Sport Fish Research Studies

Federal Aid Project F-394-R11

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Job Progress Report

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Project Title: Sport Fish Research Studies

Period Covered: July 1, 2011 – June 30, 2012

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.

Hatchery Production

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 throughout the rearing process and during on-site spawning operations 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 2011, BFRH personnel checked all of the Hofer[1] (GR), Harrison Lake (HL), Hofer × Harrison Lake (GR×HL) and brood fish (2, 3, and 4 year-olds) weekly for ripeness. Maturation is indicated by eggs or milt flowing freely when slight pressure is applied to the abdomen of the fish. The first females usually maturate two to four weeks after the first group of males. As males are identified, they are moved into a separate section of the raceway to reduce handling and fighting injuries. On November 29, 2011, the first group of GR females were ripe and ready to spawn.

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

[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.

Water-hardened fertilized (green) eggs from different crosses of the GR, HL, and GR×HL were moved to the BFRH main hatchery building. Extreme caution was used to keep each individual cross 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 12.2ºC (54ºF) of flow-through well water. The total number of eggs was calculated using number of eggs per ounce (Von Bayer trough count minus 10%) multiplied by the total ounces of eggs. Subsequent daily egg-takes and specific individual crosses were put into separate trays and recorded. To control fungus, eggs received a prophylactic flow-through treatment of formalin (1,667 ppm for 15 min) every other day until eye-up.

Eggs reached the eyed stage of development after 14 days in the incubator. The eyed eggs were removed from the trays and physically shocked to detect dead eggs, which turn white when disturbed. Dead eggs were removed (both by hand and with a Van Galen fish egg sorter, VMG Industries, Grand Junction, 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. Select groups of eggs were kept for brood stock purposes at the BFRH.

Table 1.1. Bellvue Fish Research Hatchery on-site spawning information for the Hofer (GR), Harrison Lake (HL), and Hofer × Harrison Lake (GR×HL) rainbow trout strains during the winter 2011-2012 spawning season**.**

Strain	Date Spawned	No. Spawned Females	No. Green Eggs	No. Eyed Eggs	Shipped To
100% GR	11/29/11-12/21/11	139	143,355	114,684	Fish Research Hatchery/CPW Hatcheries
100% HAR	12/21/11-1/31/12	46	81,274	70,673	Fish Research Hatchery/USFWS Hatcheries
H×H	1/11/2012	30	2000	1700	Fish Research Hatchery Brood
Total	11/29/11-1/31/12	215	226,629	187,057	82% Good Eggs to Eye-up

The FRH 2011/2012 on-site rainbow trout production spawn started on November 29, 2011, with the last groups of HL females spawned on January 31, 2012. The initial goal was to produce 154,000 eyed eggs; egg take exceeded the production needs with 187,057 eyed eggs produced (Table 1.1). With the availability of both ripe males and females from several year classes and combinations of previous years crosses of GR, HL, and GR×HL, BFRH personnel produced seven different lots during the spawn. BFRH personnel were able to fill all GR, HL, and GR×HL production and research directed project egg requests for Colorado in 2011-2012. The

GR×CRR brood stock are not mentioned in this report because they have been fully transitioned into production at the CPW Glenwood Springs Hatchery and Poudre Ponds Hatchery brood units.

Research Projects

Eggs produced specifically for research projects and brood stock management comprises 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. The bulk of these family group descriptions appear in Job No. 2: Improved Methods for Hatchery and Wild Spawning and Rearing of Sport Fish Species.

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.

Rainbow Trout Egg Formalin Sensitivity Experiment

INTRODUCTION

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).

Differential formalin sensitivity has been demonstrated for various strains of rainbow trout when exposed post-hatch (Piper and Smith 1973); however, there has been little to no research on differential strain sensitivity to formalin exposure during egg incubation. The objective of this study was to determine if there was differential sensitivity (measured by mortality) of four whirling disease resistant rainbow trout strains to varying formalin concentrations used to control fungus during egg incubation (pre-hatch), and if mortality increased post-hatch as a result of exposure during egg incubation.

METHODS

Strains and Spawning

Four whirling disease resistant rainbow trout strains and crosses were used to determine formalin sensitivity, exposed during egg incubation to varying formalin concentrations: Hofer (GR), Harrison Lake (HL), Hofer × Harrison Lake 50:50 (GR×HL 50:50), and Hofer × Harrison Lake (GR×HL 75:25). All four of these strains and crosses are maintained as brood stock at the BFRH.

Spawning occurred in late December 2011. GR egg groups were created by pooling the eggs from 13 pairs of two-year-old GR females spawned with three-year-old GR males. The eggs from two pairs of two-year-old HL females spawned with three-year-old HL males, and six pairs of three-year-old HL females spawned with two-year-old HL males, were pooled together to create the HL strain egg groups for the experiment. The GR×HL 50:50 cross egg groups were created by pooling the eggs from five pairs of three-year-old GR females spawned with two-year-old HL males. The eggs from six pairs of two-year-old GR×HL 50:50 females spawned with three-year-old GR males were pooled together to create the GR×HL 75:25 egg groups for

the experiment. Following spawning, eggs were disinfected with iodine and water hardened for one hour before being distributed in the egg tray towers for incubation and formalin exposure.

Experimental Design

Three, five gpm flow-through egg tray towers were utilized for the formalin exposure experiment, with one formalin treatment per tower. Only the top four egg trays within the seven tray towers were used for the experiment. In the control tower, the lower three trays were used to rear the hatchery's 2012 brood stock fish. Two, three inch diameter, screen-bottomed PVC inserts were placed in each of the four trays, a total of eight PVC inserts per treatment (Figure 2.1). Each PVC insert contained 500 eggs from a given strain or cross, providing two 500 egg replicates per strain or cross, per treatment. Strains and crosses were assigned to PVC inserts within a treatment using a random number generator (Table 2.1). Eggs from each strain or cross were initially counted out by hand to determine the number of ounces containing 500 eggs. This measurement was then used to distribute approximately 500 eggs to each of the PVC inserts.

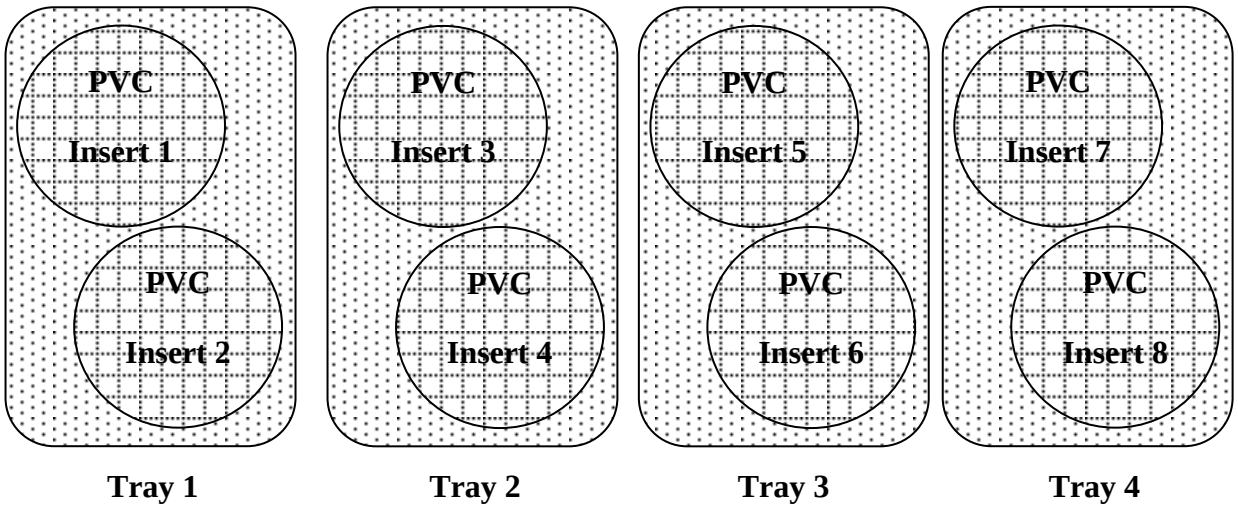

Figure 2.1. Arrangement of eight screen-bottomed PVC inserts in the four trays (1-4, from top of tower down) used in each formalin treatment group. Strains and crosses were randomly assigned to an insert, within a treatment, using a random number generator (see Table 2.1).

Table 2.1. Assignment of strain to PVC insert within a given treatment via a random number generator. Each treatment contains two 500 egg replicates per strain or cross.

PVC Insert	Control	Increased Formalin	High Formalin
1	GR×HL 75:25	GR	GR×HL 50:50
2	GR	GR×HL 75:25	GR×HL 75:25
3	GR×HL 50:50	HL	GR
4	GR	GR×HL 50:50	GR×HL 50:50
5	HL	GR×HL 75:25	GR
6	GR×HL 50:50	GR	HL
7	HL	HL	HL
8	GR×HL 75:25	GR×HL 50:50	GR×HL 75:25

Three formalin treatment levels were used to determine rainbow trout egg formalin sensitivity. The control formalin concentration was the concentration that was traditionally used to treat eggs at the BFRH. Eggs in the control treatment were exposed to 1,667 parts per million (ppm) of formalin, equating to 16 oz of formalin in a one gallon chicken feeder for an exposure period of 15 minutes with a flow of five gpm. A traditional control, consisting of no formalin treatment, was not included in this experiment because experience had shown that pre-hatch mortality would be high due to fungal infection if the eggs were not treated.

Eggs in the increased formalin concentration treatment were exposed to 2,000 ppm of formalin, equating to 19.2 oz of formalin in a one gallon chicken feeder for an exposure period of 15 minutes with a flow of five gpm. The increased formalin concentration was chosen because it is traditionally used to treat eggs in areas where higher fungal infection rates occur (Piper et al. 1982). The high formalin concentration was five times the effective treatment level (1,000 ppm) for control of fungus (Marking et al. 1994). Eggs in the high formalin concentration treatment were exposed to 5,000 ppm of formalin, equating to 48 oz of formalin in a one gallon chicken feeder for an exposure period of 15 minutes with a flow of five gpm. This concentration was thought to be a toxic concentration of formalin to rainbow trout eggs; however, in a similar experiment, toxicity to eggs (defined as a 10% or more decline in hatching rate) was not apparent at a concentration of 5,000 ppm for exposures of 15 or 30 minutes (Marking et al. 1994).

The experiment began with the distribution of eggs to the PVC inserts within each treatment. Formalin treatment began on the second day of the experiment, with treatment occurring every other day until the eggs were eyed. Once the eggs eyed, treatments ceased. Eyed eggs were physically shocked by pouring the eggs into a second tray where the dead and unfertilized eggs were identified, counted, and removed. Pre-hatch mortality was calculated using the equation (Barnes et al. 2000) $\%\, prehatch\ mortality = 100 \times \frac{mortality\ before\ hatch}{initial\ number\ of\ eggs}$. *Mortality before hatch* was calculated by summing the number of eggs that were picked-off (those eggs that turned white prior to eyeing), dead eggs that were removed following physical shock, and eggs that remained unhatched once hatching had occurred.

Upon hatching, each replicate was transferred to a labeled, two gallon tank and held until the fish swam up. Post-hatch mortality was calculated using the equation (Barnes et al. 2000) $\%\, posthatch\ mortality = 100 \times \frac{mortality\ after\ hatch}{initial\ number\ of\ eggs}$. *Mortality after hatch* was calculated by summing the number of crippled fish that did not survive to swim-up, and the number of deformed fish that were not counted as "healthy" upon completion of the experiment. These deformed fish were removed and counted as mortalities while a final count of swum-up fish was obtained. The initial number of eggs, used in both of the equations presented above, was back-calculated upon conclusion of the experiment by counting the number of fish that were remaining at the end of the experiment, and adding the number of pre- and post-hatch mortalities that occurred. Percent total mortality, including both pre-hatch and post-hatch mortality was calculated using the equation $\%\, total\ mortality = 100 \times \frac{prehatch+posthatch\ mortality}{initial\ number\ of\ eggs}$.

Statistical analyses were conducted using the GLM procedure in SAS (SAS Institute 2011). Differences in percent pre-hatch, post-hatch, and total mortality were analyzed using a two-factor

ANOVA, with strain/cross and treatment as the factors ($N = 24$). Percentages were arcsine-square root transformed prior to analysis. Values for all analyses were reported from the type III sum of squares. If significant effects were identified ($P < 0.05$), the least-squares means method with a Bonferroni adjustment was used to determine which treatments caused significant differences in mortality within a strain or cross.

RESULTS

As mentioned in the methods, 500 eggs from each strain or cross were counted by hand and measured to determine how many ounces of eggs constituted five hundred eggs. After the initial count, eggs were measured out, not counted out, using this known measurement. Using this procedure to distribute the eggs resulted in an average (± SD) of 506 (± 25) eggs per PVC insert. Average starting number of eggs did not differ among strains/crosses or treatments ($F = 0.95$, $P = 0.530$).

Average pre-hatch mortality differed both between the treatments ($F = 52.63$, $P < 0.001$), and among the strains/crosses ($F = 103.14$, $P < 0.001$); the interaction was also significant ($F = 31.38$, $P < 0.001$). Eggs within the high formalin treatment exhibited significantly higher average (± SD) percent pre-hatch mortality (31.8 ± 7.3%) than did either the increased formalin (22.9 ± 9.4%) or control treatments (23.5 ± 12.3%; $P < 0.001$); the control and increased formalin treatments did not differ ($P = 1.000$). The GR×HL 50:50 cross showed significantly lower average percent pre-hatch mortality (17.4 ± 15.2%) than all of the other strains and crosses ($P < 0.001$). Conversely, the GR×HL 75:25 cross showed significantly higher average percent pre-hatch mortality (36.4 ± 3.7%) than all of the other strains or crosses ($P < 0.001$). The two pure strains, GR and HL, did not differ from each other in average percent pre-hatch mortality (GR: 23.5 ± 3.1%, HL: 26.8 ± 1.2%; $P = 0.114$).

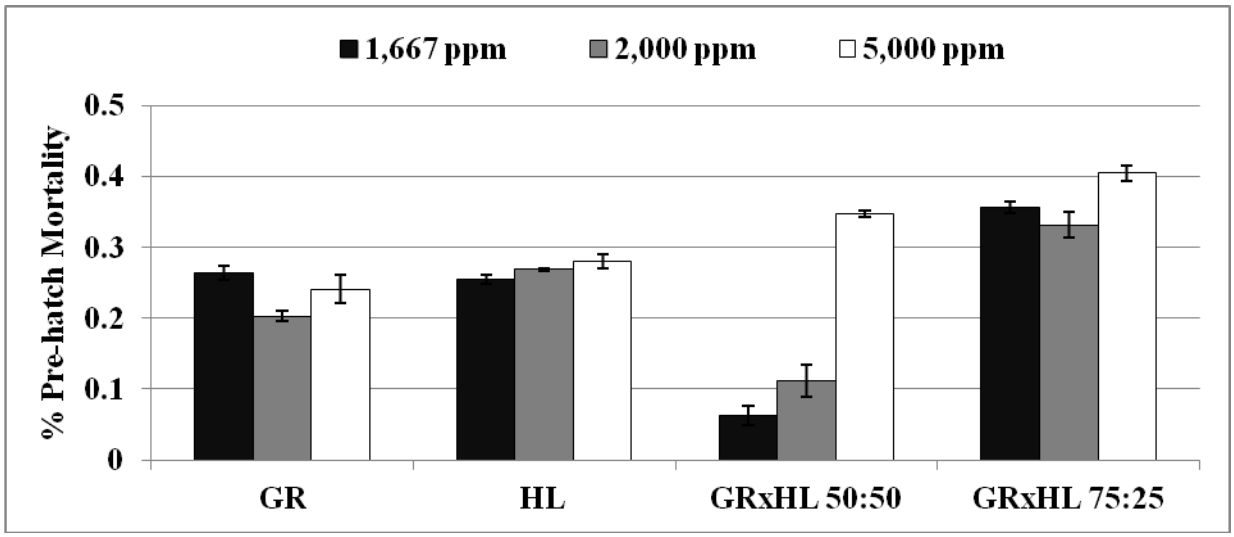

Figure 2.2. Average percent pre-hatch mortality (SE bars) by strain and treatment.

On average, the greatest mortality was observed in the form of eggs that turned white and were picked off prior to eying up (14.1 ± 5.5%), and eggs that did not survive to eye-up and were

removed following bumping of the eyed eggs (8.6 ± 4.1%). On average, only 3.3% (± 1.9%) of the eggs not removed during the physical shock removal did not survive to hatching; these were removed following hatching of all of the other eggs within a PVC insert.

Despite exhibiting the lowest average percent pre-hatch mortality, the GR×HL 50:50 cross was the only strain or cross to exhibit sensitivity to formalin, pre-hatch (Figure 2.2). GR×HL 50:50 eggs in the high formalin treatment exhibited significantly higher mortality (34.7 ± 0.6%) than did those in the increased formalin (11.2 ± 3.1%) or control treatments (6.3 ± 1.8%; $P < 0.001$); the increased formalin and control treatments did not differ ($P = 0.225$). None of the other strains or crosses exhibited a significant increase in mortality with an increase in formalin treatment concentration, pre-hatch ($P > 0.691$; Figure 2.2).

Average post-hatch mortality differed both between the treatments ($F = 4.05$, $P = 0.045$), and among the strains/crosses ($F = 89.08$, $P < 0.001$); the interaction was also significant ($F = 3.46$, $P = 0.032$). However, following Bonferroni correction of the arcsine-square root transformed values for percent post-hatch mortality, no significant differences were observed between the treatments using the least-squares means method ($P > 0.068$). Average percent post-hatch mortality was 6.8% (± 4.2%) in the control treatment, 7.1% (± 5.1%) in the increased formalin treatment, and 8.1% (± 4.4%) in the high formalin treatment. The HL strain exhibited significantly higher average percent post-hatch mortality (13.0 ± 1.3%) than any of the other strains ($P < 0.002$). The GR×HL 75:25 cross and GR strain differed from each other in average percent post-hatch mortality (GR×HL 75:25: 8.7 ± 0.4%, GR: 5.1 ± 0.5%; $P < 0.001$), and both exhibited significantly higher post-hatch mortality than the GR×HL 50:50 cross (2.6 ± 2.0%; $P < 0.002$). Following Bonferroni correction of the arcsine-square root transformed values for percent post-hatch mortality, no significant differences were observed across the treatments within a strain or cross using the least-squares means method (Figure 2.3).

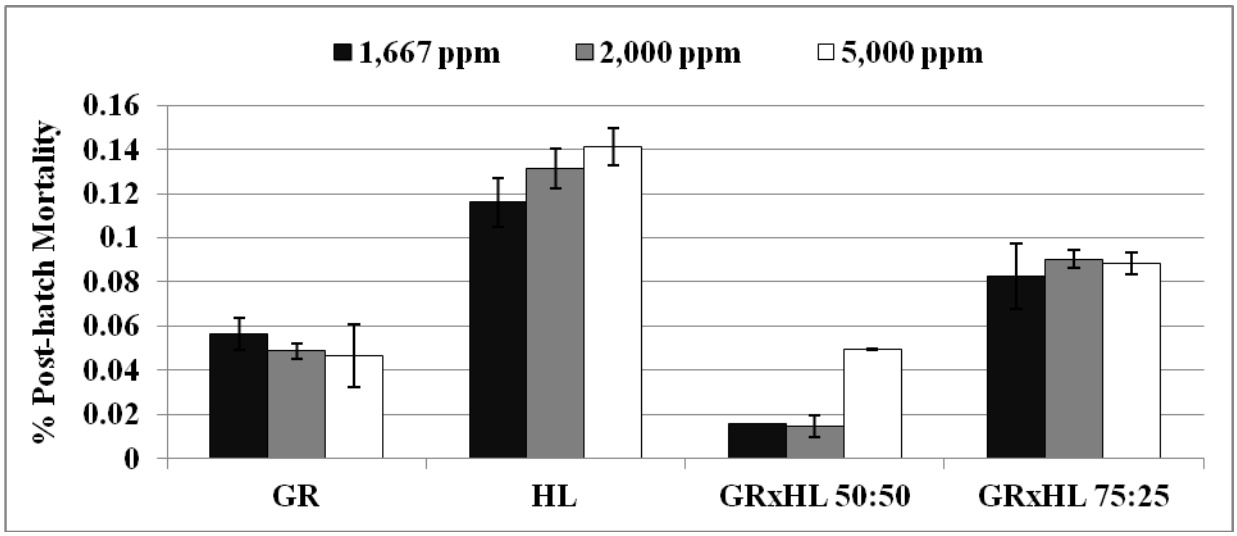

Figure 2.3. Average percent post-hatch mortality (SE bars) by strain and treatment.

On average, the greatest post-hatch mortality (5.6 ± 3.9%) was observed in the form of crippled fish that were removed either post-mortem, or pre-mortem if it was obvious that the fish was

unable to swim up due to deformities. Only a small percentage of post-hatch mortality (1.8 ± 0.7%) occurred in the form of deformed, unhealthy fish that were removed while counting healthy fish at the end of the experiment.

Average percent total mortality differed both between the treatments ($F = 64.05$, $P < 0.001$), and among the strains/crosses ($F = 187.37$, $P < 0.001$); the interaction was also significant ($F = 36.79$, $P < 0.001$). Eggs within the high formalin treatment exhibited significantly higher average (± SD) perecnt total mortality (39.9 ± 8.5%) than either the increased formalin (30.0 ± 13.8%) or control treatments (30.2 ± 15.7%; $P < 0.001$); the control and increased formalin treatments did not differ ($P = 1.000$). The GR×HL 75:25 strain exhibited significantly higher average percent total mortality (45.1 ± 3.7%) than any of the other strains or crosses ($P < 0.010$). The HL and GR strains differed from each other in average percent total mortality (HL: 39.8 ± 2.5%, GR: 28.7 ± 3.5%; $P < 0.001$), and both strains exhibited significantly higher total mortality than the GR×HL 50:50 strain (20.0 ± 17.1%; $P < 0.001$).

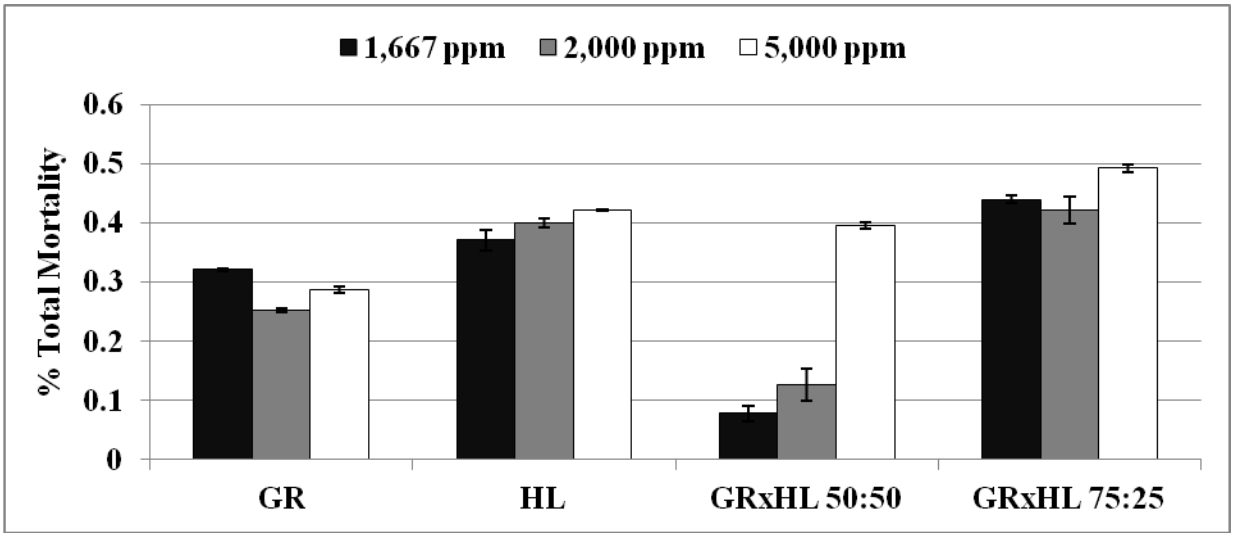

Figure 2.4. Average percent total mortality (SE bars) by strain and treatment.

Despite exhibiting the lowest average percent total mortality, the GR×HL 50:50 cross was the only strain or cross to exhibit sensitivity to formalin, as measured by percent total mortality differences among the treatments (Figure 2.4). GR×HL 50:50 eggs in the high formalin treatment exhibited significantly higher mortality (39.6 ± 0.7%) than did those in the increased formalin (12.6 ± 3.8%) or control treatments (7.8 ± 1.9%; $P < 0.001$); the increased formalin and control treatments did not differ ($P = 0.380$). None of the other strains or crosses exhibited a significant increase in total mortality with an increase in formalin treatment concentration ($P > 0.448$; Figure 2.4).

CONCLUSIONS

At the onset of this experiment, it was believed that the GR strain had a higher sensitivity to formalin treatment because large die-offs of GR strain fingerling fish had occurred in Colorado hatcheries following treatment of with formalin. However, it was unknown whether this

sensitivity was exhibited in the egg stage of the life cycle as well. The results of this experiment suggest that neither the pure GR nor HL strains are sensitive to formalin treatment during the egg life stage, as no increase in total mortality was observed with an increase in formalin treatment concentration. The same was not true, however for the GR×HL 50:50 cross, which did show an increase in egg total mortality with an increase in formalin treatment concentration, and therefore, sensitivity to formalin treatment at higher concentrations. The majority of the mortality experienced in this strain occurred pre- versus post-hatch.

Despite exhibiting an increase in mortality with an increase in formalin concentration, the results of this experiment show that GR×HL 50:50 mortality did not exceed the average "normal" mortality experienced by the other strains or crosses. In fact, at the control and increased formalin treatment concentrations, the GR×HL 50:50 strain exhibited significantly lower mortality than the other three strains and crosses. These results suggest that on average, the GR×HL 50:50 cross survives better to swim up than the other strains as long as formalin treatment concentrations remain low, a characteristic potentially explained by heterosis. The opposite was seen in the GR×HL 75:25 cross, which exhibited the highest average total mortality of all of the strains and crosses. Because there was no pattern of increasing mortality with increasing formalin concentration, this high mortality rate could not be attributed to treatment of the eggs with formalin. Mortality is more likely a result of genetic impurities in the GR×HL 75:25. For example, there are likely a number of deleterious alleles in both pure strains which cause mortality when combined correctly. The GR×HL 50:50, being heterozygous, are relatively immune to these deleterious allele combinations. However, the likelihood of deleterious alleles from both the pure strains matching up is higher in the GR×HL 75:25 cross, and the number of potentially deleterious alleles is greatly increased in this cross as it has inherited alleles from both of the parental strains. Genetic recombination of deleterious alleles could explain the higher total mortality experienced by the GR×HL 75:25 cross.

Triploid Brown Trout

There is a need in Colorado for a biological mechanism to control stunted brook trout populations and create healthier fisheries without intensive or detrimental management. So far, this has been achieved using sterile tiger trout. Tiger trout provide their own difficulties, from obtaining brook trout milt to hatching and rearing the fish. As a result of these complications, a need for an alternative management tool was needed. The solution was thought to be triploid brown trout.

The triploid brown trout experiment took place during the fall 2011 brown trout spawn at North Delaney Buttes Lake, and was designed to determine if diploid brown trout could be made using various procedures. Triploid browns were made using high pressure instead of heat, a decision that was made because a pressure chamber would already be on site for the creation of tiger trout. The study was designed to test the effectiveness of time after fertilization and under pressure (9500 psi) in creating triploid brown trout. Three treatment groups, 25, 30, and 35 minutes post-fertilization, were tested. Each group was additionally broken into two treatments of five and seven minutes under pressure. A control was used to provide diploid eggs, giving the study a total of seven different treatments that were treated identically, with the exception that treatment eggs were put under pressure for triploid creation. Data was collected regarding

percent dead, percent hatched, and numbers of cripples present (Table 2.2). A triploid test was also administered by Dr. Bonnie Brown at Virginia Commonwealth University (Table 2.3).

Table 2.2. Results of the triploid brown trout experiment conducted at North Delaney Buttes Lake on October 10, 2011. Lots are numbered such that the first number (e.g., 25) refers to the time after fertilization, and the second number (i.e., either 5 or 7) refers to the time spent under pressure (9500 psi).

Treatment	Egg Total	Dead egg	% Dead	Un-hatched	% Unhatched	Cripples
Lot 25-5	1,363	1,188	87	67	5	13
Lot 25-7	1,507	183	12	62	4	33
Lot 30-5	1,272	553	43	101	8	24
Lot 30-7	1,477	177	12	21	1	12
Lot 35-5	1,348	811	60	89	7	31
Lot 35-7	1,399	409	29	20	1	19
Diploid	1,695	400	24	43	2.5	16

Table 2.3. Triploid brown trout ploidy test results (Dr. Bonnie Brown, Virginia Commonwealth University).

Treatment	1st run % diploids	1st run % triploids	2nd run % diploids	2nd run % triploids	Average % triploids
Lot 25-5	39.73	60.27	41.80	58.20	59.24
Lot 25-7	5.44	94.56	2.45	97.55	96.05
Lot 30-5	5.90	94.10	4.88	95.12	94.61
Lot 30-7	1.89	98.11	1.22	98.78	98.45
Lot 35-5	6.72	93.28	6.74	93.26	93.27
Lot 35-7	5.21	94.79	2.84	97.16	95.97
Diploids	100	0	100	0	0

The results from this experiment were encouraging, showing that triploid brown trout could be made, and that an up to 98% success rate could be obtained. A similar experiment will be conducted again in the fall of 2012 to determine if a triploid success rate of 100% can be achieved. A success rate of 100% is desired to prevent fertile brown trout from being introduced to waters in which they are not desired. Water temperature, time after fertilization, and time under pressure will be regulated to attain desired results.

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

Job Objective: Identify and propagate whirling disease resistant domestic strains that are useful for catchable put-and-take or fingerling put-grow-and-take fisheries management applications.

