMMISSION

AQUATIC RESEARCH STAFF

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

Jim Guthrie, Federal Aid Coordinator
Kay Knudsen, Librarian

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

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

Date: 08-17-2016

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

Table of Contents

State: Colorado **Project No. F-394-R15**

Project Title: Sport Fish Research Studies

Period Covered: July 1, 2015 – June 30, 2016

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, maintain, and rear three brood stocks of *M. cerebralis*-resistant rainbow trout at the Bellvue Fish Research Hatchery through June 30, 2016.
2. Mark 3,000 *M. cerebralis*-resistant rainbow trout with coded wire tags for use in research project assessing post-stocking survival and resistance of various strains stocked as fingerlings by June 30, 2016.
3. Distribute 50,000 eggs and fish for laboratory use, fish production, and wild trout experiments by June 30, 2016.

Approach

Action #1:

- *Level 1 Action Category: Species Reintroduction and Stocking*
- *Level 2 Action Strategy: Production and stocking for 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 2015 through January 2016, and maintained and reared through June 30, 2016. 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 unique stocks are individually marked with Passive Integrated Transponder (PIT) and/or Visible Implant Elastomer (VIE) tags before leaving the main hatchery. This allows for definitive identification before the fish are subsequently used for spawning.

Starting in the middle of October 2015, BFRH personnel checked all of the Hofer (GR)[1] and Harrison Lake (HL) brood fish (2, 3, and 4 year-olds) weekly for ripeness. The third strain of GRXHL brood stock has been discontinued at this time. 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, 2015, 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 from all others. 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.

[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.

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. Eyed eggs were shipped via insulated coolers to other state and federal hatcheries three days following physical shock (see Job No. 1, Action #3). Select groups of eggs were kept for brood stock purposes at the BFRH.

Action #2:

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

Myxobolus cerebralis-resistant rainbow trout strains and crosses will be batch marked using coded wire tags for identification following collection from the field. Marked fish with be stocked as fingerlings in Parvin Lake to assess post-stocking survival and disease resistance (see Job No. 3).

Action # 2 Accomplishments

Three strains of *M. cerebralis*-resistant rainbow trout were reared at the BFRH for use in the Parvin Lake fingerling evaluation experiment (see Job No. 3, Action #1): 1) Hofer (GR), 2) Hofer by recreational greenback cutthroat trout (GR×GBN), and 3) Hofer by Colorado River Rainbow trout (GR×CRR). Three thousand fish from each of the three strains were batch marked with coded wire tags using both a handheld tagging gun and the Mark IV automatic tag injector (Northwest Marine Technology, Inc., Shaw Island, WA), and tag numbers were unique to each strain (GR: 620292; GR×GBN: 620286; GR×CRR: 620287). All three strains were stocked into Parvin Lake on April 28, 2016. At the time of stocking, GR averaged 274 mm total length (TL), GR×GBN averaged 245 mm TL, and GR×CRR averaged 265 mm TL.

Action #3:

- *Level 1 Action Category: Species Reintroduction and Stocking*
- *Level 2 Action Strategy: Production and stocking for recreational purposes*
- *Level 3 Action Activity: N/A*

Eggs spawned by the Bellvue Fish Research Hatchery will be distributed to the CPW Hatchery Section for rearing and stocking into recreational fisheries. Additional fish reared by the Bellvue Fish Research Hatchery will be used in laboratory and wild trout experiments in several locations across the state.

Action # 3 Accomplishments

The BFRH 2015/2016 on-site rainbow trout production spawn started on November 17, 2015, with the last groups of HL females spawned on January 19, 2016. The initial goal was to produce 100,000 eyed eggs; egg take exceeded the production needs with 205,181 eyed eggs produced (Table 1.3.1). BFRH personnel were able to fill all GR and HL production and research directed project egg requests for Colorado in 2015/2016.

Table 1.3.1. Bellvue Fish Research Hatchery on-site spawning information for the Hofer (GR), and Harrison Lake (HL), rainbow trout strains during the winter 2015-2016 spawning season**.**

Strain	Date Spawned	No. Spawned Females	No. Green Eggs	No. Eyed Eggs	Shipped To
100% HL	12/16/15-1/19/16	179	32,421	29,165	Fish Research Hatchery
100% GR	11/17/15-12/16/15	180	202,318	176,016	Fish Research Hatchery/CPW Hatcheries
Total	11/17/15-1/19/16	359	234,739	205,181	

Eggs produced specifically for research projects and brood stock management comprise a large proportion of the total production from the BFRH. Specific details of those individual crosses and families created for laboratory and field experiments are described in their respective sections of this report. Research projects for which fish were produced by BFRH are described in Job No. 2, Action #1, formalin sensitivity in rainbow trout; Job No. 2, Action #3, rainbow trout predator recognition and avoidance training; Job #3, Action #1, Parvin Lake fingerling plant experiments; and, Job No. 4, Action # 5, H×C fry stocking in the upper Colorado River.

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

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

Need

The methods for spawning and rearing sport fish are continuously evolving, especially as new strains or species are brought into the hatchery system. Experiments conducted under culture conditions can help improve hatchery survival, growth, the quality and quantity of fish stocked, and post-stocking survival. The use of wild brood stocks can be used to supplement, as well as introduce genetic diversity into hatchery brood stocks. Research focused on wild egg collection methods and the genetic diversity of wild brood stocks improves the utility of these brood stocks for hatchery use.

Objectives

1. Conduct four experiments examining the sensitivity of various *M. cerebralis*-resistant rainbow trout strains and crosses to formalin exposure by October 31, 2015.
2. Assist with spawning operations of two wild rainbow trout brood stocks by June 30, 2016.
3. Complete two electrofishing surveys of rainbow trout fry that were or were not exposed to chemical cues of predation by November 30, 2015.

Approach

Action #1:

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

Hofer and Harrison Lake strains, and 50:50 and 75:25 crosses of the two strains, will be reared to fingerling size at the Bellvue Fish Research Hatchery. The four experiments will consist of holding fish at four different densities and exposing them to three different concentrations (0, 167, and 250 ppm) of formalin. Formalin exposure will occur three times in each experiment, with a day in between each exposure, to determine the effects of multiple exposures on fingerling sensitivity to formalin. Endpoints will be mortalities per treatment or control group.

<u>Action #1 Accomplishments</u>

Four experiments were conducted at the CPW Bellvue Fish Research Hatchery to determine the formalin sensitivity of rainbow trout exposed multiple times and held at different densities. Formalin is one of the most effective and widely used compounds in fish culture for therapeutic and prophylactic treatment of fungal infections and external parasites of fish and fish eggs (Bills et al. 1977). Formalin has been shown to effectively prevent fungal infections on rainbow trout eggs at concentrations as low as 250 ppm. However, at 1,000 ppm, formalin not only prevented infection, but also decreased existing infection and increased hatching rates at exposure times ranging from 15 to 60 minutes (Marking et al. 1994). In addition to being a fungicide, formalin has been shown to be an egg disinfectant, reducing bacteria abundance on the surface of the egg at concentrations of up to 2,000 ppm (Wagner et al. 2008).

Formalin is effective against most ectoparasites, including *Trichodina*, *Costia*, *Ichthyophthirius*, and monogenetic trematodes (Piper et al. 1982). Typical formalin exposure concentrations range from 125 – 250 ppm for up to one hour (Piper et al. 1982). However, concentrations of up to 400 ppm have been used experimentally in toxicity tests (Wedemeyer 1971; Howe et al. 1995). A poll of CPW hatchery managers found that a range of concentrations from 130 – 250 ppm were used, with the most common treatment being 167 ppm for 30 minutes.

Differential formalin sensitivity has been demonstrated for various strains of rainbow trout when exposed post-hatch (Piper and Smith 1973) although there has been little to no research on differential strain sensitivity to formalin exposure during egg incubation. In addition, the formalin sensitivity of fingerling rainbow trout exposed to varying levels of formalin during egg incubation is unknown. Therefore, *M. cerebralis*-resistant strains and crosses of rainbow trout were exposed to various formalin concentrations at multiple life stages and under various hatchery conditions to examine if, and under what conditions, sensitivity to formalin (measured by mortality after exposure) occurs. Previous experiments conducted in 2012, 2013, and 2014 examined the formalin sensitivity of four rainbow trout strains and crosses in the egg and fingerling life stages, and included an examination of the effects of common hatchery practices on formalin sensitivity. The objective of the experiment conducted in 2015 was to determine if mortality from multiple exposures was additive, occurred with each exposure, or if sensitive fish were lost during first exposure and more tolerant fish survived the remainder of the exposures.

In addition, the objective of this experiment was to determine the effect of rearing density on formalin sensitivity.

Four *M. cerebralis*-resistant rainbow trout strains and crosses were used in the formalin sensitivity experiments: 1) Hofer (GR), 2) Harrison Lake (HL), 3) Hofer × Harrison Lake 50:50 (GR×HL 50:50), and 4) Hofer × Harrison Lake 75:25 (GR×HL 75:25). All four of these strains and crosses are maintained as brood stock at the Bellvue Fish Research Hatchery. Fish were fed a similar ration of food (i.e., 2% body weight per day), and were reared under similar environmental conditions (i.e., flows, temperatures, etc.), until they reached 3” in length.

Two weeks prior to initiation of the first formalin sensitivity experiment, all fish were marked on both sides with a visual implant elastomer (VIE) tag in the adipose tissue behind the eye, preventing misidentification if a tag was lost from one side during experimentation. VIE tags were used for individual identification upon death as fish from each of the four strains were combined in each replicate. One VIE color was used for each strain or cross (e.g., GR: red, HL: green, HxH 50:50: orange, HxH 75:25: blue; see Figure 2.1.1 for example of identification using VIE tags).

Figure 2.1.1. Visual implant elastomer (VIE) tags behind the eye of the (clockwise from the top) HL, GR×HL 50:50, GR, and GR×HL 75:25 fish, as seen fluorescing under a UV light.

Twelve 20 gallon tanks were used in each experiment (Figure 2.1.2). Flow was maintained at two gallons per minute, achieving three full turnovers during the 30 minute treatment and allowing us to reach the desired formalin concentration. Treatments were assigned to tanks using a random number generator. Five days prior to the experiment, 10 (half normal density), 20 (normal density), 40 (2x normal density), or 80 (4x normal density) fish of each strain were randomly distributed to each of the experimental tanks. The five day pre-experiment monitoring period was used to account for any mortality that occurred as a result of moving fish from inside the hatchery to FR1. Mortalities, and their lengths and weights, were recorded daily in each tank, and were identified to strain using the VIE tags. The final pre-experiment feeding occurred the day prior to conducting a trial. Fish were also fed on the days in between treatments.

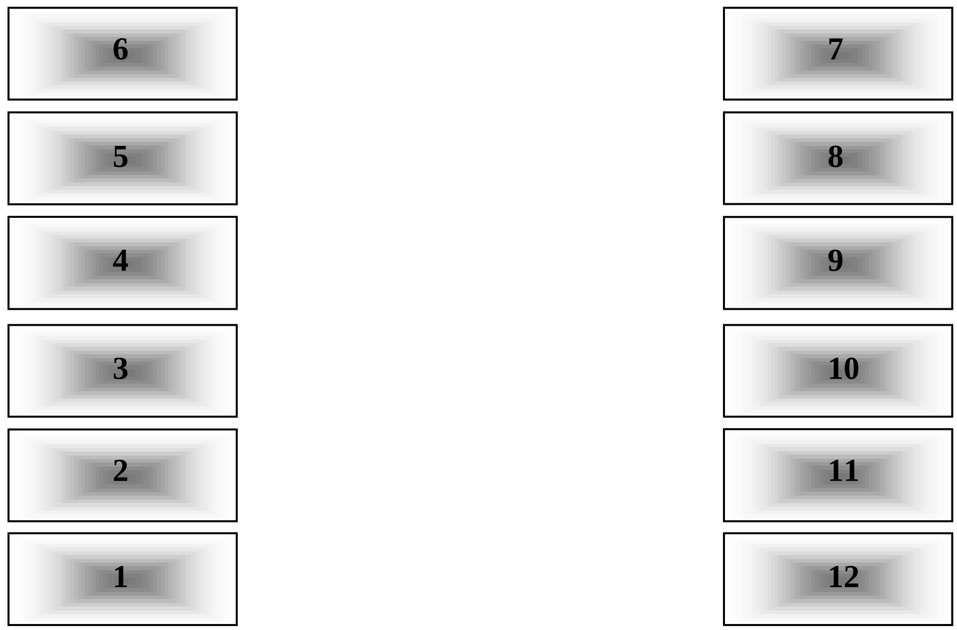

Figure 2.1.2. Arrangement and numbering of the twelve experimental tanks used in the fingerling formalin sensitivity experiments, housed in FR1 of the BFRH.

On the first, third, and fifth day of a trial, peristaltic metering pumps were used to deliver formalin at the correct rate to produce the required concentration in the tank (1.26 ml per minute for 167 ppm, 1.89 ml per minute for 250 ppm, and 3.78 ml per minute for 500 ppm). Hatchery water was similarly delivered to control tanks via the peristaltic pumps. Oxygen levels were monitored during treatment. Mortalities occurring during and after formalin exposure were recorded on a per strain basis, as were fish lengths and weights. The time at which the mortality occurred in relation to the beginning of the exposure period was also noted. Fish were retained within the experimental tanks for five days following the third and final treatment so that residual mortality could be assessed. Fish were checked in the morning and afternoon during this post-exposure monitoring period, and the time at which mortalities were found, strain, length, and weight were recorded. Fish remaining at the conclusion of the post-exposure monitoring period were euthanized using an overdose of MS-222, counted, measured and weighed. Following removal of fish, tanks were cleaned and prepared for the next experiment.

The data from the four density experiments was combined and analyzed using a general linear model implemented in SAS Proc GLM (SAS Institute, Inc. 2016). An intercept-only model was included in the model set, as were all singular, additive, and interactive combinations of factors suspected to have contributed to mortality, including strain (S), formalin concentration (C), density (D), and treatment number (one, two, or three; T). Model weights and delta Akaike Information Criterion corrected for small sample size (ΔAICc) rankings were used to determine support for each of the models included in the model set, and parameter estimates were reported from the candidate model with the lowest AICc value (Burnham and Anderson 2002).

