

Sport Fish Research Studies

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Project Title: Sport Fish Research Studies

Period Covered: July 1, 2012 – June 30, 2013

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 2012, BFRH personnel checked all of the Hofer¹ (GR), Harrison Lake (HL), Hofer × Harrison Lake (GR×HL) brood fish (2, 3, and 4 year-olds) weekly for ripeness. Maturation is indicated by eggs or milt flowing freely when slight pressure is applied to the abdomen of the fish. The first females usually mature 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 27, 2012, 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 11.1°C (52°F) 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 2012-2013 spawning season.

Strain	Date Spawned	No. Spawned Females	No. Green Eggs	No. Eyed Eggs	Shipped To
100% HL	12/11/12-1/22/13	202	19,004	15,773	Fish Research Hatchery/CPW Hatcheries
100% GR	11/27/12-12/18/13	237	143,592	120,617	Fish Research Hatchery/CPW Hatcheries
GR×HL	12/11/12	57	5697	4615	Fish Research Hatchery Brood
Total	11/27/12-1/22/13	496	168,293	141,005	83.7% Good Eggs to Eye-up

The FRH 2012/2013 on-site rainbow trout production spawn started on November 27, 2012, with the last groups of HL females spawned on January 22, 2013. The initial goal was to produce 154,000 eyed eggs; egg take exceeded the production needs with 168,283 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 2012-2013. 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 and Fingerling 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).

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

Differential formalin sensitivity has been demonstrated for various strains of rainbow trout when exposed post-hatch (Piper and Smith 1973); however, there has been little to no research on differential strain sensitivity to formalin exposure during egg incubation. In addition, the formalin sensitivity of fingerling rainbow trout exposed to varying levels of formalin during egg incubation is unknown. The objective of this study is to determine if there is differential sensitivity (measured by mortality) of four whirling disease resistant rainbow trout strains to varying formalin concentrations used to control external parasite infections as fingerlings

following exposure to varying levels of formalin used to treat fungal infections 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: 1) Hofer (GR), 2) Harrison Lake (HL), 3) Hofer × Harrison Lake 50:50 (GR×HL 50:50), and 4) 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 December, 2012. GR egg groups were created by pooling the eggs from 18 pairs of two-year-old GR females spawned with three-year-old GR males. The eggs from three pairs of two-year-old HL males spawned with three-year-old HL females, and 18 pairs of three-year-old HL males spawned with two-year-old HL females, 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 20 pairs of two-year-old GR males spawned with two-year-old HL females. The eggs from 37 pairs of two-year-old GR×HL 50:50 females spawned with two-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.

Egg Formalin Sensitivity

Two, five gpm flow-through egg tray towers were utilized for the egg formalin exposure experiment, with one formalin treatment per tower. Six egg trays within the seven tray towers were used for the experiment. Two, three inch diameter, screen-bottomed PVC inserts were placed in each of the six trays, a total of 12 PVC inserts per treatment (Figure 2.1). Each PVC insert contained 500 eggs from a given strain or cross, providing three 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.

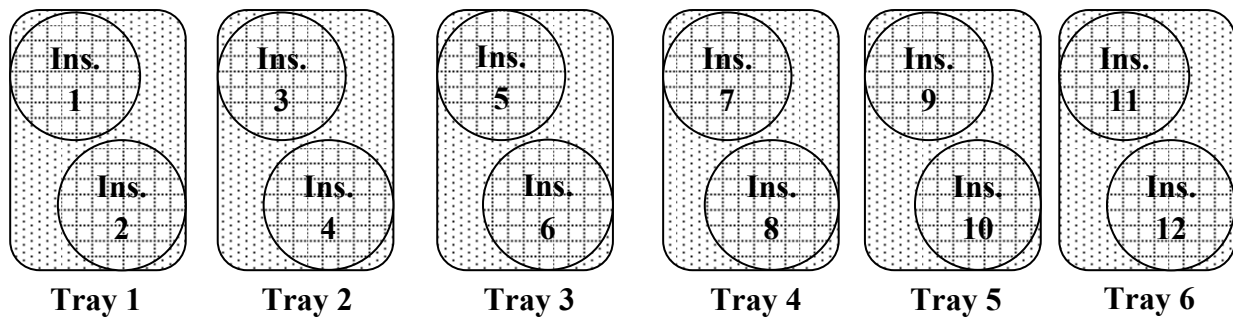


Figure 2.1. Arrangement of 12 screen-bottomed PVC inserts in the six trays (1-6, from top of tower down) used in each formalin treatment group. Strains and crosses were randomly assigned to an insert, within a treatment, using a random number generator (see Table 2.1).

Two formalin treatment levels were used to determine rainbow trout egg formalin sensitivity. The control formalin concentration was the same as that 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.

The second formalin treatment, 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 (Marking et al. 1994); 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). In a similar experiment conducted in 2012, the 5,000 ppm egg treatment was the only one of three treatments (1,667, 2,000 and 5,000 ppm) in which one strain, the GR×HL 50:50, showed increased mortality relative to the other two formalin concentrations.

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	High Formalin
1	GR×HL 50:50	GR×HL 75:25
2	GR×HL 75:25	HL
3	GR×HL 50:50	GR×HL 50:50
4	GR	HL
5	GR	GR
6	HL	GR×HL 50:50
7	GR×HL 75:25	GR×HL 75:25
8	GR	GR×HL 50:50
9	HL	GR×HL 75:25
10	GR×HL 75:25	GR
11	HL	HL
12	GR×HL 50:50	GR

The experiment started 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) $\% \text{ pre hatch mortality} = 100 \times \frac{\text{mortality before hatch}}{\text{initial number of eggs}}$. 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) $\% \text{ post hatch mortality} = 100 \times \frac{\text{mortality after hatch}}{\text{initial number of eggs}}$. 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 $\% \text{ total mortality} = 100 \times \frac{\text{pre hatch} + \text{post hatch mortality}}{\text{initial number of eggs}}$.

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 analysis of variance (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.

Fingerling Formalin Sensitivity

Upon conclusion of egg formalin sensitivity experiment, strain replicates within a formalin treatment were combined into a single rearing trough, for a total of eight troughs (1,667 GR, 1,667 HL, 1,667, GR×HL 50:50, 1,667 GR×HL 75:25, 5,000 GR, 5,000 HL, 5,000 GR×HL 50:50, and 5,000 GR×HL 75:25). All groups were fed a similar ration of 2.5% of their body weight day⁻¹ in the interim between experiments, and were reared under similar environmental conditions (i.e., flows, temperatures, etc.), until they reached 3” in length (fingerlings).

Two weeks prior to initiation of the first fingerling formalin sensitivity experiment, fish were marked with a visual implant elastomer (VIE) tag in the adipose tissue behind both the left and right eyes, preventing misidentification if a tag was lost from one of the sides during experimentation. One VIE color was used for each of the four strains, regardless of egg treatment level (GR: red, HL: green, GR×HL 50:50: orange, GR×HL 75:25: pink; Figure 2.2).



Figure 2.2. Visual implant elastomer (VIE) tags behind the eye of the (clockwise from the top) HL, GR×HL 50:50, GR, and GR×HL 75:25 fish, as seen fluorescing under a black light.

Twelve tanks (74.8 L) were used in each formalin trial (Figure 2.3), providing three replicates of each of four treatment levels: 0 ppm, 167 ppm, 250 ppm and 500 ppm. Treatment was randomly assigned to tank using a random number generator (Table 2.2). Five days prior to a trial, 20 fish of each strain were randomly distributed to each of the twelve tanks, resulting in a total of 80 fish per tank. The five day pre-experiment monitoring period was used to account for any mortality that occurred as a result of moving fish from inside the hatchery to FR1. Feeding of the fish in FR1 was ceased the day prior to conducting a formalin trial.