Field Performance Evaluations: Parvin Lake Fingerling Stocking Experiments

INTRODUCTION

Earlier experiments demonstrated that the Hofer (GR) strain and Hofer × Harrison Lake (GR×HL) crosses have excellent growth and return-to-creel when stocked as catchable-sized fish. Colorado Parks and Wildlife (CPW) is aggressively transitioning its brood facilities to produce larger numbers of GR or GR×HL fish for catchable production purposes. In addition to catchable stocking, many waters in Colorado are stocked with fingerlings or subcatchable-sized fish. These fish are subjected to greater threats from predation than catchable-sized fish and must be able to forage and survive long enough to become available to anglers. Because of the domesticated nature of the GR strain, there are reasons to be concerned about the possibility of low survival and returns when fish of the GR strain, or slightly outbred varieties of the strain, are stocked as fingerlings. An experiment was designed to evaluate the survival of these varieties as fingerling plants in a location subjected to high predation pressure.

Figure 3.1. Parvin Lake, Colorado.

Parvin Lake (Figure 3.1), located 45 miles northwest of Fort Collins, Colorado, was used for this evaluation. The reservoir is stocked annually with fingerling brown trout (*Salmo trutta*), splake (*Salvelinus namaycush x Salvelinus fontinalis*), and rainbow trout (*Oncorhynchus mykiss*). The reservoir was also stocked in 2000 through 2003 with tiger muskies (*Esox masquinongy x Esox lucius*) to control the abundant white sucker (*Catostomus commersoni*) population. An inlet trap, historically used for rainbow trout spawning operations, has also been operated more recently to remove white suckers from the reservoir during their annual spawning run up the inlet stream (May-July). Trap captured sucker and trout numbers vary from year to year, but sucker numbers appear to have been greatly reduced in recent years (Figure 3.2). In 2009, 539 white suckers, and 67 salmonids were captured in the inlet trap. One hundred seventy-six suckers and 153 salmonids were captured in the inlet trap in 2010. In 2011,121 suckers and 76 salmonids were captured in the inlet trap, although high water in May and June 2011 prevented fish from entering the trap until later than normal. In 2012, only 4 suckers and 31 salmonids were captured in the trap due to virtually non-existent runoff conditions.

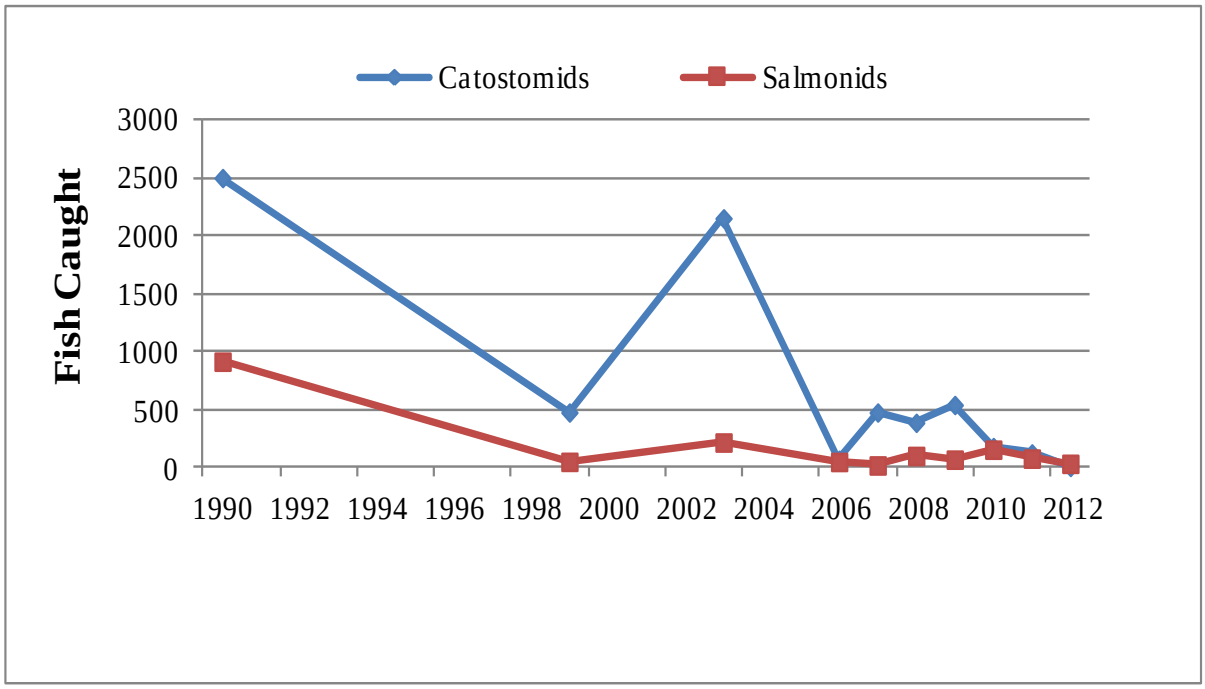

Figure 3.2. Number of catostomids and salmonids captured in the Parvin Lake inlet trap (May-July) in years where data are available.

A fall electrofishing survey has been conducted annually since 2002 to monitor species composition and growth in Parvin Lake. A shift from a population dominated by white suckers to one dominated by rainbow trout has occurred since 2006 (Figure 3.3). In 2009, 69.7% of the total catch was rainbow trout, whereas only 14.4% was white suckers. In 2010, rainbow trout comprised 76.5% of the total catch, with white suckers comprising only 3.6%. In 2011, the proportions of rainbow trout and white suckers in the total catch were 66.1% and 15.2%, respectively. The 2011 proportions compare well with the figures from 2006, when over 60% of the total catch was white suckers.

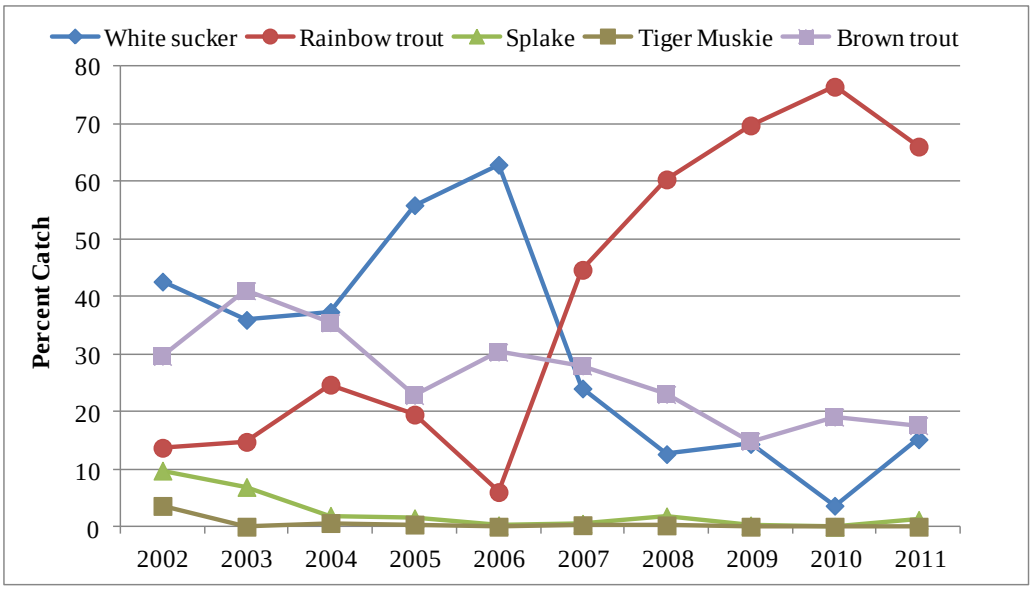

Figure 3.3. Percent of catch by species during fall electroshocking surveys for the years 2002 – 2011.

METHODS

In order to evaluate survival and growth of multiple varieties of fingerling trout, live-release experiments have been conducted on a yearly basis from 2007 to present. Preliminary returns of the different varieties, as well as fingerling strain availability, were used to determine which varieties would be used for each subsequent plant. In addition, changes to experimental groups stocked each year have been made in response to suggestions by field biologists and hatchery managers to determine if specific strains may be more or less suitable for stocking as fingerlings in lake or reservoir environments.

In 2007, 2,800 fish each of the GR, Harrison Lake (HL), GR×HL (50:50), GR×HL (75:25), and Bellaire rainbow trout × Snake River cutthroat trout 50:50 cross (RXN) varieties were batch-marked with coded-wire tags to identify fish return by variety. Fish were reared under the same conditions, and growth was matched as closely as possible before stocking. However, because of the rapid growth of the GR strain, and the relatively slow growth of the HL strain, sizes were not exactly matched (Table 3.1). All fish were stocked at the same time into the Parvin Lake inlet on August 14, 2007.

In 2008, 2,050 fish of each GR, HL, GR×HL (50:50), GR×HL (75:25), and Bellaire-Snake River RXN were again batch-marked with coded-wire tags. Similar difficulties were encountered with size matching of the HL strain compared to the other varieties during the rearing period (Table 3.1). These fish were stocked into Parvin Lake on July 31, 2008.

Table 3.1. Coded-wire tagged fish stocked in Parvin Lake during 2007 and 2008.

2007 Plants				2008 Plants			
Strain	**Lbs**	**Number**	**Length (mm)**	**Strain**	**Lbs**	**Number**	**Length (mm)**
GR	225	2800	147	GR	103	2050	127
HL	64.2	2800	97	HL	38.4	2050	91
GR×HL (50:50)	75.5	2800	104	GR×HL (50:50)	78.2	2050	117
GR×HL (75:25)	76.6	2800	104	GR×HL (75:25)	81.7	2050	117
RXN (50:50)	125	2800	122	RXN (50:50)	103	2050	127

Fish stocked in 2009 included all five varieties described for the 2007 and 2008 plants, with the addition of the pure Tasmanian rainbow trout (TAS), the GR×HL (87.5:12.5) cross, and the HHN cross (Table 3.2). The HHN is a cross between the GR×HL 75:25, currently used at the Crystal River Hatchery as brood stock for all GR×HL plants, and the Snake River cutthroat trout, also housed at the Crystal River Hatchery. Fish were stocked in the Parvin Lake inlet on August 12, 2009.

Table 3.2. Coded-wire tagged fish stocked in Parvin Lake during 2009.

2009 Plants								
Strain	HL	TAS	GR	GR×HL (50:50)	GR×HL (75:25)	GR×HL (87.5:12.5)	HHN (50:50)	RXN (50:50)
Lbs	42.2	119.6	83.7	83.7	83.7	83.7	55.8	50.3
Number	1005	1005	1005	1005	1005	1005	1005	1005
Length (mm)	117	167	150	150	150	150	132	127

Fish stocked in 2010 included two distinct lots, stocked on July 6, 2010. The first lot was the HHN variety, and the second lot was another standard cutthroat-rainbow cross (RXN) produced at the Crystal River Hatchery, created by crossing a Snake River cutthroat trout with a Tasmanian strain rainbow trout (Table 3.3).

Four varieties of fish were stocked in 2011, including the GR×HL 75:25 × Snake River (spring-spawning) HHN and Tasmanian-Snake River (spring-spawning) RXN, as well as pure GR and Hofer × Colorado River (GR×CR) cross (Table 3.3). These fish were stocked on November 3, 2011.

Table 3.3. Coded-wire tagged fish stocked in Parvin Lake in 2010 and 2011.

	2010 Plants		2011 Plants			
Strain	HHN (50:50)	RXN (50:50)	GR	GR×CR	HHN (50:50)	RXN (50:50)
Lbs	260	219	32.4	32.4	32.4	32.4
Number	7511	7380	3000	3000	3000	3000
Length (mm)	112.4	106.7	76.2	76.2	76.2	76.2

Collections of coded-wire tagged fish were obtained using boat electroshocking (and a few gill net sets to augment the catch) every two months during the open-water season in 2007 and 2008. In 2009, 2010, and 2011, fish were collected by evening boat electroshocking. Marked fish were subject to sampling for the first time in August of the year in which they were stocked. Sample goals were typically accomplished by shocking the entire perimeter of the lake over a three-hour period. Fish with coded-wire tags were identified during sampling with a hand-held tag detector. Collected fish were weighed to the nearest gram and measured to the nearest mm. Heads were removed, and coded-wire tags extracted and examined with a MagniViewer coded-wire tag reader. The remainder of the head tissues were packaged in individually numbered zip-lock bags and frozen for later myxospore count enumeration. Fish length, weight, tag number, and myxospore count for each fish was recorded in an individual database for each sampling event.

RESULTS

Samples collected in 2011 produced a representative cross-section of fish stocked in previous years. Results for each individual year-class are presented separately below, along with cumulative catch data from previous years of sampling, to provide a comprehensive overview of the results for each project year. Fish stocked in 2007 and 2008 became scarce with subsequent sampling within the 2011 sampling year, so results from these year-classes are limited.

2007 Year Class

Numbers of fish stocked in 2007 were greatly reduced during the 2011 sampling relative to previous years. Reductions may have been due to age-related mortality, or a result of anglers being more likely to harvest the larger fish in the population. Bellaire-Snake River (RXN) fish continued to be the most highly represented from this year of stocking, with nine of the 16 fish from the 2007 year class caught being of that variety in 2011. Only one fish from this year class was captured during the August and October sampling events, a single RXN that was captured in October.

Cumulative totals of fish from the 2007 plant (Figure 3.4) resulted in the RXN being consistently more abundant than the other strains, contributing to 46.7% (199 fish) of the overall catch of 426 fish. The HL strain fish constituted 20.9% (89 fish), the GR×HL (50:50) constituted 17.8% (76

fish), the GR×HL (72:25) constituted 8.2% (35 fish), and the GR strain constituted 6.3% (27 fish) of the total catch.

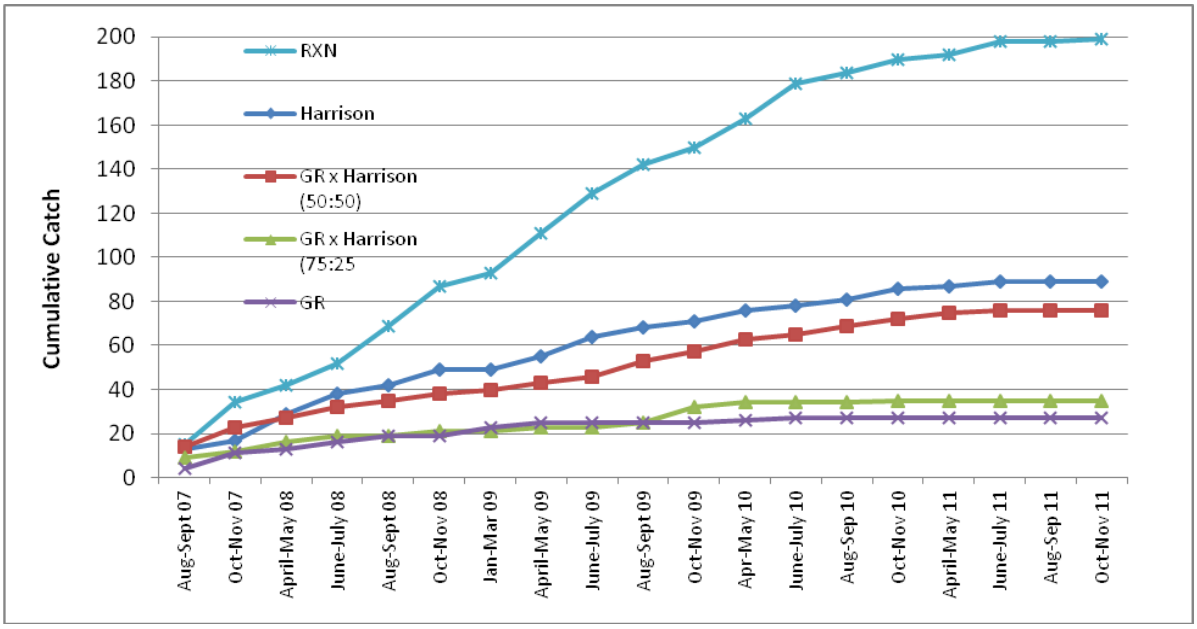

Figure 3.4. Cumulative catch for each of the five varieties of fingerling rainbow trout stocked in Parvin Lake in August 2007.

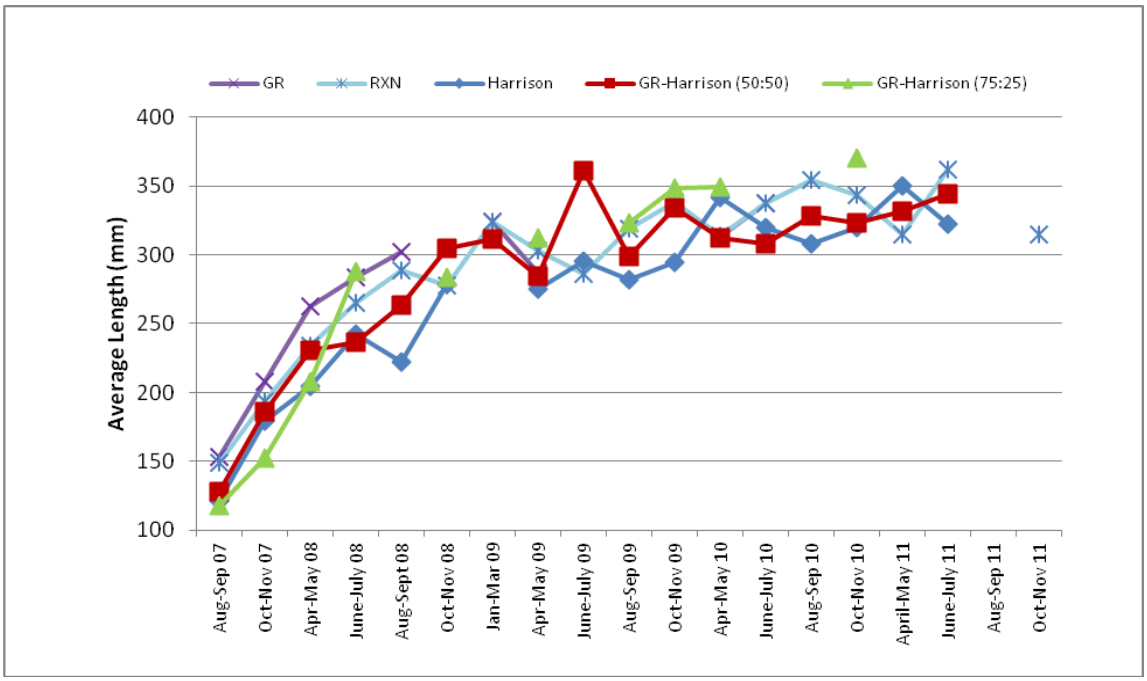

Figure 3.5. Fish length from 2007 through 2011 for each of the five varieties stocked in Parvin Lake in 2007.

Growth of the five varieties was relatively equal for all fish from the 2007 plant (Figure 3.5). The pure GR strain appeared to grow faster in the first year, but was such a small proportion of

the catch in later years that it was difficult to evaluate long-term growth. All varieties appear to plateau in growth upon reaching 305 mm, occurring about 24 months post-stocking.

2008 Year Class

As with the 2007 year class, fish from the 2008 year class were limited in number during the 2011 sampling. The collections from this year class were highly represented by the Bellaire-Snake River (RXN) cross, with 19 of the 33 fish from the 2008 year class being of that variety. Six HL fish and eight GR×HL (50:50) fish were also captured during the 2011 sampling events.

Cumulative multiple-year collections of fish from the 2008 plant resulted in the RXN and GR×HL (50:50) crosses being more abundant in the samples than the other varieties (Figure 3.6). The RXN contributed to 38.6% (97 fish) of the overall catch of 251 fish. The HL fish constituted 17.9% (45 fish), the GR×HL (50:50) constituted 29.5% (74 fish), the GR×HL (72:25) constituted 9.6% (24 fish), and the GR strain constituted 4.4% (11 fish) of the overall catch.

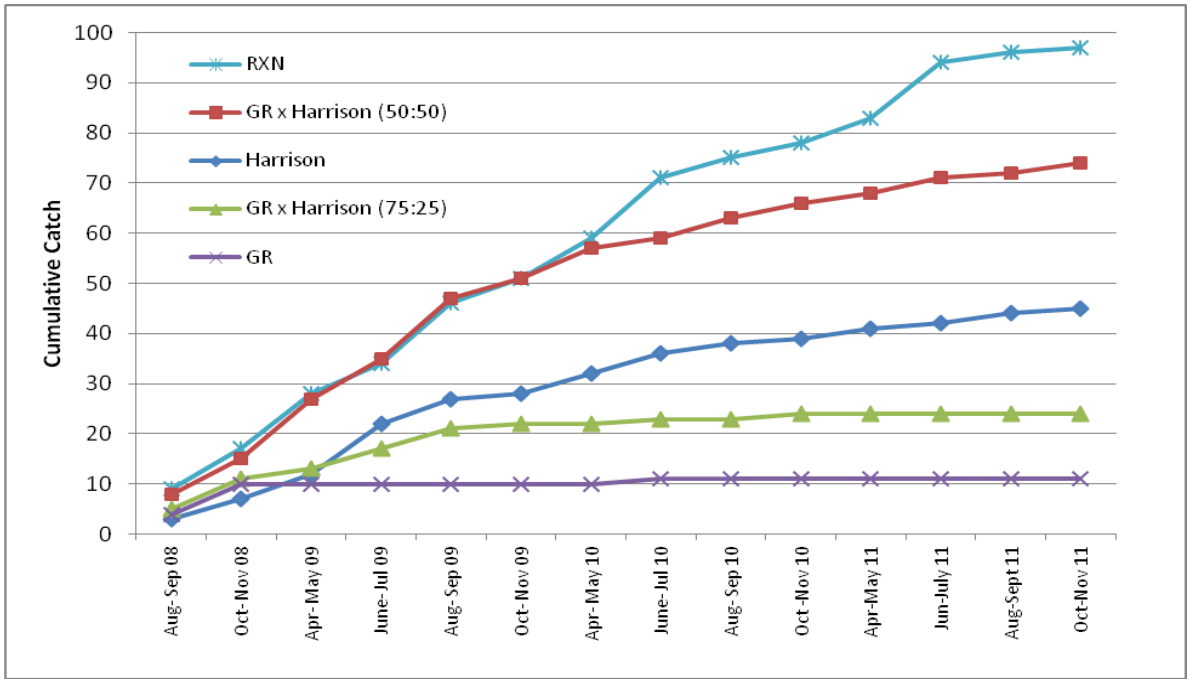

Figure 3.6. Cumulative catch for each of the five varieties of fingerling rainbow trout stocked in Parvin Lake in July 2008.

Growth of the five strains was similar to that of the 2007 plants, leveling off at 310 mm at 24 months post-stocking (Figure 3.7). The exception was the HL strain, which grew slower than the other varieties, averaging 272 mm at 24 months post-stocking. HL strain fish not only started out at a smaller size, but the rate of growth was slower; as a result, the size of the fish at which growth leveled off (270-280 mm) was lower than that of the other strains.

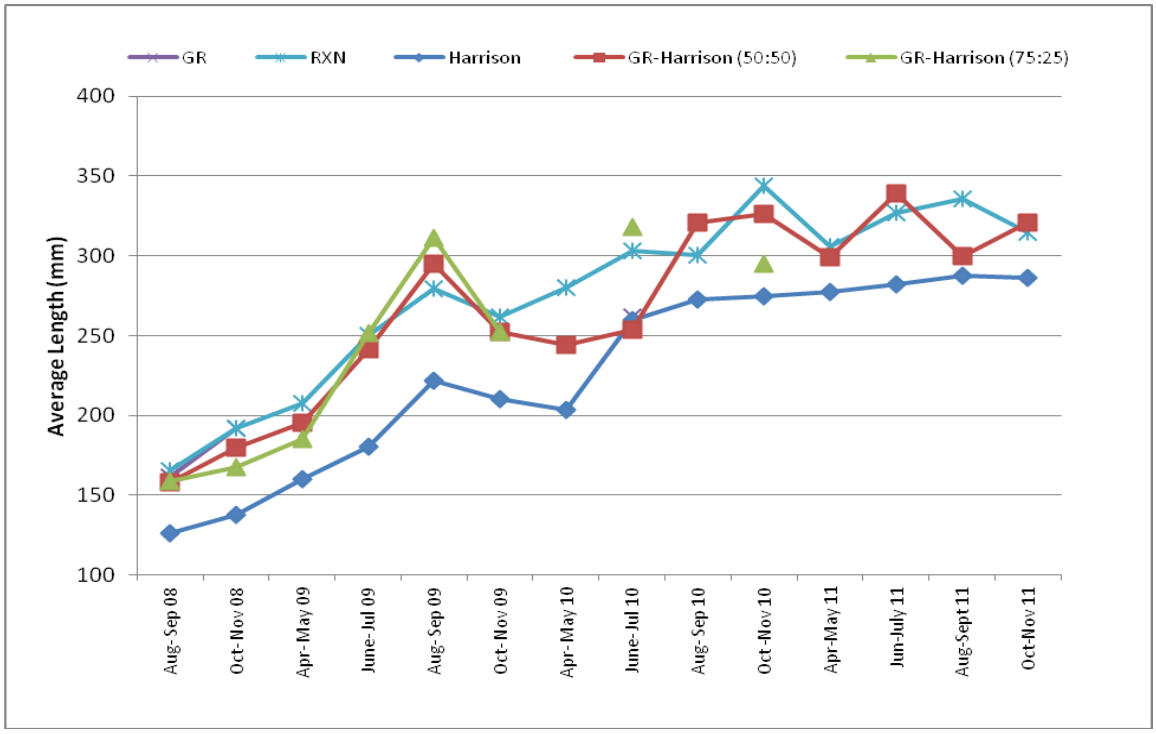

Figure 3.7. Fish length from 2008 through 2011 for each of the five varieties stocked in Parvin Lake in 2008.

2009 Year Class

The 2009 year class consisted of eight different varieties of fish. During the first year of sampling, numbers of each of the varieties in the overall catch were relatively equal. However, differentiation of variety abundance began to occur during the 2010 collections, becoming even more differentiated by the 2011 sampling season. In 2011, the HL, GR×HL (50:50), GR×HL (75:25), and TAS were all relatively equal, with seven, six, seven, and eight fish captured from each of these groups, respectively. Only one GR and two GR×HL (87.5:12.5) fish were found during the 2011 sampling events. The two varieties that were much more abundant than the other six were the RXN and HHN, of which 19 and 15 individuals were collected, respectively.

Cumulative collections of fish from the 2009 plant resulted in a total of 346 fish collected by the end of the 2011 field season (Figure 3.8). HL fish were the most abundant at 19.4% of the catch (67 fish), primarily because of the high catch rate for this variety during the 2010 field season. RXN and HHN were also present in high proportions, with 15.6% (54 fish) and 15.0% (52 fish), respectively. Catch for the three GR×HL crosses (50:50, 75:25, and 87.5:12.5) was 11.6% (40 fish), 11.6% (40 fish), and 8.1% (28 fish) of the total catch, respectively. Catch for the TAS was 11.3% (39 fish), and for the GR was only 7.5% (26 fish) of the total catch.

As in the 2008 year class, HL strain fish appeared to grow more slowly than the other varieties, which were relatively comparable in size throughout all of the sampling occasions. However, the average length at 24 months post-stocking for the HL strain (284 mm) was comparable to the

average of the other strains (299 mm) at this time period. The October 2011 data point for the HL strain is obscured in Figure 3.9.

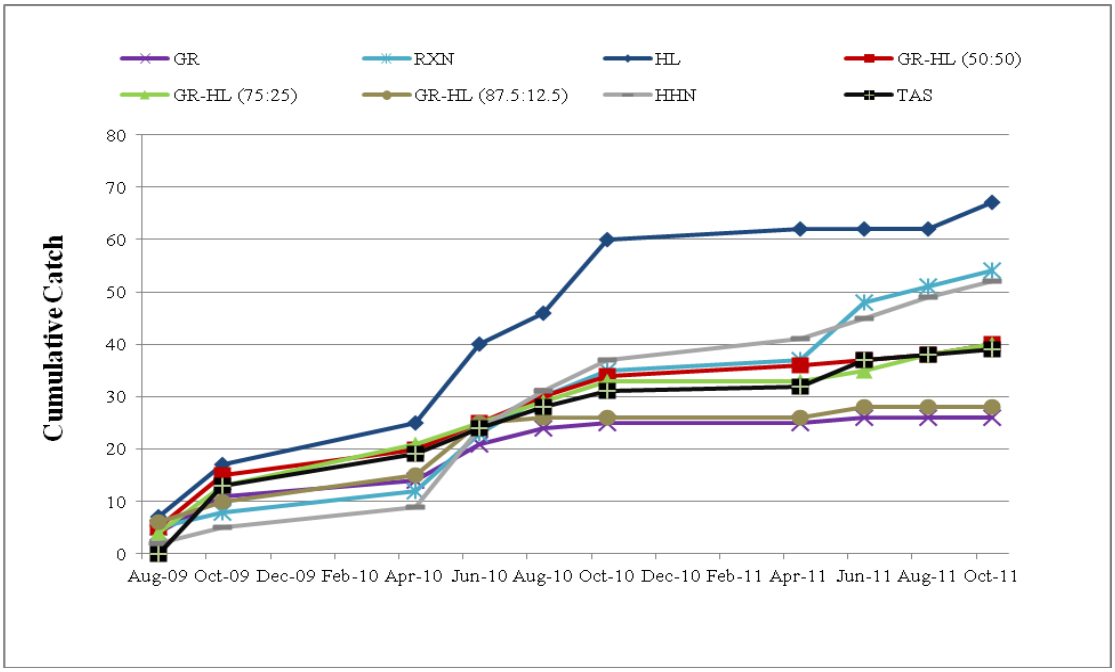

Figure 3.8. Cumulative catch for each of the eight varieties of fingerling rainbow trout stocked in Parvin Lake in July 2009.