Table 2.1.1. Model selection results for factors influencing formalin sensitivity in *Myxobolus cerebralis* resistant rainbow trout. Model set included singular, additive, and interactive effects of strain (S), formalin concentration (C), density (D), and treatment number (one, two, or three; T). The model set included 52 models; only models with weight are shown. Models are ranked by their AICc difference (Δ_i) relative to the best model in the set and Akaike weights (w_i) quantify the probability that a particular model is the best model in the set given the data and the model set.

Model	$\mathbf{R^2}$	***log(L)***	**K**	**AICc**	$\mathbf{\Delta_i}$	$\boldsymbol{w_i}$
T+(S*C*D)	0.70	1347.76	67.00	-2543.62	0.00	0.5146
S*C*D	0.70	1343.88	64.00	-2543.50	0.12	0.4850
D+(S*C)	0.63	1284.74	20.00	-2527.96	15.66	0.0002
D+T+(S*C)	0.63	1287.90	23.00	-2527.80	15.82	0.0002

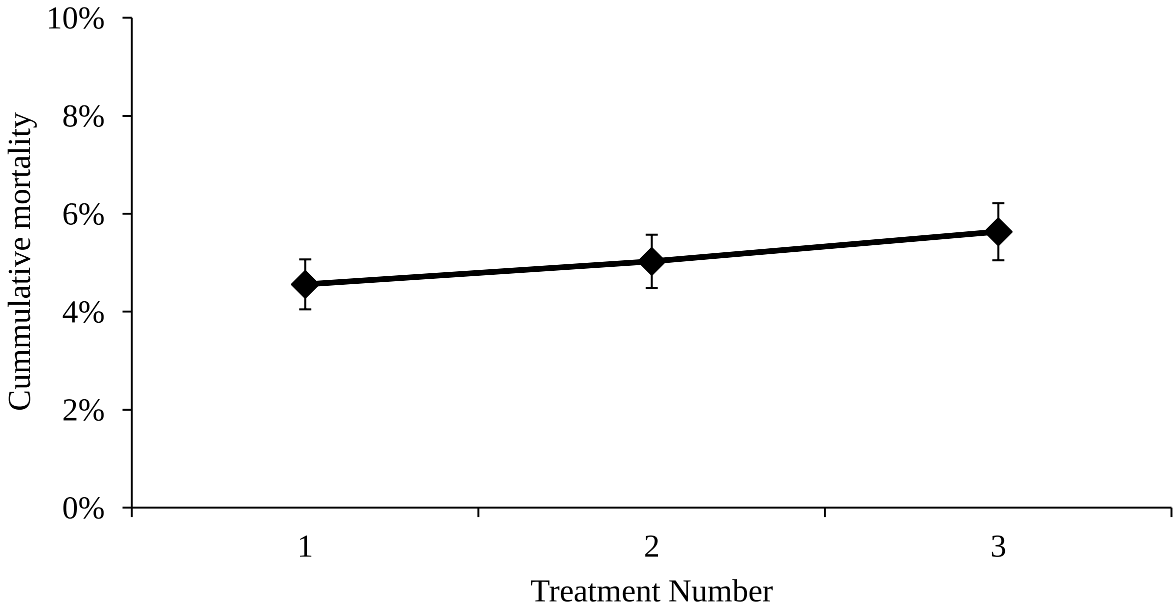

Figure 2.1.3. Cumulative mortality (SE bars) following one, two, and three exposures to formalin in the fingerling formalin sensitivity experiment. Mortality is averaged over strain, formalin concentration, and density. Note that the axis is reduced to show differences in cumulative mortality among number of exposures.

Model selection results indicated that the interaction between strain, formalin concentration, and density had a large effect on mortality, appearing additively with treatment number in the top model (AICc weight = 0.51), as well as alone in the second best model (AICc weight = 0.49; Table 2.1.1). Although treatment number appeared in the top model, there was little difference in AICc values between the top two models, suggesting that number of exposures had a relatively minor effect on mortality. Mortality was highest following the first exposure to formalin. Cumulative mortality increased by approximately half a percent with each consecutive exposure to formalin, however, the increase in mortality was not significant across the number of exposures (Figure 2.1.3).

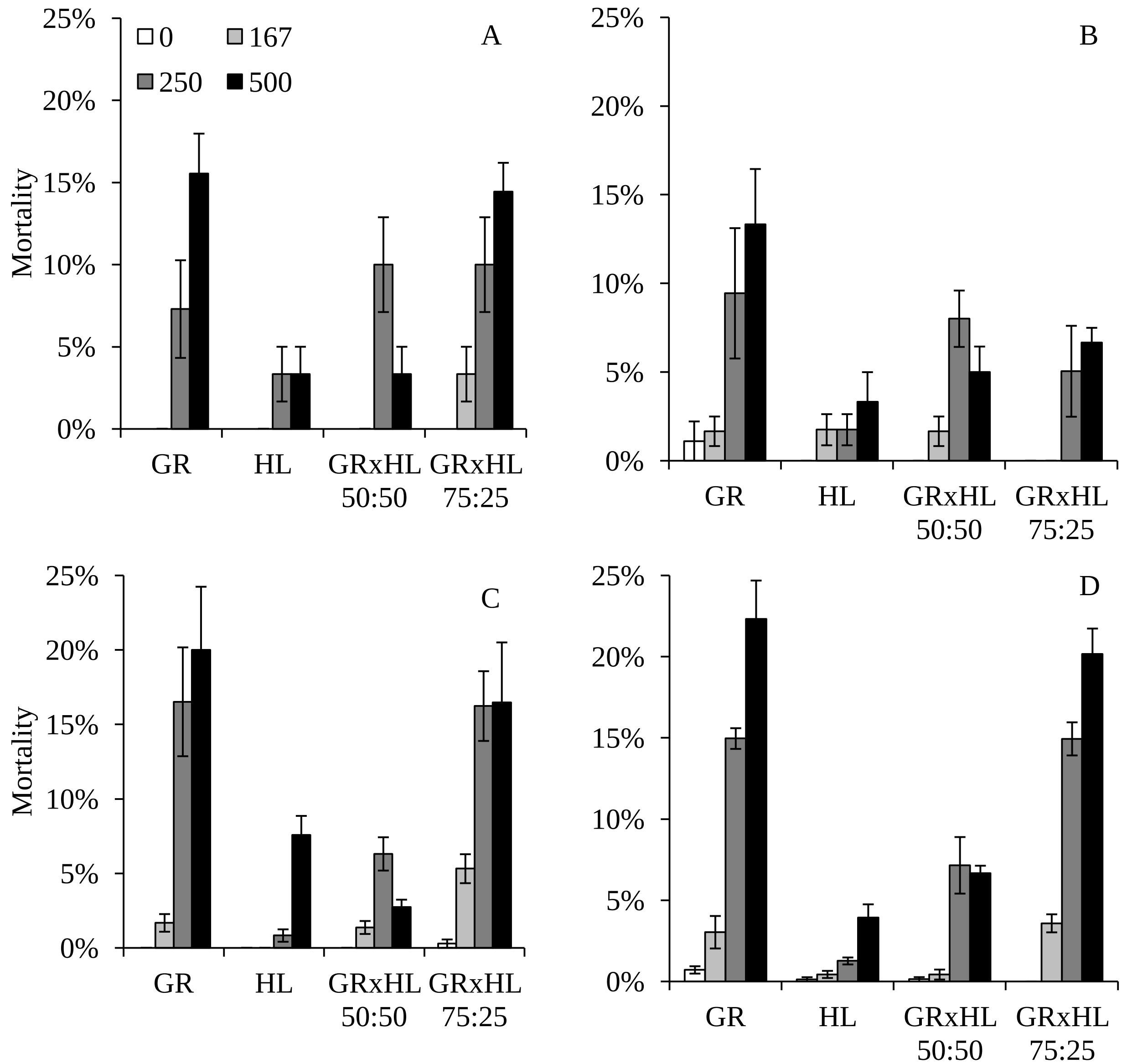

Figure 2.1.4. Mortality of four strains and crosses of *M. cerebralis*-resistant rainbow trout exposed to 0, 167, 250, or 500 ppm formalin for an exposure duration of 30 minutes, and reared at four densities: A) 10 fish per strain (40 fish total), B) 20 fish per strain (80 fish total), C) 40 fish per strain (160 fish total), and D) 80 fish per strain (320 fish total). Note that the axis is reduced to show differences among strains and crosses and formalin concentrations.

The GR and GR×HL 75:25 exhibited sensitivity to formalin, an increase in mortality with an increase in formalin concentration, at all rearing densities (Figure 2.1.4). Mortality rates were similar between the GR and GR×HL 75:25 at all formalin concentrations and rearing densities, with the exception of a rearing density of 20 fish per strain (80 fish total), where the GR exhibited higher mortality rates than the GR×HL 75:25. With the exception of an exposure of 250 ppm at a density of 10 fish per strain (40 fish total) and 20 fish per strain for the GR and GR×HL 75:25, and 500 ppm at a density of 20 fish per strain for the GR×HL 75:25, the GR and

GR×HL 75:25 exhibited higher mortality rates than either the HL or GR×HL 50:50. For both the GR and GR×HL 75:25, mortality generally increased with an increase in fish density.

The GR×HL 50:50 exhibited sensitivity to formalin at lower concentrations (0, 167, and 250 ppm), however, mortality was similar, or more often lower, between concentrations of 250 and 500 ppm (Figure 2.1.4). Although the HL strain also appeared to be sensitive to formalin exposure, mortality rates were generally less than 5% across formalin concentrations and rearing densities. At densities of 10 fish per strain and 20 fish per strain, the HL strain exhibited similar mortality rates among formalin concentrations; sensitivity among concentrations was only observed at densities of 40 fish per strain (160 fish total) and 80 fish per strain (320 fish total) for the HL strain. Neither the HL nor GR×HL 50:50 exhibited increased mortality rates with an increase in fish density.

The results suggest that the GR and higher proportion GR crosses are more sensitive to formalin than the HL and lower proportion GR crosses. Similarly, the GR and higher proportion GR crosses also exhibit higher mortality rates at higher rearing densities. This confirms what Colorado hatchery managers have observed during formalin treatment with these strains and crosses. The density results suggest that fish should be maintained at lower densities to prevent increased mortality during formalin treatment. Maintaining lower densities may have the added benefit of lowering fish stress and the potential for disease outbreaks, reducing the need to treat fish with formalin. If fish need to be treated multiple times for heavy infections, results suggest that the more sensitive fish will be lost during the first treatment. Fish remaining during subsequent treatments appear to be more tolerant of formalin, reducing further losses.

Bills, T. D., L. L. Marking, and J. H. Chandler Jr. 1977. Formalin: its toxicity to nontarget aquatic organisms, persistence, and counteraction. Investigations in Fish Control Number 73, U. S. Department of the Interior, Washington, D. C.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach. Second edition. Springer-Verlag, New York, New York.

Howe, G. E., L. L. Marking, T. D. Bills, and T. M. Schreier. 1995. Efficacy and toxicity of formalin solutions containing paraformaldehyde for fish and egg treatments. The Progressive Fish-Culturist 57:147-152.

Marking, L. L., J. J. Rach, and T. M. Schreier. 1994. Evaluation of antifungal agents for fish culture. The Progressive Fish-Culturist 56:225-231.

Piper, R. G., and C. E. Smith. 1973. Factors influencing formalin toxicity in trout. The Progressive Fish-Culturist 35:78-81.

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, Inc. 2016. SAS system software, Release 9.4. Cary, North Carolina.

Wagner, E. J., R. E. Arndt, E. J. Billman, A. Forest, W. Cavender. 2008. Comparison of the efficacy of iodine, formalin, salt, and hydrogen peroxide for control of external bacteria on rainbow trout eggs. North American Journal of Aquaculture 70:118-127.

Wedemeyer, G. 1971. The stress of formalin treatments in rainbow trout (*Salmo gairdneri*) and coho salmon (*Oncorhynchus kisutch*). Journal of the Fisheries Research Board of Canada 28:1899-1904.

Action #2:

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

Wild brood stocks will be spawned in April to May 2016. Genetic samples will be collected from the wild brood stocks to determine the genetic background of fish returning to spawn. Methods for egg collection will be assessed, including fish capture, egg treatment and hardening, and transportation to hatcheries.

<u>Action #2 Accomplishments</u>

Genetic samples were not collected from wild brood stocks in 2016. Genetic data collected from the rainbow trout population in the East Portal of the Gunnison River between 2012 and 2015 revealed that there was no detectable level of Hofer genetic contribution in the spawning population. This was either due to failure of the H×C fish stocked in this location to mature and reproduce successfully, or due to insufficient sensitivity of the genetic test to detect reproductive contributions by the H×C strain. As such, collection of genetic samples was not warranted in 2016.

A late runoff combined with spring rains made for unsafe sampling conditions in Harrison Creek, preventing genetic sample collection from the H×H brood stock in that location. Although genetic samples were not collected in this cycle, progress was made regarding the incorporation of these brood stocks into Colorado's hatchery program.

In the fall of 2015, Bacterial Kidney Disease (BKD) was discovered in hatchery brood stocks maintained at the CPW Glenwood Springs Hatchery, resulting in depopulation of the hatchery and the loss of Colorado's H×C brood stock. Because the pure CRR brood stock had been discontinued in 2010, a source of CRRs was needed to create the new H×C brood stock. Genetic data collected from the East Portal of the Gunnison River suggested that H×Cs stocked in that location between 2006 and 2013 had not recruited to the spawning population, possibly due to low survival or retention. Alternatively, the contribution of Hofer genetics to the spawning population may have been below detectable levels. Genetic data also suggested that the East Portal rainbow trout, though genetically similar to pure CRR, appeared notably different from the CRR brood stock previously maintained in CPW hatcheries, resulting in a change in the name from CRR to Gunnison River Rainbow, or GRR.