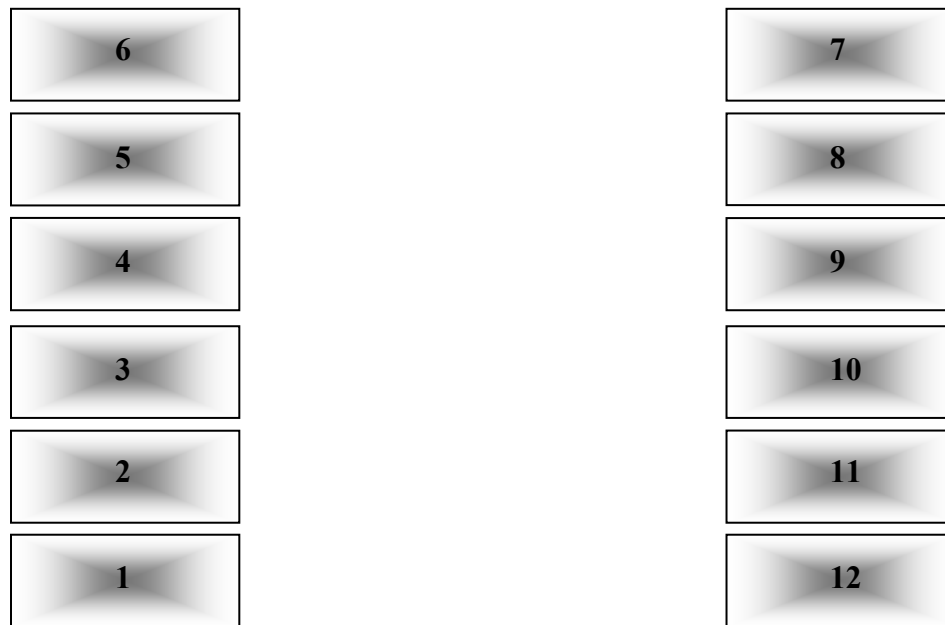


Figure 2.3. Arrangement and numbering of the twelve experimental tanks used in the fingerling formalin sensitivity experiments, housed in FR1 of the Bellvue Fish Research Hatchery.

Peristaltic meter pumps were used to deliver the formalin at the correct rate to produce the desired formalin concentration in each tank; formalin was delivered at a rate of $1.26 \text{ ml minute}^{-1}$ for the 167 ppm treatment, $1.89 \text{ ml minute}^{-1}$ for the 250 ppm treatment, and $3.78 \text{ ml minute}^{-1}$ for the 500 ppm treatment. Because formalin is known to remove oxygen from the water (1 ppm oxygen removed for every 5 ppm formalin within 30-36 hours; Piper et al. 1982), oxygen levels were monitored during treatment. Treatments occurred for either 30 or 60 minutes, and treatment time was the same across all tanks within a trial. As a result, four trials were conducted: fish treated at 1,667 ppm as eggs treated for 30 minutes as fingerlings, fish treated at 1,667 ppm as eggs treated for 60 minutes as fingerlings, fish treated at 5,000 ppm as eggs treated for 30 minutes as fingerlings, and fish treated at 5,000 ppm as eggs treated for 60 minutes as fingerlings. Mortalities that occurred during and after a trial were identified using the VIE tags, and the length (mm), weight (g), and time and date found were recorder for each mortality.

It is known that fish treated with excessive concentrations of formalin may suffer delayed mortality, with the onset of death occurring within 1 to 24 hours of treatment, but potentially occurring up to 48 to 72 hours later depending on size and condition of fish, and water temperatures (Piper et al. 1982). Therefore, fish were retained within the experimental tanks for five days following formalin exposure so that residual mortality could be recorded. Fish were checked in the morning and afternoon during this post-exposure monitoring period; the time at which mortalities were found, as well as the strain, length, and weight of each fish was recorded. Fish remaining at the conclusion of the post-exposure monitoring period were euthanized using an overdose of MS-222, counted, measured and weighed. Following removal of fish, tanks were cleaned and prepared for the next formalin trial.

Table 2.2. Assignment of treatment to tank and order in which the experimental treatments were applied in the four formalin trials (fish treated at 1,667 ppm as eggs treated for 30 minutes as fingerlings [1,667 for 30 Min], fish treated at 1,667 ppm as eggs treated for 60 minutes as fingerlings [1,667 for 60 Min], fish treated at 5,000 ppm as eggs treated for 30 minutes as fingerlings [5,000 for 30 Min], and fish treated at 5,000 ppm as eggs treated for 60 minutes as fingerlings [5,000 for 60 Min]).

Treatment (ppm)	1,667 for 30 Min		1,667 for 60 Min		5,000 for 30 Min		5,000 for 60 Min	
	Tank	Order	Tank	Order	Tank	Order	Tank	Order
0	9	10	5	10	2	3	8	4
0	10	2	1	8	4	7	10	1
0	5	8	12	5	6	10	5	7
167	7	5	8	12	9	4	2	11
167	12	11	6	9	7	2	7	5
167	2	1	11	6	3	9	9	10
250	1	12	10	4	11	5	1	9
250	3	4	4	1	1	6	3	3
250	6	7	7	2	5	11	12	8
500	4	9	9	3	8	1	4	6
500	11	6	3	7	10	8	11	2
500	8	3	2	11	12	12	6	12

Statistical analyses were conducted using the GLM procedure in SAS (SAS Institute 2011). Differences in mortality were analyzed using a two-factor ANOVA, with strain/cross and treatment as the factors ($N = 48$). 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

Egg Formalin Sensitivity

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 500 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 (\pm SD) of 506 (\pm 29) eggs per PVC insert. Average number of eggs did not differ among strains/crosses or treatments ($F = 0.86$, $P = 0.560$).

Average pre-hatch mortality differed both between the treatments ($F = 9.37$, $P = 0.008$), and among the strains/crosses ($F = 29.54$, $P < 0.001$); the interaction was also significant ($F = 7.73$, $P = 0.002$). Eggs within the 5,000 ppm treatment experienced significantly higher average (\pm SD) percent pre-hatch mortality ($32.4 \pm 13.3\%$) than did the control treatment ($26.8 \pm 6.5\%$). The GR \times HL 75:25 exhibited significantly higher percent pre-hatch mortality ($43.0 \pm 13.0\%$) than all of the other strains and crosses ($P < 0.001$). The HL strain exhibited significantly higher average percent pre-hatch mortality ($30.0 \pm 2.0\%$) than the GR strain ($P = 0.012$), but did not

differ from the GR×HL 50:50 cross. The GR×HL 50:50 cross and the GR strain did not differ from each other in average percent pre-hatch mortality (GR×HL 50:50: $23.9 \pm 2.7\%$, GR: $21.5 \pm 3.0\%$; $P = 1.000$).

On average, the greatest mortality was observed in the form of eggs that turned white and were picked off prior to eyeing up ($14.6 \pm 7.8\%$), and eggs that did not survive to eye-up and were removed following bumping of the eyed eggs ($11.4 \pm 4.3\%$). On average, only $3.6\% (\pm 1.6\%)$ of the eggs not removed during the physical shock removal did not survive to hatching; these were removed following hatching of all of the eggs within a PVC insert.

In addition to exhibiting a higher average percent pre-hatch mortality than the other strains and crosses, the GR×HL 75:25 cross was the only strain or cross to exhibit sensitivity to formalin, pre-hatch (Figure 2.4). GR×HL 75:25 eggs in the high formalin treatment exhibited significantly higher mortality ($53.4 \pm 2.4\%$) than did those in the control treatment ($32.6 \pm 5.3\%$; $P = 0.001$). None of the other strains or crosses exhibited a significant increase in mortality with an increase in formalin treatment concentration, pre-hatch ($P = 1.000$; Figure 2.4).