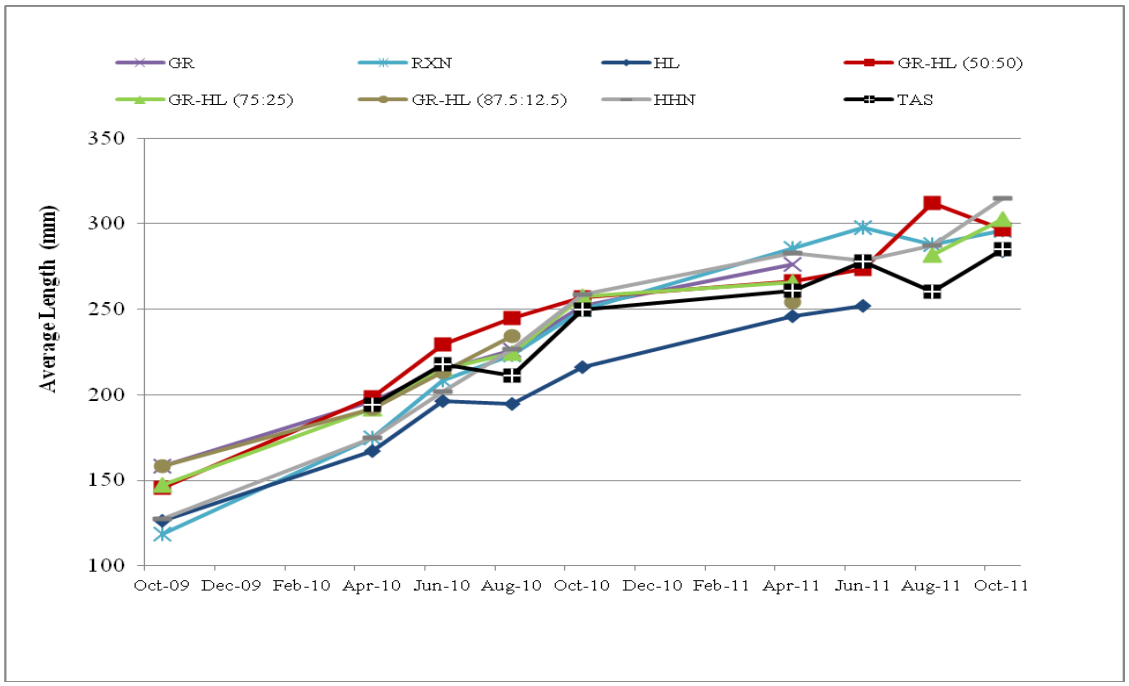

Figure 3.9. Fish length from 2009 through 2011 for each of the eight varieties stocked in Parvin Lake in 2009.

2010 Year Class

Sampling of the 2010 year class during 2010 and 2011 resulted in relatively equal numbers of fish of the HHN and TAS-Snake River RXN varieties. Sample numbers collected in 2010 were nearly identical, consisting of 33 HHN and 29 RXN. During 2011, 97 HHN and 127 RXN were collected. Collective sums were 130 HHN (45.5%) and 156 RXN (54.5%), for a total of 286 fish (Figure 3.10). Growth did not differ between the two strains. Average length at the end of the 2011 sampling season was 278 mm for the HHN strain and 274 for the RXN strain (Figure 3.11).

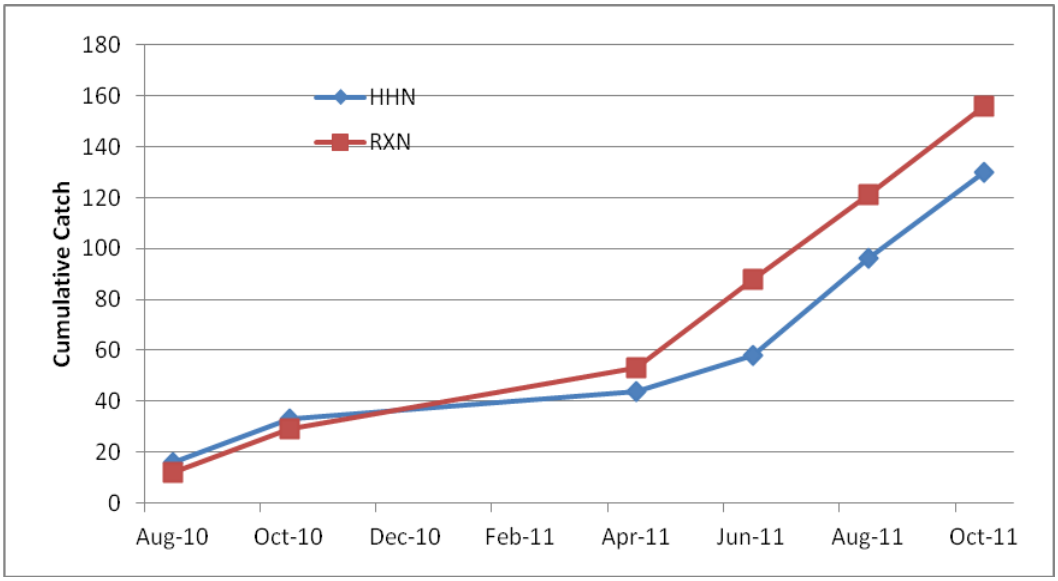

Figure 3.10. Cumulative catch for the two varieties of fingerling rainbow trout stocked in Parvin Lake in July 2010.

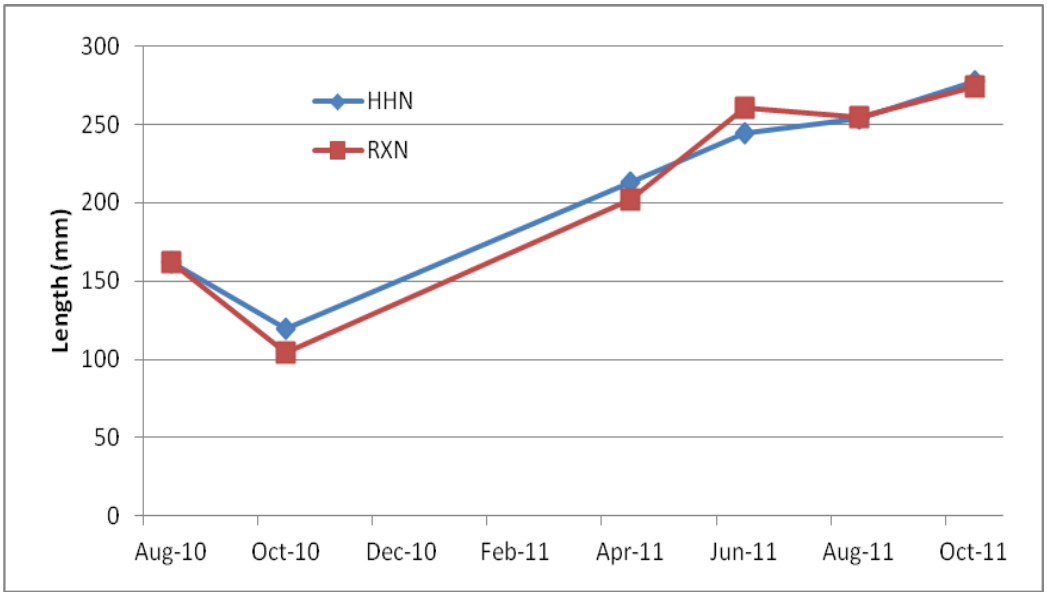

Figure 3.11. Fish length in 2010 to 2011 for the two varieties stocked in Parvin Lake in 2010.

Myxospore Counts

A sub-set of fish from the 2007 and 2008 plants collected during the open-water season in 2009 and 2010 were submitted for *M. cerebralis* testing. In April 2009, only samples from the 2007 plant were submitted. In the following 2009 and 2010 collections, fish from both the 2007 and 2008 plants were submitted for testing. These samples provided a very good overview of the infection severity in the various varieties of fish that had been released into this *M. cerebralis*-positive environment (Table 3.4). Figure 3.12 provides a consolidated average of the myxospore count data for the fish planted in 2007 and 2008 and consists of 80 RXN, 38 pure HL, 42 GR×HL (50:50), 20 GR×HL (75:25), and two GR rainbow trout collected in 2009 and 2010.

Table 3.4. Myxospore results for five strains stocked in 2007 and 2008 for each collection period in 2009-2010. 'NC' means no samples were collected for that strain and sample time.

	RXN		**HL**		**GR×HL (50:50)**		**GR×HL (75:25)**		**GR**	
	2007	**2008**	**2007**	**2008**	**2007**	**2008**	**2007**	**2008**	**2007**	**2008**
April 2009	40,150	NC	80,909	NC	3,756	NC	0	NC	0	NC
June 2009	30,370	28,975	39,698	96,069	1,209	5,218	NC	17,281	NC	NC
Aug 2009	11,333	71,967	94,857	20,529	18,909	3,507	0	1,101	NC	NC
Oct 2009	79,081	112,149	50,644	0	22,142	3,667	994	0	NC	NC
April 2010	36,645	25,400	16,640	8,317	1,580	10,989	0	NC	0	NC
June 2010	NC	4,733	NC	1,204	0	0	NC	NC	NC	0
Aug 2010	NC	NC	NC	6,344	NC	NC	NC	NC	NC	NC
Oct 2010	24,464	90,968	15,669	0	0	1,748	0	0	NC	NC
Overall Averages	**36,221**	**57,883**	**47,989**	**42,804**	**9.905**	**4,990**	**497**	**7,573**	**0**	**0**

In 2011, samples from each year class were submitted. TAS had the highest myxspore counts among those varieties stocked in 2009, averaging nearly 150,000 myxospores per fish. The RXN had consecutively lower myxospore counts. Overall myxospore counts among HL strain fish were lower than the RXN in the 2011 samples. This differed from the 2009 and 2010 samples in which HL myxospore counts were not significantly different from those of the RXN fish.

The GR and GR-cross myxospore counts were quite low in the 2011 samples, similar to the 2009 and 2010 samples. This was also true of the HHN strain, which averaged 22,644 myxospores in fish collected from the 2009 year class, and 102 myxospores in fish collected from the 2010 year

class. In comparison, RXN averaged of 54,716 myxospores in fish collected from the 2009 year class and 9,665 myxospores in fish collected from the 2010 year class (Figure 3.13). Increasing levels of GR in the varieties resulted in lower myxospore counts across all year classes.

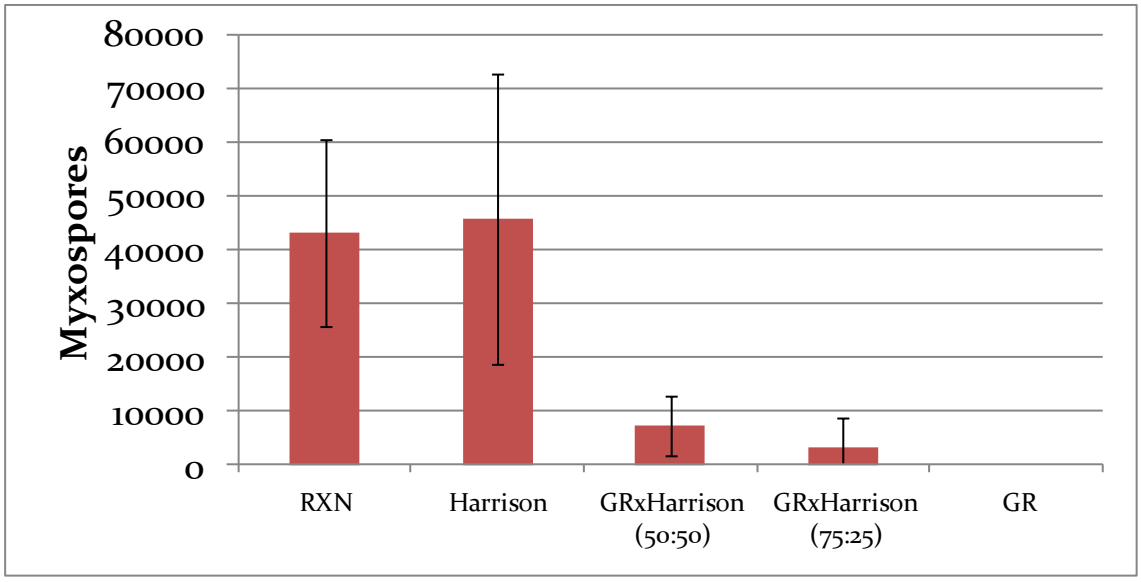

Figure 3.12. Overall averages of myxospore counts for the 2007 and 2008 plants of five strains of trout during 2009 and 2010.

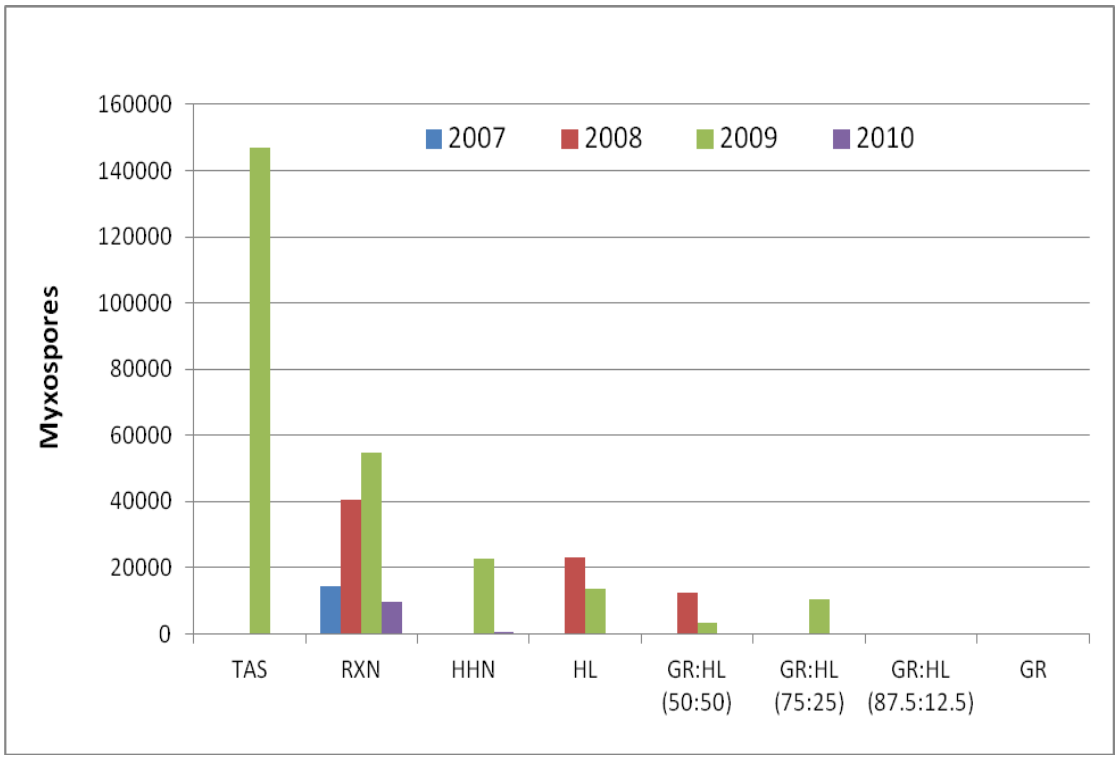

Figure 3.13. Myxospore count results by year class and strain for 2011 samples.

DISCUSSION

It is important to consider all year class stocking returns to fully understand the differences in returns and myxospore counts for the different varieties of rainbow trout. While some varieties had relatively consistent performance in different stocking years, some did not. Performance differences could have been related to environmental conditions favoring some varieties over others in some years, condition of the fingerlings in a given year class, or a host of other factors related to the year of stocking.

The pure GR strain fish were present only in very low numbers in each year class during the sampling events. Very few GR strain fish were found in the first year post-stocking, and were essentially absent from the samples following the first year. The early growth of the GR strain fish was very good, specifically in 2007, when reasonable numbers of the strain could be recaptured. No *M. cerebralis* myxospores were found in any of the pure GR fish collected in any of the sampling events. However, the lack of fright response evident in these fish when reared in a hatchery setting (Schisler and Fetherman 2009) clearly has an effect on the survival ability of this strain in an environment such as Parvin Lake where predators such as cormorants, osprey, and tiger muskie are present.

The GR×HL (87.5:12.5) cross was only stocked during the 2009 season. This cross exhibited slightly better survival than the pure GR strain, but was less abundant than all of the other strains stocked that year. Growth, to the extent that it could be evaluated, was consistent with the other GR-crosses. Like the pure GR strain, there were no myxospores found in any of the fish collected of this strain.

The GR×HL (75:25) cross had poor survival compared with most other strains with which it was planted. Like the pure GR, they were typically found in lower numbers during collection events, but survived much better than the pure GR strain. Low survival was likely due to the higher proportion of GR alleles in this variety, resulting in poor predator avoidance. However, the cross was found in much later sampling events than the pure GR strain, and also survived better than the GR×HL (87.5-12.5) cross when both were planted in the same year. Wagner et al. (2012) found that this variety survived better than the Ten Sleep variety rainbow trout in Porcupine and Hyrum Reservoirs, Utah.

The GR×HL (50:50) cross performed relatively well with respect to both myxospore count and survival. The cross consistently survived better than the pure GR, and other GR-crosses, with the exception of the HHN variety. Myxospore counts were higher than in the other GR-crosses, also with the exception of the HHN variety. Growth was consistent with the other varieties. In general, it appears that a higher ratio of HL to GR in the crosses is advantageous to post-stocking survival of fingerling fish, albeit increasing the HL component results in higher myxospore counts, which could also lead to increased parasite loading in receiving waters.

The HL strain was at a distinct disadvantage in the three years in which it was stocked due to smaller size, particularly in the 2007 stocking event. However, this strain performed well with respect to survival, consistently surviving at a higher rate than the pure GR or GR×HL (75:25) cross. Through 2011, the HL strain was the most abundant strain from the 2009 stocking event.

Growth of the HL strain, in general, was slower than the other varieties. Myxospore counts in the HL strain were relatively high compared to the GR-crosses, but didn't differ significantly from the RXN fish in the 2007 and 2008 year classes. These results are consistent with those of previous laboratory experiments in which HL strain fish developed higher myxospore counts than either the pure GR strain or GR-crosses (Schisler et al. 2011).

The RXN fish survived very well in every year they were stocked. They were much more abundant in the catch from the 2007 plant than the other varieties. In the 2008 plant, however, the RXN and GR×HL (50:50) varieties performed equally well. In the 2009 plant, the HL, RXN, and HHN varieties performed the best of the eight varieties stocked, with the RXN and HHN appearing in nearly identical numbers in the cumulative catch; the HL variety survived better than either of these varieties. Myxospore counts in each year of collections of the RXN were higher than any of the other varieties stocked in 2009, with the exception of the TAS which was much higher than all of the other varieties.

The HHN fish survived equally well as the RXN in both the 2009 and 2010 stocking events. These strains were out-survived only by the HL strain in the 2009 plant. The HHN and RXN had nearly identical growth rates as well. The real difference between the two strains was apparent in the myxospore counts. In both the 2008 and 2009 year classes, the average myxospore count was substantially higher in the RXN fish than in fish of the HHN variety. This was expected, due to the GR-genetic background of the HHN variety. With the survival of the two strains being nearly equal, the use of the HHN as a replacement cutbow for recreational stocking is a valuable option.

CONCLUSIONS

Given the relatively high survival of the GR×HL (50:50) cross in both the 2007 and 2008 plants, and the low myxospore counts compared to the pure HL and the RXN varieties, the GR×HL (50:50) appears to be a very good fit for fingerling reservoir plants to optimize survival and minimize *M.cerebralis* infection rates in areas where *M. cerebralis* and high predation pressure exists. However, the RXN consistently exhibited better survival than the GR×HL (50:50) cross. The RXN and HL varieties survived very well in these experiments, and would likely be preferred lake strains if not for the higher myxospore counts produced by these varieties. Samples collected from the later year classes of fish identify the HHN strain as a very good option for these types of environments as well, and will provide a high survival, whirling disease-resistant alternative for cutbow stocking.

Strains stocked in 2011 (pure Hofer, GR×CR, HHN and RXN) will be evaluated in the next few years to determine if relative survival between the pure Hofer, HHN and RXN strains remains consistent, and to evaluate the performance of the GR×CR plant, previously evaluated as a lotic rainbow trout strain, as a fingerling plant in a lentic environment.

References

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Schisler, G. J., E. R. Fetherman, and B. Neuschwanger. 2011. Salmonid Disease Studies. Federal Aid Project F-394R-10 Final Project Report July, 2011. 145 pp.

Wagner, E. J., M. Bartley, R. Arndt, R. W. Oplinger, and M. D. Routledge. 2012. Comparison of hatchery and field performance between a whirling-disease-resistant strain and the Ten-Sleep strain of rainbow trout. Journal of Aquatic Animal Health 24:110-120

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

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.

Upper Colorado River

INTRODUCTION

The upper Colorado River downstream of Windy Gap Reservoir is known to be one of the most heavily infected river segments with whirling disease in the state of Colorado. The 26 km (16.2 mi) reach, downstream of the reservoir to the Kemp-Breeze State Wildlife area (Figure 4.1) has been an area of particular interest with respect to whirling disease investigations. Historically, prior to the introduction of whirling disease, this area had been used as a source of eggs to maintain Colorado River Rainbow (CRR) trout brood stock. However, since the introduction of whirling disease, no natural recruitment of rainbow trout has occurred in the upper Colorado River, leading to severe population declines (Figure 4.2).

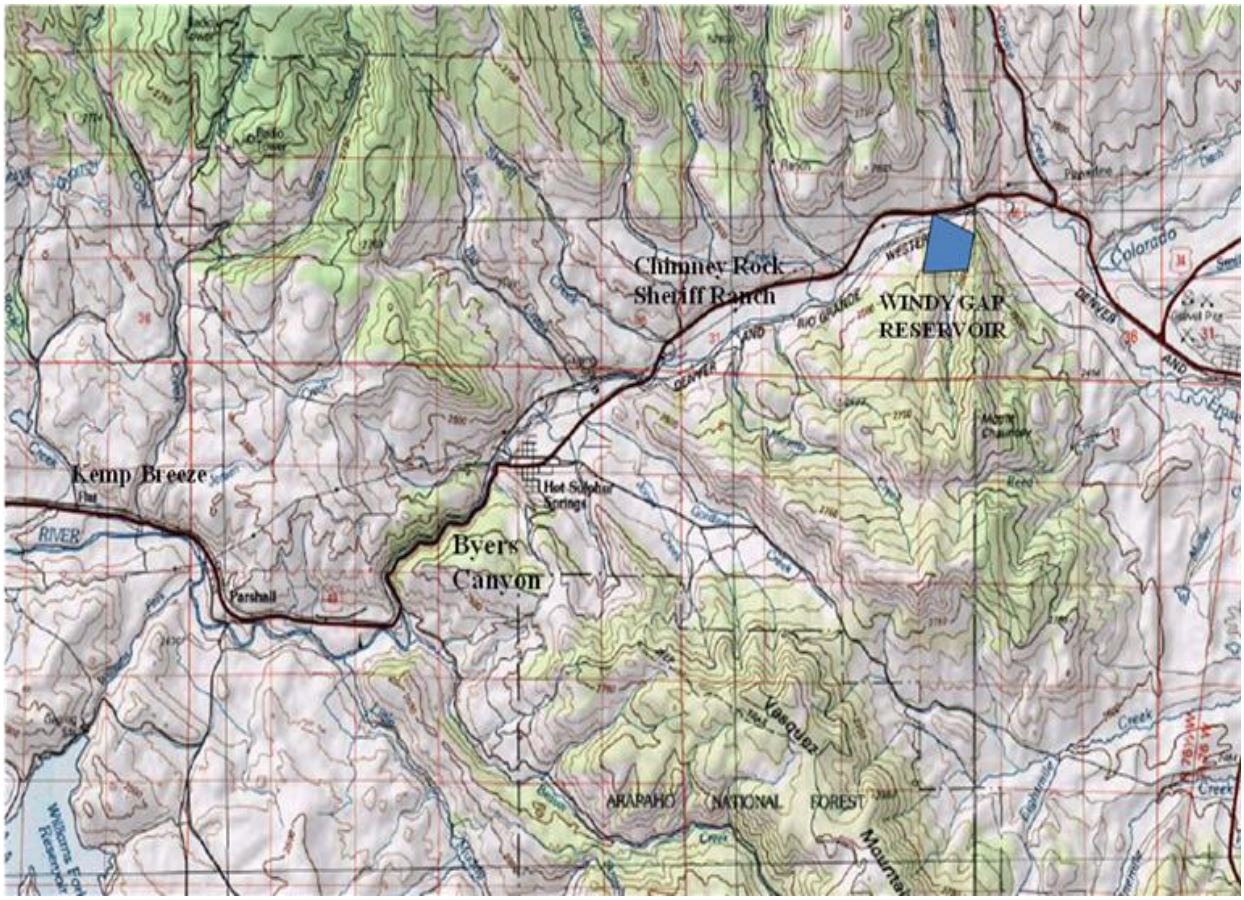

Figure 4.1. Upper Colorado River study area.

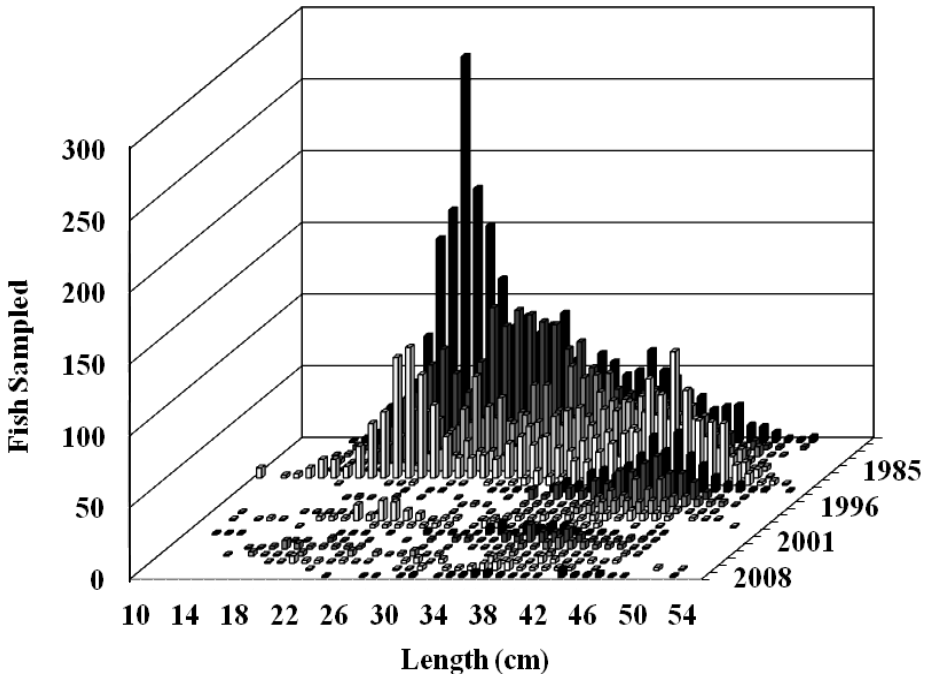

Figure 4.2. Upper Colorado River historic rainbow trout length-frequencies at Kemp-Breeze State Wildlife Area.

INTRODUCTION OF H×C STRAINS

In 2006, a single lot of Hofer × Colorado River Rainbow 50:50 (referred to as H×C by CPW biologists) was stocked into the upper Colorado River at 23.5 cm (9.4 in) total length (TL) to evaluate the survival of these larger fish (relative to previous plants) in an area dominated by brown trout, and with an extremely high prevalence of *Myxobolus cerebralis*. All introduced rainbow trout were tagged with an individually numbered fine-filament Floy tag, and secondarily adipose clipped for identification in the event of tag loss. Yearly adult population estimates, beginning in 2008, were used to monitor the survival and spawning status of these introduced fish. Estimates indicated that survival was relatively low for the introduced fish. Fry estimates, conducted on a monthly basis June through October, indicated that the introduced rainbow trout were reproducing; however, recruitment to the adult population remained low to nonexistent.

A second introduction of 5,000 H×Cs, averaging 20.9 cm (8.2 in) TL, occurred in the upper Colorado River in January 2009. Again, all introduced rainbow trout were tagged with an individually numbered fine-filament Floy tag, and secondarily adipose clipped for identification in the event of tag loss. Approximately two-thirds of the H×Cs were introduced to the river via the Windy Gap Reservoir bypass flume, in which water was open and flowing, while the other third were introduced through a hole in the ice below Hitchin' Post Bridge, approximately one mile downstream of Windy Gap Reservoir. The objective of this second introduction was to increase the adult *M. cerebralis* resistant rainbow trout population in the upper Colorado River. Unfortunately, none of the fish from the 2009 introduction were observed alive during adult population estimates conducted in this section of river in 2010 and 2011, indicating that the introduction had been unsuccessful.

In June 2010, 1,947 H×C rainbow trout, averaging 17.2 cm (6.8 in) TL and 58.1 g (0.1 lbs), were introduced to the upper Colorado River. Prior to being introduced, rainbow trout were tagged with an individually numbered fine-filament Floy tag, and secondarily adipose clipped for identification in the event of tag loss. Approximately one-third of the fish were introduced at each of three locations: the Sheriff Ranch, located at the lower end of the Chimney Rock/Sheriff Ranch section, Red Barn, located in the middle of the Chimney Rock/Sheriff Ranch section, and Hitchin' Post Bridge, located toward the upstream end of the Chimney Rock/Sheriff Ranch section. This plant was used to boost adult *M. cerebralis* resistant rainbow trout numbers throughout this section of river, following the unsuccessful introduction in the winter of 2009.

Population estimates, conducted in spring 2011, indicated that despite these introductions, the adult rainbow trout population remained low (fish from the 2006 and 2010 introductions were present at a density of only four fish per km [six fish per mi]). In addition, survival of the stocked rainbow trout was estimated to be about 0.017 (± 0.12), which is fairly low. Despite the low numbers of adult rainbow trout in the Chimney Rock Ranch section of the upper Colorado River, rainbow trout fry continued to be encountered during monthly fry estimates in 2011. Unfortunately, unusually low water levels prevented an adult population estimate from being completed in the Chimney Rock/Sheriff Ranch section of the upper Colorado River in spring 2012.