Two *M. cerebralis* exposure experiments conducted in 2012 and 2014 showed that myxospore development was low in GRR fish originating from the East Portal of the Gunnison River, especially compared to Puget Sound rainbow trout controls (Figure 2.2.1). GRRs developed an average of 38,063 myxospores per fish, exhibited low mortality rates (4%), and a low incidence of deformities (16%) compared to pure CRRs tested in previous laboratory experiments (average myxospore count: 187,595, mortality: 13% [Fetherman et al. 2012]; incidence of deformities: 96% [Fetherman et al. 2011]). Results suggested that either the GRRs had naturally developed resistance to *M. cerebralis* over 20 years of continuous exposure, or that resistance had been imparted to the population by the repeated stocking of Hofer rainbow trout. Because *M. cerebralis* prevalence is low in the East Portal, exposure levels were not high enough to cause a complete collapse of the rainbow trout population, and natural reproduction continued to occur on annual basis, the combination of which may have resulted in the development of resistance. The results from research conducted between 2012 and 2014 were presented to the CPW Aquatic Senior Staff in December 2015, and the decision was made to use the GRR to recreate the H×C (now H×G) brood stock at the CPW Glenwood Springs Hatchery. The H×G brood stock presents a possible advantage over the H×C brood stock in that the GRR are more resistant to whirling disease than the CRR. By spawning the GRR with the Hofer, resistance of the brood stock is likely to be higher than that of the previously maintained H×C brood stock.

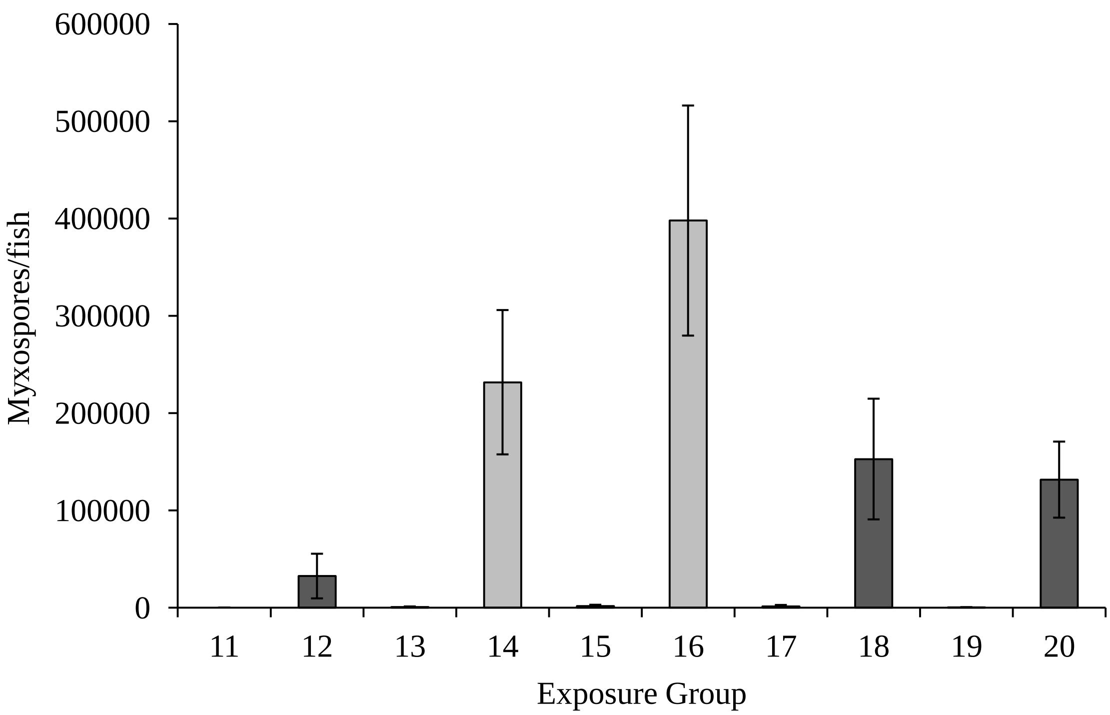

Figure 2.2.1. Average number of myxospores per fish in eight families of rainbow trout originating from the East Portal of the Gunnison River (GRR; dark bars; families 11-13, 15, and 17-20) and two families of Puget Sound rainbow trout (light bars; families 14 and 16).

In the spring of 2016, GRR milt was collected during wild spawning operations in the East Portal of the Gunnison River. Milt was transported to the CPW Poudre Rearing Unit and mixed with eggs from Hofer females. Low egg survival resulted in the loss of some of the unique male-female families. However, eggs from several families were sent to the CPW Glenwood Springs Hatchery to start the new H×G brood stock. This same process will be used to create multiple year classes of H×G brood stock in 2017 and 2018. As the new brood stock is developed, exposure experiments and genetic analyses will be used to confirm that the H×G brood stock is maintaining *M. cerebralis* resistance, and stocking experiments will be used to monitor H×G survival in comparison to the H×C fish previously stocked by the CPW hatchery system.

Fetherman, E. R., D. L. Winkelman, G. J. Schisler, and C. A. Myrick. 2011. The effects of *Myxobolus cerebralis* on the physiological performance of whirling disease resistant and susceptible strains of rainbow trout. Journal of Aquatic Animal Health 23:169-177.

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

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*

Two strains of rainbow trout, the Hofer × Colorado River Rainbow (H×C) and Gunnison River Rainbow (GRR; origin: East Portal of the Gunnison River) will be reared in the CPW Glenwood Springs Hatchery. Prior to stocking, half of each strain will be exposed to chemical cues of predation, and the other half will not be exposed to chemical cues. Four batch marks of coded wire tags will be used to differentiate the four groups (H×C and GRR exposed and not exposed to chemical cues). All four groups will be stocked into a one mile section of the Gunnison River near Pleasure Park. Following stocking, two removal electrofishing fry surveys will be conducted to determine the change in abundance of the four groups over time, evaluating the effectiveness of chemical cue exposure on post-stocking survival of stocked rainbow trout. The majority of the pre-stocking chemical cue exposure and tagging will be conducted by a research associate (Christopher Kopack) with Colorado State University. Post-stocking evaluations will be conducted by CPW personnel.

Action #3 Accomplishments

Prior to the start of this field experiment, it was determined that not enough was known about the effectiveness of training protocols for changing the behavior of hatchery-reared, predator-naïve rainbow trout strains to conduct a large-scale management experiment. As such, a laboratory experiment was conducted by Christopher Kopack at the Colorado State University Foothills Fisheries Laboratory to determine if training rainbow trout using multiple exposures to conspecific alarm cues and predator kairomones would increase the survival of rainbow trout. This experiment is described in detail below.

Hatcheries are a crucial tool for the management of wild fish populations, rearing and stocking fish for both angling opportunities and for conservation of native fish populations. Unfortunately, hatchery-reared fish, especially domestic brood stocks reared solely in hatchery environments, are subject to artificial selection for the hatchery environment. A single generation of artificial selection can result in differential expression of hundreds of genes involving responses in wound healing, immunity, and metabolism (Christie et al. 2016). In addition, targeted selection for specific characteristics such as growth or disease resistance can result in genetically depauperate populations. Genetic and behavioral changes induced by hatchery environments have the potential to contribute to low survival in the wild, resulting in little to no genetic contribution of hatchery fish to wild populations (Fitzpatrick et al. 2014).

The Hofer rainbow trout is known to be domesticated and exhibits low post-stocking survival in the wild when stocked at sizes larger than 100 mm total length. Anecdotal evidence from tiger muskie predation studies conducted in Parvin Lake (Red Feather Lakes, Colorado) suggested that predators were selectively preying upon pure Hofer rainbow trout compared to Hofer crosses or other wild strains. It was thought that the Hofer rainbow trout may have lost its ability to recognize danger and behave accordingly to avoid predation. However, Kopack et al. (2015) showed that the Hofer rainbow trout expressed behavioral changes necessary for survival and predator avoidance following a single exposure to conspecific alarm cues. Unfortunately, this ability to react to alarm cue did not translate to increased survival rates of Hofer rainbow trout when exposed on a hatchery truck en route to their stocking location (Kopack et al. *In review*). The question that remained following these two experiments was whether a single exposure to alarm cue would be adequate for fish to learn to avoid predators, or if multiple exposures would increase their chances of survival. The objectives of this experiment were to determine if fish exposed multiple times exhibited increased predator avoidance behaviors and survived longer in the presence of a brown trout predator compared to fish exposed only once or not at all.

Prior to the start of a trial, 56 Hofer rainbow trout were marked in the tissue on either side of the dorsal fin with Visual Implant Elastomer (VIE) tags to indicate treatment type and number of times exposed to cues or a water control. Two colors were used on one side of the fin to indicate whether the fish were exposed one or three times. Four colors were used on the opposite side of the fin to indicate treatment type: 1) control (exposed to distilled water), 2) conspecific alarm cue, 3) predator model only, or 4) predator model and conspecific alarm cue.

Fifty six ten-liter tanks were used to complete the exposures, ten tanks at a time. Each tank was painted on three sides to prevent fish from observing the behaviors of fish in adjacent tanks. GoPro cameras were mounted above each tank to record fish behavior. To start, the ten-liter tanks, housing individual fish, were transferred from a holding rack to a treatment table and allowed to acclimate for 30 minutes to recover from handling. Following the acclimation period, fish behavior was recorded for five minutes to provide a baseline for pre-exposure behaviors, including the amount of time a fish was active, exploratory behavior (measured by the number of "blocks" a fish passed through, represented by a grid drawn on the bottom of the tank), and time spent frozen or unmoving. Distilled water (control) or conspecific alarm cue (treatment) was administered to each tank using a 10 mL syringe for one minute following the pre-exposure monitoring period. The predator model, a three inch long brown trout rapala tied to a stick with fishing line, was also swam in the predator model only, and predator model and conspecific alarm cue treatment tanks in a fish-chasing motion during the one minute exposure period. Fish behavior was again recorded for five minutes following exposure to determine if behaviors had changed as a result of treatment.

Fish treated three times were exposed on Monday, Wednesday and Friday, and were returned to the holding rack following treatment on Monday and Wednesday. Fish treated once were exposed on Friday only, remaining on the holding rack during the Monday and Wednesday treatments. Once all fish had completed treatment on Friday, they were immediately introduced to a raceway containing two brown trout predators, ranging between 335 and 370 mm total length, and starved for 48 hours prior to the introduction of rainbow trout. All 56 rainbow trout, including all combinations of treatment and number of exposures, were included in the same predator arena to determine if brown trout selectively consumed fish from a given treatment. Survival was estimated by capturing rainbow trout from the tank and counting the number of fish remaining from each treatment type and exposure duration combination. To prevent increased mortality rates or susceptibility to predation from handling, the first sampling for survival occurred one week following the introduction of rainbow trout to the tank. Sampling occurred every four days after the first sampling event until no rainbow trout remained in the predator arenas. In total, two trials with 56 rainbow trout each were run during the experiment. At the time that this report was written, the videos of the behavioral data were still being analyzed, so only the survival results from the predator arenas are presented herein.

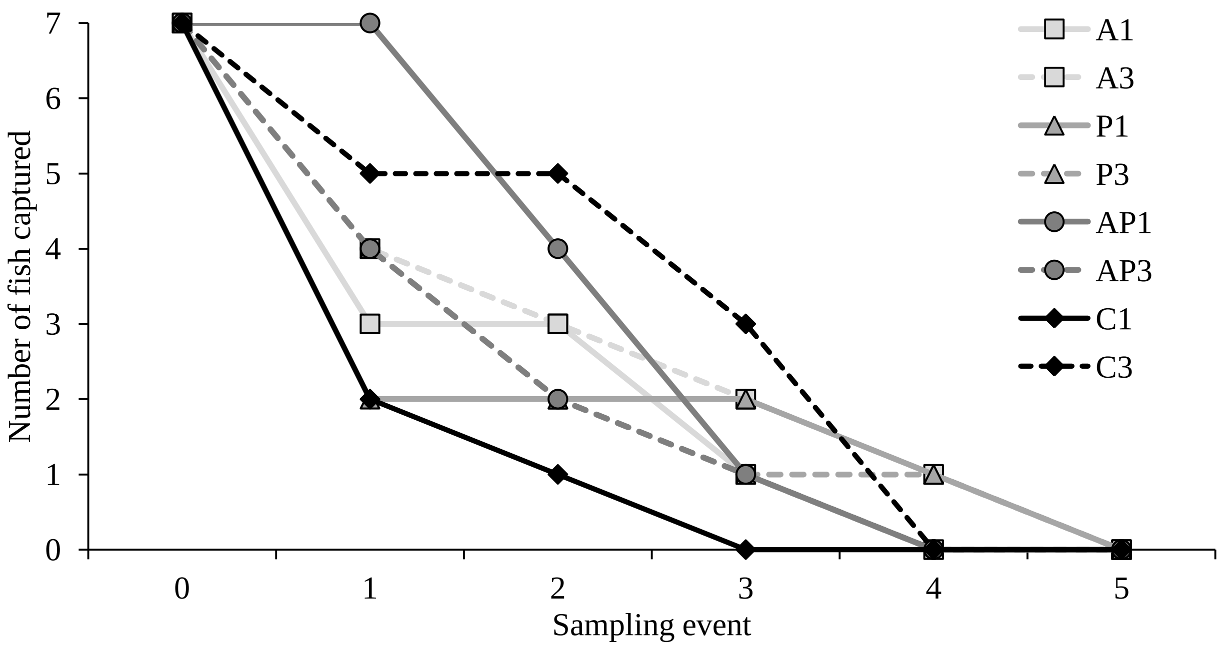

Figure 2.3.1. Number of fish sampled of each treatment (alarm cue [A], predator model [P], alarm cue and predator model [AP], and control [C]) and exposure time (1 or 3) combination at five sampling events in the first predator trial.

In the first predator trial, no differences in survival were observed between fish that had been exposed three times compared to those only exposed once from the predator model only and conspecific alarm cue treatments. There was a difference in survival between fish exposed one time and fish exposed three times from the treatment containing both the conspecfic alarm cue and predator model, but contrary to expectations, fish exposed only once exhibited higher survival rates in the first two sampling events than did fish exposed three times. Only in the control treatment was survival rates higher in the fish exposed three times versus those exposed once (Figure 2.3.1). In the second predator trial, fish in all four treatments exposed only once exhibited higher survival than fish exposed three times at each sampling event (Figure 2.3.2).

Several factors could have resulted in the lack of difference in survival across treatments. First, tanks did not contain any structure to aid in evading predators so survival was primarily based on physiological performance, i.e., the ability to get away from a predator, rather than behavioral differences in predator recognition and evasion. Second, social learning between treatment and control fish could have caused fish from all treatments to exhibit similar predator naïve or evasion behaviors. Finally, small sample sizes resulted in low statistical power to detect differences between treatment groups.