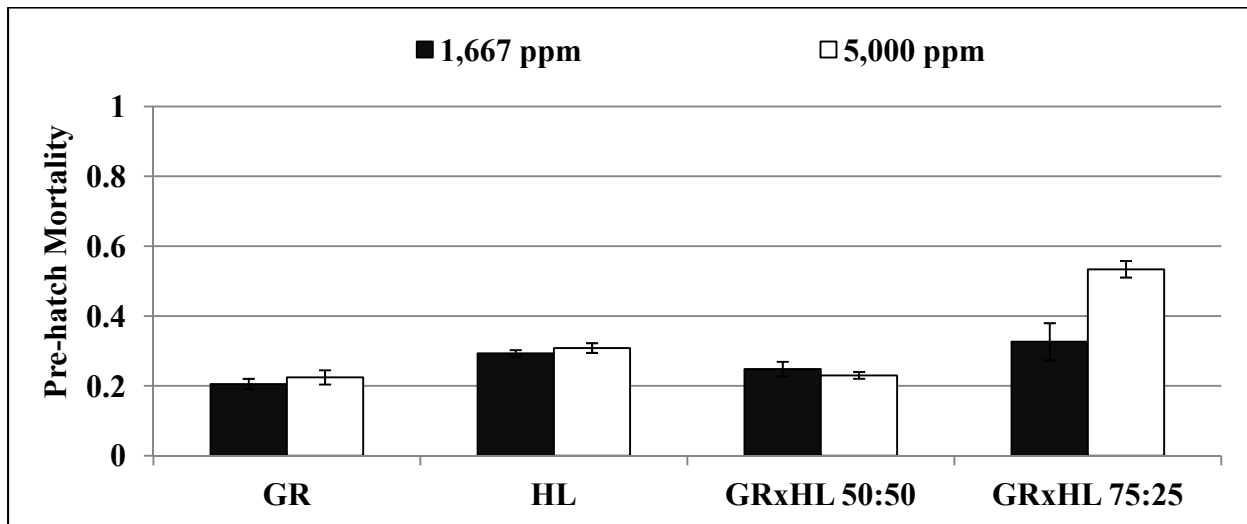


Figure 2.4. Average percent pre-hatch mortality (SE bars) by strain and treatment.

Average post-hatch mortality differed only among the strains ($F = 4.18$, $P = 0.023$); post-hatch mortality did not differ among formalin treatments ($F = 0.69$, $P = 0.419$), and the interaction between treatment and strain was not significant ($F = 0.49$, $P = 0.695$). The GR×HL 50:50 cross exhibited significantly higher average percent post-hatch mortality ($9.9 \pm 2.6\%$) than the GR strain ($4.6 \pm 3.2\%$; $P = 0.038$), but did not differ significantly from the GR×HL 75:25 cross ($6.3 \pm 2.9\%$) or HL strain ($4.7 \pm 3.0\%$; $P > 0.053$). The GR, GR×HL 75:25, and HL did not differ from each other in average percent post-hatch mortality ($P = 1.000$; Figure 2.5).

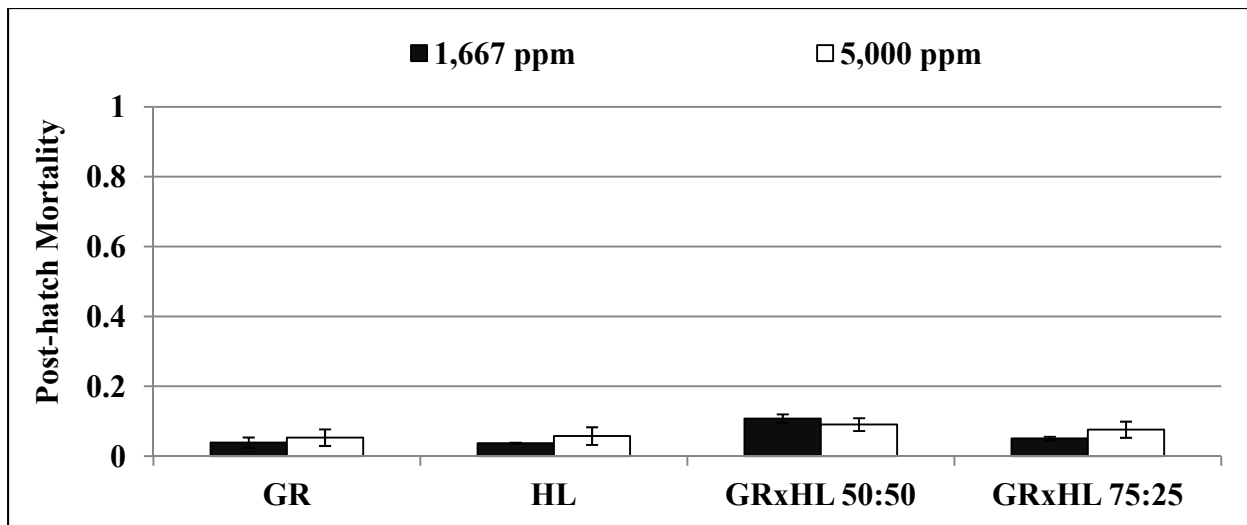


Figure 2.5. Average percent post-hatch mortality (SE bars) by strain and treatment.

On average, the greatest post-hatch mortality ($4.2 \pm 2.9\%$) 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 ($1.7 \pm 1.1\%$) occurred in the form of deformed, unhealthy fish that were removed while counting fish at the end of the experiment.

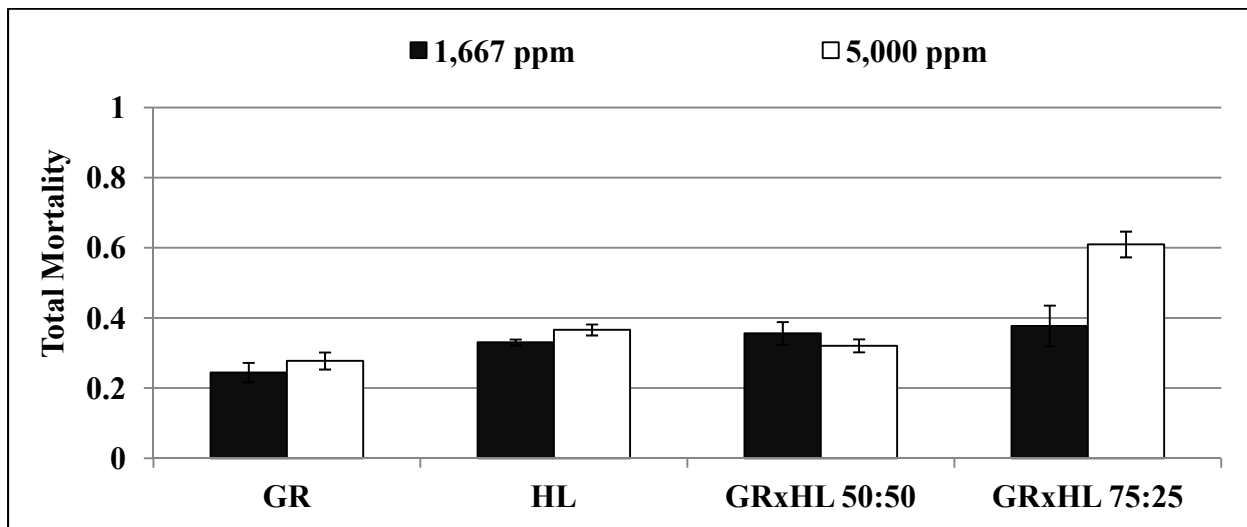


Figure 2.6. Average percent total mortality (SE bars) by strain and treatment.

Average percent total mortality differed both between the treatments ($F = 8.70$, $P = 0.009$), and among the strains/crosses ($F = 18.70$, $P < 0.001$); the interaction was also significant ($F = 6.33$, $P = 0.005$). Fish within the high formalin treatment exhibited significantly higher average percent total mortality ($39.3 \pm 13.9\%$) than the control treatment ($32.7 \pm 7.5\%$; $P = 0.009$). The GRxHL 75:25 cross exhibited significantly higher average percent total mortality ($49.3 \pm 14.8\%$) than any of the other strains or crosses ($P < 0.002$). The other three strains did not differ

significantly from each other in average percent total mortality (GR: $26.1 \pm 4.4\%$, GR×HL 50:50: $33.8 \pm 4.5\%$, HL: $34.8 \pm 2.7\%$; $P > 0.062$).

In addition to exhibiting the highest average percent mortality, the GR×HL 75:25 cross was the only strain or cross to exhibit sensitivity to formalin, as measured by percent total mortality differences among the treatments. GR×HL 75:25 fish in the high formalin treatment exhibited significantly higher mortality ($61.0 \pm 3.7\%$) than did those in the control treatment ($37.7 \pm 5.8\%$; $P = 0.003$). None of the other strains or crosses exhibited a significant increase in total mortality with an increase in formalin treatment concentration ($P = 1.000$; Figure 2.6).