FRY POPULATION SAMPLING

Fry estimates were conducted once a month, June through October 2011. Unfortunately, unusually high water prevented population estimates from being conducted in June 2011, so the data presented is from July to October 2011. Standard, three-pass, 50 ft removal estimates were conducted at seven stations throughout the upper Colorado River, with three sites downriver of Byers Canyon (Kemp-Breeze, Lone Buck, and Paul Gilbert State Wildlife Areas), and four sites within the 6.28 km (3.9 mi) study reach on the Chimney Rock and Sheriff Ranches (Sheriff Ranch, Lower and Upper Red Barn, and Hitchin' Post Bridge). Two LR-24 Smith-Root backpack electrofishing units were used to complete the fry estimates. All fry caught within the 50 ft sections were identified as brown trout or rainbow trout, measured, and examined for signs of whirling disease. In addition, spot shocking was conducted to collect rainbow trout for additional disease status information. Fin clips were taken from all rainbow trout fry for genetic analysis. During the October 2011 fry estimates, 30 brown trout and 29 rainbow trout were collected for myxospore enumeration.

Two hundred and fifty-nine rainbow trout fry were encountered over the four month fry evaluation period, 238 in the three sites below Byers Canyon, and 21 in the four sites above Byers Canyon. For comparison, 375 rainbow trout fry were encountered over the five month sampling period in 2010, 77 in 2009, 22 in 2008, and 14 in 2007. Numbers of fry encountered increased significantly in 2010 and 2011 as a result of an introduction of approximately 60,000 rainbow trout fry along the margins of the river below Byers Canyon in July 2010 and 100,000 fry in August 2011. Of the 259 rainbow trout fry encountered in 2011, 245 were encountered within the 50 foot study sections, and 14 were encountered in areas outside of the study sections

during spot shocking. Spot shocking occurred only in October, both above and below Byers Canyon, to increase genetic and myxospore enumeration sample sizes.

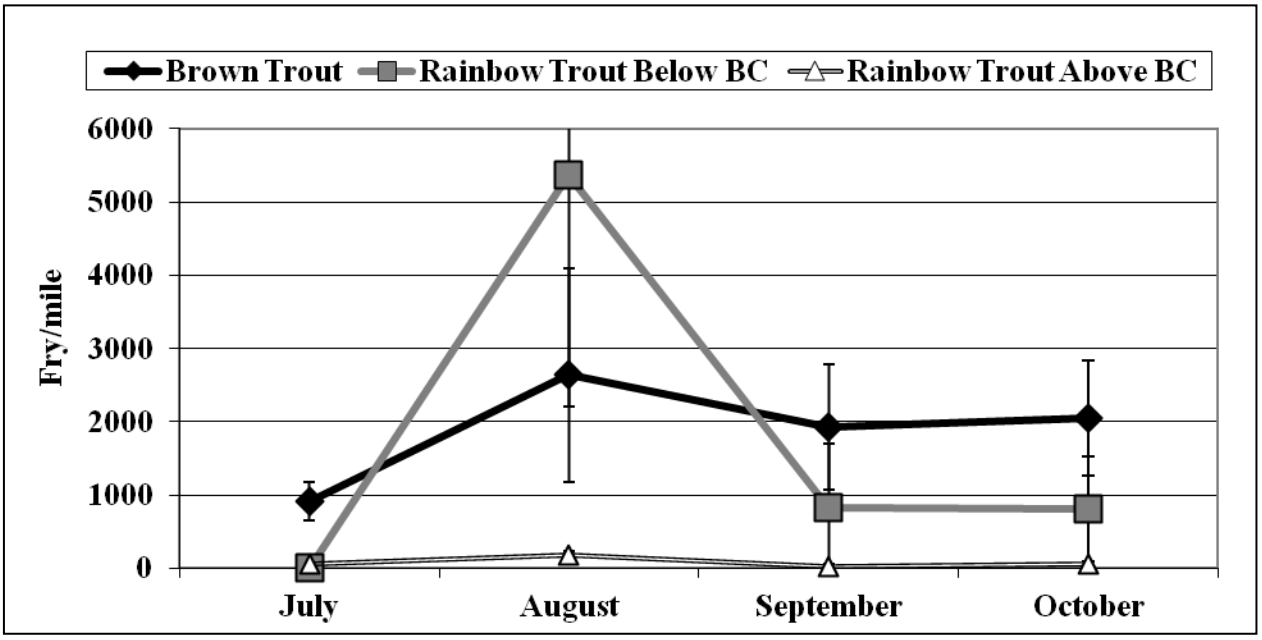

Figure 4.3. Upper Colorado River brown trout and rainbow trout fry density estimates (fry/mile), above and below Byers Canyon (BC), for the months of July to October 2011.

Fry density estimates were calculated using the three-pass removal equations of Seber and Whale (1970). Brown trout fry densities peaked in August, with an estimate of 1,645 fry per km (2,640 fry per mi), dropping to an estimate of 1,277 fry per km (2,050 fry per mile) in October. Rainbow trout fry densities similarly peaked in July, both above and below Byers Canyon, with an estimate of 3,346 fry per km (5,371 fry per mi) below Byers Canyon, and an estimate of 115 fry per km (184 fry per mi) above Byers Canyon. These estimates dropped to 505 fry per km (811 fry per mi) below Byers Canyon, and 33 fry per km (53 fry per mile) above Byers Canyon, by the end of October (Figure 4.3).

Seven percent of the brown trout fry encountered during the fry estimates showed signs of whirling disease, whereas 7.7 percent of the rainbow trout fry encountered showed signs of disease. The average myxospore count of the brown trout fry collected in the Chimney Rock/Sheriff Ranch stretch of the upper Colorado River in October was 31,526 myxospores per fish, compared with 5,535 myxospores per fish for the rainbow trout fry. Rainbow trout fry stocked below Byers Canyon did not show any myxospores in the 15 fish tested, likely a result of these fish not reaching the number of degree-days required for full myxospore development and detection by the PTD method by the time they were collected in October. Brown trout below Byers Canyon exhibited an average myxospore count of 31,553 myxospores per fish.

CONCLUSIONS

Survival of the introduced adult rainbow trout population remains low in the upper Colorado River. Previous estimates indicate that survival of the introduced rainbow trout is about 0.017. Despite the low survival, the introduced rainbow trout do appear to be reproducing. Rainbow trout fry numbers, however, continue to be low in October, indicating that not many of the fry are surviving to the fall; recruitment to the adult population is likely low as a result. Interestingly, rainbow trout fry myxospore counts were lower in 2011 than they were in previous years. In addition, rainbow trout fry myxospore counts were lower than brown trout in the same stretch of river, suggesting that these fry may have been produced by *M. cerebralis* resistant parental crosses (see the Genetic Techniques section of Job 4 for further conclusions related to genetic resistance in the rainbow trout fry in the upper Colorado River). Because estimates were not conducted in 2012 due to low water, survival of the introduced rainbow trout population, population size, and recruitment from the fry to juvenile and adult life stages is unknown at this time.

Introducing *M. cerebralis* resistant rainbow trout to the river as fry does appear to be a promising management option for increasing survival and retention of the introduced rainbow trout in the upper Colorado River. Fry estimates indicate that by October there were 15 times more fry in locations where rainbow trout fry introduced, compared to places where rainbow trout fry production occurred naturally. In addition, the number of rainbow trout fry remaining in locations where fry had been stocked is comparable to the number of brown trout fry present in the same location. Anecdotal evidence from population estimates conducted in locations where rainbow trout fry had been stocked suggests that there is some recruitment from the fry to adult population in these locations, as an increase in the number of rainbow trout in the 152 to 203 mm (6 to 8 in) size class has been observed in this section of the river. The benefits of introducing *M. cerebralis* resistant rainbow trout as fry on recruitment and survival will continue to be examined during future research projects.

Gunnison River

INTRODUCTION

The rainbow trout population in the Gunnison River has dramatically declined since the introduction of whirling disease in the early 1990's (Figure 4.4). Like the upper Colorado River, multiple years of stocking pure Colorado River rainbow trout fingerlings has not resulted in any measurable increase in rainbow trout density or biomass. In fact, rainbow trout numbers have continued to decline, and brown trout numbers have increased to historical highs. A series of stocking events in the Gunnison River study area (Figure 4.5) have occurred since 2004 in which equal numbers of pure CRR and H×C fish have been differentially marked and stocked together to evaluate relative survival rates of the strains, and as an attempt to re-establish a wild self-sustaining population in this location. Additional management options, such as brown trout fry removals and rainbow trout fry stocking, have been evaluated for their effectiveness at increasing the survival and retention of introduced rainbow trout in the Gunnison River study area.

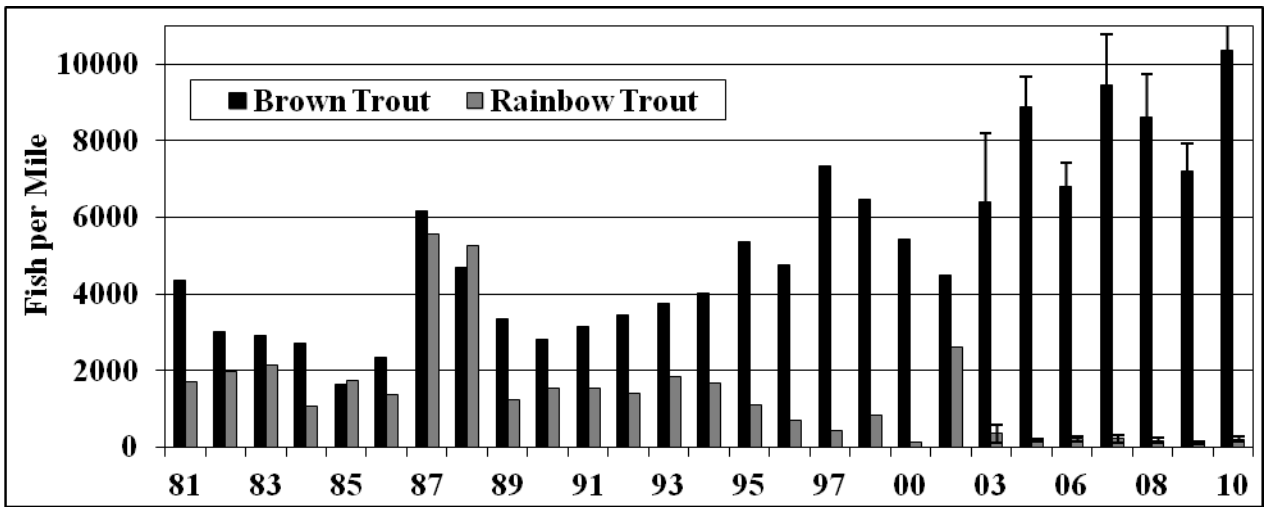

Figure 4.4. Historic rainbow trout and brown trout population estimates (fish per mile) for the Ute Park section of the Gunnison Gorge.

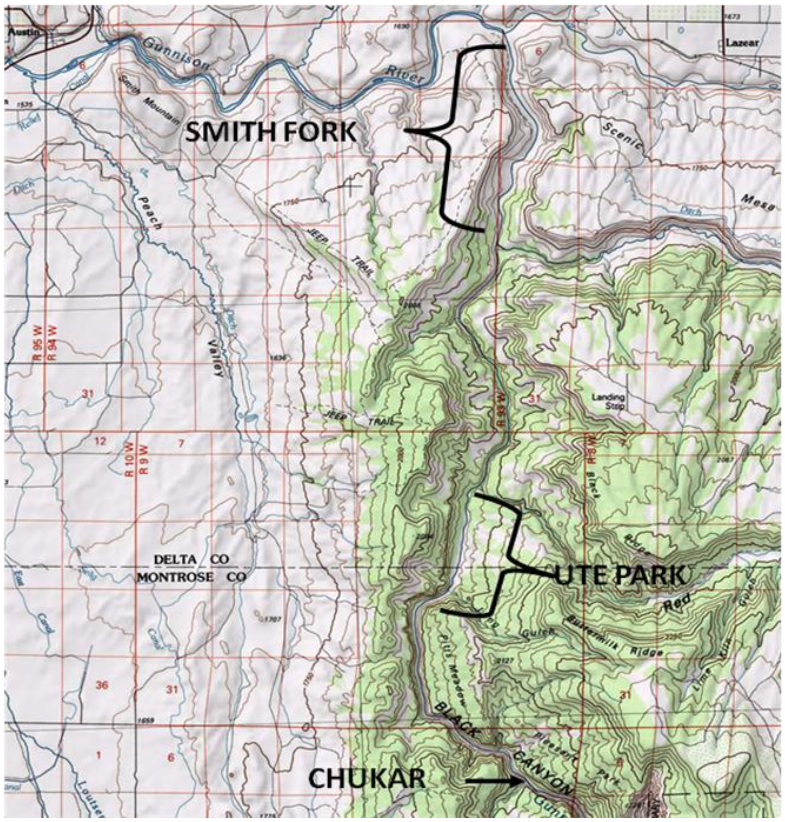

Figure 4.5. Gunnison River study area.

BROWN TROUT FRY REMOVAL

A large-scale brown trout fry removal project was initiated in the lower Smith Fork section of the Gunnison Gorge, just upstream of Pleasure Park and the confluence of the North Fork of the

Gunnison River, in July 2011. A one mile section of the Gunnison River was selected for the experimental manipulation. The downstream end of the section was located just upstream of the confluence with the North Fork of the Gunnison River, and extended nearly one mile (5,122 ft) upstream (Figure 4.6).

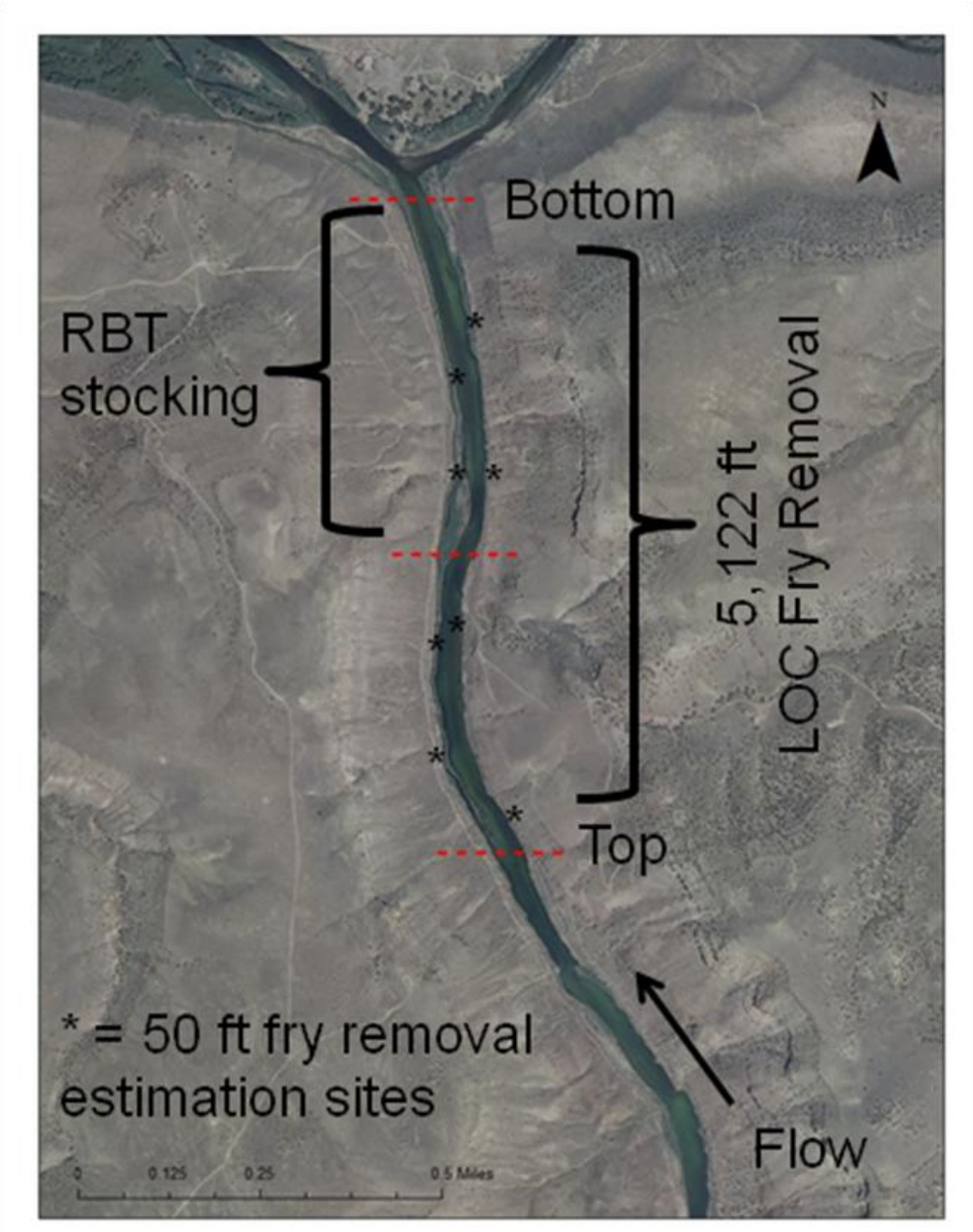

Figure 4.6. Map of the brown trout fry removal experiment conducted in the lower Smith Fork section of the Gunnison Gorge. The two asterisks within each of the treatment areas represent the fry population estimation sites established in July 2011, and resampled in September 2011.

Removal of brown trout fry occurred over the full length of the section on the east side of the river; no removal occurred on the west side of the river. It was assumed that the river was wide and swift enough to prevent recolonization of brown trout fry from the west side of the river. In addition, the section was split into two half-mile sections; rainbow trout fry were stocked in the lower half-mile section, and were not stocked in the upper half-mile section. This provided four treatment areas: (1) no brown trout fry removal and no rainbow trout fry stocking (NR, NS), (2) no brown trout fry removal and rainbow trout fry stocking (NR, S), (3) brown trout fry removal and no rainbow trout fry stocking (R, NS), and (4) brown trout fry removal and rainbow trout fry stocking (Figure 4.6).

Brown trout fry removal occurred during the first week of July 2010. Prior to the removal, two fry population estimation sites, 50 ft in length, were established in each of the four treatment areas; the first represented "good" fry habitat (many fry expected prior to estimates) and the second represented "moderate" fry habitat (fewer fry expected prior to estimates), to determine the range of fry distribution throughout the study section. Three-pass removal population estimates were conducted using two Smith-Root LR-24 backpack electrofishing units running side-by-side. Total lengths were obtained from all fish encountered during the population estimates. The removal was accomplished using two Smith-Root LR-24 backpack electrofishing units, and one cat-raft-mounted bank electrofishing unit. The cat-raft and backpack shocking crews started in two different locations, marked by a flag; once a crew reached the other's start flag, they moved above where the crew upstream of them was working, conducting the removal in a leap-frog-like fashion. Over the two-and-a-half day removal efforts, the entire removal section was shocked twice by at least one of the crews. All rainbow trout fry encountered during the removal were immediately returned to the river; brown trout fry were not. A total of 4,694 brown trout fry were removed over the course of the two-and-a-half day removal, 3,463 removed on the first complete pass through the section, and 1,231 removed on the second. Using a two-pass estimate from the removal numbers, and including the data from the 50 ft removal section estimates, over 80% of the brown trout fry population was removed from the east side of the river.

Rainbow trout fry were stocked on July 8, 2012. A total of 20,000 rainbow trout fry were brought from the Hotchkiss National Fish Hatchery in 12 bags. Three bags were taken to each of the four 50 ft population estimation sections in the lower half mile of the river for stocking. The 50 ft sections were used as focal points for the stocking to ensure that rainbow trout were introduced to the sections were the fry population estimates would be repeated in September. The fish were distributed both up and downriver from the 50 ft sections, with the rainbow trout being introduced in groups of 10 to 50 every couple of feet. After stocking, rainbow trout were observed swimming in the margins of the river, feeding, and reacting to shadows normally.

Fry population estimates were conducted in September to evaluate the success of the brown trout fry removal and rainbow trout fry stocking. Two LR-24 backpack electrofishing units were used to complete a three-pass removal in each of the eight previously established sites (two in each of the treatment areas). Lengths were taken from all fish encountered during the sampling. In addition, fin clips were taken for genetic analysis from the sites in which rainbow trout fry were not stocked in June (sites within the NR, NS and R, NS treatment areas). Fry density estimates were calculated using the three-pass removal equations of Seber and Whale (1970).

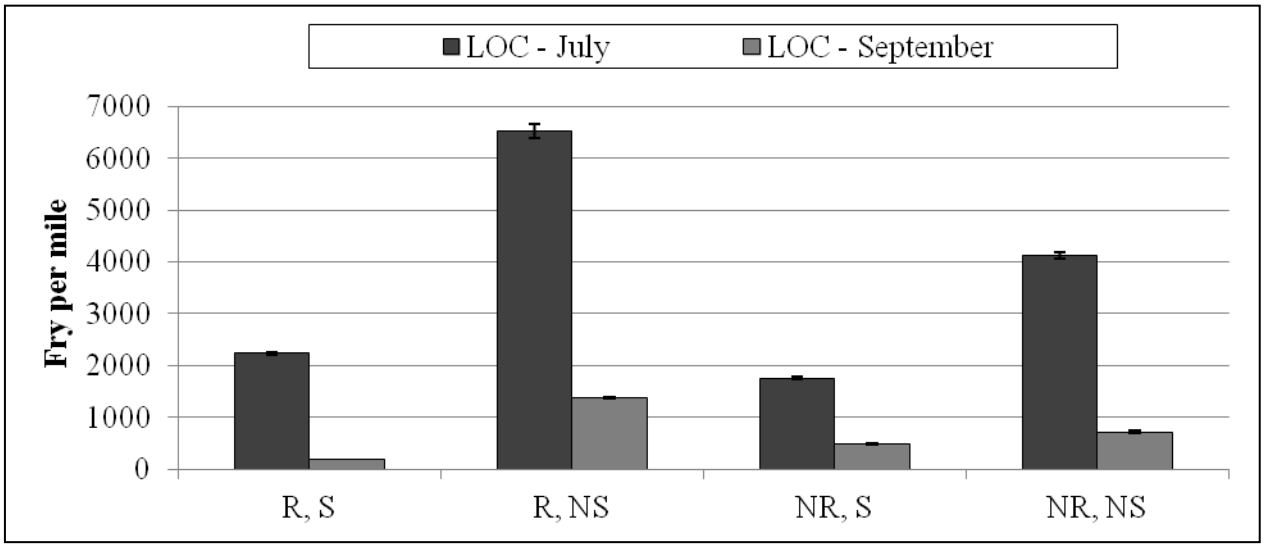

Figure 4.7. Estimated brown trout (LOC) fry abundance (per mile) in the four treatment sections, for the months of July and September, in the lower Smith Fork section of the Gunnison Gorge.

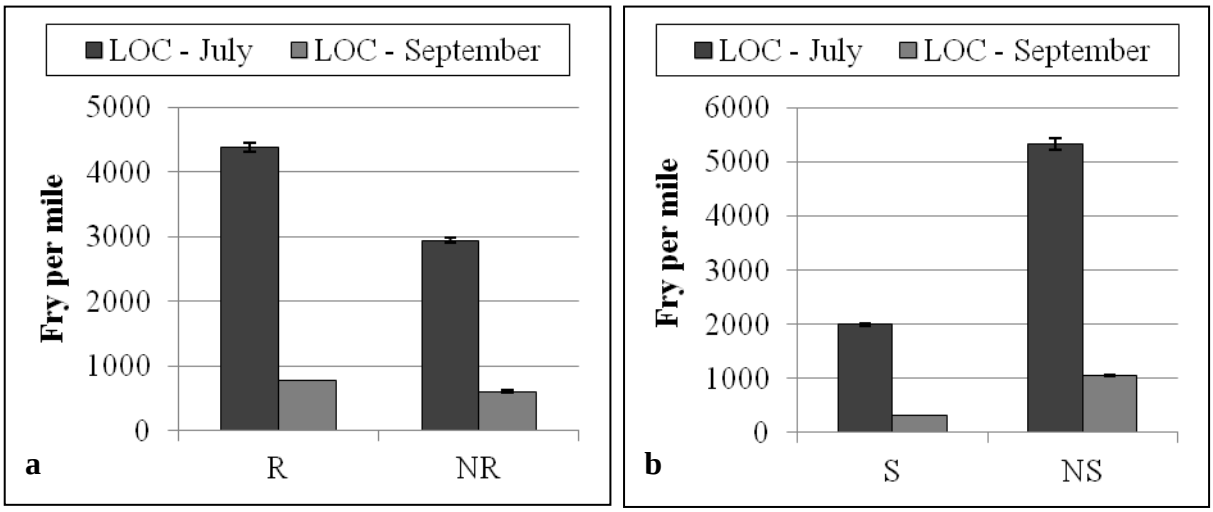

Figure 4.8. Estimated brown trout (LOC) fry abundance (per mile) in the (a) removal (R) and non-removal (NR) sections, and (b) the sections stocked (S) and not stocked (NS) with rainbow trout fry, for the months of July and September, in the lower Smith Fork section of the Gunnison Gorge.

Brown trout fry abundance decreased in all four of the treatment sections between July and September (Figure 4.7). There was a larger proportional decline in brown trout abundance between July and September in the sections in which brown trout were removed, compared to those in which they were not removed. Despite this, brown trout fry abundances did not differ between the removal and non-removal sections in September. There was a larger proportional decrease in brown trout fry between July and September in sections in which rainbow trout were

not stocked in comparison to sections in which they were. However, the abundance of brown trout fry was lower in the sections in which rainbow trout fry were stocked in September, suggesting that rainbow trout fry stocking may have had an effect on brown trout fry abundance (Figure 4.8).

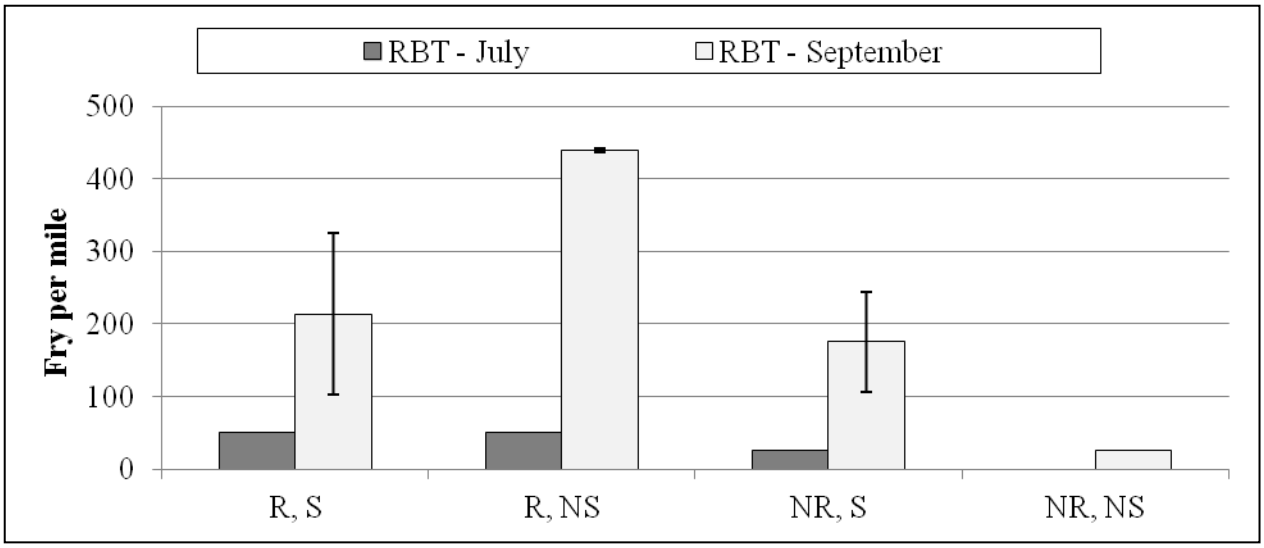

Figure 4.9. Estimated rainbow trout (RBT) fry abundance (per mile) in the four treatment sections, for the months of July and September, in the lower Smith Fork section of the Gunnison Gorge.

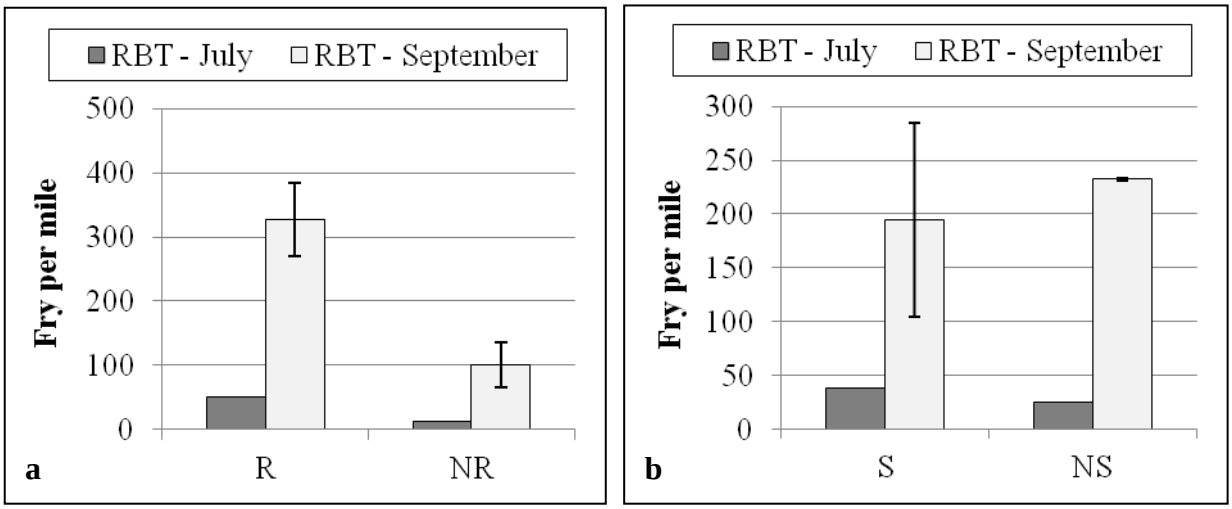

Figure 4.10. Estimated rainbow trout (LOC) fry abundance (per mile) in the (a) removal (R) and non-removal (NR) sections, and (b) the sections stocked (S) and not stocked (NS) with rainbow trout fry, for the months of July and September, in the lower Smith Fork section of the Gunnison Gorge.