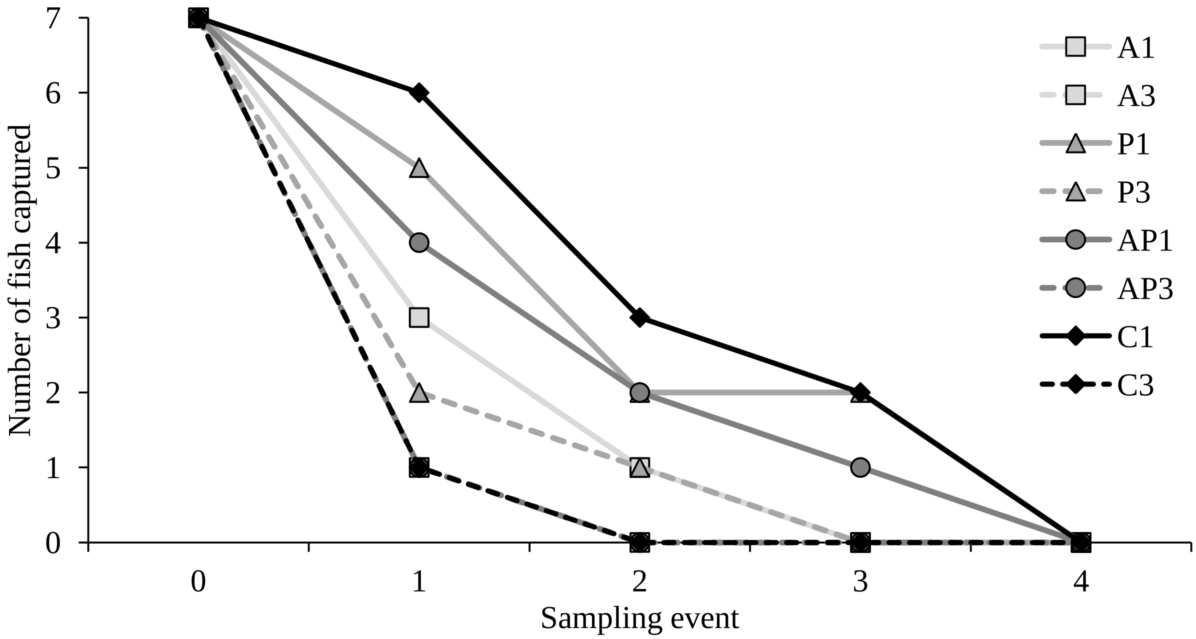

Figure 2.3.2. Number of fish sampled of each treatment (alarm cue [A], predator model [P], alarm cue and predator model [AP], and control [C]) and exposure time (1 or 3) combination at five sampling events in the second predator trial.

The number of exposures was directly correlated with poor survival, with fish handled and exposed three times surviving at a much lower rate than those handled and exposed only once. Handling fish more than once could have resulted in conditioning fish for the wrong predator (humans versus brown trout). Additionally, it is possible that multiple exposures to the same stimulus, especially without negative consequences, could have resulted in overstimulation and habituation, and prevented fish from associating brown trout kairomones with the threat of predation. The results suggest that fish should only be handled and trained once prior to being stocked, which ultimately would be labor and cost effective compared to multiple exposures over multiple days.

Although this experiment suggested that there was no difference in survival between treated and control fish, more information is now known regarding training fish to recognize and avoid predation, and warrants further investigation. Future experiments should train fish only once prior to exposing them to a predator. In addition, the potential for social learning to result in equivocal survival rates among treatments suggests that treatment groups should be exposed to the predator individually, and that other metrics, such as time to a predation event or number of strikes a predator takes at prey before successful consumption, should be measured in addition to prey survival. The potential for large variability in both predator and prey response behaviors also suggests that sample size should be increased in future experiments to have a higher statistical power for detecting subtle differences among the treatment groups.

Christie, M. R., M. L. Marine, S. E. Fox, R. A. French, and M. S. Blouin. 2016. A single generation of domestication heritability alters the expression of hundreds of genes. Nature Communications 7:1-6.

Fitzpatrick, S.W., H. Crocket, and W. C. Funk. 2014. Water availability strongly impacts populations genetic patterns of an imperiled Great Plains endemic fish. Conservation Genetics 15:771-788.

Kopack, C. J., E. D. Broder, E. R. Fetherman, J. M. Lepak, and L. M. Angeloni. *In review*. The effects of a single pre-release exposure to conspecific alarm cue on post-stocking survival in three strains of rainbow trout. Canadian Journal of Zoology.

Kopack, C. J., E. D. Broder, J. M. Lepak, E. R. Fetherman, and L. M. Angeloni. 2015. Behavioral responses of a highly domesticated, predator naïve rainbow trout to chemical cues of predation. Fisheries Research 169:1-7.

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, 2015.
2. Conduct one study examining the survival, infection severity, and return to creel of fish reared in an *M. cerebralis*-positive hatchery environment by December 31, 2015.

3. Produce 3,000 rainbow trout for fingerling survival and infection severity experiments to be conducted in Parvin Lake in 2017 by June 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 2015, and summer of 2016. 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

Four different varieties of fish that had been grown to catchable size at the BFRH were stocked into Parvin Lake in spring 2015. Samples were collected by night electrofishing events in May and October 2015, and June 2016. Each fish was weighed and measured, and tags were extracted for strain identification. Heads were submitted to the Brush Fish Health Laboratory for myxospore enumeration. The results for the May and October samples are complete. Analysis of survival for multiple year-classes of fish stocked into the reservoir is ongoing.

Action #2:

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

Fish from an M. cerebralis-positive hatchery will be stocked in the summer of 2015 into Chatfield Reservoir, a put-and-take fishery located in the western suburbs of Denver. A creel survey will be conducted by CPW staff to determine the return to creel of the stocked rainbow trout. Heads of the fish caught by anglers will be returned to CPW staff on a volunteer basis for coded wire tag recovery and assessment of infection severity via myxospore enumeration. Electrofishing surveys will be conducted in late fall to assess survival and collect samples for infection severity from fish remaining in the reservoir.

Action # 2 Accomplishments

Four different varieties of fish that had been grown to catchable size at the CPW Poudre Rearing Unit were stocked into Boyd Lake in Larimer County on May 27, 2015. This lake was used, rather than Chatfield Reservoir in Denver due to availability and demand for the fish at time of stocking. Each of the four varieties, including Snake River cutthroat trout (SR2), Hofer × Colorado River rainbow trout (H×C), Hofer × Snake River cutthroat trout (HN2), and pure Hofer (HOF or GR), had been previously batch-marked with coded-wire tags (62-2323, 62-2424, 62-

2525, and 62-2626, respectively). A total of 1,750 fish of each variety were stocked. A creel survey was performed on one weekday and one weekend day per week for six weeks post-stocking. Heads were collected from harvested fish to obtain a sub-sample of total harvest. Tags were extracted from the sub-sample of thirty-five fish to estimate total harvest by strain. Samples were submitted to the Brush Fish Health Lab for myxospore enumeration.

Action #3:

- *Level 1 Action Category: Species Reintroduction and Stocking*
- *Level 2 Action Strategy: Production and stocking for recreational purposes*
- *Level 3 Action Activity: Put-grow-and-take*

Rainbow trout to be used in the fingerling survival and infection severity experiments conducted in Parvin Lake in 2017 will be produced by CPW hatcheries in early 2016. Eggs from strains not spawned on-site will be shipped to the Bellvue Fish Research Hatchery, where they will be hatched and reared.

Action #3 Accomplishments

Fish to be used in the 2016 Parvin Lake fingerling experiment were reared at the BFRH. Seven hundred and forty fish each of pure Hofer (HOF or GR), Hofer × Colorado River rainbow (H×C) and Hofer × cutthroat trout (HGBN), were reared to size and batch marked with coded wire tags (62-0292, 62-0187, and 62-0286, respectively) to distinguish the fish by strain. Total fish stocked (2,250) was slightly less than the original target of 3,000 because the lots were reared to a larger size than originally planned. These lots were stocked into Parvin Lake on April 28, 2016 at 254 mm, 203 mm, and 178 mm TL, respectively.

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, 2015.
2. Conduct one fry abundance estimate in the Gunnison River by November 30, 2015.
3. Conduct one adult abundance estimate in the upper Colorado River by June 30, 2016.
4. Conduct five fry abundance estimates in the upper Colorado River by November 30, 2015.
5. Stock 100,000 H×C fry into the upper Colorado River by August 30, 2015.
6. 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, 2016.
7. Complete sampling for one study designed to examine the side-by-side survival of pure Hofer and H×C stocked as fry in the Cache la Poudre, Colorado, and South Platte drainages by June 30, 2016.

Approach

Action #1:
- *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*

The adult abundance estimate in the Gunnison River will occur in fall 2015. 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 not conducted in the Gunnison River in the fall of 2015. Getting all of the necessary equipment and personnel into the Gunnison Gorge requires a helicopter transport. Unfortunately, the helicopter service usually hired for this trip was unavailable during the week that the population estimates were scheduled to be conducted. Last minute hiring of a different helicopter service proved to be cost prohibitive. Adult population estimates are scheduled to occur in the fall of 2016, and results of that sampling will be available in the next reporting cycle.

Action #2:
- *Level 1 Action* *<u>Category</u>*: *Data Collection and Analysis*
- *Level 2 Action* *<u>Strategy</u>*: *Research, survey or monitoring – fish and wildlife populations*
- *Level 3 Action* *<u>Activity</u>*: *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 2015. 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

Gunnison Gorge fry estimates are being used to monitor long-term trends in natural reproduction following the introduction of *M. cerebralis*-resistant rainbow trout strains to the Gunnison River in the mid- to late-2000's. Fry population estimates were conducted at eight sites in the Gunnison Gorge between August 24 and 27, 2015. Two sites, Chukar and Bobcat, are located in the upper Gunnison Gorge upstream of Ute Park. Three sites, Cowboy, Ute, and Caddis, are located in Ute Park. In 2013, a new site, T-Dyke, was added in the interior section of the Gunnison Gorge to determine if rainbow trout were spawning in other sections of the river outside of the Ute Park introduction sites. This site was resampled in 2015. The Rainbow Wall and Gold Mine sites are located in the Pleasure Park section of the Gunnison Gorge between the Smith Fork confluence on the upstream end and the confluence with the North Fork of the Gunnison River on the downstream end.

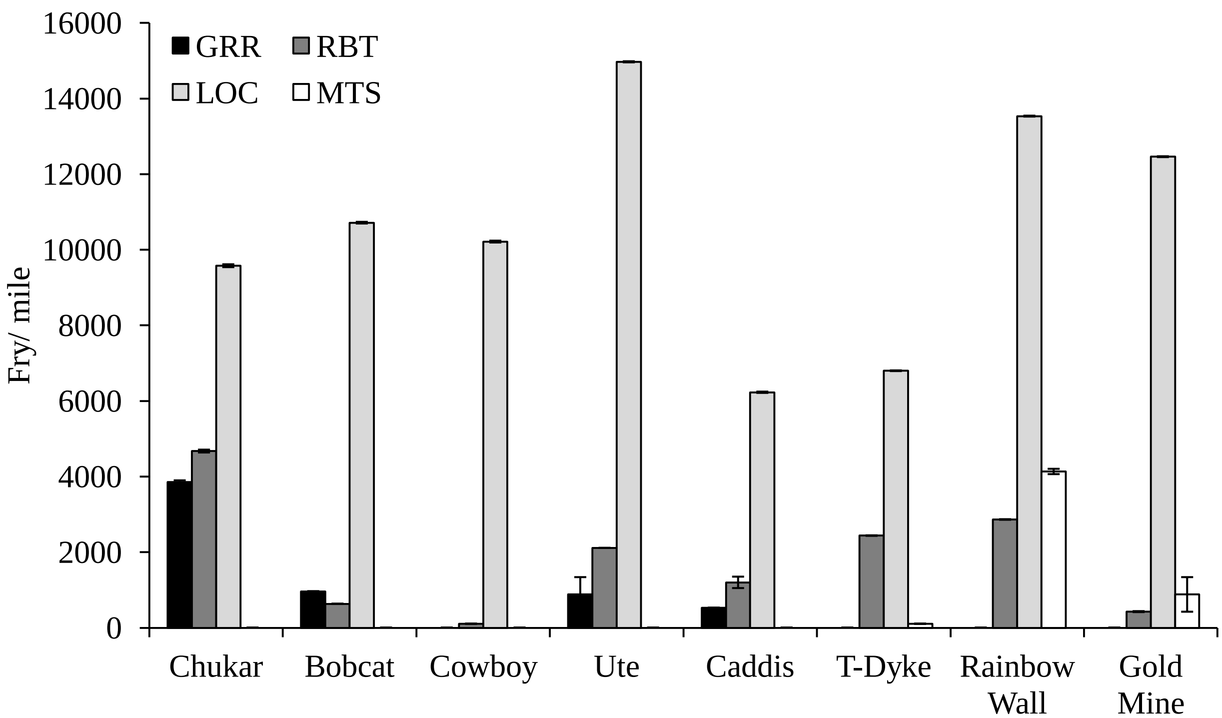

Figure 4.2.1. Brown trout (LOC), Gunnison River Rainbow (GRR), wild rainbow trout (RBT), and mottled sculpin (MTS) density estimates (fry/mile; SE bars) at eight sites in the Gunnison River through the Gunnison Gorge. Sites are ordered upstream to downstream from left to right.

Fry estimates were accomplished using a three electrode bank electrofishing unit. The three electrodes were deployed such that all available fry habitat was covered. Three passes were completed through each of the 50 foot long study sites, and fry were removed on each pass. All salmonid fry encountered during the removal estimates, as well as sculpins in the sites in which they were encountered, were measured and returned to the site. A subset of fry were also weighed at each site. Genetic samples were taken from a subset of up to ten wild rainbow trout fry at each site (see Job No. 4, Action #6) to determine the percentage of GR genes present in the fry population. Fry density estimates were calculated using the three-pass removal equations of Seber and Whale (1970).