Fingerling Formalin Sensitivity

Only two of the four trials were completed at the time of this report. One treatment was fish treated at 1,667 ppm as eggs, and treated for 30 minutes as fingerlings. The other treatment was fish treated at 5,000 ppm as eggs, and treated for 30 minutes as fingerlings. For the fish treated at 1,667 ppm as eggs treated for 30 minutes as fingerlings, average percent mortality differed among the treatments ($F = 18.41$, $P < 0.001$), but did not differ among the strains or crosses ($F = 2.45$, $P = 0.081$); however, the interaction between treatment and strain/cross was significant ($F = 3.42$, $P = 0.005$). Fish in the 500 ppm treatments exhibited significantly higher average (\pm SD) mortality ($15.4 \pm 13.0\%$) than any of the other treatments ($P < 0.001$). Fish in the control (0 ppm), 167 ppm, and 250 ppm treatments did not differ from each other in average mortality (0 ppm: $0.4 \pm 0.8\%$, 167 ppm: $0.8 \pm 0.9\%$, 250 ppm: $3.3 \pm 2.4\%$; $P > 0.186$).

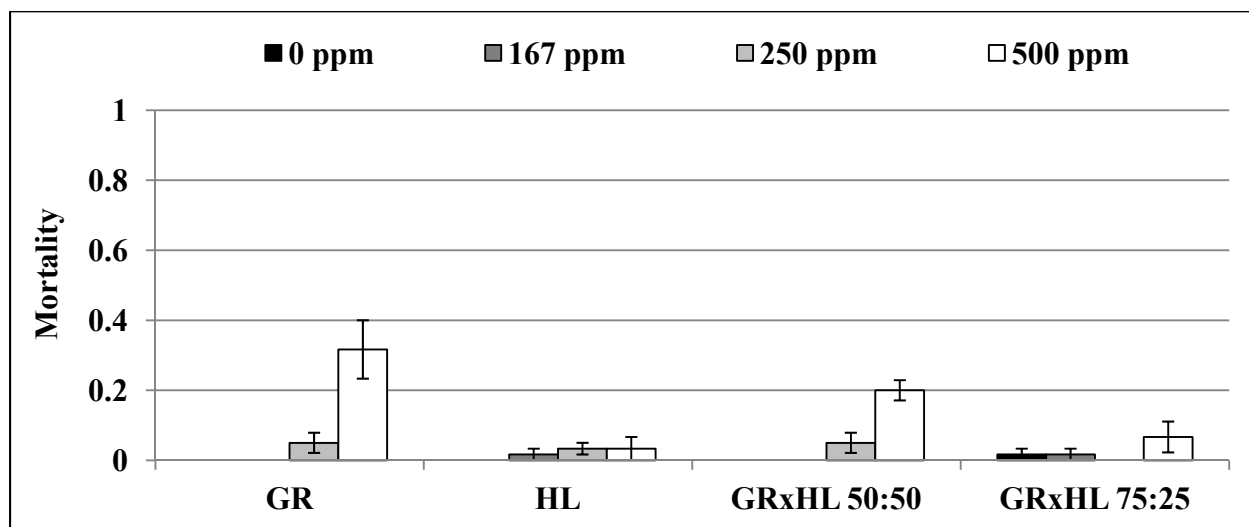


Figure 2.7. Average percent mortality (SE bars) by strain and treatment for fish treated at 1,667 ppm as eggs, and treated for 30 minutes as fingerlings.

The GR strain and GR×HL 50:50 cross both exhibited increases in mortality with an increase in formalin concentration. In the GR strain, fish within the 500 ppm treatment exhibited significantly higher average mortality ($31.7 \pm 14.4\%$) than GR strain fish within the other three formalin treatments ($P < 0.001$). GR strain fish within the 0 ppm, 167 ppm, and 250 ppm treatments did not differ in average mortality ($P = 1.000$; Figure 2.7). In the GR×HL 50:50

cross, fish within the 500 ppm treatment similarly exhibited significantly higher average mortality ($20.0 \pm 2.9\%$) than GR×HL 50:50 cross fish in the other three formalin treatments ($P = 0.005$). GR×HL 50:50 fish within the 0 ppm, 167 ppm, and 250 ppm treatments did not differ in average mortality ($P = 1.000$; Figure 2.7).

For the fish treated at 5,000 ppm as eggs, and treated for 30 minutes as fingerlings, average percent mortality differed among the treatments ($F = 30.73$, $P < 0.001$), and among the strains and crosses ($F = 3.63$, $P = 0.023$); however the interaction between treatment and strain/cross was not significant ($F = 1.54$, $P = 0.176$). Fish within the 250 ppm and 500 ppm treatments exhibited significantly higher average mortality than fish within the 0 ppm and 167 ppm treatments ($P < 0.001$), but did not differ from each other in average mortality (250 ppm: $8.3 \pm 5.9\%$, 500 ppm: $12.9 \pm 6.4\%$; $P = 0.749$). Fish within the 0 ppm and 167 ppm treatments did not exhibit any mortality (Figure 2.8). GR strain fish exhibited significantly higher average mortality ($8.8 \pm 10.6\%$) than the GR×HL 75:25 cross ($2.1 \pm 3.1\%$; $P = 0.021$), but did not differ in average mortality from the GR×HL 50:50 cross ($5.8 \pm 6.9\%$) or the HL strain fish ($4.6 \pm 6.3\%$; $P > 0.574$). The HL strain, GR×HL 50:50 cross, and GR×HL 75:25 cross did not differ from each other in average mortality ($P > 0.161$).

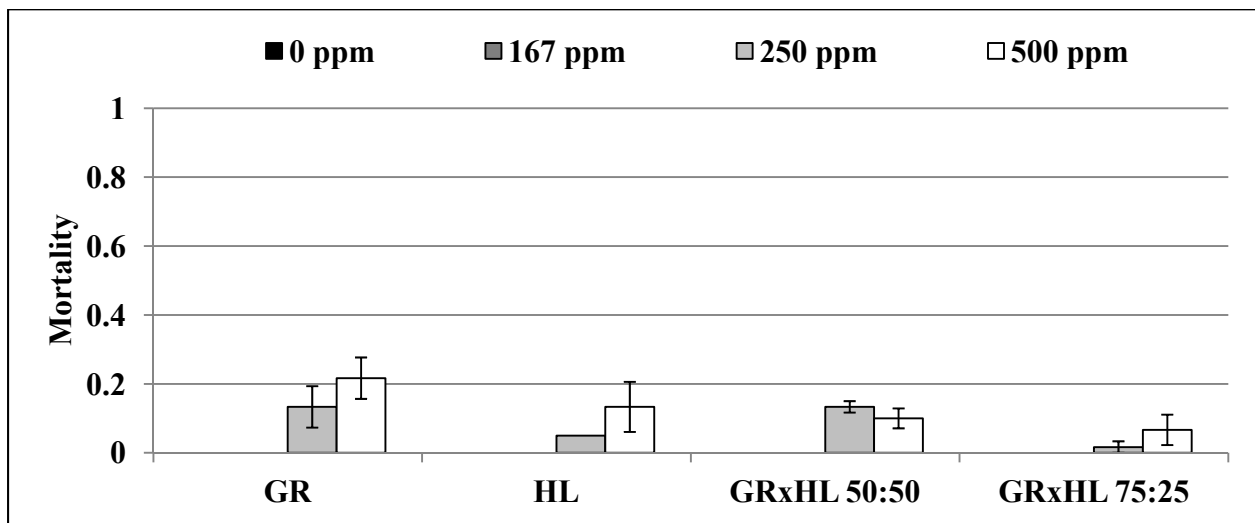


Figure 2.8. Average percent mortality (SE bars) by strain and treatment for fish treated at 5,000 ppm as eggs, and treated for 30 minutes as fingerlings.

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 the egg formalin sensitivity 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 75:25 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. In a similar experiment conducted in 2011, the GR×HL 50:50 cross also showed an increase in mortality with an increase in formalin concentration. Taken together, these results suggest that crosses between the two pure strains are more likely to exhibit a formalin sensitivity during egg treatments than either of the pure strains, and caution should be used when using higher concentrations of formalin to treat GR-cross eggs.