Rainbow trout fry abundance increased in all four of the treatment sections between July and September. The smallest increase in rainbow trout fry abundance was in the section in which no brown trout fry removal and no rainbow trout fry stocking occurred (Figure 4.9). There was a larger proportional increase in rainbow trout fry abundance in the sections in which brown trout fry were removed, in comparison to the section in which brown trout fry were not removed. In September, there were significantly more rainbow trout fry per mile in the brown trout fry removal sections, in comparison to the non-removal sections. Sections stocked with rainbow trout fry experienced a similar proportional increase in rainbow trout fry abundance in comparison to sites that were not stocked with rainbow trout fry; stocked and non-stocked sites did not differ in rainbow trout fry abundance in September (Figure 4.10).

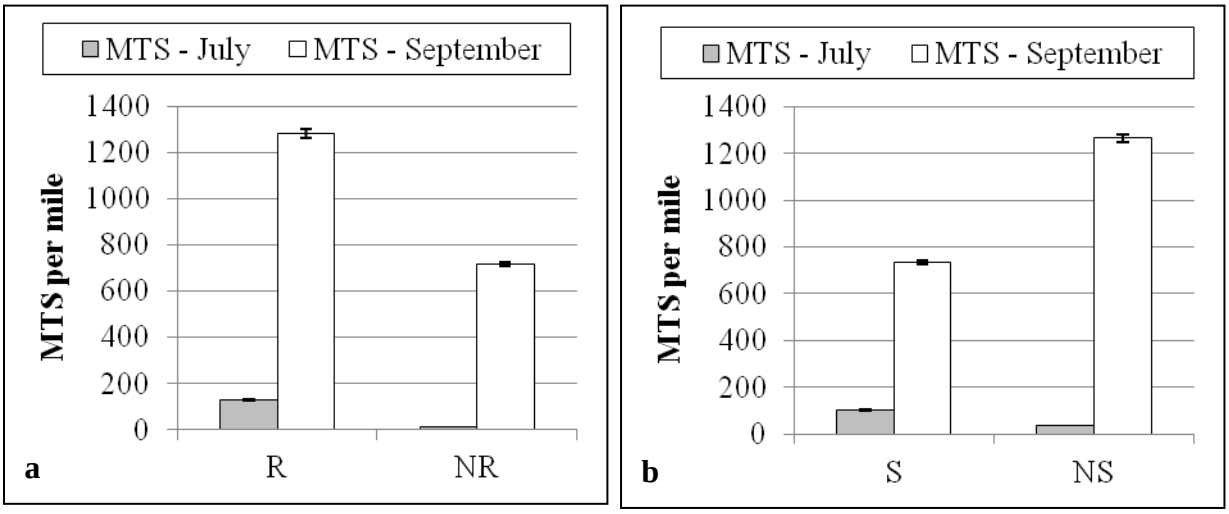

Figure 4.11. Estimated mottled sculpin (MTS) abundance (per mile) in the (a) removal (R) and non-removal (NR) sections, and (b) the sections stocked (S) and not stocked (NS) with rainbow trout fry, for the months of July and September, in the lower Smith Fork section of the Gunnison Gorge.

Mottled sculpin (MTS) fry and adults appeared to be affected by both the brown trout removal and rainbow trout fry stocking. Mottled sculpin abundances increased significantly in all sections of the Gunnison River between July and September. A significantly higher abundance of mottled sculpin was observed in September in the sections where brown trout fry were removed, in comparison to those in which they were not. Rainbow trout fry stocking appeared to negatively affect mottled sculpin abundance, with significantly lower mottled sculpin abundances in sections in which rainbow trout fry were stocked, in comparison to those sections in which rainbow trout fry were not stocked (Figure 4.11).

CONCLUSIONS

Overall, brown trout fry removal did not appear to significantly affect brown trout fry abundance in the Gunnison River. Though a higher proportional decline in brown trout fry abundance was experienced in the removal section, brown trout fry abundance did not differ significantly between the removal and non-removal sections. Removal, however, did appear to positively

affect rainbow trout fry retention and survival. In addition, brown trout fry removal appeared to positively affect other species of fish in the Gunnison River, specifically, mottled sculpin.

Rainbow trout fry stocking did not appear to positively affect rainbow trout fry abundance, as both the stocked and non-stocked sections had similar abundances in September. The error associated with the stocked section estimates suggest that rainbow trout fry are likely unevenly distributed among sampling sites, providing less accurate abundance estimates. In addition, the lack of difference between the sections could suggest a high rate of movement from the stocked sections to other sections of the river. Despite the non-stocked section being located upstream of the stocked section, rainbow trout of the same size as those stocked, compared to wild fry, were found in the non-stocked section. Finally, the lack of difference could be explained by a rise in river discharge (re-peak at ~4,000 cfs) the week following rainbow trout fry introduction. Rainbow trout fry introductions appears to negatively affect both brown trout fry and mottled sculpin, as both species had lower abundances in sections in which rainbow trout fry were stocked; this was likely due to increased competition for resources.

The expectation that a combination of both brown trout fry removal and rainbow trout fry stocking would result in higher abundances of rainbow trout fry was not met in this experiment. However, the lowest rainbow trout fry abundance was observed in the section in which rainbow trout fry were not stocked, and brown trout fry were not removed. These results indicate that rainbow trout fry abundance is positively affected by stocking and/or removal, and that a lack of implementation of at least one of these management options results in lower rainbow trout fry abundances.

Cache la Poudre River

INTRODUCTION

Brown trout are relatively resistant to whirling disease (Hedrick et al. 1999), and their populations have increased greatly in many rivers across Colorado since the introduction of whirling disease and decline in rainbow trout populations (Figure 4.12). Despite repeated introductions of both whirling disease susceptible and resistant rainbow trout, rainbow trout populations continue to be low, and self-sustaining rainbow trout populations have mot recovered in many rivers across the state. It is believed that the increases in the brown trout populations led to increases in competition with introduced and residual rainbow trout for habitat and food resources. This competition in turn is leading to low survival and recruitment in reintroduced whirling disease resistant rainbow trout populations. Brown trout removal was proposed as a management strategy that may reduce competition and predation of introduced rainbow trout, as well as open up habitat for the introduced rainbow trout to establish themselves within a section of river or stream.

The Cache la Poudre River (Poudre River) was selected for this experiment because of its history of maintaining self-sustaining rainbow trout populations prior to the introduction of whirling disease. Rainbow trout and brown trout were historically present in the river in proportions of 60:40, rainbow trout to brown trout. Like many rivers across the state, the rainbow trout population declined significantly with the introduction of whirling disease in the early 1990s.

Despite several introductions of rainbow trout to the river (667,500 rainbow trout introduced over the last 20 years), the wild rainbow trout population remains low, and little natural reproduction and recruitment is occurring.

The primary objective of the brown trout removal experiment was to evaluate if brown trout removal increases retention and survival of introduced whirling disease resistant rainbow trout. The study was designed to estimate the rate and magnitude of rainbow trout emigration in areas with ambient levels of brown trout and in areas where brown trout numbers have been reduced. Brown trout reinvasion and movement in both removal and control reaches was also estimated. In addition, differences in retention and survival were compared between two resistant rainbow trout strains as an evaluation of which strain is better for use in reintroductions in rivers and streams.

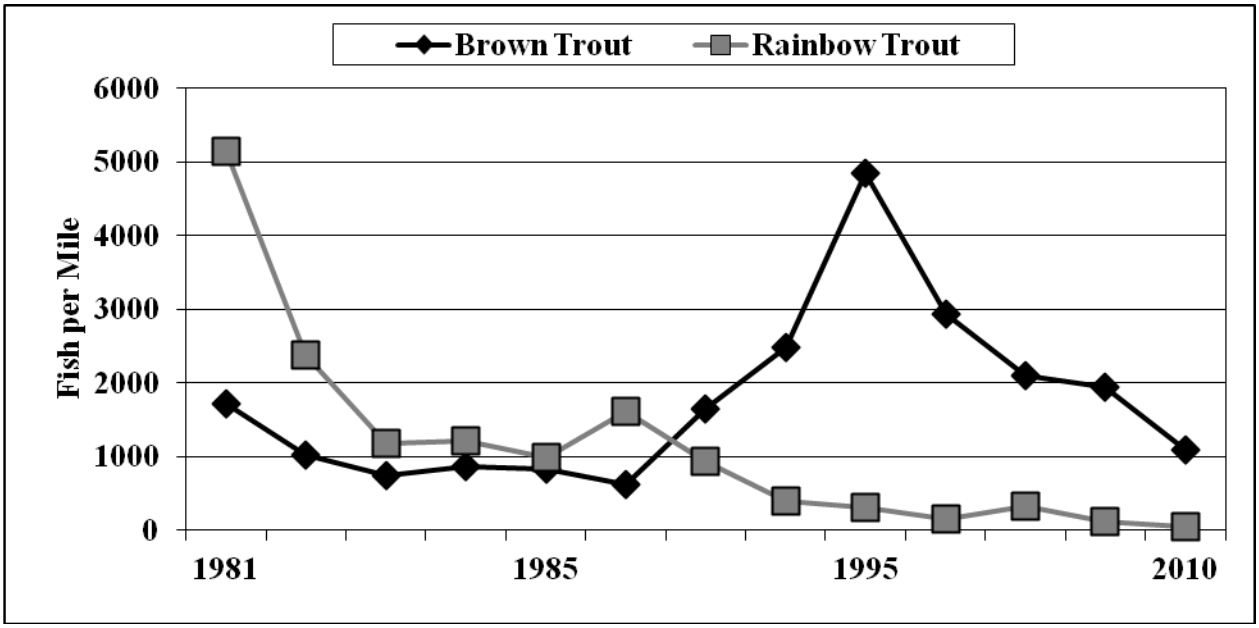

Figure 4.12. Estimated number of rainbow trout and brown trout per mile in the upper Colorado River between 1981 and 2010. Notice the large increase in the brown trout population in the early 1990s as the rainbow trout population declines due to the introduction of whirling disease.

STUDY DESIGN

Figure 4.13. Location of the control and treatment sections within the Poudre River.

Two sections of the Poudre River were used for the brown trout removal experiment. The first section, the control section, was located in Indian Meadows, about two miles downstream from the town of Rustic. No removal of brown trout occurred in this section. The second section, the treatment section, was located in the Black Hollow section of the Poudre River, about 1.5 miles downstream of the CPW Poudre Rearing Unit (Figure 4.13). Removal of brown trout occurred in this section. Removed brown trout were relocated approximately ten miles downstream of the control section, below the Narrows, a narrow, fast moving section of the Poudre River thought to be somewhat of a barrier to upstream movement. Both sections were located in public catch and release waters so that introduced and tagged fish were not removed from the sections by anglers. Only 5.7% of the 10 miles of public catch and release waters, equating to about 1.2% of the 50 miles of public water within the Poudre River, were affected by the removal.

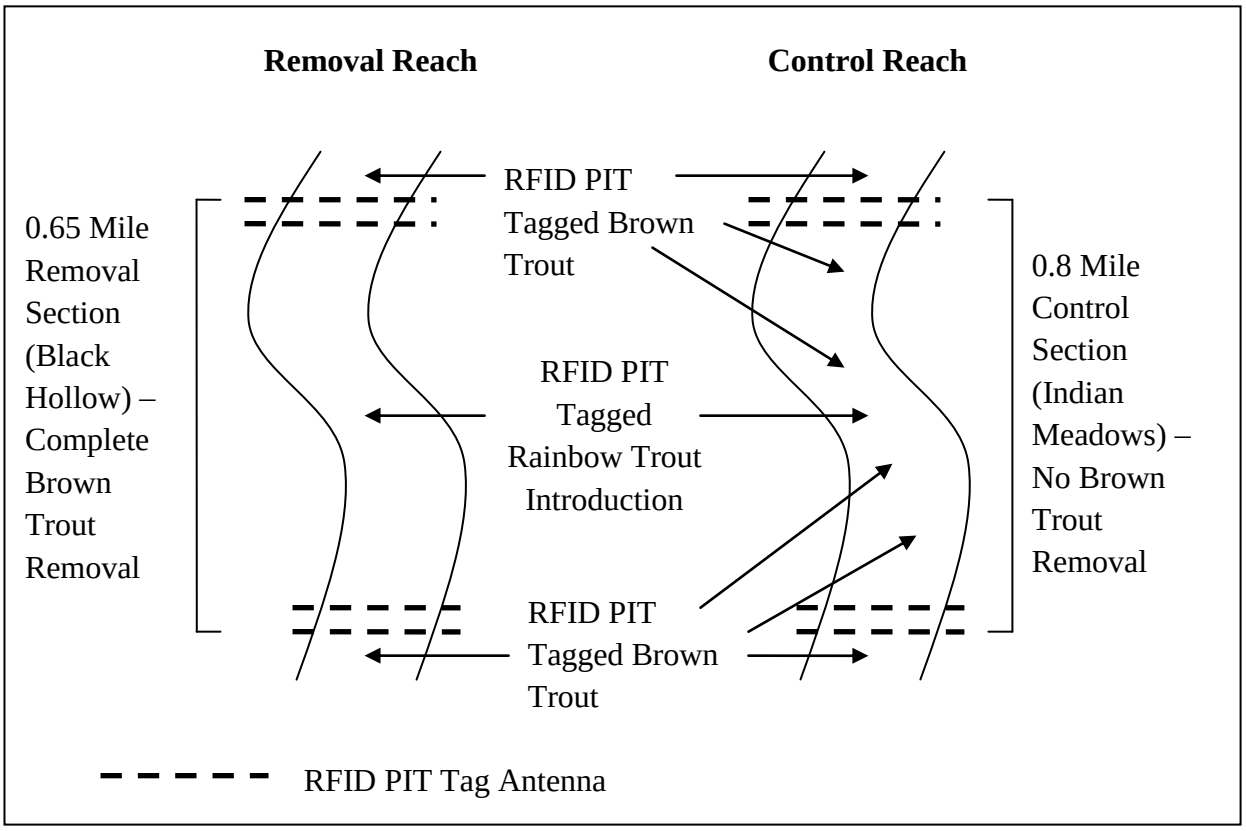

Figure 4.14. Details regarding the reaches, RFID PIT tag antenna locations, and introduction locations for the brown trout removal experiment conducted in the Poudre River in 2010.

The removal section (Black Hollow) was 0.65 miles in length; the control section (Indian Meadows) was 0.8 miles in length. Length was determined by the optimal location for the half-duplex Radio Frequency Identification (RFID) flatbed antenna arrays, located in pairs at both ends of the sections (Figure 4.14). Antennas were paired to determine directionality of movement of RFID Passive Integrated Transponder (PIT) tagged rainbow trout and brown trout as they moved in and out of the sections. Brown trout were PIT tagged on both the upstream and downstream ends of the removal section, and throughout the removal section. After the removal

PIT tagged rainbow trout were introduced to both sections (Figure 4.14). Two strains of whirling disease resistant rainbow trout were stocked, the H×C and the Hofer × Harrison Lake 50:50 (referred to as H×H by CPW biologists) rainbow trout strains, and were compared for their survival and retention in the river environment.

The brown trout removal project was designed to answer four research questions:

- How quickly do brown trout adjacent to the removal section reoccupy the area?
- Do removed brown trout, moved several miles downstream of the removal section, return? How quickly do they return?
- What is the survival and retention of rainbow trout in sections where brown trout have, or have not, been removed?
- Is there a difference in survival and retention between the HxC and HXH strains of rainbow trout

In addition, two overarching management questions were to be answered by this research:

- Does the removal of brown trout lead to the successful reintroduction of a whirling disease resistant rainbow trout population?
- Which strain of rainbow trout is best for successful reintroductions of rainbow trout to Colorado's rivers?

PRE-REMOVAL EXPERIMENTS, SET-UP, TAGGING, AND POPULATION ESTIMATION

Vertical and Horizontal Detection Probabilities

Experiments were conducted at the CPW BFRH to determine vertical and horizontal detection probabilities of the flatbed antennas arrays, and ultimately, optimal antenna location within the Poudre River. Thirty rainbow trout were RFID PIT tagged prior to assembly of the antenna array, allowing two days for recovery prior to the experiments. Three groups of 10 fish each were randomly chosen, and a single group was used for each trial so that use in a previous trial did not influence results. The antenna array was assembled, placed in the raceway, and tuned prior to the start of a trial. The antenna array was constructed using the same 8-gauge speaker as the antenna arrays in the Poudre River. Because of the small size of the array (to fit in the raceway) two loops were needed to have a high enough inductance to tune the antenna. The array itself was one foot wide by three feet long.

Vertical detection distance of the antennas was tested at three different heights: 0-1 feet, 1-2 feet, and 2-3 feet. All vertical detection experiments were conducted at the lowest velocity setting for the raceway. Prior to each experiment, flash boards were used to adjust water height. For the 0-1 foot experiment, maximum vertical swimming height was restricted to one foot above the antenna array. For the 1-2 foot experiment, water height was adjusted so that maximum vertical swimming height above the antenna array was two feet. In addition, a net was placed horizontally across the raceway, restricting minimum swimming height above the antenna array to one foot. For the 2-3 foot experiment, water height was adjusted so that maximum vertical swimming height above the antenna array was three feet. Similar to the 1-2 foot experiment, a net was placed horizontally across the raceway, restricting minimum swimming height above the antenna array to two feet (Figure 4.15). Because the expected vertical detection distance of a flat

bed antenna is 45 cm (1.5 ft; Oregon RFID 2009), we expect detection to be 1.0 in the 0-1 foot increment, high but less than 1.0 in the 1-2 foot increment, and low in the 2-3 foot increment.

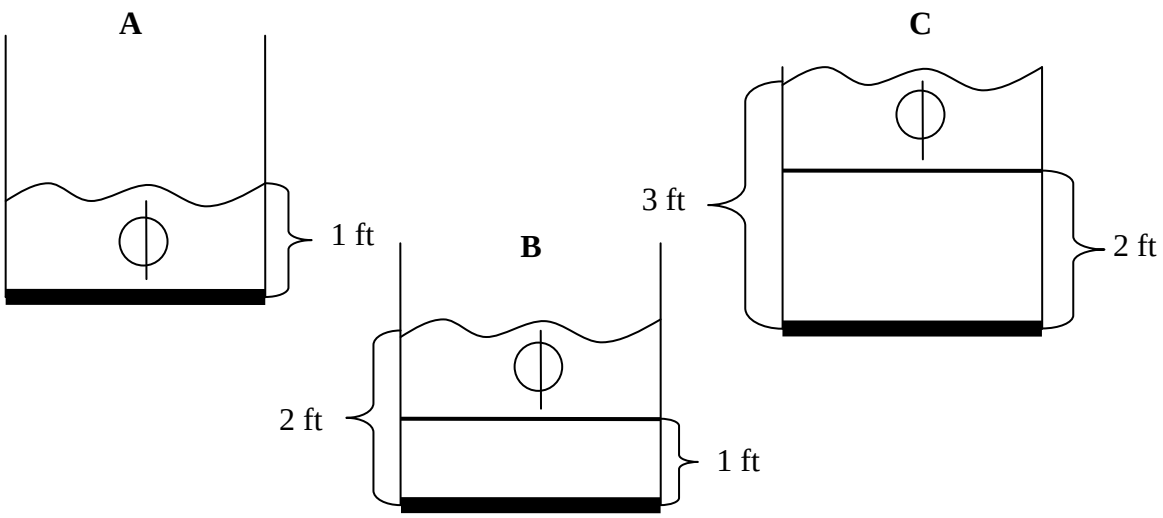

Figure 4.15. Maximum vertical detection distance experiments conducted at three height increments: 0-1 feet (A), 1-2 feet (B) and 2-3 feet (C).

Horizontal detection was tested using the 0-1 foot increment as detection at this depth was expected to be 1.0. Velocity was increased using a pump to push water through the raceway. Three velocities were tested: 0.10 m/s, 0.25 m/s and 0.5 m/s; 0.5 m/s was the maximum speed that could be reached within the raceway. During the horizontal detection experiments, fish were encouraged to move over the antenna array from both upstream and downstream of the antenna to determine if direction of movement affected detection probability at the different velocities.

Detection experiments were conducted for a duration of two hours at each height or velocity setting. Fish were crowded down to the lower end of the raceway prior to the start of the experiments, with their release signifying the beginning of the experiment. During the experiment, fish were allowed to move over the antenna array at will. If fish movement over the antenna array did not occur for longer than 15 minutes, fish were encouraged to move over the antenna array by passing an object through the water. An observer was present for the duration of the experiment to record movement over the antenna array. Positive detection by the array was aided by the use of a piezoelectric beeper, which emitted a whistle when a tag was detected by the antenna. A video camera was used to record movement over the antenna that may have escaped the observer, or to help determine the number of fish that passed over the antenna array if the fish moved in groups. At the end of all of the experiments, detection data from the antenna was downloaded and compared to both observer and camera recorded movement over the antenna array to calculate detection probability for each of the height and velocity settings.

Detection probability for the 0-1 foot height increment was 1.0, dropping to a detection probability of 0.89 at the 1-2 foot height increment, and a detection probability of 0.004 at the 2-3 foot height increment. Detection probability for all three of the velocities (0.10, 0.25, and 0.50 m/s) was 1.0. These results indicated that the antennas placed in the Poudre River should be put in a location where average maximum water height was two feet, and if possible, a location

where velocities were less than 0.50 m/s within two feet of the river bottom. Several locations that met these criteria in the Poudre River were examined, and the distance between the most ideal sites was maximized to have the largest sections possible for the removal experiment.

Hatchery PIT Tagging, Tag Retention and Mortality

Two thousand H×Cs were RFID PIT tagged at the CPW Glenwood Springs Hatchery and two thousand H×Hs were RFID PIT tagged at the CPW BFRH 1.5 months prior to their introduction to the Poudre River. PIT tags were 32 mm in length, and were inserted into the peritoneal cavity, under the right pectoral fin, using a PIT tag insertion gun; insertion opening was not stitched or glued closed following insertion. Weight, length, and PIT tag number were recorded for each individual. Fish were also differentially fin clipped for secondary identification if the PIT tag was lost after being stocked; H×Cs were adipose clipped, whereas H×Hs were adipose and right pelvic clipped. Fish were separated into two groups of 1,000 during tagging operations and kept separate through stocking so that tag numbers for the fish stocked in each section were known.

One month after tagging, two weeks prior to their introduction to the Poudre River, tag retention and mortality from the tagging procedure were estimated in both crosses. Tag retention was estimated in two ways: (1) from lost tags found in the raceway over the month monitoring period, and (2) by scanning 100 fish in each group of 1,000 fish and recording the number that had retained their tags. Mortality was estimated by the number of mortalities collected over the one month monitoring period. In the H×Cs, 15 tags were found in the bottom of the raceway, providing an estimate (1) of tag retention of 99.25%. However, upon scanning the fish, 98 and 99 had retained tags in the two groups of 100, providing an estimate (2) of average tag retention of 98.5%. Overall, 59 fish died over the month monitoring period, presumably from the tagging procedure, providing an estimate of mortality of 2.95% for the H×Cs. In the H×Hs, only one tag was found in the bottom of the raceway, providing an estimate (1) of tag retention of 99.95%. Similarly, only one untagged fish was found upon scanning providing an estimate (2) of average tag retention of 99.5%. Overall, 11 fish died over the month monitoring period, providing an estimate of mortality of 0.55% for the H×Hs. The results of the hatchery monitoring indicated that mortality was low, and tag retention high, using the PIT tag insertion gun and not closing the insertion opening; similar results, therefore, were expected for fish tagged in the wild using the same procedure.

Antenna Array Construction and Deployment

Antennas, deployed two weeks prior to the removal, consisted of a single loop of high strand count 8-gauge copper speaker wire. Three of the four antennas were anchored to the substrate using Duckbill Anchors. Two antennas, the lower removal and lower control antennas, were anchored using a jackhammer. Anchors for the upper removal antenna were pounded in by hand. The substrate around the upper control antenna was too armored for pounding in anchors by hand, and the site was not jackhammer accessible. Therefore, the antenna was threaded through large landscaping bricks for anchoring; after the bricks were set, other rocks from the substrate were rolled on top of them to secure them to the bottom of the river. Half-duplex antennas run electromagnetic pulses through the wire; therefore, it was not necessary to enclose the wire to maintain rigidity or protect it from water vibrations.

The antenna loop was connected to a tuner box on shore, mounted in bushes or trees above bankfull height of the river, allowing them to remain deployed during high water conditions. A tuner box translates the electromagnetic pulses from a detected PIT tag into the unique numeric code stored by the antenna reader. Twin-axial cable connected the tuner box to the reader, translating the detection signal for storage. The power source was two 12 volt, 120 Ah marine deep-cycle batteries, connected in parallel. The reader and batteries were placed in a lock box, anchored to the ground using ground augers, located above bankfull height of the river.

Wild Fish PIT Tagging and Initial Population Size Estimation

Wild fish were tagged throughout the control section the week prior to the removal. Three passes were made upstream, and between (within) the antenna arrays on three consecutive days, with the downstream section being sampled the last day; the downstream section was also sampled a second time the week of the removal. The goal was to tag an approximately equal number of fish per mile throughout the three sections. All fish encountered on the first day of sampling were tagged, measured (mm), and weighed (g). Fish encountered on the second and third days were checked for tags; if present, the number was recorded for population estimation purposes, and if absent, the fish was tagged, measured (mm), and weighed (g). A total of 676 brown trout were tagged throughout the three portions of the control section; 222 upstream of the upper antenna array, 270 between the antenna arrays, and 184 downstream of the lower antenna array. Brown trout averaged 276 (± 50.1) mm TL, and weighed an average of 224 (± 106) g. Additionally, 35 wild rainbow trout, averaging 286 (± 66) mm TL and 274 (± 173) g, two mountain whitefish, averaging 225 (± 78) mm TL and 147 (±127) g, and one cutbow, 285 mm TL and 238 g, were tagged throughout the control section.

Population estimates were obtained using a Huggins closed capture-recapture estimator in program MARK (White and Burnham 1999). For the upstream and within portions of the section, a three pass model was used, whereas a two pass model was used for the downstream portion; all three portions were estimated separately. Models in which *p*, *c*, and *N* varied by group (species), or were continuous, were run in all possible combinations. In addition, models in which *p* and *c* were equal were also run. Models were ranked using AICc, and estimates of *p*, *c*, and *N* were obtained using model averaging.

The brown trout population was estimated to be 1,673 (± 453) brown trout within the 0.8 mile control section. Therefore, 16% of the brown trout population in the control section was PIT tagged for this experiment. The rainbow trout population was estimated at 59 fish within the control section. Therefore, 24% of the wild rainbow trout population in the control section was PIT tagged.

BROWN TROUT REMOVAL

Brown trout removal occurred in the Poudre River August 16-18, 2010. Prior to removal, both ends of the section were blocked using a medium-mesh chicken wire fence to prevent movement of adult fish during the removal. The fence was monitored continuously to prevent build-up of debris and collapse. The removal was accomplished using 14 backpack electrofishing units, four electrofishing rafts, one three-electrode cat-raft, and over 100 volunteers. The backpack and cat-

raft crews proceeded upstream in a line through the section, completing one pass the first day of the removal, and two passes each in the second and third days of the removal. The boat crews made several passes each within a day. All fish caught were measured (mm) and weighed (g), and all fish encountered during the removal were removed from the section. Brown trout were transported approximately 15 miles downstream and returned to the river below the Narrows. All other fish were returned to the river downstream of the section. Of the brown trout removed, 200 were PIT tagged prior to transport to track return to the removal section.

On day one of the removal, 726 brown trout were removed from the 0.65 mile removal section. This number decreased to 429 removed on day two, and to 263 removed on day three. The percentage of the brown trout population removed was estimated by dividing the total number of fish caught during the removal by the estimated size of the brown trout population prior to the removal. A Huggin's closed capture model in program MARK (White and Burnham 1999), using a five pass (number of passes made by the backpack/cat-raft crews) removal estimate, was used to estimate the size of the brown trout population prior to the removal. The fish from the boat crews, who made several passes through the section per day, were grouped on a per pass basis with the fish collected by the backpack and cat-raft crews, to produce five distinguishable removal passes. Models in which detection probability differed by group (four groups: brown trout greater than 150 mm, brown trout less than 150 mm, rainbow trout greater than 150 mm, and rainbow trout less than 150 mm), pass, and length were run, as were all additive combinations. Models were ranked using AICc, and estimates of *p* and *N* were obtained through model averaging.