Fry estimates were highly variable among sites, especially those located in Ute Park (Figure 4.2.1). Population estimates were combined to examine patterns in abundance in the upper gorge (Chukar and Bobcat), Ute Park (Cowboy, Ute, and Caddis), inner gorge (T-Dyke), and lower gorge (Rainbow Wall and Gold Mine; Figure 4.2.2). Brown trout densities were similar between the upper gorge and Ute Park, lower in the inner gorge, and higher in the lower gorge. Though spawning habitat can be found in the inner gorge, spawning habitat is sparser compared to the other three sections, potentially resulting in lower numbers of fry in this section of the Gunnison River. On average 10,562 (± 1,085) brown trout fry were present per mile. Brown trout fry averaged 79.69 (± 0.55) mm total length (TL) and 4.96 (± 0.11) g, and did not differ in size across the four sections of the Gunnison River. Mottled sculpin were only encountered in the inner gorge and lower gorge fry sites. In these two sections, mottled sculpin averaged 1,707 (± 1,234) sculpin per mile. This estimate represents all life classes of mottled sculpin, from fry to adult, as all life stages occupy similar habitat to that of the salmonid fry. Mottled sculpin averaged 35.37 (± 1.81) mm TL. Not enough sculpin were weighed to produce a reliable estimate of average weight.

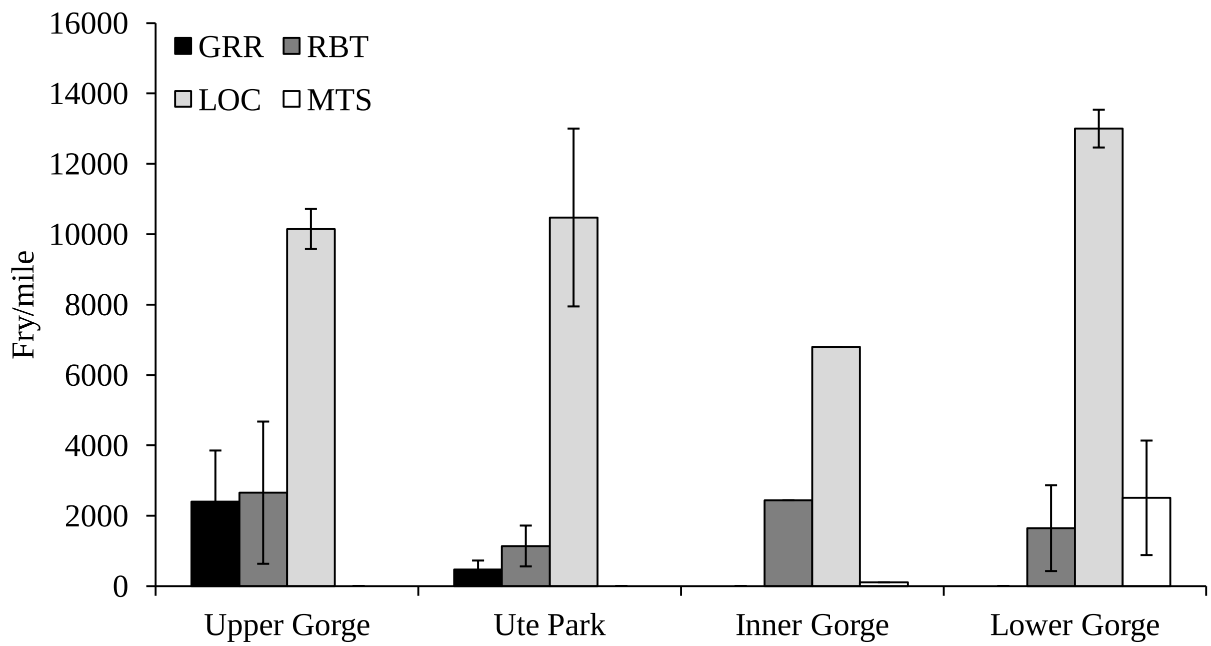

Figure 4.2.2. Brown trout (LOC), Gunnison River Rainbow (GRR), wild rainbow trout (RBT), and mottled sculpin (MTS) density estimates (fry/mile; SE bars) in the upper gorge, Ute Park, inner gorge, and lower gorge sections of the Gunnison River through the Gunnison Gorge.

Two types of rainbow trout fry were encountered in the Gunnison River. One week prior to conducting the fry population estimates, Gunnison River Rainbow (GRR) fry, originating from the East Portal of the Gunnison River, were stocked throughout the upper sections of the Gunnison Gorge. The GRR were only encountered in the upper gorge and Ute Park sections of the Gunnison River (Figure 4.2.2), and averaged 1,234 (± 673) fry per mile in the sections in which they were found. Stocked shortly after swim-up, GRR fry averaged only 34.28 (± 0.40) mm TL and 0.39 (± 0.01) g. This made them fairly easy to differentiate from rainbow trout fry originating from natural reproduction in the Gunnison River. Wild rainbow trout averaged 51.07 (± 1.88) mm TL and 1.51 (± 0.19) g, and average fry size increased from upstream to downstream. The lower gorge is warmer due to the influence of the Smith Fork. Fry in the lower gorge could have been larger than those upstream due to warmer water temperatures contributing to increased growth rates. Alternatively, warmer water temperatures could have resulted in earlier spawning and/or emergence in the lower gorge. Despite differences in size, rainbow trout abundances were similar across the four sections of the Gunnison River, with wild rainbow trout fry averaging 1,806 (± 541) fry per mile.

Sampling results indicate that rainbow trout are spawning in the Gunnison River throughout the Gunnison Gorge. The presence of rainbow trout fry in the inner gorge indicates that rainbow trout have dispersed throughout the gorge, and are able to survive and reproduce in all sections of the Gunnison River. The GRR fry appear to be surviving well in the sections they were stocked, and constitute roughly 50% of the rainbow trout fry population in those sections of the Gunnison River. Future sampling efforts will continue to monitor natural reproduction in the Gunnison River, as well as the genetic contribution of the adult rainbow trout population to the wild fry population.

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 2016. 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 section of the upper Colorado River in May 2016, with the mark run occurring on May 3, 2016, and the recapture run occurring on May 5, 2016. 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 a subset of five fish per species and ten millimeter size class were weighed to the nearest gram. In addition, 35 genetic samples were collected from adult rainbow trout (see Job No. 4, Action #6) encountered throughout the study reach to determine the percentage of GR genes in the adult spawning population. 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.

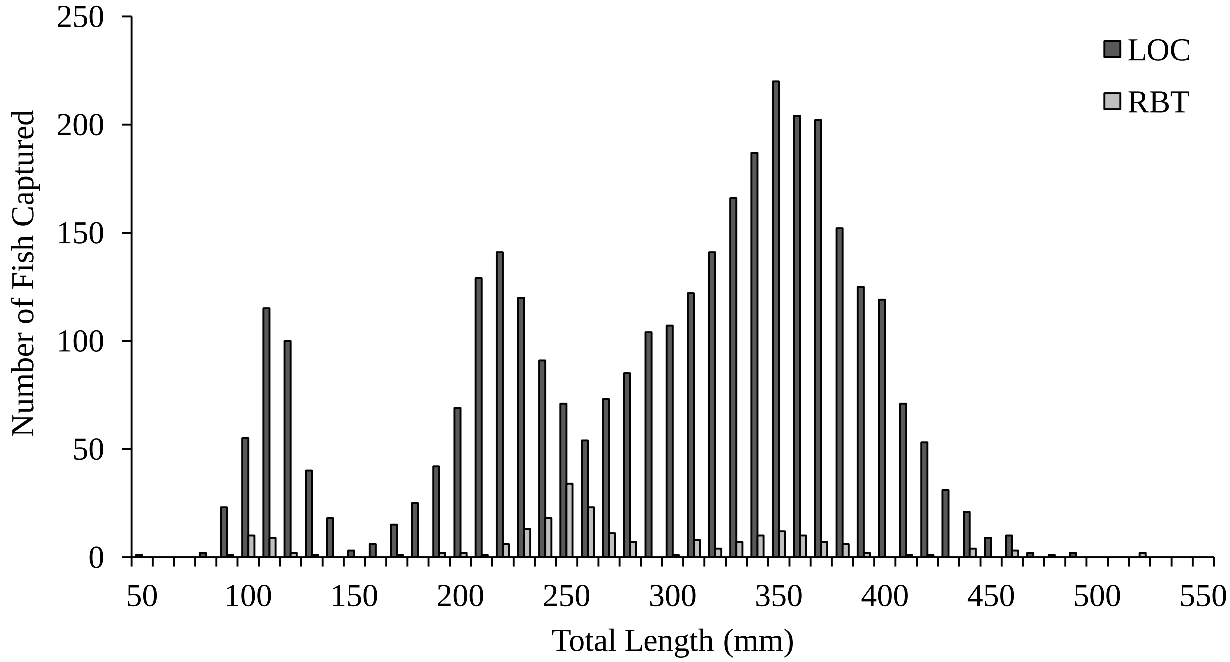

Figure 4.3.1. Number of brown trout (LOC) and rainbow trout (RBT) captured by total length (mm) during the 2016 adult population estimates in the Chimney Rock/Sheriff Ranch study section.

An estimated 6,671 (± 321) adult brown trout were present in the Chimney Rock/Sheriff Ranch study section in 2016, nearly 2,000 more than 2015 (Fetherman and Schisler 2015). Overall, 1,710 (± 83) brown trout were present per mile in the study section, averaging 313 (± 67) mm total length (TL) and 311 (± 210) g. All age classes of brown trout were represented in the sample, including several juvenile (≤ 150 mm TL) brown trout, but the majority of the brown trout captured were age 3+ (Figure 4.3.1).

Rainbow trout densities tripled between 2015 and 2016, with an estimated 113 (± 31) adult rainbow trout present in the study section in 2015 (Fetherman and Schisler 2015), and 355 (± 56) present in the study section in 2016. The rainbow trout population in the upper Colorado River has exhibited an exponential increase in abundance since 2013, with an estimated 91 (± 14) present per mile in the study section in 2016 (Figure 4.3.2). Adult rainbow trout averaged 270 (± 84) mm TL and 255 (± 175) g, smaller than the average size rainbow trout encountered in 2015, likely a result of the number of smaller age 2 rainbow trout captured during the 2016 population estimates (Figure 4.3.3). Fry stocked in 2015 were represented in the smaller length classes (90-130 mm TL), the presence of which indicated that fry stocked in 2015 survived the winter and were recruiting. Very few fish were captured between 110 and 230 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 more prevalent in the population than in previous years, and also made up a larger proportion of the total rainbow trout population, suggesting a strong age class that will recruit to the adult spawning population in 2017. The age 3+ rainbow trout population also increased in 2016 suggesting that age 2 fish from 2015 had recruited to the adult spawning population (Figure 4.3.4).

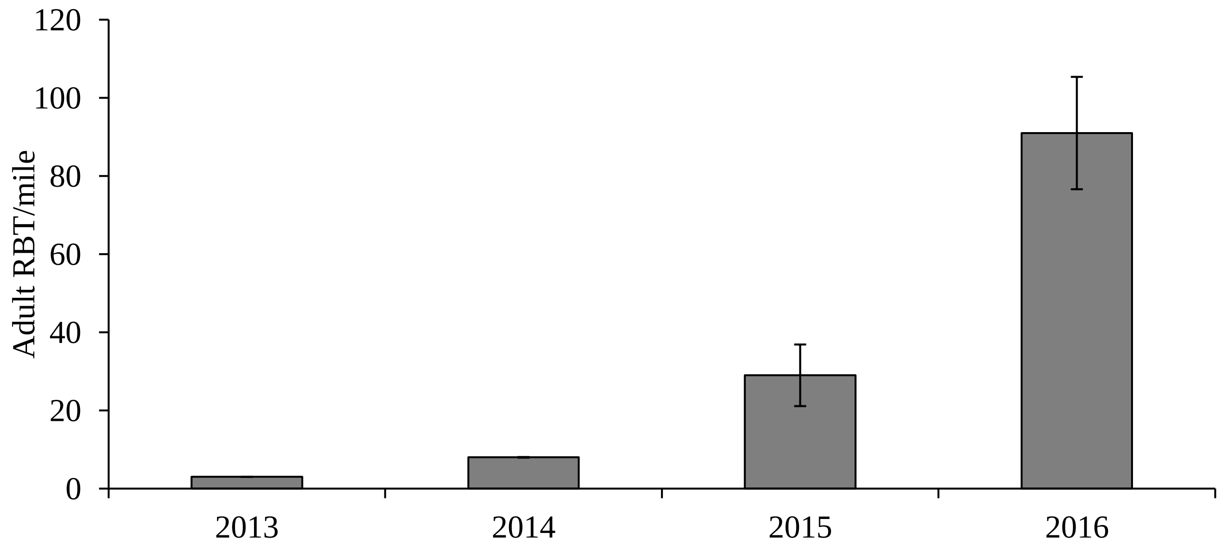

Figure 4.3.2. Estimated number of adult rainbow trout (RBT) per mile in the Chimney Rock/Sheriff Ranch study section between 2013 and 2016.

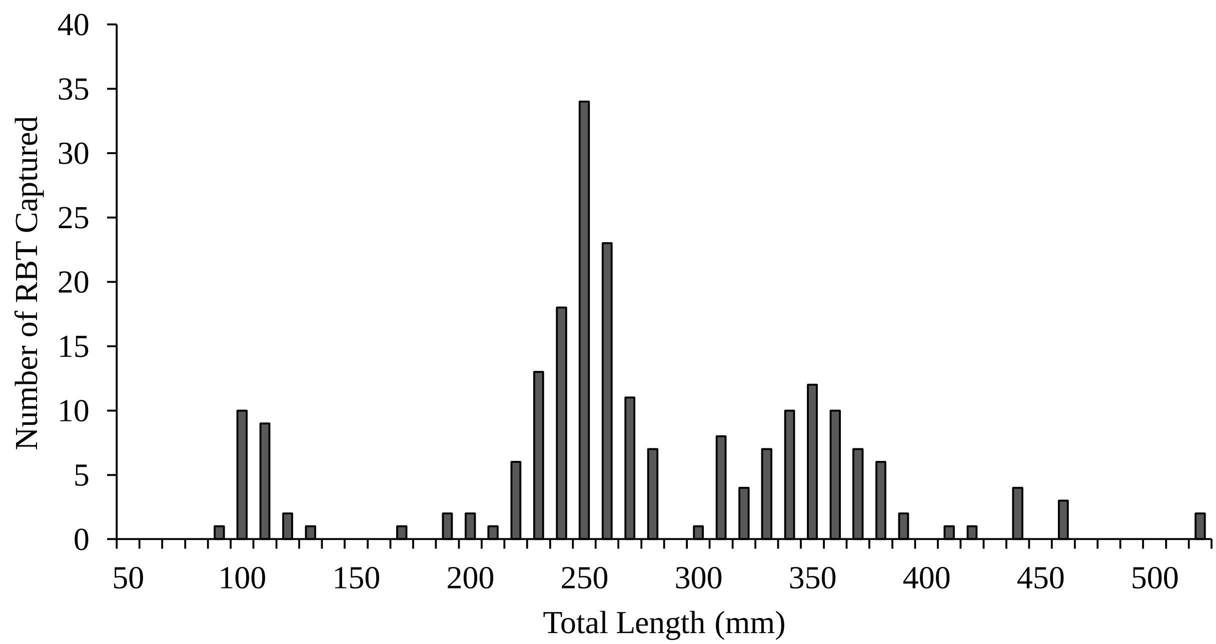

Figure 4.3.3. Number of rainbow trout (RBT) caught by total length (mm) during the 2016 adult population estimates in the Chimney Rock/Sheriff Ranch study section.