The results of the fingerling formalin exposure experiment suggest that the GR strain does exhibit sensitivity to formalin when treated as fingerlings. In addition, the GR×HL 50:50 strain also showed some sensitivity to formalin. Currently, it is difficult to determine what may have caused the sensitivity in the GR×HL 50:50 cross. If sensitivity were genetically determined, and the GR strain was more sensitive than the HL strain, we would have expected to see higher mortality in the GR×HL 75:25 as it contains a higher proportion of GR genes than the GR×HL 50:50 cross; further investigation is needed. The results do suggest, however, that caution should be used when using increased formalin concentrations to treat heavy infestations by external parasites on GR and GR-cross fish. The results of both trials conducted thus far suggest that if formalin treatments are necessary, a concentration of 167 ppm for 30 minutes should result in little to no mortality; however, mortality may increase even with this concentration if environmental or health stressors are elevated at the time of treatment.

Dissolved Oxygen Tolerance of Rainbow Trout Strains

INTRODUCTION

Whirling disease-resistant rainbow trout strains have been developed for production in Colorado's hatchery system and use in wild reintroductions. However, information on culturing these strains and potential deviations from the norm in the hatchery environment is still being gathered. One of the questions of interest is whether these strains and crosses exhibit differences in dissolved oxygen minimum tolerances, and how these differences may affect hatchery culture practices. There has been little work dedicated to determining if differences in dissolved oxygen tolerances exist among rainbow trout strains; however, dissolved oxygen tolerances have been examined in stocks of cutthroat trout (Wagner et al. 2001). The objectives of this experiment were to determine the critical dissolved oxygen minimum for four strains of rainbow trout currently cultured in Colorado, and to determine if there are differences in dissolved oxygen tolerance among the strains.

In addition, chemical treatment, for example using formalin to treat external parasite infestations, can change dissolved oxygen levels during treatment. Formalin is known to remove oxygen from the water (1 ppm oxygen for every 5 ppm formalin within 30-36 hours; Piper et al. 1982). Therefore, treatment with formalin could change dissolved oxygen tolerances in cultured rainbow trout. A third objective of this experiment was to determine if there was an interactive effect of formalin treatment and dissolved oxygen on the critical dissolved oxygen minimum in the four strains of rainbow trout.

METHODS

Strains and Rearing Procedures

Four whirling disease-resistant rainbow trout strains and crosses were used to determine differences in critical dissolved oxygen minima: 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.

Rainbow trout were spawned and reared at the CPW Bellvue Fish Research Hatchery, where they were held until they reached 30, 60, 90, or 120 days post-swim-up; critical dissolved oxygen trials were conducted on fish at 30, 60, 90, and 120 days post-swim-up to determine if age/fish size played a role in dissolved oxygen tolerance. One week prior to commencement of the dissolved oxygen trials, fish were transported from the Research Hatchery to the CPW Aquatic Toxicology Lab in Fort Collins, Colorado. Fish were transported in five gallon water coolers filled with hatchery water and supplied with air from a pump connected to the vehicle auxiliary power system; total loading and transport time was approximately 30 minutes. Upon arrival at the Aquatic Toxicology Lab, water from within the lab was mixed with water from the hatchery to allow fish to slowly acclimate to the water temperature and conditions of the lab.

Fish were held in four, 29 gallon aquaria, one for each strain or cross. Well water was supplied to the tanks at a flow of five gpm and maintained at a temperature of 12°C. Thirty and 60 day old fish were fed a diet of Rangen size 0 soft moist feed, and 90 and 120 day old fish were fed a diet of Rangen size 1 or 2 soft moist feed. Fish were fed at 2.5% of their body weight to maintain both fish size and health with minimal growth, and feeding proportions were recalculated on a daily basis according to tank density. When fish were not being used in the dissolved oxygen trials, they were fed four times a day (0900, 1200, 1500, and 1800) using Fish Mate automatic feeders. Fish used in the trials were transferred out of the holding tanks before the 0900 feeding and were not fed on the day in which they were used in a trial.

Experimental Procedures

Dissolved oxygen experiments were conducted in two, 2.5 liter glass aquariums insulated on four of the six sides to prevent temperature fluctuations over the course of the two hour trial period (Figure 2.9); having two aquariums allowed two trials to be conducted simultaneously. Prior to commencement of a trial, aquariums were filled with 200 ml of 12°C water supplied from the holding tanks of the strain being used in that trial. Tank temperatures were maintained at 12°C using in-tank customized cooling systems (Figures 2.9, 2.10). The cooling system consisted of a custom-shaped titanium chilling rod, and used peristaltic pumps to move ice water from a two gallon water cooler containing an ice bath through the chilling rod when needed. Temperature regulators monitored tank temperatures using temperature probes. If water temperatures increased to 12.1°C or greater, temperature regulator sensors would initiate the peristaltic pumps, running ice water through the chilling rod until temperatures returned to 12°C. In addition, Corning magnetic stirrers and Teflon-coated magnetic stir bars were used to circulate the water in the tanks and maintain temperature.

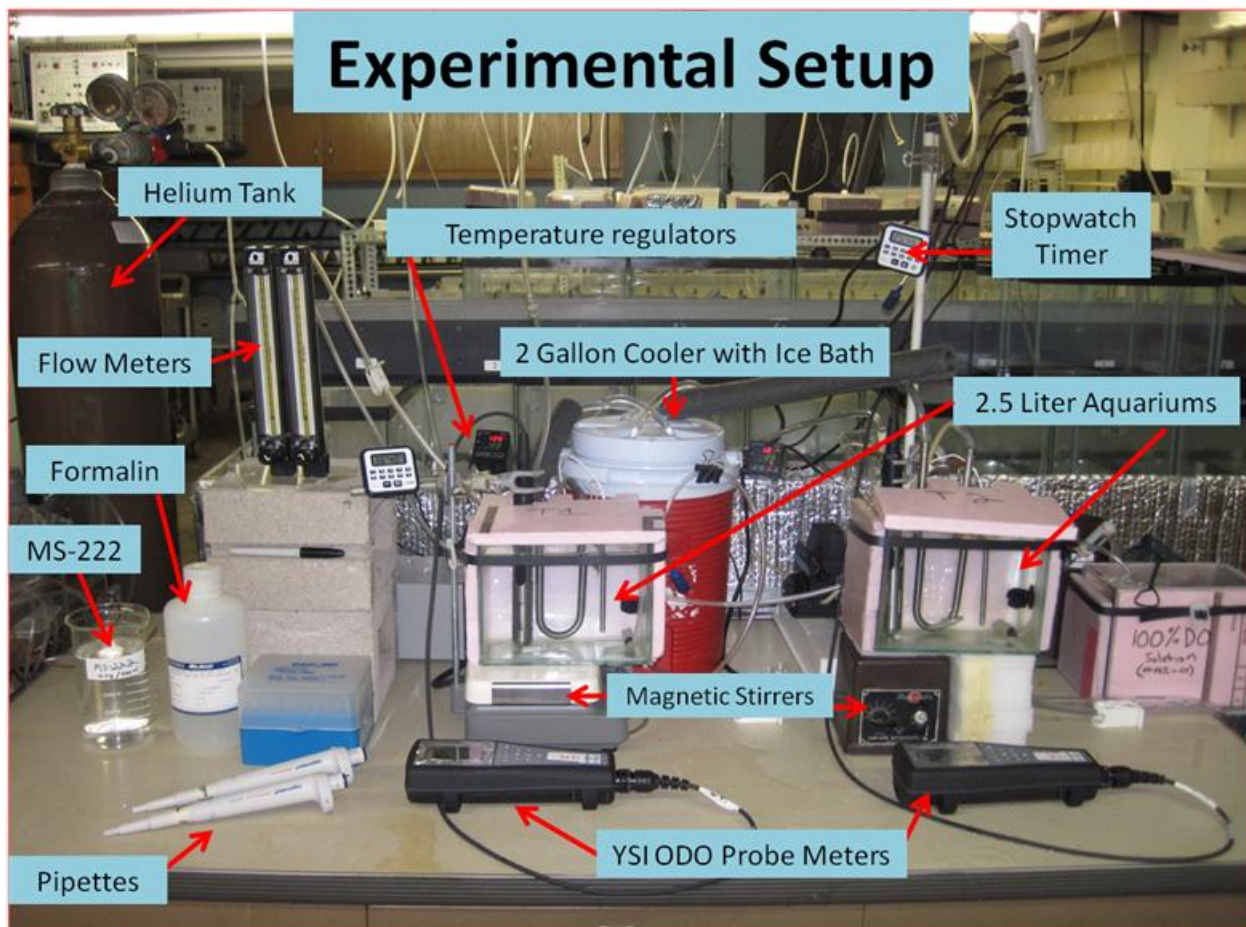


Figure 2.9. Experimental setup for the rainbow trout strain dissolved oxygen tolerance experiments conducted in the CPW Aquatic Toxicology Lab.