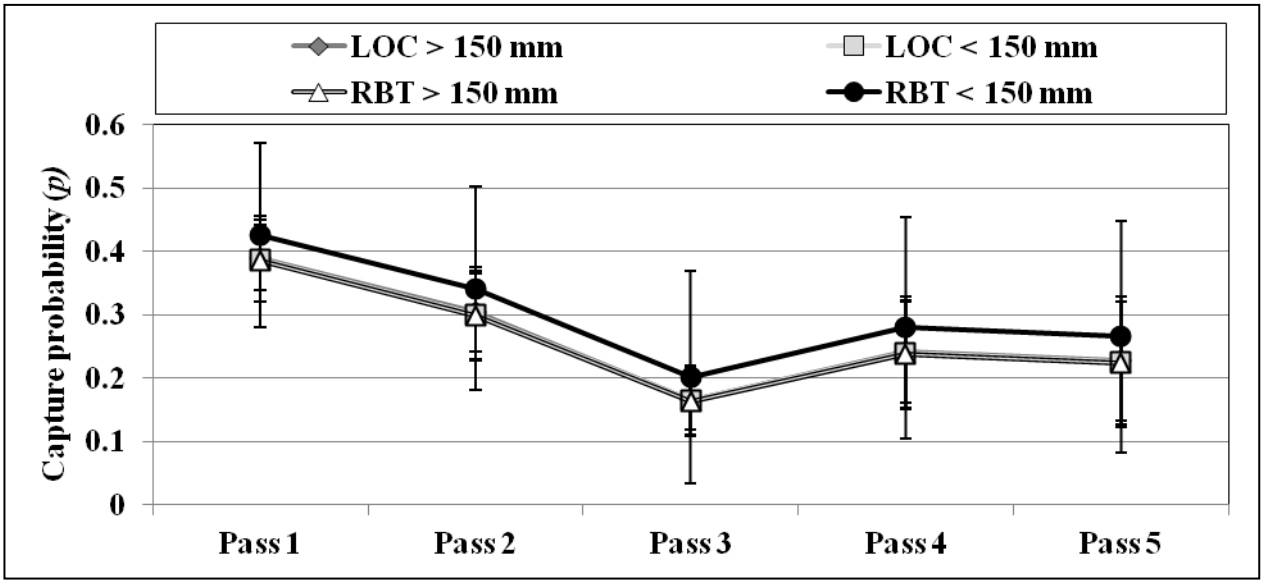

Figure 4.16. Detection probability, by pass, for brown trout and rainbow trout, greater than and less than 150 mm in size, encountered in the removal section (August 2010).

Both top models had *p* varying by pass. Length was also included in both of the top models, whereas group was only included in the second best model. In general, detection probability did not differ among the species or sizes caught. For both sizes of brown trout, and rainbow trout

greater than 150 mm, detection probability was significantly higher on the first pass than on the third pass. Detection probability did not differ among the other passes (Figure 4.16).

A total of 1,399 brown trout were removed from the section. It was estimated that 1,975 brown trout were present in the section prior to the removal; therefore, 71% of the brown trout were removed. Seven hundred and forty-four of the estimated 834 brown trout greater than 150 mm (adults) were removed, equating to about 89% of the adult brown trout population, indicating that the removal was successful in eliminating a large percentage of the adult brown trout, and therefore competition, for the introduced rainbow trout. In contrast, 655 of the estimated 1,141 brown trout less than 150 mm (fry/juvenile) were removed, equating to only about 57% of the fry and juvenile brown trout population.

PIT tagged rainbow trout were introduced to both the control and removal sections of the Poudre River on August 19, 2010, the day after the completion of the brown trout removal. In the removal section, 1,975 PIT tagged rainbow trout, 979 H×Cs and 996 H×Hs, were stocked via buckets. Fish were distributed evenly throughout the section by spreading fish from three stocking locations within the section. The control section received 1,964 PIT tagged rainbow trout, 972 H×Cs and 992 H×Hs, using rafts to distribute the fish throughout the section. The number of fish stocked into both sections was fewer than expected due to mortality and tag loss during loading and transportation. H×Cs stocked in the control section (199.5 mm TL, 92.8 g) were significantly longer ($p < 0.001$), and weighed significantly more ($p < 0.001$), than the H×Hs stocked in the control section, and both the H×Cs and H×Hs stocked in the removal section. H×Cs stocked into the removal section (195.6 mm TL, 86.8 g) were significantly longer ($p < 0.001$), and weighed significantly more ($p < 0.001$), than the H×Hs stocked into either the control or removal sections. H×Hs stocked in the control section (156.9 mm TL, 41.2 g) and the removal section (157.7 mm TL, 40.3 g) did not differ from each other in either length ($p = 0.328$) or weight ($p = 0.334$).

Other than passive detection of PIT tagged fish as they moved past the stationary antenna arrays, no further manipulation or population estimation was conducted in either of the sections for nearly two months. This provided ample time for the rainbows to establish themselves without disturbance.

POPULATION ESTIMATION – OCTOBER 2010

Population estimates were conducted at nine historic and six experimental sites throughout the Poudre River, from Lee Martinez Park in Fort Collins, to Bliss State Wildlife Area, located upstream of the Poudre Fish Hatchery. A two-pass removal estimate was conducted at 13 sites using a four-electrode truck-mounted bank electrofishing unit; a three-electrode cat-raft was used at two of sites where access by truck was not possible. All fish encountered during the estimates were measured (mm), weighed (g), scanned for a PIT tag (regardless of secondary fin clip), and examined for secondary marks (i.e., adipose or pelvic fin clips).

Abundance (N) was estimated using a Huggin's closed capture model in program MARK (White and Burnham 1999). Detection probability (p) was modeled using a continuous detection model. The model set also included p varying by electrofishing gear (EFgear; differentiating those sites

in which an electrofishing cat-raft was used [two sites], versus those in which a bank shocking unit mounted on the back of a truck was used [all others]), species, boulder/riffle (differentiating boulder and riffle dominated habitats from cobble and pool dominated habitats), site length, site width, length, interactions between electrofishing gear and habitat, interactions between electrofishing gear and species, interactions between electrofishing gear and length, and all additive combinations therein. Models were ranked using AICc, and estimates of *p* and *N* were obtained using model averaging.

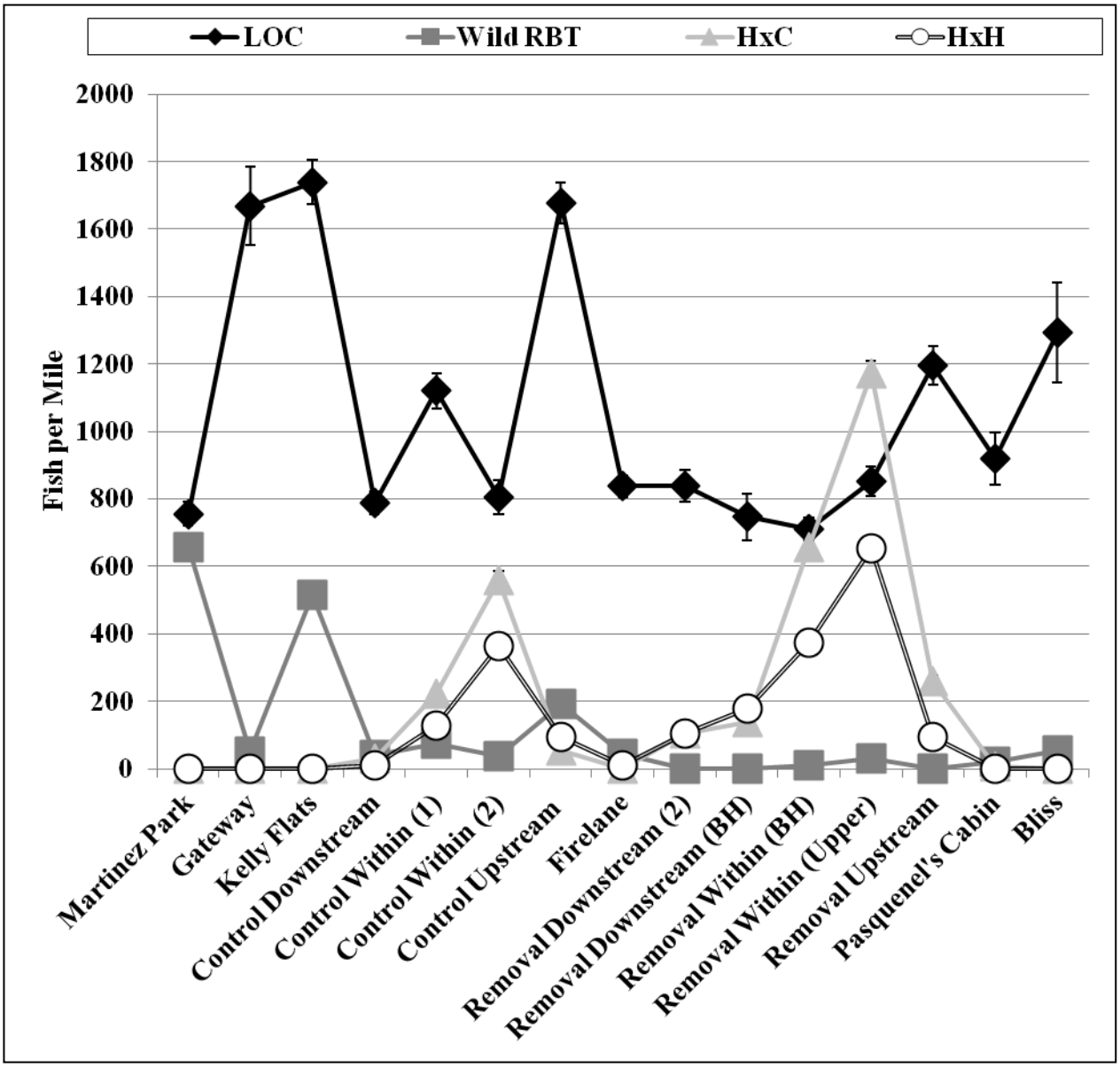

Figure 4.17. Estimated number of fish per mile for brown trout, wild rainbow trout, and introduced HxCs and HxHs, at fifteen sites throughout the Poudre River in October 2010. Sites are arranged from downstream to upstream moving from left to right in the figure.

Overall, brown trout numbers in the Poudre were high across the sections, rarely dropping below 800 fish per mile, and estimated at over 1,500 fish per mile in some sites (Gateway Park, Kelly Flats, and Bliss State Wildlife Area). Wild rainbow trout were found throughout the Poudre in low abundances (less than 100 fish per mile), with the exception of Lee Martinez Park (653 ± 27 fish per mile) and Kelly Flats (524 ± 24 fish per mile). Introduced rainbow trout abundances were highest in the two experimental sections, with abundances higher in the removal section than in the control section; Firelane and Pasquenel's Cabin were the only two sites in which introduced rainbow trout were found outside of the study sections (Figure 4.17).

The brown trout population in the control section was estimated at 776 (± 55) fish in October 2010, a loss of 54% from the estimated 1,673 (± 453) in August. Significant changes in population size indicate that a net movement of brown trout out of the control section occurred between August and October. In contrast, brown trout in the removal section increased from an estimated 95 (± 50) fish remaining in the section after the removal in August to an estimated 770 (± 41) fish in October. Significant changes in population size indicate that a net movement of brown trout into the removal section occurred between August and October (Figure 4.18).

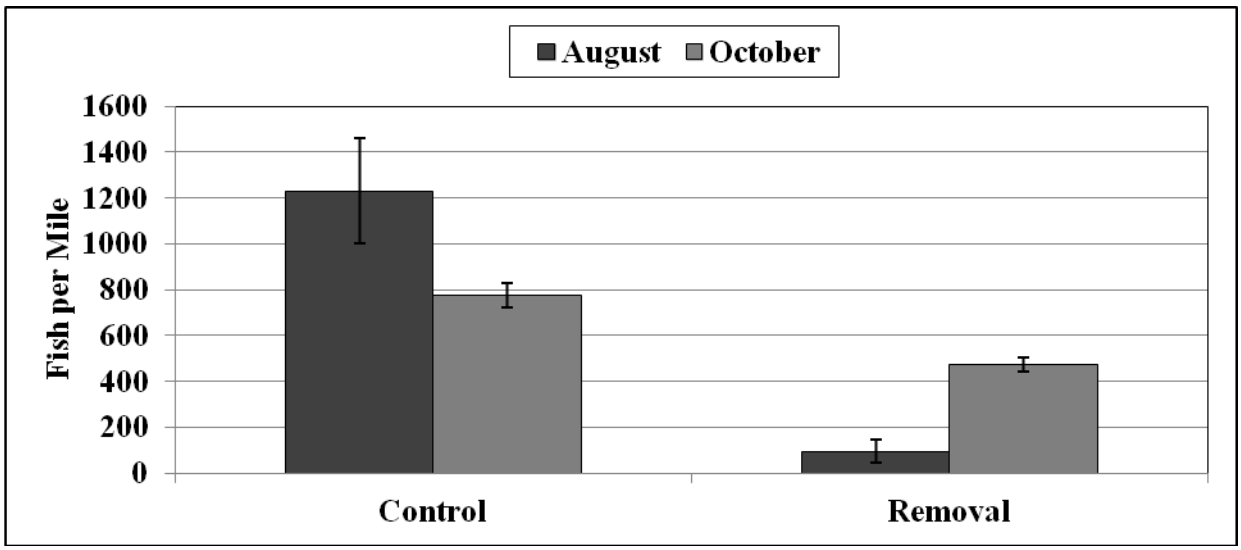

Figure 4.18. Brown trout population estimates in August and October in both the control and removal sections of the Poudre River (2010).

The total stocked rainbow trout population in the control section was estimated to be 503; therefore, only an estimated 26% of the 1,964 rainbow trout stocked in August remained in October. Of those, 308 were H×C, 31% of the 979 stocked in August, and 195 were H×H, 20% of the 996 stocked in August. The total stocked rainbow trout population in the removal section was estimated to be 854; therefore, 43% of the 1,975 rainbow trout stocked in August remained in the section in October. Of those, 545 were H×C, 56% of the 972 stocked in August, and 308 were H×H, 31% of the 992 stocked in August (Figure 4.19).

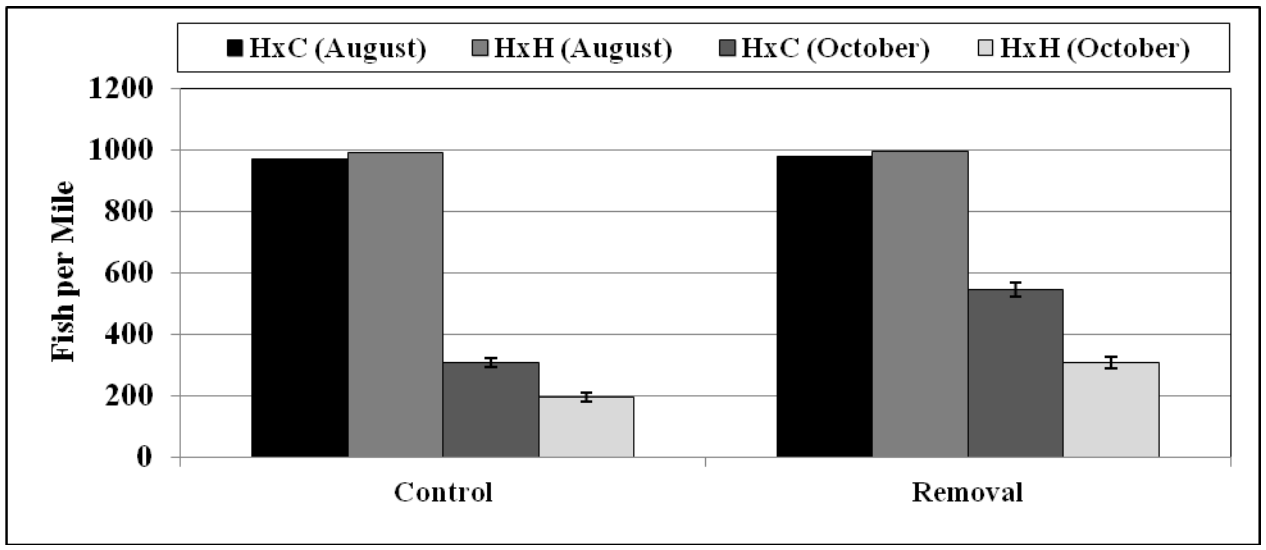

Figure 4.19. Number of H×C and H×H rainbow trout introduced to both the control and removal sections in August, and estimates of the H×C and H×H populations in the control and removal sections in October (2010).

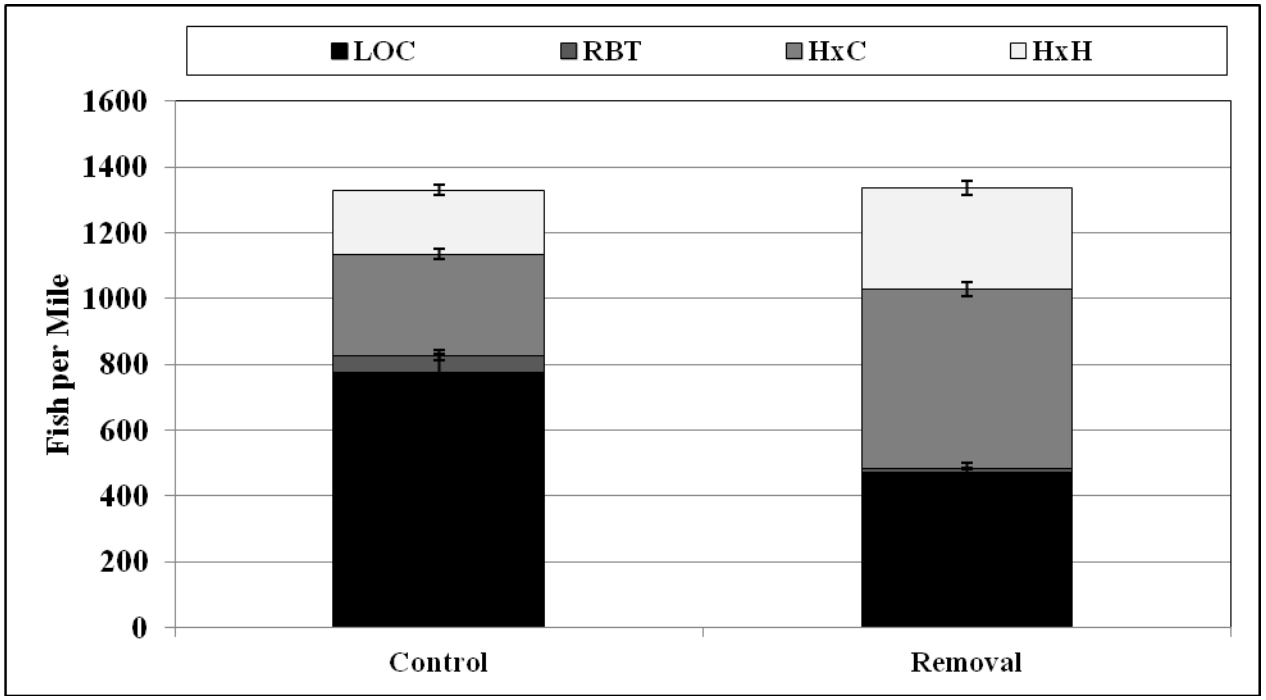

Figure 4.20. Total number of fish per mile in the control and removal sections in October, and proportion comprised of brown trout, wild rainbow trout, H×Cs, and H×Hs (2010).

The total number of fish in the control section did not change significantly between August and October, with an estimated 1,290 fish in the section in August, and an estimated 1,330 fish in October. In contrast, the removal section increased significantly in total number of fish between

August and October, with an estimated 865 fish in August (estimate prior to removal), increasing to an estimated 1,337 fish in October. These estimates suggested that more fish existed in the removal section than can normally be supported by that section (assuming that the system was at an "equilibrium" prior to the removal) and that a reduction in total fish through mortality or emigration was likely. Total number of fish per mile did not differ between the control and removal sections in October (Figure 4.20). However, the proportions of the brown trout and rainbow trout comprising the total did differ between the two sections. The ratio of brown trout to rainbow trout in the control section in October was 58:41, whereas in the removal section, the ratio of brown trout to rainbow trout was 35:65, which is close to the historical ratio of the Poudre River of 40:60.

Rainbow trout showed significant increases in length between August and October ($p < 0.001$). H×Cs exhibited similar length gains between the two sections ($p = 0.552$; Figure 4.43). H×Cs in the control section (229.8 mm TL) did not differ in length from H×Cs in the removal section (233.3 mm TL; $p = 0.636$). H×Cs in both sections did not differ from brown trout in either the control (233.6 mm TL; $p > 0.626$) or removal (233 mm TL; $p > 0.685$) sections. Despite a non-significant change in length between the H×Cs in both sections and the H×Hs in the control section ($p > 0.345$; Figure 4.21), H×Hs in the removal section (188.1 mm TL) were significantly smaller than H×Cs and brown trout in both sections ($p < 0.001$). Despite a significantly smaller gain in length in the removal H×Hs ($p = 0.024$; Figure 4.21), they did not differ significantly in length (186.1 mm TL) from the control H×Hs ($p = 0.831$). In comparison, brown trout in the control section gained only 5.6 mm TL, and only 1.3 mm TL in the removal section, both growing significantly less than either rainbow trout cross in either section ($p < 0.001$).

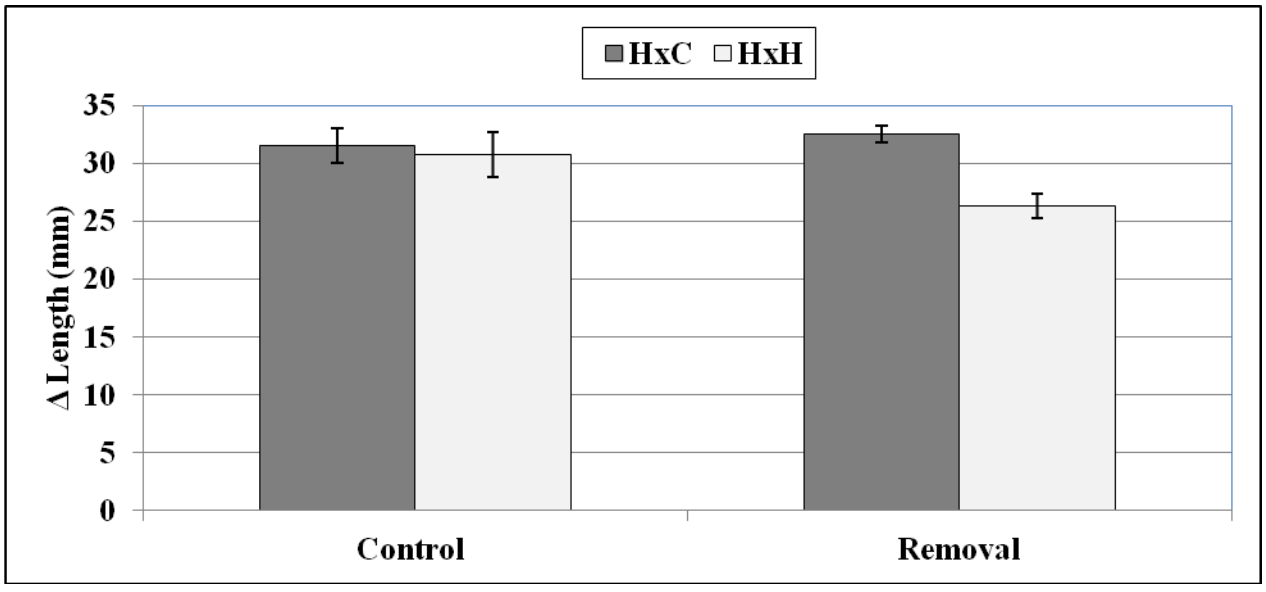

Figure 4.21. Change (Δ) in length of the H×Cs and H×Hs in the control and removal sections of the Poudre River between August and October 2010.

H×Cs exhibited similar weight gains between the two sections ($p = 0.895$; Figure 4.44). H×Cs in the control section (119 g) did not differ from H×Cs in the removal section (123.3 g; $p = 0.724$);

however, H×Cs in the control section weighed significantly less than control brown trout (145.4 g; $p = 0.030$), and H×Cs in the removal section weighed significantly less than removal brown trout (153 g; $p < 0.001$). Despite a non-significant change in weight between the H×Cs and H×Hs in the control section ($p = 0.091$; Figure 4.22), H×Hs in the removal section (65.8 g) were significantly smaller than the H×Cs in the control section ($p < 0.001$). There was not a significant difference in weight gain between the control and removal H×Hs ($p = 0.346$). In comparison, brown trout lost 0.3 g in the control section, but gained 5.8 g in the removal section, both growing significantly less than either rainbow trout cross in either section ($p < 0.001$).

During the population estimates, 12 brown trout had rainbow trout PIT tags in their stomachs, indicating that predation of rainbow trout by brown trout was occurring. Of these, eight were H×Hs, three were H×Cs, and one was a wild rainbow trout that had been tagged in the control section in August. Rainbow trout consumed averaged 51% of brown trout length, ranging from 41 to 78% of brown trout length.

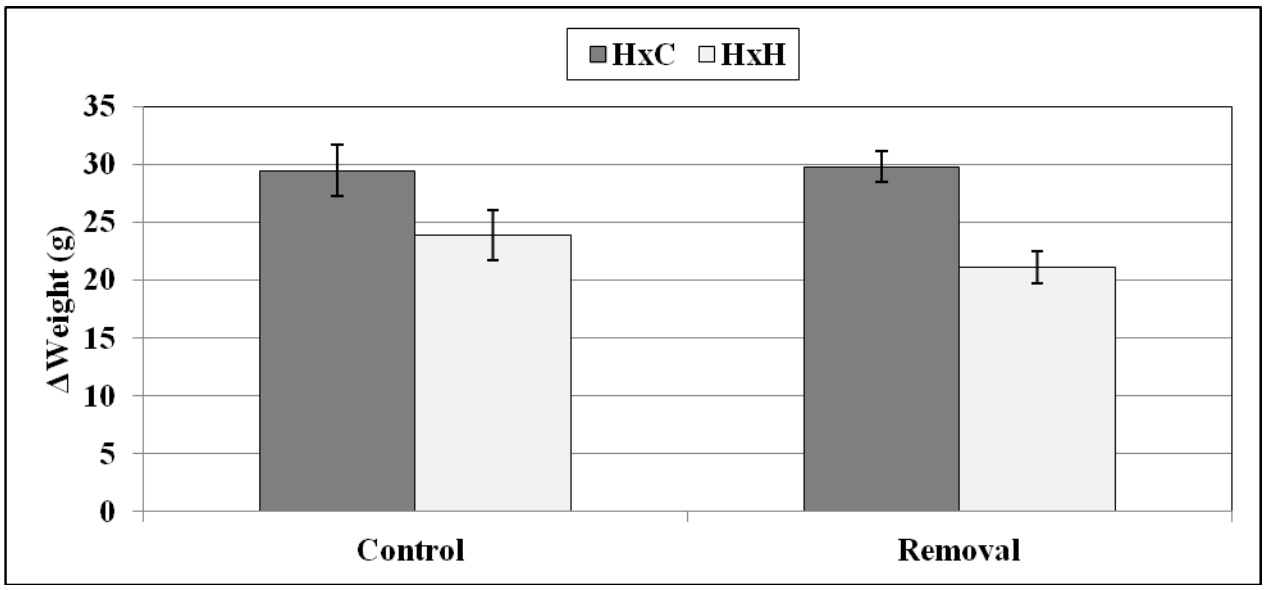

Figure 4.22. Change (Δ) in weight of the H×Cs and H×Hs in the control and removal sections of the Poudre River between August and October 2010.

POPULATION ESTIMATION – APRIL 2011

Population estimates in April 2011 were conducted at two historic and six experimental sites. A two pass removal estimate was conducted at six of the eight sites; two sites required a third pass to achieve acceptable depletion for population estimation. A four-electrode truck-mounted bank electrofishing unit was used at two of the eight sites; the other six sites were sampled using a three-electrode cat-raft. All fish encountered during the estimates were measured (mm), weighed (g) scanned for a PIT tag, and examined for secondary marks.

Abundance (*N*) was estimated using a Huggin's closed capture model in program MARK (White and Burnham 1999). The two and three pass estimates were modeled separately. Model sets

included a model in which *p* was constant, as well as models in which *p* varied by electrofishing gear (EFgear; only included in the two pass model set), habitat, site length, site width, length, the interaction between electrofishing gear and habitat (for the two pass model set only), and all additive combinations therein. Models were ranked using AICc and estimates of *p* and *N* were obtained using model averaging.

The brown trout population in the control section was estimated at 809 (± 103) fish in April 2011, a non-significant change from the estimates obtained in October 2010. However, a significant reduction in the brown trout population was observed in the removal section, with a 49% decline in the population between October 2010 (770 ± 41) and April 2011 (400 ± 42; Figure 4.23).

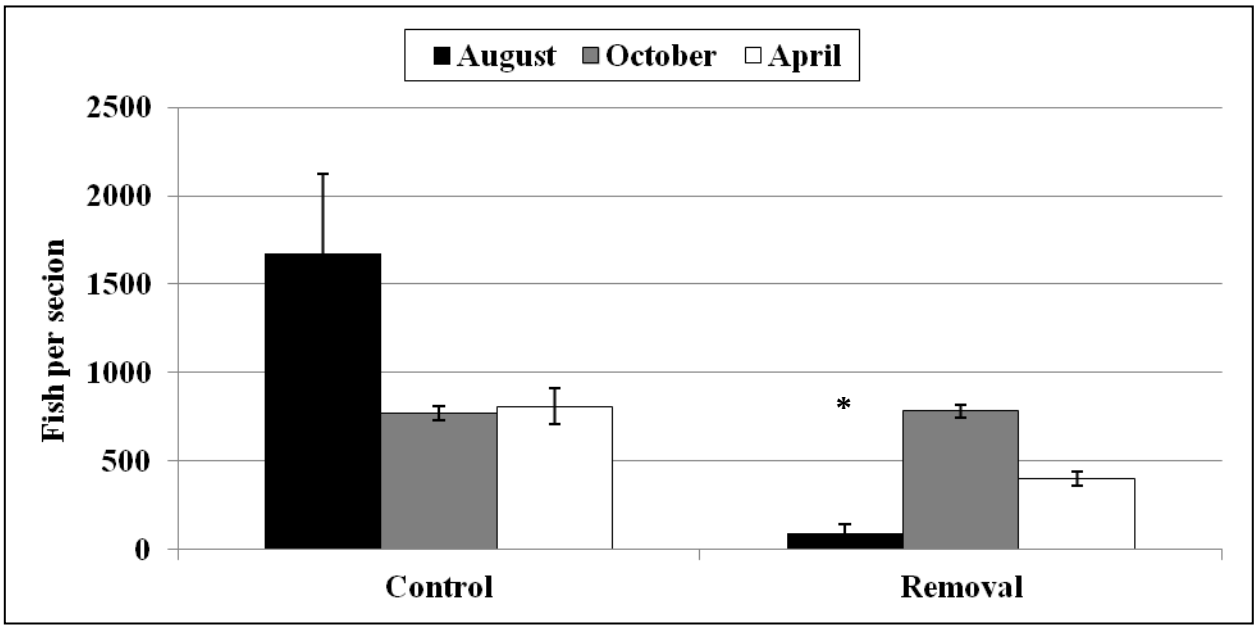

Figure 4.23. Brown trout population estimates within the control and removal sections in August 2010, October 2010, and April 2011. Note: the asterisk indicates the estimated size of the brown trout population prior to the August 2010 removal.