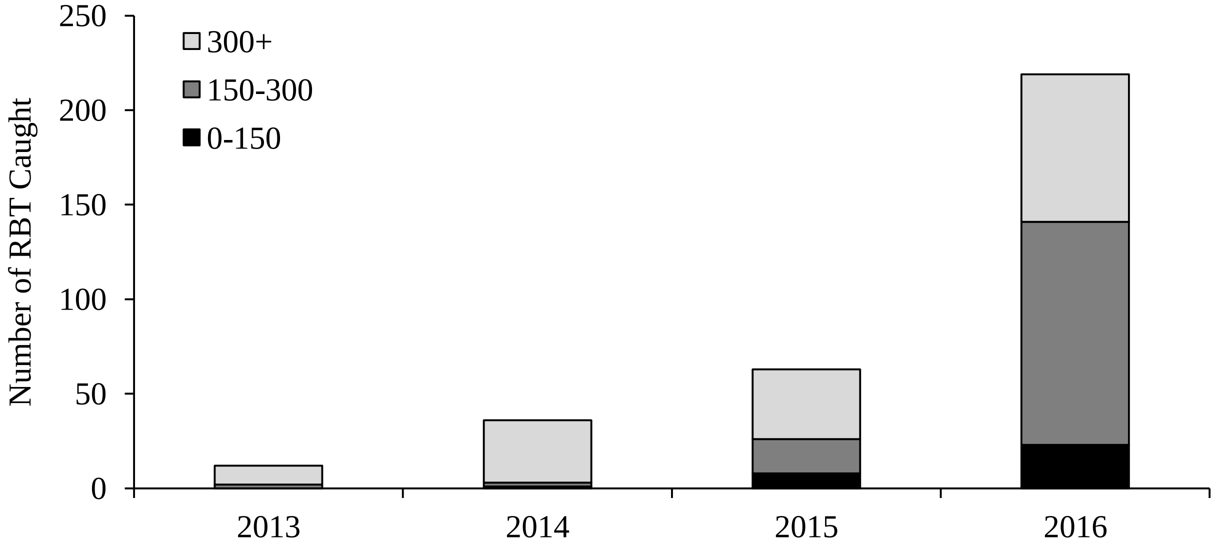

Figure 4.3.4. Number of age 1 (0-150 mm TL), age 2 (150-300 mm TL) and age 3+ (300+ mm TL) rainbow trout (RBT) captured in the Chimney Rock/Sheriff Ranch study section every year between 2013 and 2016.

The adult rainbow trout population in the upper Colorado River continues to grow at an exponential rate since fry stocking began in 2013. The presence of both age 1 and age 2 fish in the population suggests that fish are recruiting, and will continue to contribute to the adult rainbow trout population. The rainbow trout population increased in 2016 despite a 2,000 fish increase in the adult brown trout population between 2015 and 2016, likely increasing predatory and competitive interactions between the two species. Pure Hofer rainbow trout were stocked in the upper Colorado River in July 2016 due to a shortage of H×C fish in the state hatchery system. However, results from a masters experiment conducted by Colorado State University graduate student Brian Avila suggest that pure Hofer rainbow trout survive just as well as H×Cs when stocked as fry, and have the potential to reduce infection levels in the river even further due to their increased resistance to *M. cerebralis*. Given the positive results, we suggest that fry stocking continue to be used as a management option for increasing the adult rainbow trout population until significant natural reproduction is observed and fish produced by wild spawns survive and recruit to the adult population.

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. 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.

Action #4:

- *Level 1 Action* *Category*: *Data Collection and Analysis*
- *Level 2 Action* *Strategy*: *Research, survey or monitoring – fish and wildlife populations*
- *Level 3 Action* *Activity*: *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 2015. 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. Abundance estimates conducted in June and July will provide information on the number of fry produced naturally in the river by spawning rainbow trout, whereas estimates conducted in August, September, and October will provide information on the survival of stocked rainbow trout fry (see Action #5).

<u>Action # 4 Accomplishments</u>
Fry population estimates were conducted at seven sites in the upper Colorado River in June, July, August, September, and October 2015. Fry estimates completed in June and July provided information on the number of fry produced naturally in the river by spawning rainbow trout and brown trout, whereas the estimates completed in August, September, and October provided information regarding the post-stocking survival of the H×C fry stocked into the upper Colorado River on July 29, 2015 (see Job No. 4, Action #5) and survival of brown trout fry naturally produced in the river. Although this current study is focused on the Chimney Rock/Sheriff Ranch study section, H×C fry have also been stocked on an annual basis below Byers canyon, and as such, three reference sites below Byers Canyon were used to compare survival in the two sections of the river. Sampling sites (n = 3) below Byers Canyon include the Kemp-Breeze, Lone Buck, and Paul Gilbert State Wildlife Areas, and 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).

Fry estimates were accomplished using two Smith-Root LR-24 backpack electrofishing units running side-by-side to cover all 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 during the removal estimates were measured and returned to the site. In October 2015, genetic samples were taken from five rainbow trout fry at each site (see Job No. 4, Action #6), and five brown trout and five rainbow trout were collected from each site to obtain myxospore counts. Myxospore enumeration was completed by the CPW staff at the Bruch Fish Health Lab in Brush, Colorado. Fry density estimates were calculated using the three-pass removal equations of Seber and Whale (1970).

In June 2015, the Colorado River was running out of its banks at many of the fry sites. Although estimates were still attempted, only one brown trout fry and no rainbow trout fry were captured in June. Brown trout fry estimates were variable, but did not differ between July and October 2015, with an estimated 4,421 (± 916) brown trout fry per mile in July 2015, and an estimated 3,456 (± 1,002) brown trout fry per mile still present in October 2015. Prior to the introduction of the H×C fry, an estimated 177 (± 128) and 183 (± 86) wild rainbow trout fry were present per mile below and above Byers Canyon, respectively. Rainbow trout fry densities peaked in August following the introduction of H×C, with an estimated 5,593 (± 3,382) rainbow trout fry per mile below Byers Canyon, and an estimated 1,932 (± 854) rainbow trout fry per mile in the Chimney Rock/Sheriff Ranch study section above Byers Canyon. In previous years, a large decline in the rainbow trout fry population followed the initial peak immediately after stocking (Fetherman and Schisler 2015). That relatively consistent trend did not occur in 2015. Although the rainbow trout fry population declined in both below and above Byers Canyon between August and October, rainbow trout fry density estimates did not differ between August and October, with an estimated 2,479 (± 2,178) rainbow trout fry per mile still present below Byers Canyon and 1,163 (± 557) rainbow trout per mile still present in the Chimney Rock/Sheriff Ranch study section above Byers Canyon in October 2015 (Figure 4.4.2).

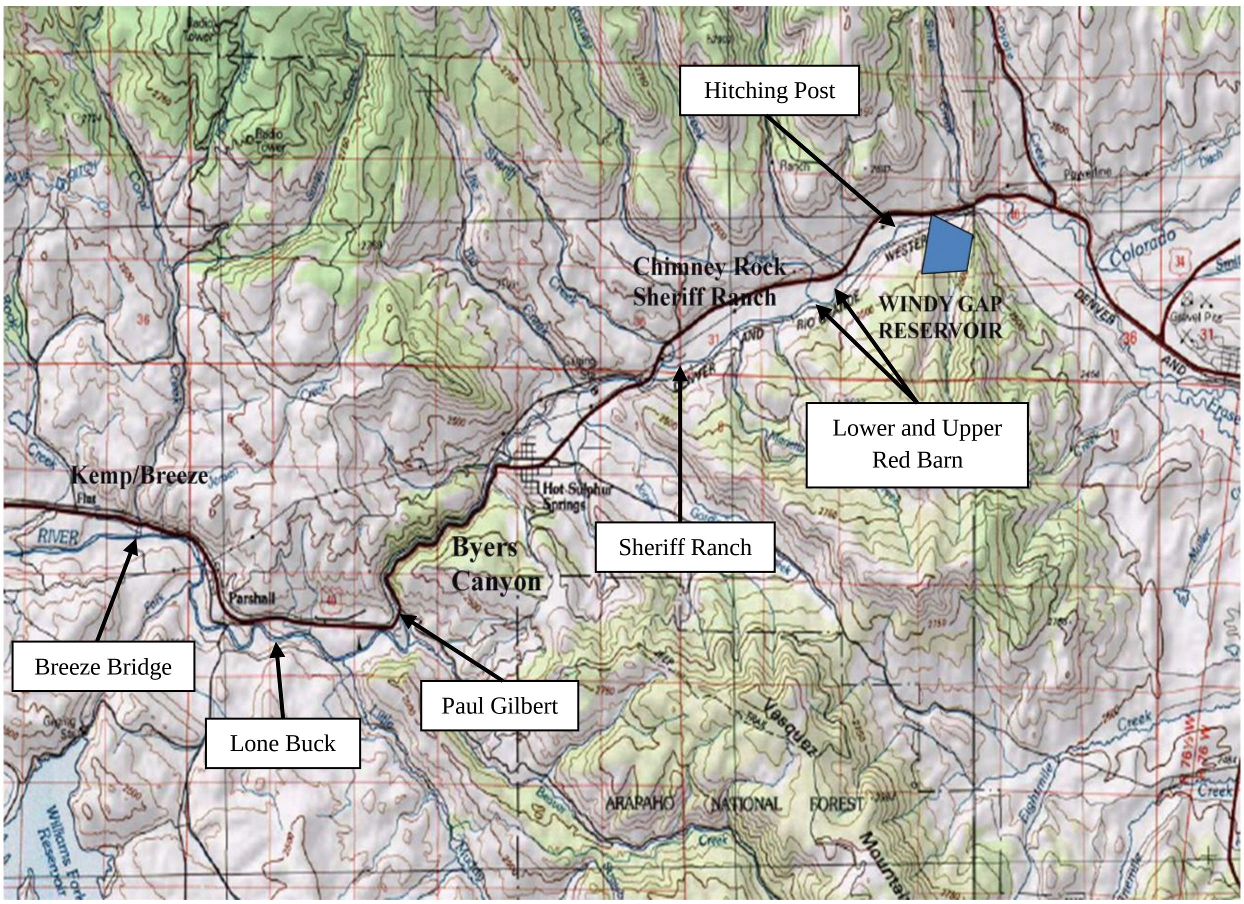

Figure 4.4.1. Map of the upper Colorado River study area showing the seven sites at which fry population estimates were conducted in June, July, August, September, and October 2015.

One potential benefit of using H×C for fry introductions is the reduction in infection severity, both in the rainbow trout and brown trout fry, and in the river as a whole. Both brown trout and rainbow trout have exhibited lower myxospore counts following the initiation of rainbow trout fry stocking in October 2013 than in the years prior to 2013 (Fetherman et al. 2014; Fetherman and Schisler 2015). Myxospore counts remained low in both rainbow trout and brown trout in 2015, with rainbow trout collected in October exhibiting an average myxospore count of 339 (± 97) myxospores per fish, and brown trout exhibiting an average myxospore count of 3,617 (± 1,007) myxospores per fish.

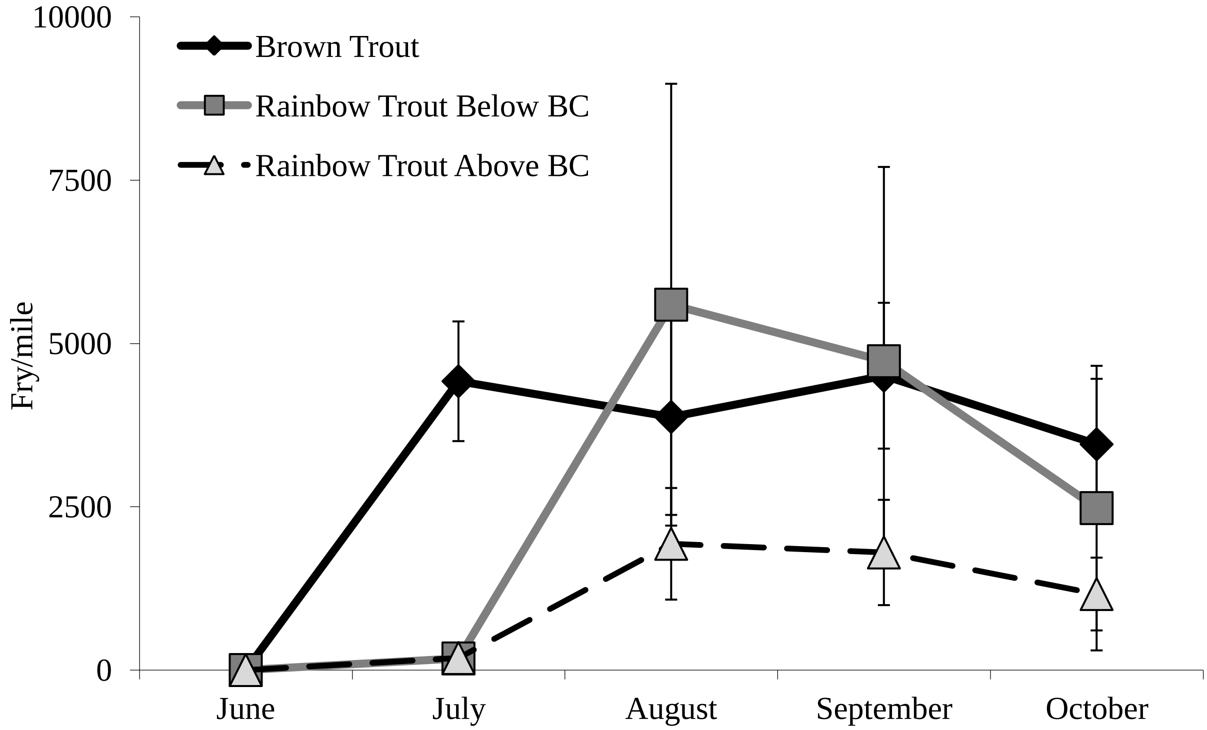

Figure 4.4.2. Upper Colorado River overall brown trout density estimates and rainbow trout density estimates above and below Byers Canyon (BC; fry/mile; SE bars) for the months of June to October 2015.