Dissolved oxygen levels at the start of each trial were maintained at 100% saturation using an oxygen diffuser. Helium, bubbled into the tanks through a diffuser stone, was used to reduce dissolved oxygen levels at an exponential rate over the course of the one hour trial (Figure 2.9, 2.10). The objective was to reduce dissolved oxygen levels to less than 10% saturation over the course of the hour trial, which was accomplished using a helium delivery rate of 125 cc per minute. Dissolved oxygen concentration (ppm) and saturation (%) were measured during the experiment using YSI Optical Dissolved Oxygen (ODO) sensors, which logged both quantities, as well as temperature and barometric pressure, every minute throughout the experimental trial.

Experiments were conducted at each of four ages (30, 60, 90 and 120 days post-swim-up), and critical dissolved oxygen minimums were measured in the absence (0 ppm) and presence of formalin (167 and 250 ppm). Ten replicates from each strain were tested at each formalin concentration for a total of 30 trials per strain at each of the four ages. Formalin treatment and strain were randomly assigned to a tank, and the order in which the trials were conducted was randomized prior to initiation of the trials within an age group.

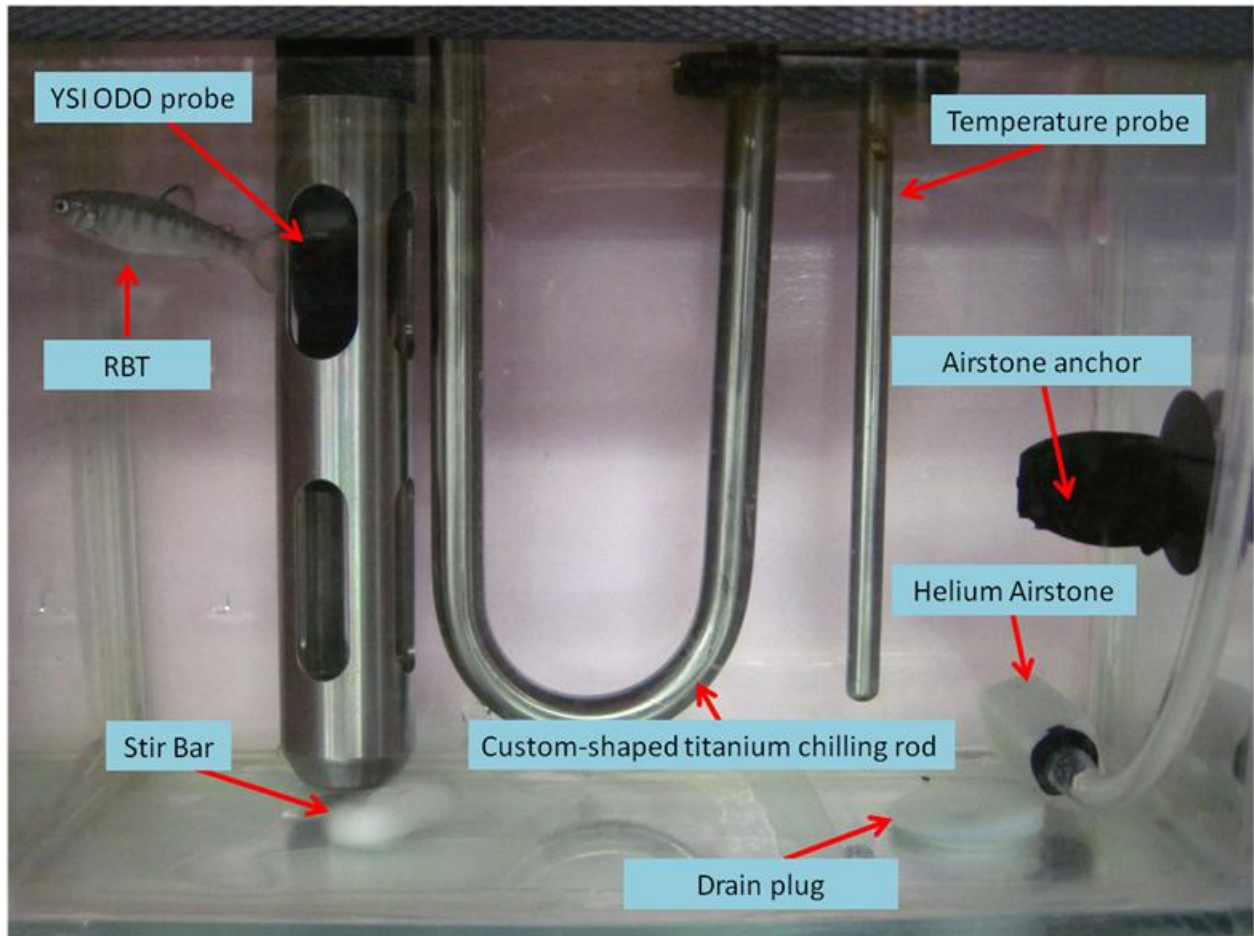


Figure 2.10. Tank cooling system, helium delivery system, and temperature and dissolved oxygen monitoring systems.

To begin each trial, a fish was randomly selected from the strain holding tank and placed into the experimental tanks where they were allowed to acclimate for one hour post-handling; water temperature and dissolved oxygen levels within the experimental tank were maintained at 12°C and 100% saturation, respectively, during the one hour acclimation period. Following the one hour acclimation period, helium regulated to 125 cc per minute was injected into the tanks to reduce the dissolved oxygen concentration within the tank. If the trial included exposure to formalin, formalin was also added at the end of the one hour acclimation period.

Critical minimums were defined as the point at which a fish lost its equilibrium for a period of ten seconds (final loss of equilibrium; FLOE). Once a fish achieved FLOE, the dissolved oxygen levels were recorded from the YSI ODO meters, and fish were immediately transferred to water held at 100% saturation to recover. Prior to the start of the next trial, tanks were flushed and rinsed twice to ensure that no formalin residue remained. Fish used in the experimental trials were held for 24 hours after experimentation to determine if delayed mortality occurred following exposure to formalin and or critical dissolved oxygen minimums. After 24 hours, fish

were removed from their holding tanks, euthanized using an overdose of MS-222, measured, and weighed.

Statistical analyses were conducted using the GLM procedure in SAS (SAS Institute 2011). Differences in dissolved oxygen critical minimums were analyzed within each age group using a two-factor analysis of covariance (ANCOVA), with strain/cross and formalin treatment as the factors, and weight as the covariate ($N = 120$). Values for all analyses were reported from the type III sum of squares. If significant ($P < 0.05$) effects were identified, the least-squares means method with a Bonferroni adjustment was used to determine which treatments caused significant differences in dissolved oxygen minimums within a strain or cross.

RESULTS

At thirty days post-swim-up, there were significant ($F = 27.53$, $P < 0.001$) differences in weight among the experimental rainbow trout used in the dissolved oxygen critical minimum trials; therefore, weight influenced critical dissolved oxygen minimums at 30 days post-swim-up. However, at 60, 90, and 120 days post-swim-up, weight did not differ significantly ($F < 0.96$, $P > 0.329$) among the experimental fish, and therefore did not influence critical dissolved oxygen minimums at these ages.