Both the H×Cs and H×Hs experienced population declines in both the control and removal sections. In the removal section, the H×C declined from an estimated 914 (± 33) fish within the section in October 2010, to only 198 (± 14) in April 2011. The H×H experienced a similar decline in the removal section, declining from an estimated 513 (± 30) fish within the section in October 2010, to an estimated 101 (± 14) fish within the section in April 2011. The decline in the control section was less severe for both species, with the H×C declining from 312 (± 18) to 237 (± 29) fish, and the H×H declining from 197 (± 17) to 127 (± 27) fish within the section between October 2010 and April 2011. Overall, the H×C was more abundant in both the removal and control sections than the H×H (Figure 4.24). In April, only 24% of the H×C and 13% of the H×H stocked in the control section remained, whereas, in the removal section, only 20% of the H×C and 10% of the H×H stocked remained.

H×Cs and H×Hs did not exhibit a significant increase in length between October 2010 and April 2011; however, they did exhibit a significant decrease in weight (average of 8 g) between October 2010 and April 2011. Conversely, the rainbow trout within the removal section did not experience a significant change in weight between October 2010 and April 2011; however, the length of both crosses increased significantly between October 2010 and April 2011.

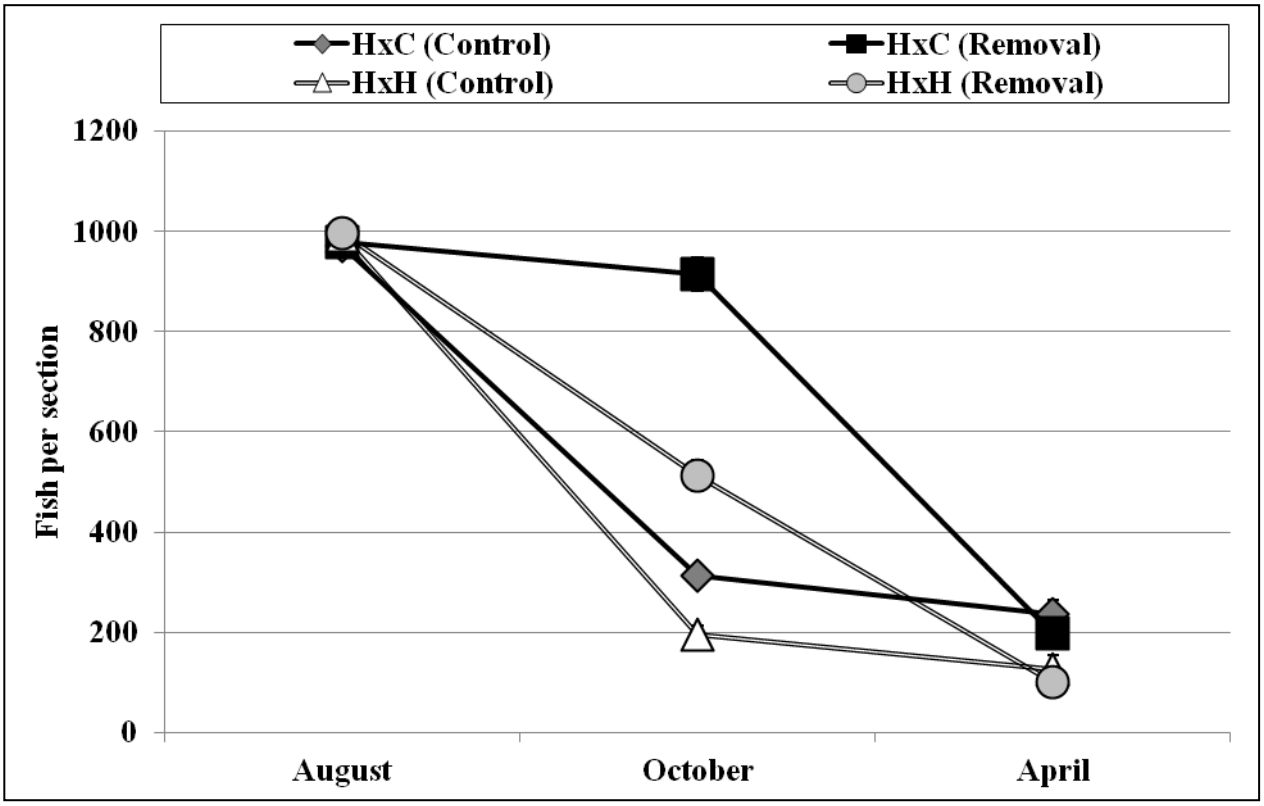

Figure 4.24. Rainbow trout (H×C and H×H) population estimates within the control and removal sections in August 2010, October 2010, and April 2011.

POPULATION ESTIMATION – OCTOBER 2011

Population estimates were conducted at the same fifteen sites sampled in October 2010. A two-pass removal estimate was conducted at nine sites using a four-electrode truck-mounted bank electrofishing unit; a three-electrode cat-raft was used at six of the sites where access by truck was limited. All fish encountered during the estimates were measured (mm), weighed (g), scanned for a PIT tag, and examined for secondary marks.

Abundance (*N*) was estimated using a Huggin's closed capture model in program MARK (White and Burnham 1999). The model set included a model in which *p* was constant, as well as models in which *p* varied by electrofishing gear (EFgear), species, habitat, site length, site width, length, interactions between electrofishing gear and habitat, interactions between electrofishing gear and species, interactions between electrofishing gear and length, and all additive combinations therein. Models were ranked using AICc and estimates of *p* and *N* were obtained using model averaging.

The brown trout population in the control section was estimated to be 643 (± 47) fish per mile in October 2011, a significant 20% decline in the population between April and October 2011. Brown trout population size did not show a significant change in the removal section, with the population estimated at 419 (± 22) fish within the removal section; this is in comparison to an estimated 400 (± 42) fish within the section in April 2011 (Figure 4.25).

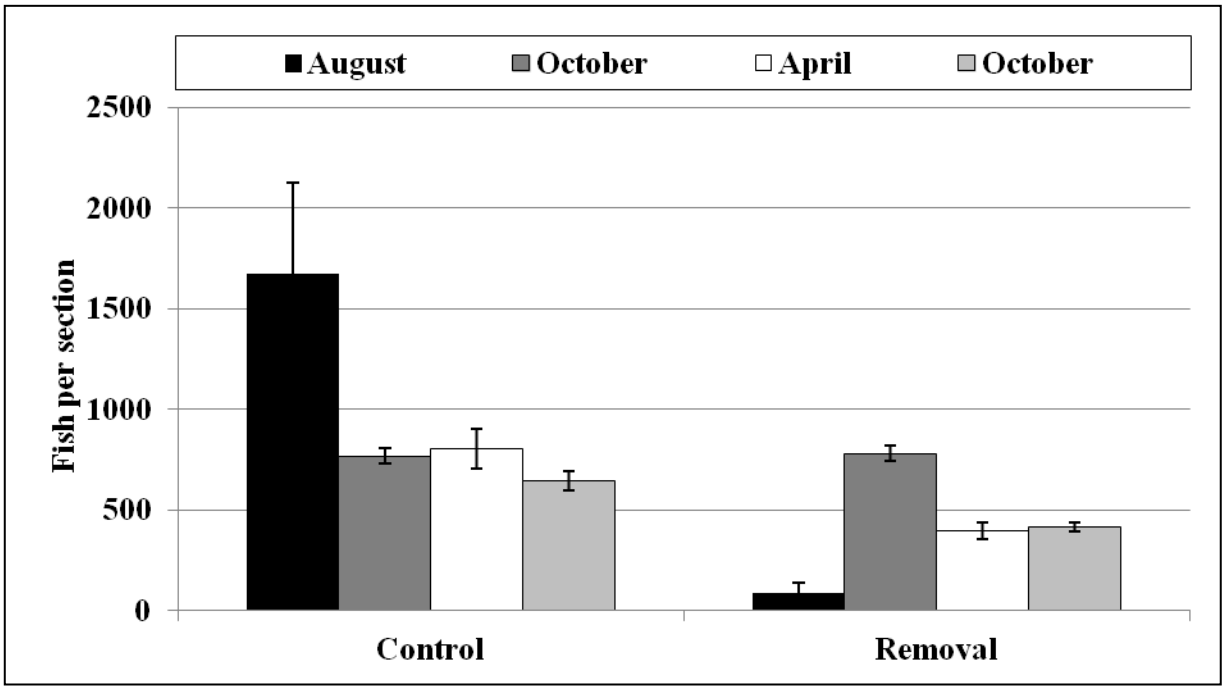

Figure 4.25. Brown trout population estimates within the control and removal sections in August 2010, October 2010, April 2011, and October 2011. The bar for the August 2010 estimate in the removal section represents the population remaining after the removal.

Both the H×C and H×H rainbow trout crosses experienced population declines in both the control and removal sections. In the removal section, the H×C declined from an estimated 198 (± 14) fish within the section in April 2011, to only 21 (± 4) in October 2011. The H×H experienced a similar decline in the removal section, declining from an estimated 101 (± 14) fish within the section in April 2011, to an estimated 12 (± 2) fish within the section in October 2011. The decline in the control section was similar for both species, with the H×C declining from 237 (± 29) to 55 (± 14) fish, and the H×H declining from 127 (± 27) to 21 (± 5) fish within the section between April and October 2011. Overall, the H×C was more abundant in both the removal and control sections than the H×H (Figure 4.26). In October 2011, only 6% of the H×C and H×H stocked in the control section remained, whereas, in the removal section, only 2% of the H×C and H×H stocked remained.

Rainbow trout of both crosses experienced similar length and weight gains in both the control and removal sections between being stocked in August 2010 and sampled in October 2011. On average, the H×C gained 74 mm in length and 101g in weight, and the H×H gained 80 mm in

length and 99 g in weight. Between April and October 2011, fish grew over 30 mm TL and over 60 g in weight (Figures 4.27 and 4.28, respectively).

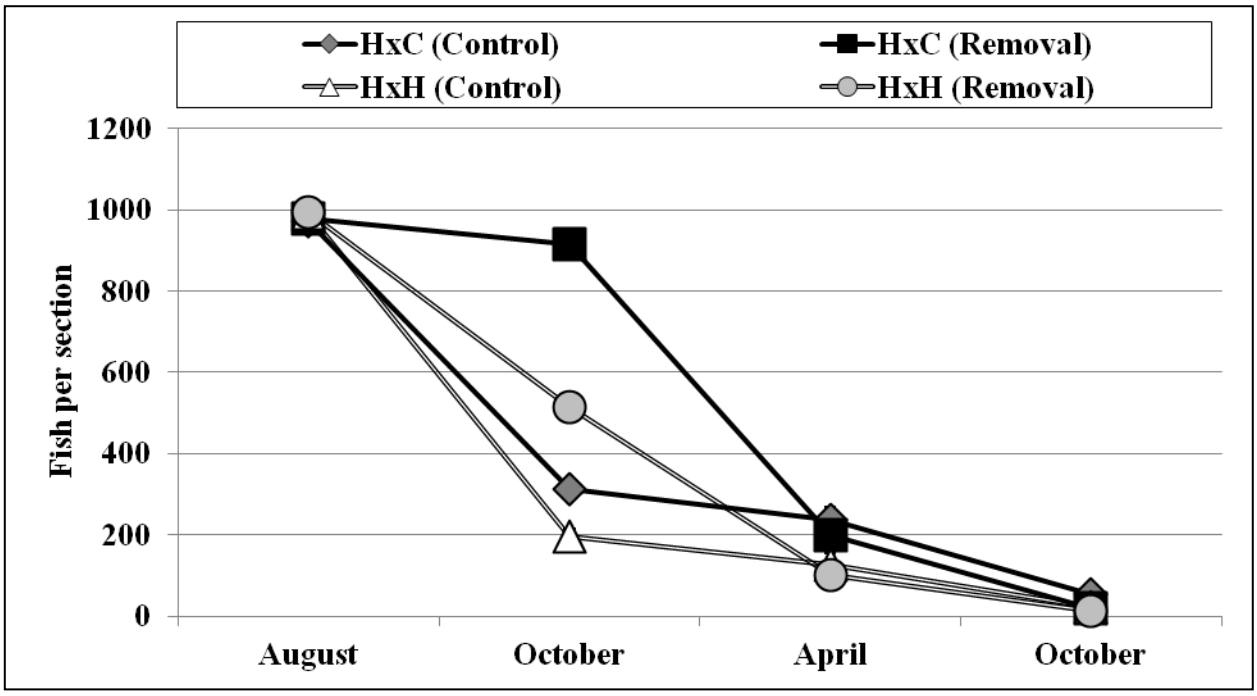

Figure 4.26. Rainbow trout (H×C and H×H) population estimates within the control and removal sections in August 2010, October 2010, April 2011, and October 2011.

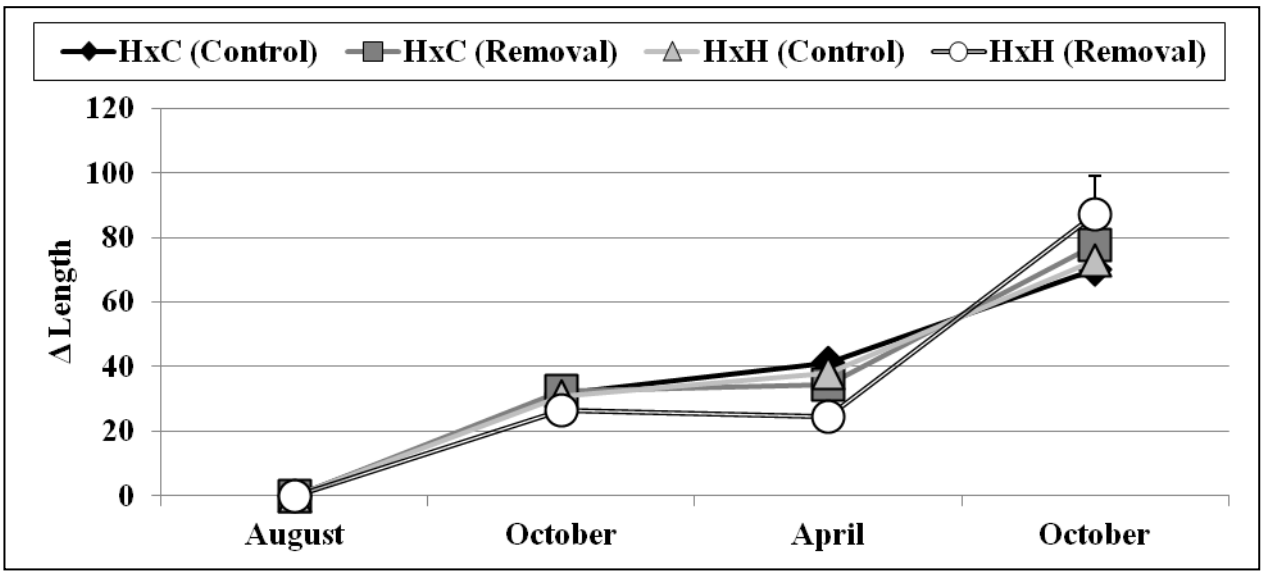

Figure 4.27. Rainbow trout (H×C and H×H) change in length (mm) between being stocked in August 2010 and sampled in October 2011.

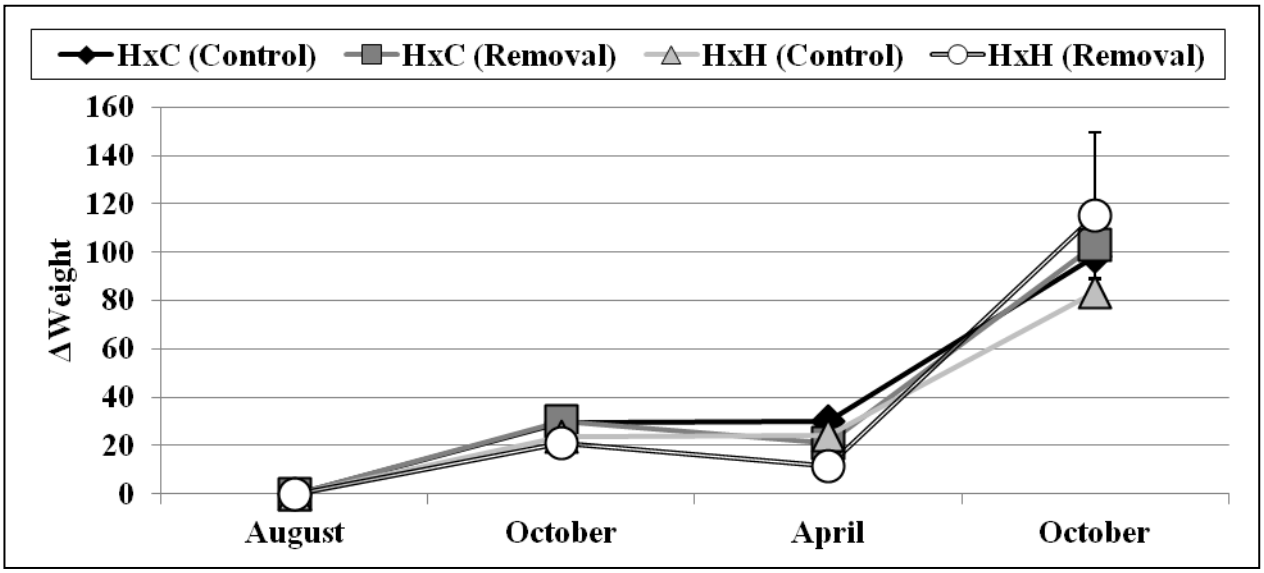

Figure 4.28. Rainbow trout (H×C and H×H) change in weight (g) between being stocked in August 2010 and sampled in October 2011.

CONCLUSIONS

On the short-term, brown trout removal appeared to increase rainbow trout survival and/or retention. Over 50% of the H×C and 40% of the H×H remained in the removal section two months following introduction; less than 35% of either cross was retained in the control section two months following introduction. Interestingly, predation of rainbow trout by brown trout does appear to contribute to mortality of the introduced rainbow trout populations. It was thought that stocking rainbow trout at about seven inches in length would prevent predation, with the major source of mortality being competition for limited resources, such as habitat and food. However, our results indicate that not only are brown trout capable of consuming the introduced rainbow trout, but they are capable of consuming a much larger percentage of their body length in prey than was previously expected. Strain comparisons showed that the H×C strain appeared to perform better on the short term, both in terms of growth and survival/retention, than the H×Hs when stocked into a river system.

On the long-term, brown trout removal did not appear to increase rainbow trout survival and/or retention. One year following their introduction, in October 2011, rainbow trout numbers did not differ significantly between the control and removal sections. One possible reason for this is the environmental conditions experienced by the fish in 2011. Winter conditions in 2011 were relatively harsh, with one of the largest snow accumulations on record in the upper sections of the Cache la Poudre watershed. The removal section especially receives very little solar input, and the conditions caused the formation of thick ice throughout the section. This may have reduced the survival of the rainbow trout, or conversely, caused the fish to move out of the section. Movement data from the antennas is still being analyzed to determine if the rainbow trout moved out of the section as winter conditions worsened. It is likely that if fish did move out, that they did not return to the removal section, but may present in other locations throughout the Poudre River. Growth data supports the difference in conditions between the two sections, as

fish located within the removal section lost an average of 8 grams of mass between October 2010 and April 2011, whereas the weight of the fish in the control section did not change over this time period.

In addition to the harsh winter conditions, the excess snow accumulation caused high water conditions in the spring of 2011. High flows, in combination with stress from harsh winter conditions, could have caused a further reduction in survival in the rainbow trout population within the removal section. Less severe declines in the rainbow trout population were observed within the control section in the period between October 2010 and October 2011, supporting the conclusion that the combination of winter and spring conditions resulted in lower survival in the removal section. Based on the results of this experiment, it does not appear that brown trout removal is a viable management option for increasing the survival and retention of reintroduced rainbow trout populations. However, a similar experiment conducted in a location where winter conditions do not cause emigration or lower survival of the introduced rainbow trout population, or conducted in years with less unusual environmental conditions, would provide more information on the utility of brown trout removals for the purpose of increasing the survival and retention of introduced rainbow trout.

In general, though numbers of both crosses are low, the H×C exhibited higher survival and/or retention in both the control and removal sections in comparison to the H×H. Both crosses appeared to grow well, and did not differ in length or weight gained between when they were stocked and sampled in October 2011. Based on survival and retention characteristics, we would recommend using the H×C for future river introductions of rainbow trout.

Genetic Techniques

HOFER VERSUS COLORADO RIVER RAINBOW DIFFERENTIATION TEST

A suite of microsatellite markers capable of distinguishing fish of the GR lineage, including pure GR, F1, F2, and backcross generations (B2 – F1×CRR – and BC1 – F1×GR), from the CRR strain, have recently been developed and tested. These markers were developed to genetically screen wild rainbow trout to detect and differentiate offspring from the GR strain from other rainbow trout strains. Known samples of GR and CRR crosses were used to identify which microsatellite markers were the most effective at differentiating between the two pure strains and their crosses, based on their frequency of appearance in the pure strains. Using the NewHybrids software program, the probability of being correctly assigned to a certain strain or cross (pure GR, F1, F2, B2, BC1, pure CRR) is provided for each unknown individual collected from the field. These results are used to determine if successful reproduction and recruitment of GR-cross rainbow trout has occurred in locations where these fish have been stocked.

Tests for Accuracy

Initial tests for accuracy were run using known samples and the NewHybrids program to determine how often an individual was correctly assigned to the known GR-cross, with a probability of 80% or greater. One hundred percent of the GR strain individuals were correctly assigned as pure GR, whereas 93.5 % of the pure CRR individuals were correctly assigned as

pure CRR; most commonly, pure CRR individuals were misidentified as B2 individuals. For the GR-crosses, 87.5% of the F1 individuals were correctly assigned as F1s, and were most commonly misidentified as F2s. Similarly, 87.2% of the B2 individuals were correctly assigned as B2s, and were most commonly misidentified as F2s. Finally, 80% of the F2 individuals were correctly assigned as F2s, and both individuals incorrectly assigned were misidentified as B2s. These results indicated that the microsatellite markers and associated NewHybrids probability tests were capable of distinguishing between pure and hybrid individuals (99% of the time), and that the majority of F1, F2 and B2 individuals could be correctly assigned.

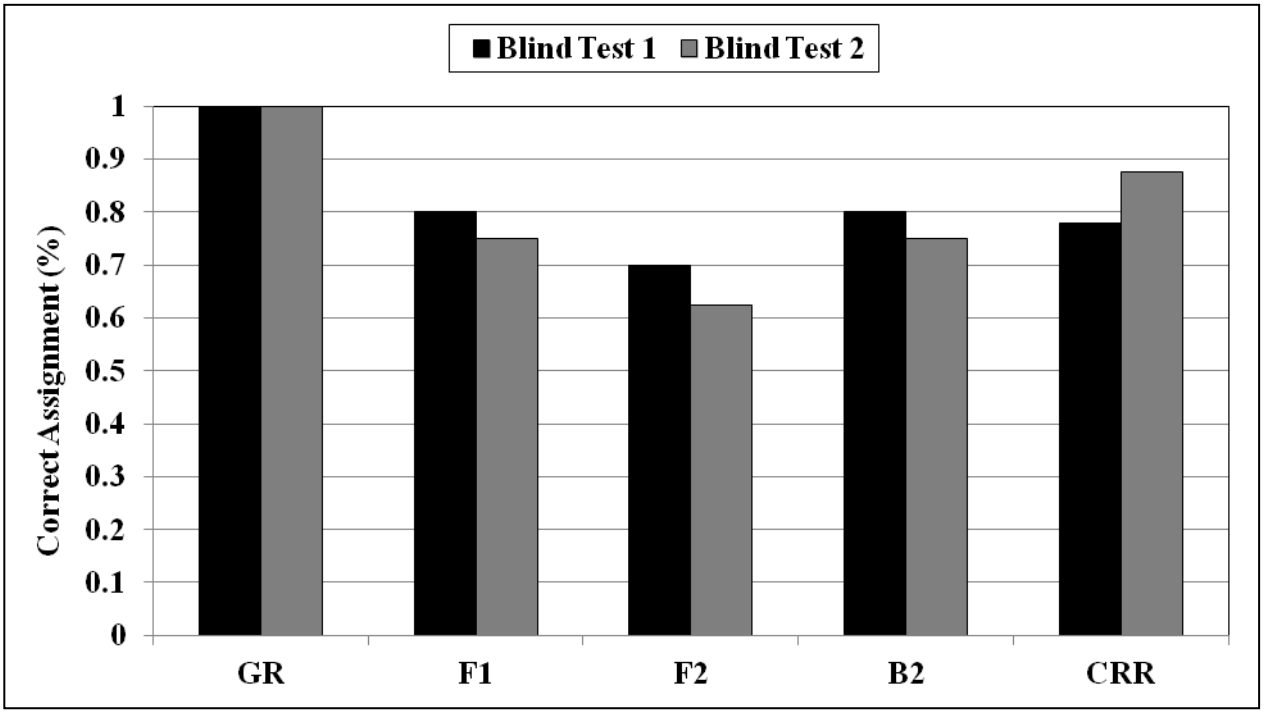

Figure 4.29. Percent of fish correctly assigned to strain or cross in the two blind tests for accuracy of the microsatellite marker development, and assignment by the NewHybrids program.

Subsequent tests (2) for accuracy used forty-eight known samples from a laboratory experiment involving the pure strains and their crosses (GR, F1, F2, B2, and CRR) run through the probability tests as blind samples. In both tests, 100% of the GR individuals were correctly assigned as pure GRs. Averaging between the two tests, 82% of the CRR individuals were correctly assigned as CRRs (Figure 4.29), with the large majority of the incorrect assignments misidentified as B2s. Similarly, when B2s were misidentified, they were most commonly assigned as CRRs. This was not entirely unexpected, considering that a B2 individual could genetically resemble a CRR individual 50% of the time. The F2 individuals were most commonly misidentified, which also was not unexpected considering they could resemble either a pure GR or pure CRR 25% of the time, respectively, and could resemble everything from an F1 to a B2 the other 50% of the time. Due to the accuracy of the test to identify the pure strains greater than 80% of the time, and the lack of a need for a test that identified an individual to a specific cross (the fact that an individual fish possesses GR genetics was sufficient for our needs), it appeared that the test was ready to use for wild fish testing.

Wild fish samples collected between 2007 and 2011 were submitted for analysis through 2010 and 2011. Results are presented for the two major rivers (Colorado River and Gunnison River), and the East Portal brood stock location in the Gunnison River. For the purposes of this analysis, genetic results were not broken down to cross (e.g., F1 or F2), rather are presented as GR-cross, CRR or unknown.

Colorado River

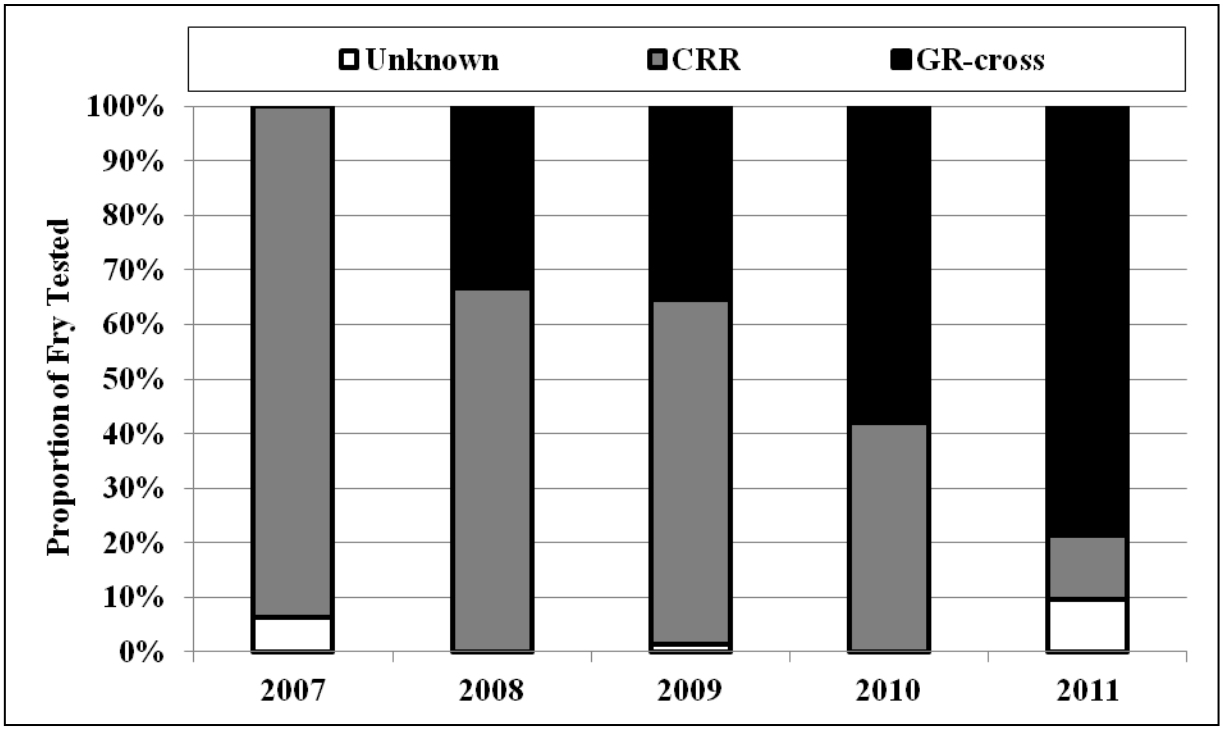

Figure 4.30. Proportion of fry categorized as unknown, pure CRR, or GR-cross fish, collected from the upper Colorado River, 2007-2011.