The introduction of *Myxobolus cerebralis*-resistant rainbow trout fry continues to be successful. Rainbow trout fry densities are consistently higher in October following fry stocking than they were when fry were produced solely by natural reproduction (Fetherman et al. 2014; Fetherman and Schisler 2105). Additionally, myxospore counts continue to be low in salmonid fry in the upper Colorado River, which is partially attributable to the rainbow trout fry stocking, and partially attributable to other cause including *M. cerebralis*-resistant rainbow trout introductions upstream of Windy Gap Reservoir and the change in composition of worm lineages in Windy Gap Reservoir (Nehring et al. 2013; Fetherman and Schisler 2015). The adult rainbow trout population in the upper Colorado River has also increased in recent years as a result of the fry stocking, increasing from three adult rainbow trout per mile in 2013 (Fetherman and Schisler 2015) to 91 fish per mile in 2016 (see Job No. 4, Action #3). Pure Hofer rainbow trout fry will be stocked into the Chimney Rock/Sheriff Ranch section of the upper Colorado River in the summer of 2016, and post-stocking survival and recruitment evaluations will be continued in the upper Colorado River in the coming years.

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

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

Nehring, R. B., B. Hancock, M. Catanese, M. E. T. Stinson, D. Winkelman, J. Wood, and J. Epp. 2013. Reduced *Myxobolus ecerbralis* actinospore production in a Colorado reservoir may be linked to changes in *Tubifex tubifex* population structure. Journal of Aquatic Animal Health 25(3):205-220.

Action #5:

- *Level 1 Action Category: Species Reintroduction and Stocking*
- *Level 2 Action Strategy: Production and stocking for recreational purposes*
- *Level 3 Action Activity: Put-grow-and-take*

Rainbow trout fry, hatched and reared by the CPW Rifle Falls Hatchery, will be stocked into the upper Colorado River in summer 2015. Rainbow trout will be stocked by raft in the margins of the river to increase post-stocking survival. Following stocking, survival will be monitored using fry abundance estimates (see Action #4).

Action #5 Accomplishments

Approximately 250,000 H×C fry were stocked by raft in the 3.9 mile stretch of the upper Colorado River between Hitching Post Bridge on the Chimney Rock Ranch and the Sheriff Ranch (see Figure 4.4.1) on July 29, 2015. Half of the rainbow trout fry (~125,000) 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 1.75 mile stretch between Hitching Post Bridge and the irrigation diversion structure located at Red Barn. The second half of the rainbow trout fry were loaded onto the raft below the Red Barn irrigation diversion, and fry were similarly stocked on both sides of the river between Red Barn and the Sheriff Ranch. Post-stocking survival evaluations were conducted in August, September, and October 2015 (see Job No. 4, Action #4).

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: 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 differentiate between the pure Hofer and pure Colorado River Rainbow trout strains and their crosses.

Action #6 Accomplishments
Genetic samples were collected from rainbow trout fry and adults captured during fry and adult population estimates on the upper Colorado River (see Job No. 4, Action #3 and Action #4), and the Gunnison River in the Gunnison Gorge (see Job No. 4, Action #2). Genetic samples consisted of fin clips taken from the upper caudal fin. Fins were preserved in 95% ethanol alcohol and stored in a freezer for future analysis. Samples for analysis were sent to the Genomic Variation Lab at the University of California Davis. Single nucleotide polymorphisms (SNPs) were used to differentiate pure Hofer (GR) from pure Colorado River Rainbow (CRR) trout, and their crosses.

In addition to the traditional SNP panel, which allowed differentiation to a specific strain or cross (e.g., CRR, F1, B2, etc.), a new system of identification was developed to allow quantification of the proportion of Hofer rainbow trout genes in any given individual. This new system of identification was necessary since many rivers have been stocked with a number of different *M. 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.

Before this system of identification could be applied to unknown wild samples, the robustness of the system needed to be tested. Known samples from hatchery populations of GR crosses and pure strains maintained in Colorado hatcheries were run through the SNP marker set, and proportion of GR markers in each strain or cross were identified and plotted. Proportions of GR in the strains and crosses were similar to those expected to occur via Mendelian inheritance (Figure 4.6.1). The pure GR strain maintained a high proportion of genes identified as GR (0.98 [± 0.02]), whereas the Harrison Lake (HL or HAR) and CRR strains maintained a low proportion of GR genes (0.01 [± 0.02] and 0.02 [± 0.02], respectively). First and second generation GR crosses maintained a 50:50 proportion of GR and CRR genes, whereas backcrosses and outcrosses with the pure strains showed an increase or reduction in GR genes, respectively (Figure 4.6.1). The results suggested that the methods used to identify GR markers and assign proportions were accurate based on expectations.

Although the new system of identification performed well with known samples, the ability of this system to determine proportion GR in unknown samples, those originating from wild populations with much intermixing, needed to be tested prior to use. Current wild rainbow trout populations consist not only of various crosses of rainbow trout, but also rainbow trout crossed with cutthroat trout (cutbows), primarily Snake River cutthroat trout (SRN). To start, known samples were run to determine if the proportion of GR genes in pure strains, GR, HAR, and CRR, as well as SRN and their crosses with GR and HAR, matched expectations (Figure 4.6.2).

After confirming that expectations were met, simulations were run to create “populations” of intermixed rainbow trout and cutthroat trout. Expectations based on Mendelian inheritance were developed to predict the proportion of GR in various crosses, including several backcrosses and outcrosses of GR, HAR, CRR, and SRN. In all cases, the expected proportion of GR genes in each cross fell within the range of error for the simulated populations (Figures 4.6.3, 4.6.4, and 4.6.5) suggesting that proportion of GR genes could be accurately assigned to individual fish originating from intermixed wild populations.

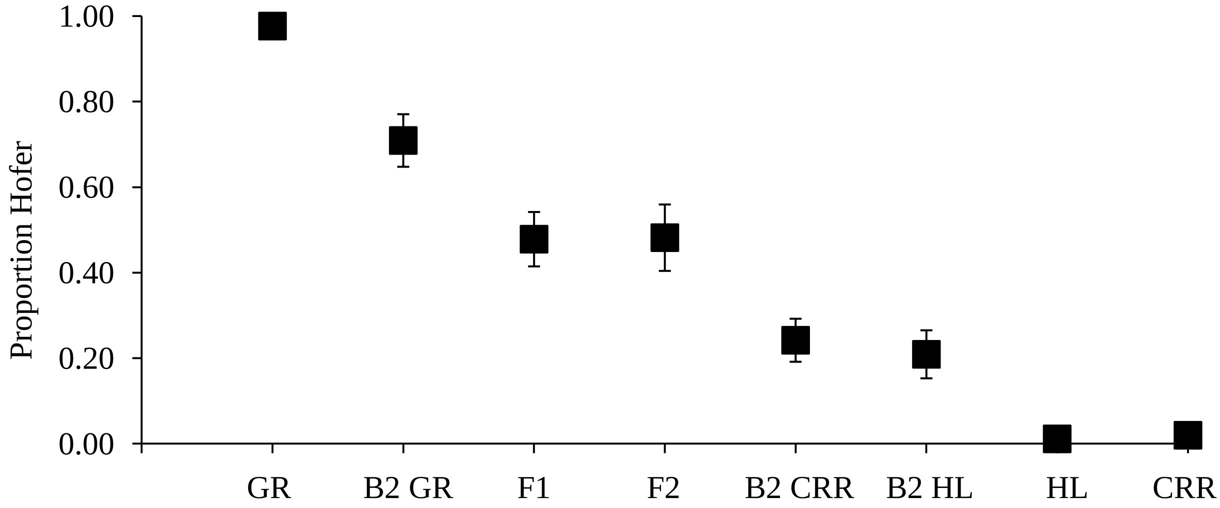

Figure 4.6.1. Proportion Hofer genes identified in fish known to be pure Hofer (GR), pure CRR, pure Harrison Lake (HL), or crosses therein. In general, proportions of GR are similar to those expected to occur via Mendelian inheritance.

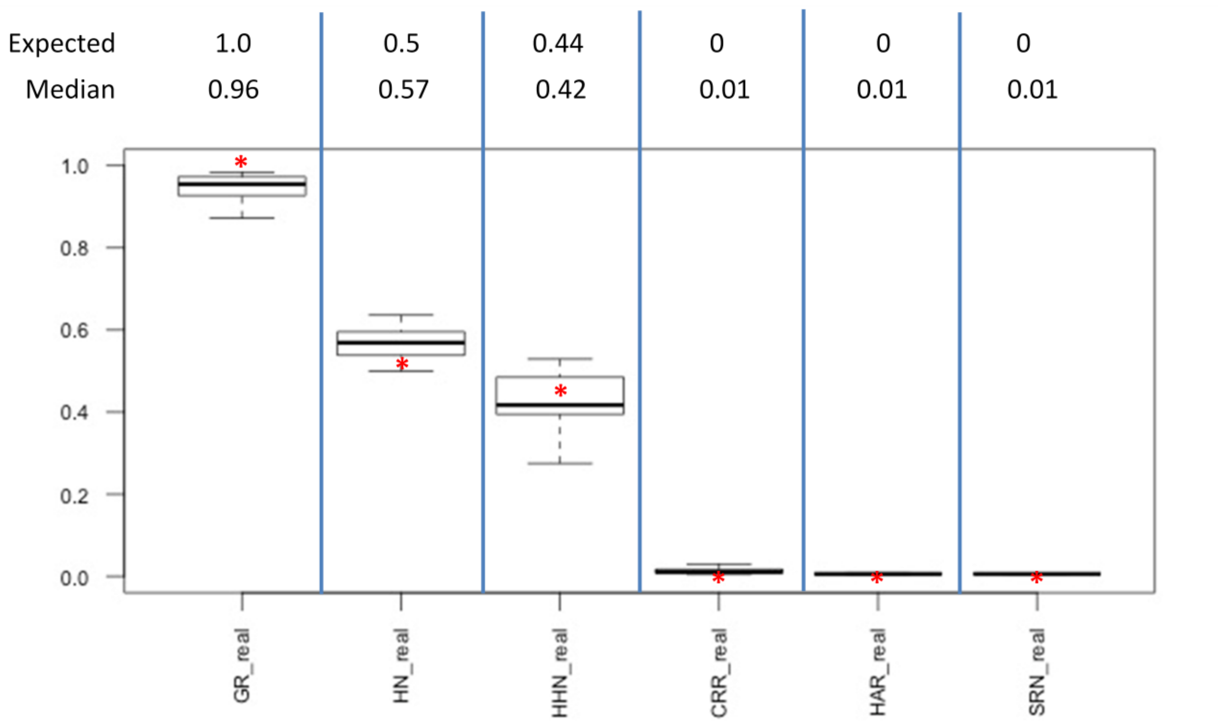

Figure 4.6.2. Proportion (simulated = box plot; expected = *) Hofer (GR) genes identified in fish known to be pure GR, pure Harrison Lake (HAR), pure Colorado River Rainbow (CRR), pure Snake River cutthroat trout (SRN), and crosses of the GR and SRN (HN) and GR, HAR, and SRN (HHN). Developed by Dr. Melinda Baerwald, UC Davis.

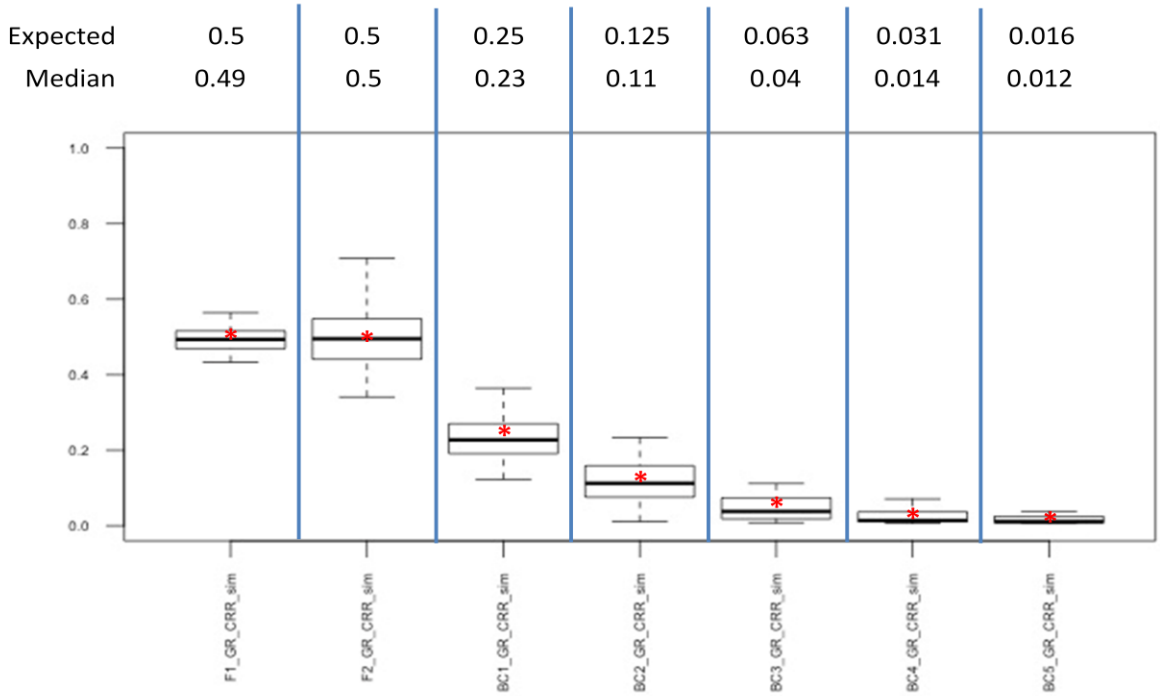

Figure 4.6.3. Proportion (simulated = box plot; expected = *) Hofer (GR) genes identified in simulated populations of crosses of GR and Colorado River Rainbow (CRR) trout. Developed by Dr. Melinda Baerwald, UC Davis.