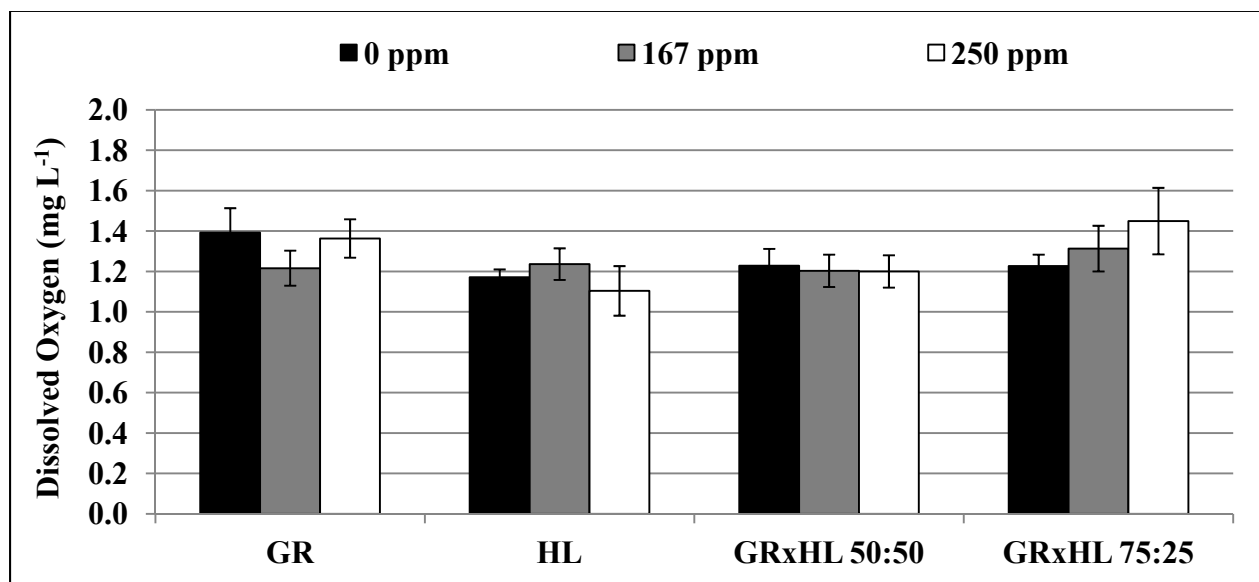


Figure 2.11. Average critical dissolved oxygen minimums (mg L^{-1}) for the GR strain, HL strain, GR \times HL 50:50 cross, and GR \times HL 75:25 cross exposed to 0, 167, or 250 ppm formalin at 30 days post-swim-up.

At 30 days post-swim-up, critical dissolved oxygen minimums differed among the strains and crosses ($F = 4.95$, $P = 0.003$), but did not differ among the formalin treatments ($F = 2.12$, $P = 0.126$), and the interaction between strain/cross and formalin treatment was not significant ($F = 0.58$, $P = 0.747$; Figure 2.11). Average critical dissolved oxygen minimum (\pm SD) was significantly lower in the HL strain ($1.17 \pm 0.07 \text{ mg L}^{-1}$) than the H \times H 75:25 cross (1.33 ± 0.11

mg L⁻¹; $P = 0.031$) or the GR strain (1.32 ± 0.09 mg L⁻¹; $P = 0.007$); however, dissolved oxygen minimum did not differ from the GR×HL 50:50 cross (1.21 ± 0.02 mg L⁻¹; $P = 1.000$). The GR strain, GR×HL 50:50 cross, and GR×HL 75:25 cross did not differ from each other in average critical dissolved oxygen minimums ($P > 0.088$).

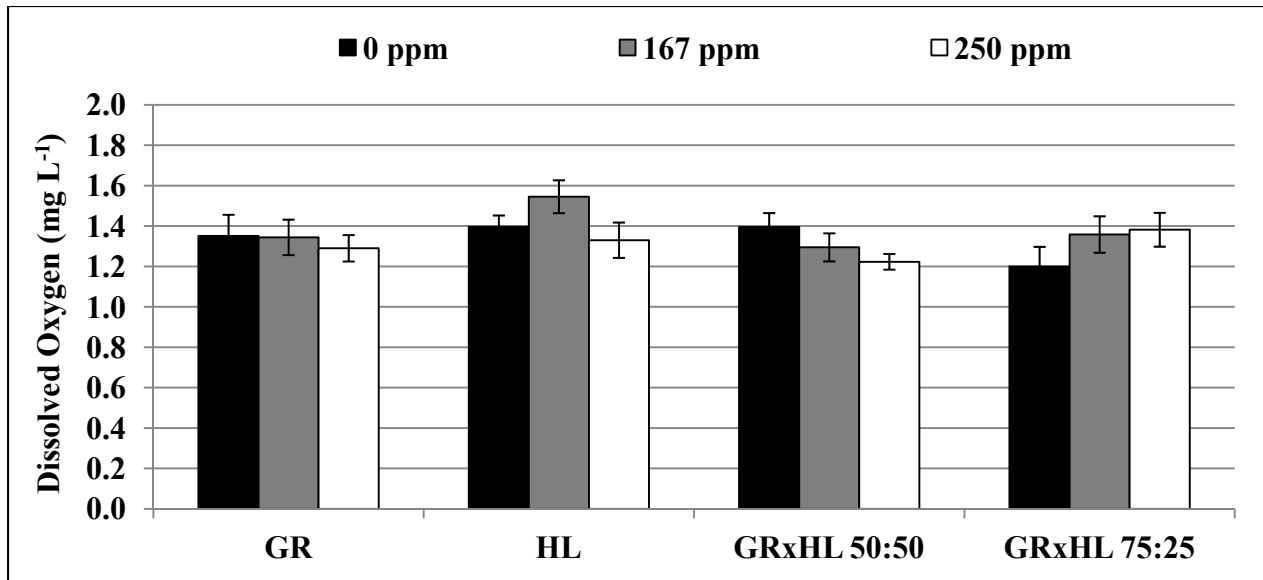


Figure 2.12. Average critical dissolved oxygen minimums (mg L⁻¹) for the GR strain, HL strain, GR×HL 50:50 cross, and GR×HL 75:25 cross exposed to 0, 167, or 250 ppm formalin at 60 days post-swim-up.

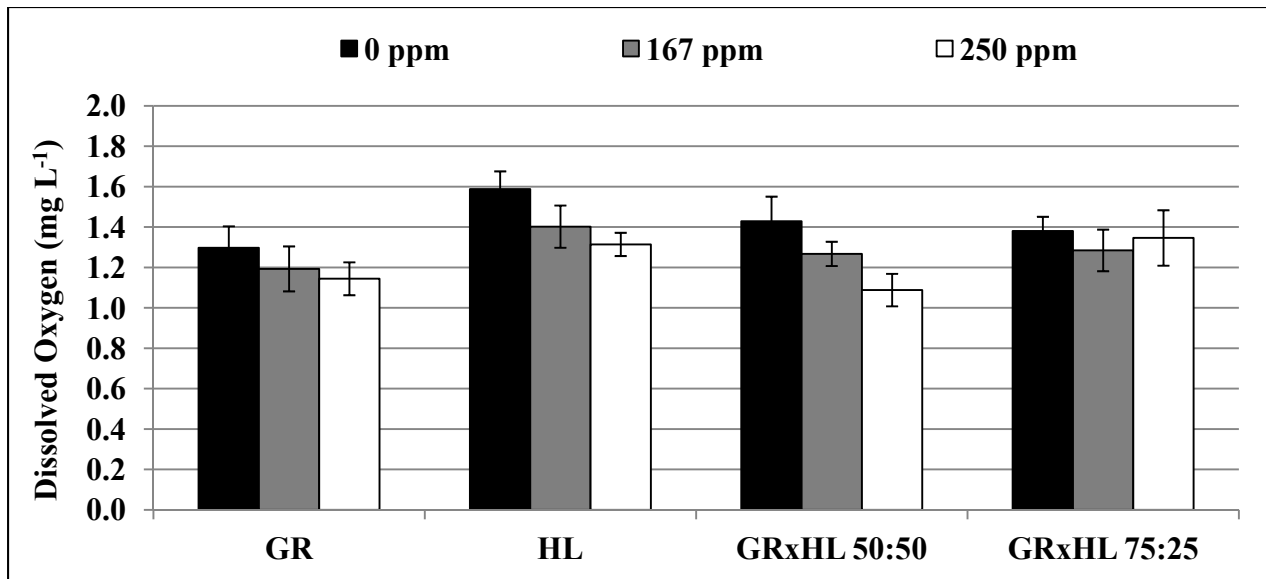


Figure 2.13. Average critical dissolved oxygen minimums (mg L⁻¹) for the GR strain, HL strain, GR×HL 50:50 cross, and GR×HL 75:25 cross exposed to 0, 167, or 250 ppm formalin at 90 days post-swim-up.