Genetic samples were collected from rainbow trout fry encountered during electrofishing efforts in 2007-2010. In 2007 (n = 16), all positively identified fry were identified as CRR; the genetic background of one individual was unknown. The proportion of fry in the sample that were positively identified as CRR dropped below 70% in 2008 (n = 21), remaining below 70% in 2009 (n = 76), dropping to almost 40% in 2010 (n = 57), and to less than 15% in 2011 (n = 42). A larger proportion of the fry (close to 10%) were classified as unknown in 2011, more than in the previous years combined. GR-cross fish began to appear in the sample in 2008 with a similar proportion of fry assigning as GR-cross fish in 2009. Over 50% of the fry sample in 2010 consisted of GR-cross fish, and this proportion increased to close to 80% in 2011 (Figure 4.30).

The proportion of CRR and GR-cross fry in the sample not only changed across years, but also across months within a year. In June 2009, five rainbow trout fry, all less than 30 mm in length, were encountered during the fry population estimates; all five were identified as pure Hofer, and remain the only pure Hofer fry encountered in the upper Colorado River. July 2009, positively

identified CRRs comprised over 75% of the sample; this proportion was reduced to just over 60% in August and September 2009, and was lowest in October 2009 at just over 50%. GR-cross fish were positively identified in the samples in July, August, September and October. The proportion of positively identified GR-cross fish was highest in August at just under 40%; however, 20% of the sample in October still consisted of positively identified GR-cross fish (Figure 4.31). The reduction in the proportion of CRR in the sample from July to October was expected as these fish are most susceptible to whirling disease infection, and are subject to increasing mortality over time as a result, a pattern that has been observed in the upper Colorado River since the introduction of whirling disease to the river in the early 1990s.

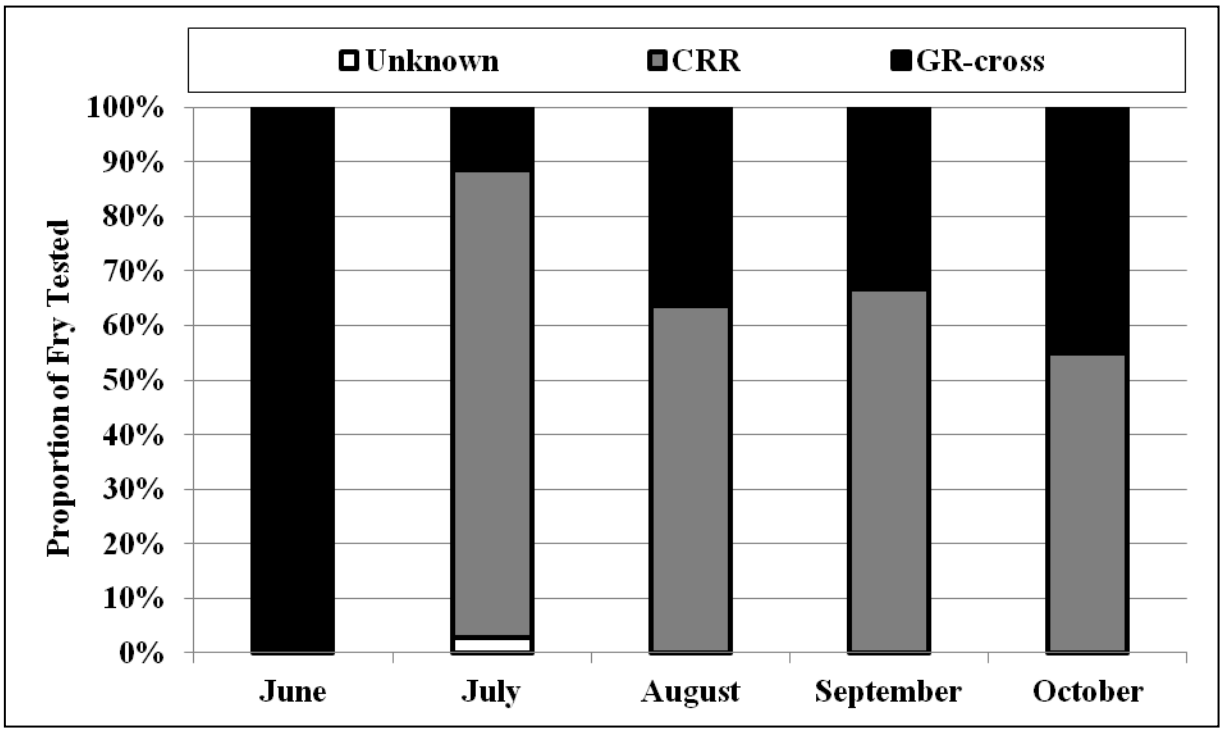

Figure 4.31. Proportion of fry categorized as unknown, pure CRR, or GR-cross fish, collected from the upper Colorado River, July – October, 2009.

In 2010, no rainbow trout fry were collected in June or October. The proportion of positively identified CRR individuals in the sample was lowest in July at less than 20%; this proportion increased to around 50% in August, and remained at around 50% in September. GR-cross fish represented the largest proportion of the sample in July, comprising over 80% of the sample; this proportion decreased to just under 50% GR-cross fish in August, remaining the same in September (Figure 4.32). The decrease in the proportion of GR-cross fish in August and September was unexpected, as these fish are more resistant to whirling disease infection, and less susceptible to mortality due to infection over time. The decrease in GR-cross fish could have resulted from a number of factors, including mortality from causes other than whirling disease, or low detection of rainbow trout in general. Despite the decrease in proportion of GR-cross fish, these fish were present in a higher proportion of the sample in September 2010 then they were in 2009.

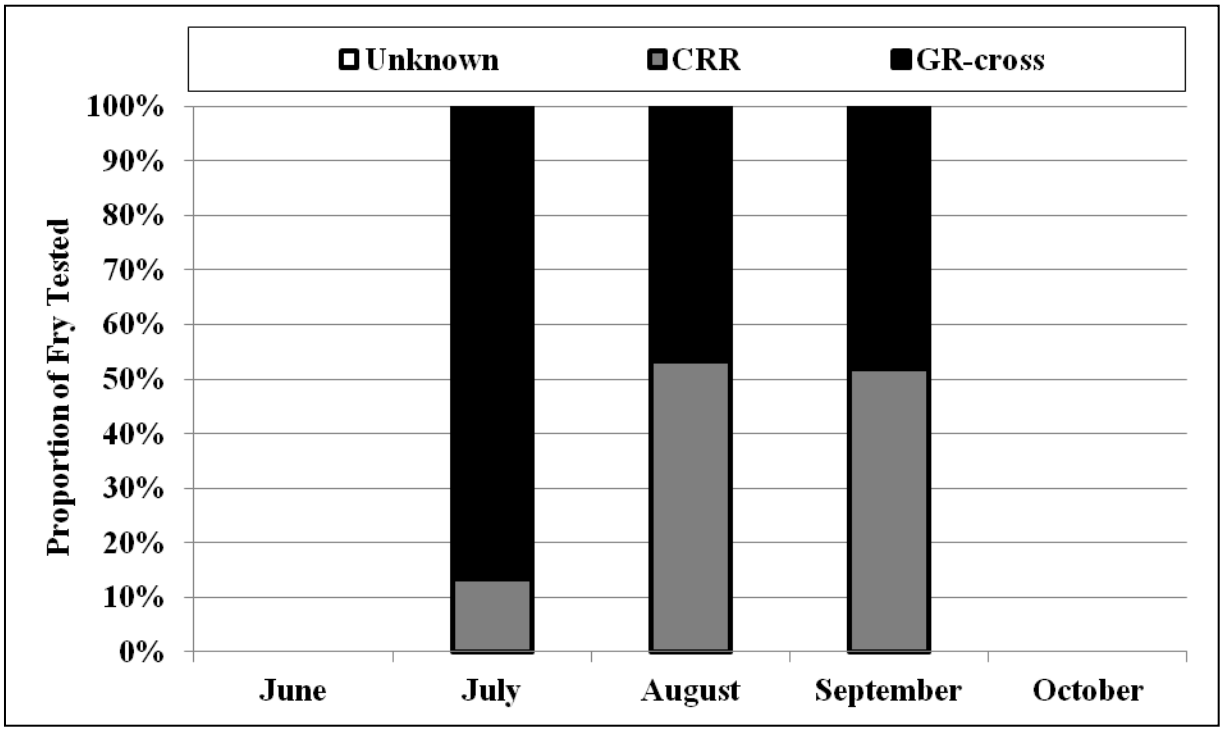

Figure 4.32. Proportion of fry categorized as unknown, pure CRR, or GR-cross fish, collected from the upper Colorado River, July – October, 2010.

In 2011, no rainbow trout fry were collected in June as a result of high water preventing fry population sampling. The proportion of CRR individuals in the sample was just under 50 % in July, and decreased to less than 30% in August. No CRR fry were encountered in September, and only one CRR fry was encountered in October, comprising less than 10% of the October sample. The proportion of GR-cross fry increased from July to September. Only one rainbow trout fry was encountered during the population estimates in September, however, that one individual was identified as a GR-cross fish. GR-cross fry dominated the sample in October as well, with over 70% of the fry identified as GR-cross fish. Interestingly, three of the fish encountered in October were unidentifiable (Figure 4.33).

The increase in the proportion of the GR-cross fish in the fry population between 2007 and 2011 is encouraging, and indicates that the H×C are reproducing in the upper Colorado River. A sample of the age-1 and older population is planned for spring 2013 to determine if the GR-cross fry are surviving and recruiting to the juvenile and adult population. Though the proportion of CRR fry declined between 2007 and 2011, it appears that there are still some residual CRR fish remaining in the adult population. CRR fry may continue to be identified in the fry population in the upper Colorado River; genetically, a pure CRR individual could result from the union of a pure CRR with a GR-cross fish or the union of two GR-cross fish.

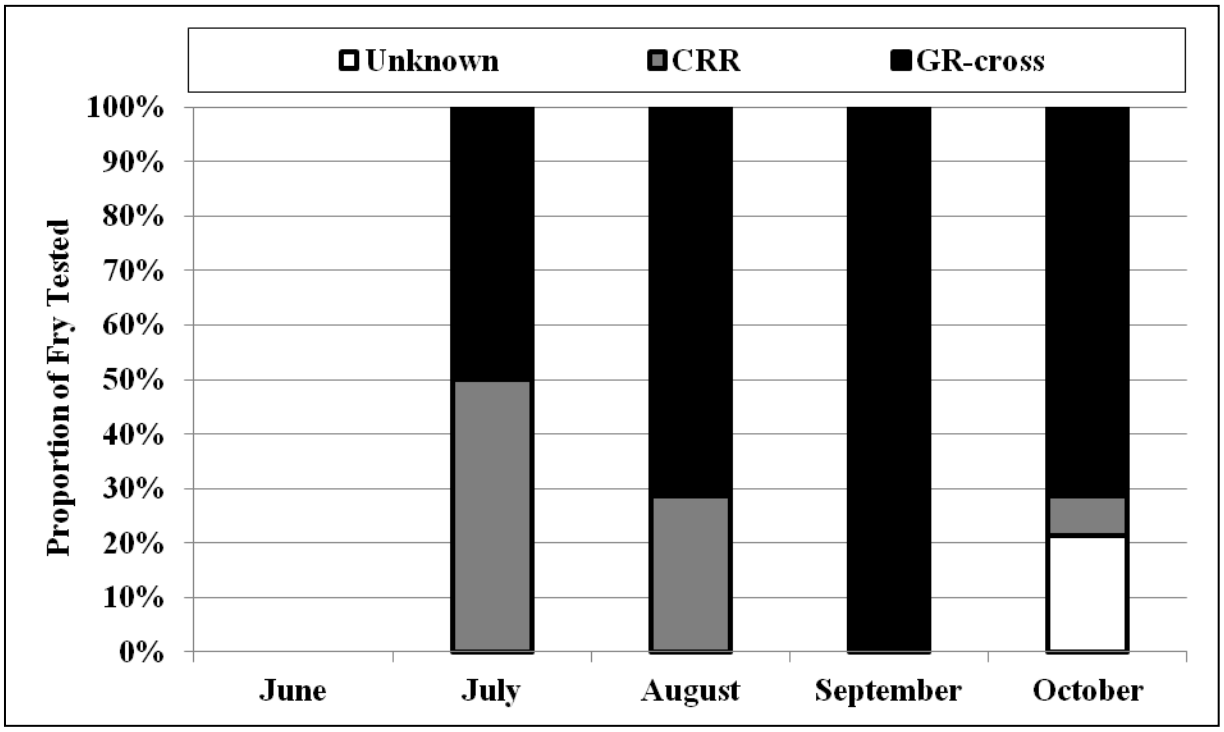

Figure 4.33. Proportion of fry categorized as unknown, pure CRR, or GR-cross fish, collected from the upper Colorado River, July – October, 2011.

Using genetic assignment, average myxospore count was calculated for CRR, GR-cross, and unknown fry. The CRR fry (n = 11) averaged (± SE) 40,388 (±11,875) myxospores per fish. The GR-cross fish (n = 13) exhibited lower average myxospore counts, averaging 24,287 (± 11,758) myxospores per fish. The fry classified as unknown (n = 3) exhibited the lowest average myxospore counts, averaging 1,061 (±767) myxospores per fish. Patterns of myxospore count suggest that the GR-cross fish, on average, are more resistant to *M. cerebralis* than CRR fish produced in the same reach of the upper Colorado River. Though unknown fish are unable to be classified by the genetic assignment test, myxospore count patterns suggest that these unknown fry may be GR-cross fish.

Gunnison River

Genetic samples were collected from fry during fry population estimates conducted in July and/or October between 2007 and 2010. In 2007 (n = 102), over 80% (87) of the fry were identified as pure CRR; only 7 fry were identified as unknown (~5%) and 8 fry as GR-cross fish (just under 10%). Over 95% of the fry encountered in 2008 (n = 58) were identified as pure CRR; only 2 fry were identified as GR-cross fish in 2008. The proportion of the fry population identified as pure CRR remained high in 2009 (over 80%), declining slightly in 2010, but still comprising over 75% of the fry population (Figure 4.34).

Despite GR-cross fish being present in the fry population between 2007 and 2011, no age-1 GR-cross fish were encountered during yearly population estimates. All age-1 fish were identified as puree CRR in 2009 and 2010.

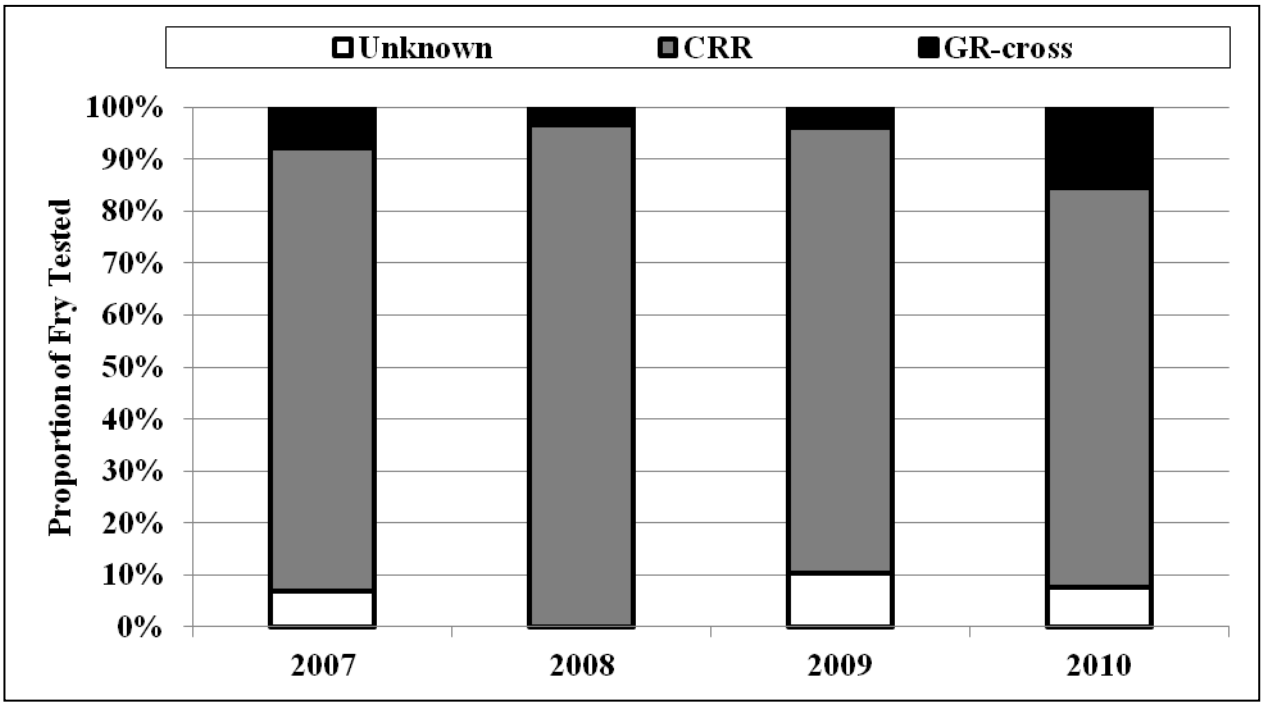

Figure 4.34. Proportion of fry categorized as unknown, pure CRR, or GR-cross fish, collected from the Gunnison River in the Gunnison Gorge, 2007-2010.

Despite a large number of introductions of F1 and B2 fish to the Ute Park section of the Gunnison River, GR-cross offspring are still poorly represented in the rainbow trout fry population. CRR offspring appear to dominate the rainbow trout fry community, indicating that residual and stocked CRR are spawning, and their offspring are surviving. Based on the genetic results from the age-1 fish collected in 2009 and 2010, it appears that survival of the GR-cross fry is low or non-existent, as they don't appear to be recruiting to the population. Though only a small number of CRR age-1 fish were encountered in 2009 and 2010, all of the fry were identified as pure CRR, indicating that at least some of the CRR fry are surviving exposure to *M. cerebralis* and recruiting to the population.

East Portal

The East Portal of the Gunnison River is currently being managed as a wild brood stock location for the H×C rainbow trout. H×C fingerlings have been stocked in the East Portal of the Gunnison River every year since 2006. In 2009, a population estimate was conducted in the East Portal to determine the size and age distribution of the introduced rainbow trout. In 2011, 60 rainbow trout were collected for a disease inspection. Fins were collected from all 60 age-1 fish used for the disease inspection. In addition, fins were collected from adult fish (ranging in size from 150 to 510 mm) captured during the elctrofishing efforts used to obtain the 60 fish disease sample. Finally, the shoreline just downstream of the boat ramp was shocked, and fin clips were obtained from the 40 rainbow trout fry encountered.

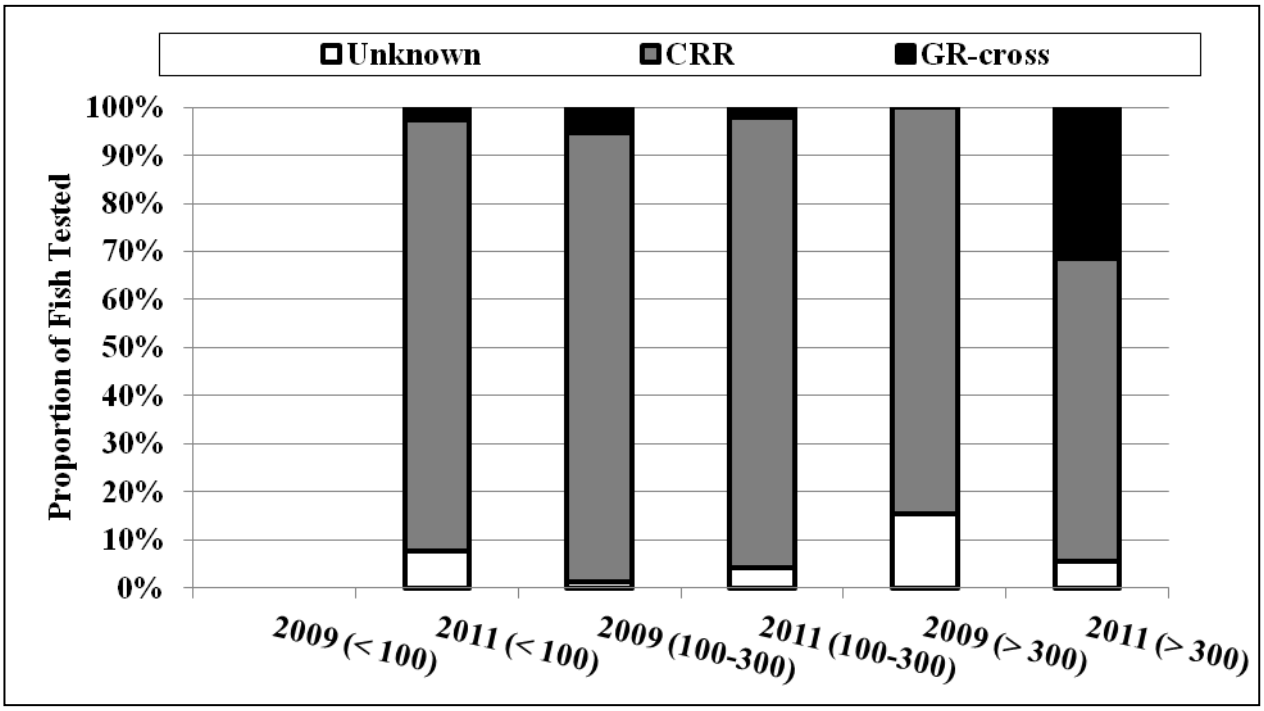

Figure 4.35. Proportion of fry (< 100 mm), juvenile, and adult (100-300 and > 300 mm) rainbow trout, encountered during the East Portal of the Gunnison River population estimate in 2009 and disease inspection in 2011, categorized as unknown, pure CRR, and GR-cross fish.

Less than 3% of the fry encountered in 2009 were identified as GR-cross fish, with the majority of the fry encountered (90%) identified as pure CRR. In the 100-300 mm size class, GR-cross fish only comprised 5% or less of the population in 2009 and 2011; the majority of the fish in this size class (> 90%) were identified as pure CRR. In 2009, none of the fish encountered over 300 mm were identified as GR-cross fish. However, over 30% of the rainbow trout greater than 300 mm in length encountered in 2011 were identified as GR-cross fish (Figure 4.35).

The genetic results described above were unexpected for this location. GR-cross fish had been the only rainbow trout stocked into the East Portal of the Gunnison River since 2006 in an effort to create a wild GR-cross brood stock. However, even with the 2011 results for the 300+ mm size class showing an increase of GR-cross fish in the population, the population as a whole could not be classified as a GR-cross brood stock. Therefore, egg collection for hatchery production, which was scheduled to begin in 2012, has been postponed until further research can be conducted on genetic and resistance characteristics of the East Portal rainbow trout.

In an experiment with multiple parts, the East Portal rainbow trout brood stock is being investigated in more depth in 2012. Eggs were collected at three different times, early spawn, mid-spawn, and late-spawn, during the spring 2012 rainbow trout spawning period. Fin clips were collected from all fish spawned during the egg collection, and groups of fish were kept separate so that parental genetic information could later be paired with offspring genetic information. The offspring collected will be used in exposure experiment to determine the disease resistance characteristics of the East Portal brood stock. In addition, fry population

estimates are being conducted at six different locations, once a month June through October 2012. Genetic samples are being collected from all rainbow trout fry encountered during the estimates, and 30 fry are being sacrificed each month for otolith collection and aging to determine hatch timing. The combination of the laboratory and wild fish information being collected during the experiments conducted in 2012 should provide a better understanding of the genetic composition and disease characteristics of the East Portal brood stock, helping guide future management decisions about hatchery egg collection from this location.

HOFER VERSUS HARRISON DIFFERENTIATION TEST

Use of H×H cross of rainbow trout is increasing in Colorado. As more of these fish are stocked in various locations across the state, it is important to have a test, similar to the Hofer versus Colorado River Rainbow differentiation test described above, to identify H×H fry and adults. A Hofer versus Harrison Lake differentiation test is currently being developed by Melinda Baerwald at the University of California Davis.

To develop the test, known samples from a wide variety of individuals was needed to establish a baseline microsatellite marker set. In December, 2011, fin clips were collected from pure Hofer, pure Harrison Lake, H×H 50:50 and H×H 75: 25 (Hofer:Harrison Lake) fish held at the CPW BFRH. In addition, two other crosses, the F2 H×H 50:50 and H×H 25:75 crosses were created for the first time to obtain genetic samples that spaned a range of possible crosses between the Hofer and the Harrison Lake. Offsring from eight families of the F2 H×H 50:50 and H×H 25:75 cross were reared at the hatchery until swim up (genetic material could not be taken during egg or sac fry stages because the oil could distort the results), and collected as known samples for the test.

The differentiation test is nearly completely developed. The current list of markers displays dramatically different allele frequencies between the pure Hofer and pure Harrison Lake strains. The test looks to be able to distinguish these two strains with high confidence. The ability of the test to distinguish between crosses of the two strains is still unknown, but is in the process of being tested. Blind samples (N = 96) have been sent to UC Davis to confirm the accuracy of the test in differentiating the pure strains and their crosses. The results of the differentiation test will be reported in the next reporting cycle.

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Job No. 5 Technical Assistance

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

Technical Assistance Milestones

Major contributions in the area of technical assistance included various public and professional meeting presentations, including the following:

1. Fetherman, E. R., D. L. Winkelman, and G. J. Schisler. 2011. Brown trout removal in the Cache la Poudre River, Colorado: Managing whirling disease resistant rainbow trout introductions in brown trout dominated streams. National American Fisheries Society Annual Meeting. Seattle, Washington. September 4-9, 2011.

2. Fetherman, E. R., D. L. Winkelman, G. J. Schisler, L. Bailey, and W. L. Kendall. 2011. Multistate modeling approach for analyzing survival and movement of Radio Frequency Identification (RFID) PIT tagged trout in rivers. National American Fisheries Society Annual Meeting. Seattle, Washington. September 4-9, 2011.

3. Fetherman, E. R and G. J. Schisler. 2012. Whirling disease resistant rainbow trout 2011 project updates. Annual Meeting of the Colorado Aquaculture Association. Mt. Princeton Hot Springs, Colorado. February 3, 2012.

4. Fetherman, E. R., D. L. Winkelman, G. J. Schisler, L. Bailey, and W. L. Kendall. 2012. Multistate modeling approach for analyzing survival and movement of Radio Frequency Identification (RFID) PIT tagged trout in rivers. 2012 Annual Meeting of the Western Division of the American Fisheries Society. Jackson, Wyoming. March 26-29, 2012.

5. Kowalski, D., E. R. Fetherman, G. J. Schisler, and R. B. Nehring. 2012. Introduction of whirling disease resistant rainbow trout in the Gunnison River, Colorado. 2012 Annual Meeting of the Western Division of the American Fisheries Society. Jackson, Wyoming. March 26-29, 2012.

6. Fetherman, E. R. 2012. Whirling disease: life cycle, disease signs, and impacts to Colorado's fisheries. Whirling Disease Panel Discussion (members E. R. Fetherman, K. Davies, and P. Walker). Colorado State University Student Chapter of the Wildlife Disease Association. Fort Collins, Colorado. April 5, 2012.

7. Fetherman, E. R. 2012. Tracking fish survival and movement using stationary and portable PIT tag antenna arrays. Colorado Parks and Wildlife 2012 Southwest Region Biology Days. Gunnison, Colorado. May 2, 2012.

8. Kowalski, D., E. R. Fetherman, G. J. Schisler, and R. B. Nehring. 2012. Introduction of whirling disease resistant rainbow trout in the Gunnison River, Colorado. Colorado Parks and Wildlife 2012 Southwest Region Biology Days. Gunnison, Colorado. May 2, 2012.

In addition to these presentations, an interview and material was provided for a popular article entitled "Rainbow Trout Experiment on Poudre River Shows Promise," published in the North Forty News (August 2011).

Technical milestones also included a USGS Federal review of a manuscript authored by Jesse M. Lepak, C. Nathan Cathcart, and Mevin B. Hooten, entitled "Otolith Weight as a Predictor of Age in Kokanee Salmon (O*ncorhynchus nerka*) from Four Colorado Reservoirs," intended for publication in the Canadian Journal of Fisheries and Aquatic Sciences, and the publication of two peer-reviewed journal articles:

- 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.

- Lepak, J. M., K. D. Kinzli, E. R. Fetherman, W. M. Pate, A. G. Hansen, E. I. Gardunio, C. N. Cathcart, W. L. Stacy, Z. E. Underwood, M. M. Brandt, C. A. Myrick, and B. M. Johnson. 2012. Manipulation of growth to reduce mercury concentrations in sport fish on a whole-system scale. Canadian Journal of Fisheries and Aquatic Sciences 69(1):122-135.

In addition to those manuscripts published in peer-reviewed journals, three other manuscripts have been submitted for publication:

- Fetherman, E. R., D. L. Winkelman, G. J. Schisler and M. M. Antolin. In review. Heritability of myxospore count and genetic correlations in whirling disease (*Myxobolus cerebralis*) resistant and susceptible strains of rainbow trout (*Oncorhynchus mykiss*). Submitted to Diseases of Aquatic Organisms.

- Fetherman, E. R., and J. M. Lepak. In review. Back-calculation of capture probability and estimating gear efficiency using known population abundances. Submitted to the North American Journal of Fisheries Management.

- Lepak, J. M., E. R. Fetherman, W. M. Pate, C. Craft, and E. I. Gardunio. In Review. An experimental approach to determine esocid prey preference in replicated pond systems. Submitted to Lake and Reservoir Management.