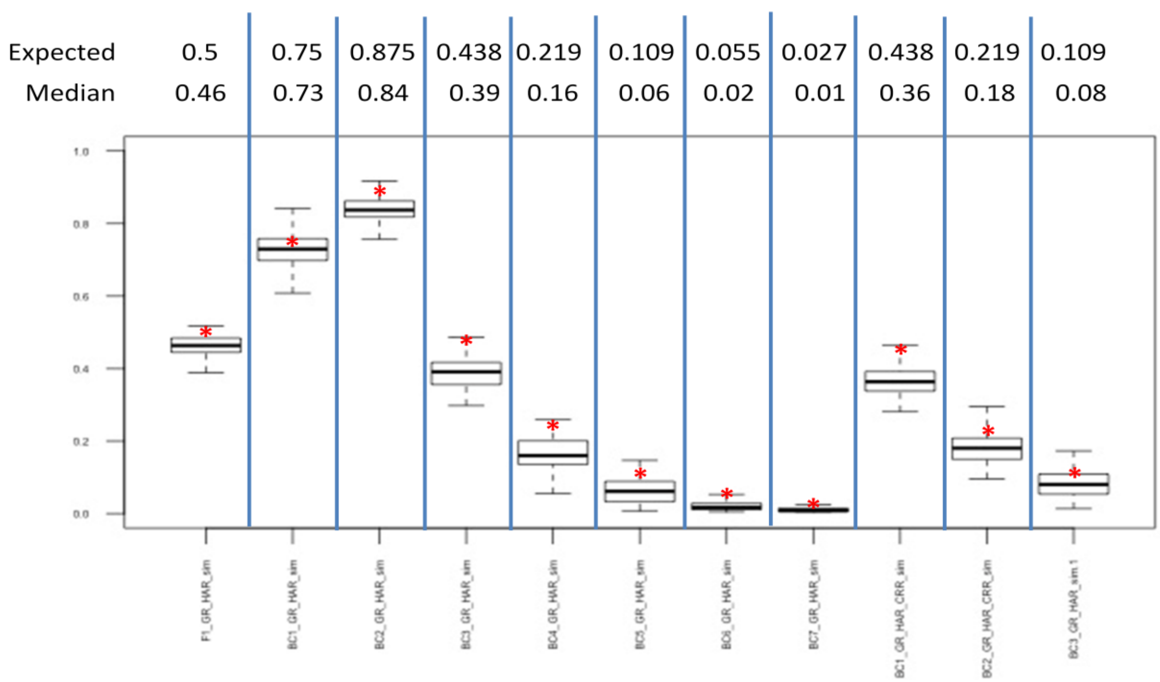

Figure 4.6.4. Proportion (simulated = box plot; expected = *) Hofer (GR) genes identified in simulated populations of crosses of GR, Harrison Lake (HAR), Colorado River Rainbow (CRR), and Snake River cutthroat (SRN) trout. Developed by Dr. Melinda Baerwald, UC Davis.

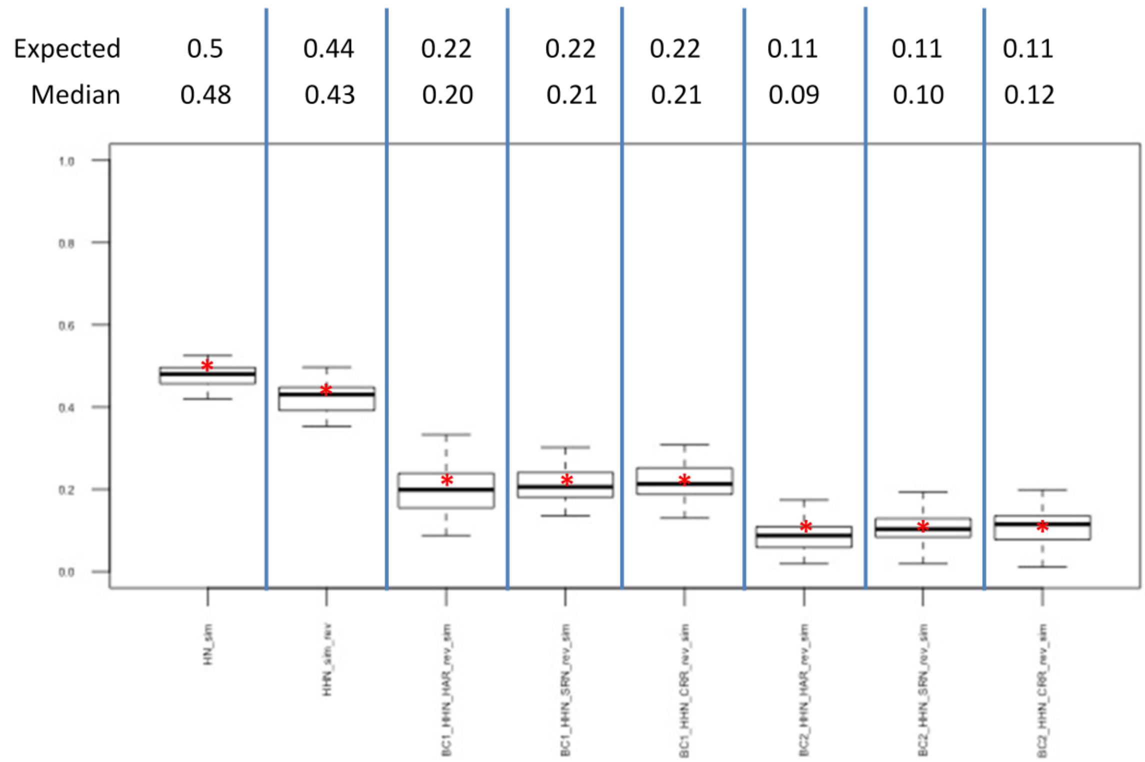

Figure 4.6.5. Proportion (simulated = box plot; expected = *) Hofer (GR) genes identified in simulated populations of crosses of GR, Harrison Lake (HAR), Colorado River Rainbow (CRR), and Snake River cutthroat (SRN) trout. Developed by Dr. Melinda Baerwald, UC Davis.

Genetic samples (940) collected from rivers across Colorado in 2013, 2014, and 2015 were rerun using this new system of identification to determine the proportion of GR genes in wild rainbow trout populations. The information gained from this study will be used to determine if wild populations are maintaining GR genes, and therefore resistance to *M. cerebralis*, despite intermixing, outcrossing, and backcrossing. Additionally, the data will be used to assess how the genetics of newly established rainbow trout populations change over time. Data analysis is pending, and results will be available within the next year.

Action #7:

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

H×C rainbow trout 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. A graduate student (Brian Avila) from Colorado State University will be evaluating 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. Three pass removal estimates, accomplished using three Smith-Root LR-24 backpack electrofishing units, will occur in July/August 2015, and will be used to determine the number of fish of each strain that survived and recruited in each stream.*

<u>Action #7 Accomplishments</u>
Three pass removal estimates were conducted in two sites (220 ft, on average) within nine streams located in three major river drainages in August 2015: 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) Mystery Creek (Colorado), 6) Rock Creek (Colorado), 7) Tarryall Creek (South Platte), 8) Michigan Creek (South Platte), and 9) Jefferson Creek (South Platte). 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 complete, at which time fish were measured, weighed, and returned to the creek within the study 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. Stream environmental characteristics thought to affect rainbow trout survival, such as temperature, width, velocity, and flood plain connectivity (i.e., entrenchment ratio) were also measured at each site.

Rainbow trout were found in eight of the nine streams in August 2015, one year post-stocking as fry. Rainbow trout were not observed in Jefferson Creek suggesting that fish had either not survived or moved out of the study section prior to sampling. Annual (12 month) rainbow trout survival was not affected by either stream environmental characteristics or strain, suggesting that GR survived just as well as H×C when stocked as fry across a range of stream types. Average annual survival rates were between 0.5 and 1.0 percent. The results presented herein are a brief summary of the results obtained by Colorado State University graduate student Brian Avila. The thesis for this project is expected to be completed in Fall 2016.

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, 2016.
2. Complete one peer review of a manuscript submitted to a scientific journal by June 30, 2016.

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 *M. cerebralis*-resistant rainbow trout, the use of predator recognition and training protocols to increase rainbow trout post-stocking survival, and the use of a portable RFID-GPS system used to track the movements of PIT-tagged fish in rivers. Five presentations were given at the CPW aquatic senior staff meeting, aquatic biologist meeting, and plains fish research meeting:

- Fetherman, E. R. 2015. East Portal of the Gunnison River rainbow trout (GRR). Colorado Parks and Wildlife Aquatic Senior Staff Meeting. Fort Collins, Colorado. December 16, 2015.
- Kopack, C., L. Angeloni, R. Fitzpatrick, D. Broder, and E. Fetherman. 2016. Training fish to survive: A hatchery life. 2016 Annual Colorado Parks and Wildlife Plains Fish Research Meeting. Fort Collins, Colorado. January 5, 2016.
- Richer, E., M. Kondratieff, and E. Fetherman. 2016. RFID-GPS system development and issues with PIT tag retention. 2016 Annual Colorado Parks and Wildlife Plains Fish Research Meeting. Fort Collins, Colorado. January 5, 2016.
- Fetherman, E. R. 2016. Wild rainbow trout brood stock development and maintenance. Colorado Parks and Wildlife Aquatic Biologist Meeting. Loveland, Colorado. January 21, 2016.
- Avila, B. W., D. L. Winkelman, and E. R. Fetherman. 2016. Evaluation of resistant rainbow trout fry stocking in Colorado. Colorado Parks and Wildlife Aquatic Biologist Meeting. Loveland, Colorado. January 21, 2016.

External presentations provided an opportunity to give research updates to other fishery managers both within and outside of the state of Colorado. Four presentations were given at the Colorado Aquaculture Association, a joint meeting of Colorado's public and private hatchery managers, as well as at chapter and division meetings of the American Fisheries Society (AFS):

- Fetherman, E. R., and G. J. Schisler. 2016. Hofer rainbow trout research update. 2016 Annual Colorado Aquaculture Association Meeting. Mount Princeton Hot Springs, Colorado. January 29, 2016.
- Avila, B. W., D. L. Winkelman, and E. R. Fetherman. 2016. Evaluation of resistant rainbow trout fry stocking in Colorado. 2016 Annual Meeting of the Colorado/Wyoming Chapter of the American Fisheries Society. Laramie, Wyoming. March 3, 2016.
- Avila, B. W., D. L. Winkelman, and E. R. Fetherman. 2016. Evaluation of whirling disease resistant rainbow trout fry stocking in Colorado. 2016 Annual Meeting of the Western Division of the American Fisheries Society. Reno, Nevada. March 23, 2016.
- Hodge, B, E. R. Fetherman, K. B. Rogers, and R. Henderson. 2016. Effectiveness of a fishway for restoring passage of Colorado River cutthroat trout. 2016 Annual Meeting of the Western Division of the American Fisheries Society. Reno, Nevada. March 24, 2016.

Disease research, and our understanding of specific diseases and how to manage for them, is constantly evolving. Continuing education sessions at national meetings help update fishery managers on the current state of knowledge regarding diseases. One presentation was given as part of the continuing education session entitled "What's bugging my fish? Detection, pathology, impacts, and treatment of problematic parasites" at the AFS 56th Annual Western Fish Disease Workshop:

- Fetherman, E. R., G. J. Schisler, and B. W. Avila. 2015. Post-stocking survival of whirling disease resistant rainbow trout, and changes in fish health before and after stocking whirling disease resistant rainbow trout. Continuing Education session of the 2015 Western Fish Disease Workshop. Steamboat Springs, Colorado. June 2, 2015.

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 2016:

- Fetherman, E. R. 2015. Salmonid disease research in Colorado. Front Range Community College, Fisheries Management class. Fort Collins, Colorado. March 9, 2016.

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:

- Fetherman, E. R., J. M. Lepak, B. L. Brown, and D. J. Harris. 2015. Optimizing time of initiation for triploid walleye production using pressure shock treatment. North American Journal of Aquaculture 77:471-477. DOI:10.1080/15222055.2015.1040568.
- Stout, J. B., B. W. Avila, and E. R. Fetherman. 2016. Efficacy of commercially available quaternary ammonium compounds for controlling New Zealand mudsnails, *Potamopyrgus antipodarum*. North American Journal of Fisheries Management 36:277-284. DOI:10.1080/02755947.2015.1120830.

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

- Fetherman, E. R., J. A. Wardell, C. J. Praamsma, and M. K. Hura. *In press*. Critical dissolved oxygen tolerances of whirling disease-resistant rainbow trout. North American Journal of Aquaculture.
- Kopack, C. J., E. D. Broder, E. R. Fetherman, J. M. Lepak, and L. M. Angeloni. *In review*. The effects of a single pre-release exposure to conspecific alarm cue on post-stocking survival in three strains of rainbow trout. Canadian Journal of Zoology.

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

- Calculated and compared detection range and detection probability of PIT tag antennas installed in Poose Creek used to track the movements of PIT-tagged Colorado River cutthroat trout through a recently installed fish ladder near Yampa, Colorado. Additionally, movement probabilities were calculated using Program MARK.
- Used Program MARK to calculate stonefly abundances within and between sites on the Rio Grande River near Creed, Colorado.
- 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:

- Lepak, J. M., M. B. Hooten, C. A. Eagles-Smith, M. A. Lutz, M. T. Tate, J. T. Ackerman, J. J. Willacker, Jr., D. C. Evers, J. Davis, C. Flanagan Pritz, and J. G. Wiener. Assessing mercury concentrations in fish across western Canada and the United States: potential health risks to fish and humans. Submitted to Science of the Total Environment.
- Anonymous. Temporal patterns in fish community structure: environmental perturbations from a well-mixed tropical estuary. Submitted to the Proceedings of the National Academy of Sciences, India Section B: Biological Sciences.
- Schmidt, T., C. Löb, B. Schreiber, and R. Schulz. A pitfall with PIT tags: Reduced detection efficiency of half-duplex passive integrated transponders in groups of marked fish. Submitted to the North American Journal of Fisheries Management.

In addition to peer reviews of manuscripts submitted to scientific journals, a funding proposal review was completed for the Austrian Science Fund (similar to the National Science Foundation) regarding the role of specific proteins in the fish immune system affecting whirling disease pathogenesis in rainbow trout.