At 90 days post-swim-up, critical dissolved oxygen minimums did not differ among the strains or crosses ($F = 1.52, P = 0.215$), but did differ among the formalin treatments ($F = 4.97, P = 0.009$); the interaction between strain/cross and formalin treatment was not significant ($F = 0.55, P = 0.767$; Figure 2.13). Fish within the 0 ppm formalin treatment had significantly higher average critical dissolved oxygen minimums ($1.42 \pm 0.12 \text{ mg L}^{-1}$) than did fish within the 250 ppm formalin treatment ($1.22 \pm 0.13 \text{ mg L}^{-1}; P = 0.009$). Fish within the 167 ppm formalin treatment ($1.29 \pm 0.09 \text{ mg L}^{-1}$) did not differ in critical dissolved oxygen minimums from those in either the 0 ppm or 250 ppm formalin treatments ($P > 0.081$).

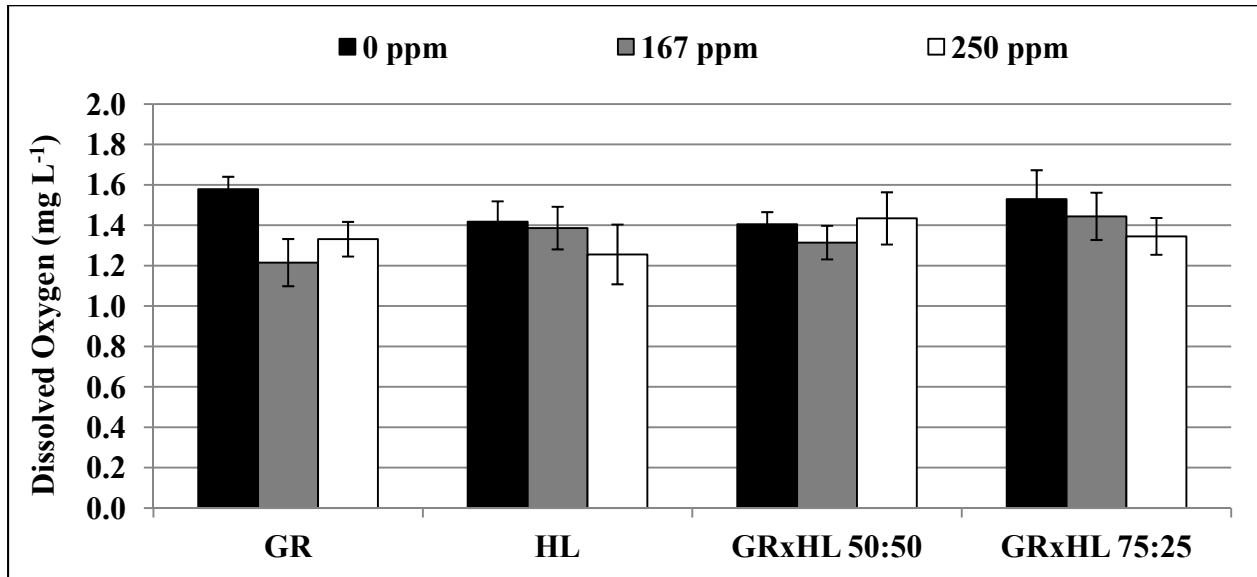


Figure 2.14. Average critical dissolved oxygen minimums (mg L^{-1}) for the GR strain, HL strain, GR \times HL 50:50 cross, and GR \times HL 75:25 cross exposed to 0, 167, or 250 ppm formalin at 120 days post-swim-up.

At 60 days post-swim-up, critical dissolved oxygen minimums did not differ among the strains or crosses ($F = 1.52, P = 0.215$) or among the formalin treatments ($F = 1.12, P = 0.330$), and the interaction between strain/cross and formalin treatment was also not significant ($F = 1.22, P = 0.300$; Figure 2.12). Similarly, at 120 days post-swim-up, critical dissolved oxygen minimums did not differ among the strains or crosses ($F = 0.27, P = 0.849$) or among the formalin treatments ($F = 2.44, P = 0.092$), and the interaction between strain/cross and formalin treatment was also not significant ($F = 0.76, P = 0.599$; Figure 2.14).

CONCLUSIONS

Overall, other than at 30 days post-swim-up when differences in weight influenced differences in critical dissolved oxygen minimums, there did not appear to be strain or cross differences in dissolved oxygen minimums. This was not entirely unexpected; in a similar experiment conducted with cutthroat trout, strain differences in dissolved oxygen minimums were also not apparent (Wagner et al. 2001). Treatment with formalin did not seem to influence critical dissolved oxygen minimums at most life stages. Interestingly, at 90 days post-swim-up, when differences among formalin treatments were observed, fish within the 0 ppm formalin treatment

exhibited higher critical dissolved oxygen minimums than those within the high (250 ppm) formalin treatment, suggesting that the presence of formalin may increase the tolerance of hypoxia at some life stages.

In culture systems, equipment can fail, often during the worst possible times (e.g., during treatment with formalin). This experiment demonstrates that dissolved oxygen levels must get below 2.0 mg L⁻¹ before potential problems may be observed during equipment failure, whether or not equipment failure occurs during formalin treatment.

References

- Barnes, M. E., K. Winterstein, W. A. Slayer, and R. J. Cordes. 2000. Use of formalin during incubation of rainbow trout eyed eggs. *North American Journal of Aquaculture* 62:54-59.
- Bills, T. D., L. L. Marking, and J. H. Chandler Jr. 1977. Formalin: its toxicity to nontarget aquatic organisms, persistence, and counteraction. *Investigations in Fish Control Number 73*, U. S. Department of the Interior, Washington, D. C.
- Howe, G. E., L. L. Marking, T. D. Bills, and T. M. Schreier. 1995. Efficacy and toxicity of formalin solutions containing paraformaldehyde for fish and egg treatments. *The Progressive Fish-Culturist* 57:147-152.
- Marking, L. L., J. J. Rach, and T. M. Schreier. 1994. Evaluation of antifungal agents for fish culture. *The Progressive Fish-Culturist* 56:225-231.
- Piper, R. G., and C. E. Smith. 1973. Factors influencing formalin toxicity in trout. *The Progressive Fish-Culturist* 35:78-81.
- Piper, R. G., I. B. McElwain, L. E. Orme, J. P. McCaren, L. G. Fowler, and J. R. Leonard. 1982. *Fish hatchery management*. U. S. Fish and Wildlife Service, Washington, D. C.
- SAS Institute, Inc. 2011. *SAS system software, Release 9.1*. Cary, North Carolina.
- Wagner, E. J., R. E. Arndt, and M. Brough. 2001. Comparative tolerance of four stocks of cutthroat trout to extremes in temperature, salinity, and hypoxia. *Western North American Naturalist* 61(4):434-444.
- Wagner, E. J., R. E. Arndt, E. J. Billman, A. Forest, W. Cavender. 2008. Comparison of the efficacy of iodine, formalin, salt, and hydrogen peroxide for control of external bacteria on rainbow trout eggs. *North American Journal of Aquaculture* 70:118-127.
- Wedemeyer, G. 1971. The stress of formalin treatments in rainbow trout (*Salmo gairdneri*) and coho salmon (*Oncorhynchus kisutch*). *Journal of the Fisheries Research Board of Canada* 28:1899-1904.

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.

INTRODUCTION

Earlier experiments demonstrated that the Hofer (GR) and Hofer \times Harrison Lake (GR \times HL) crosses have excellent growth and return-to-creel when stocked as catchable-sized fish. Colorado Parks and Wildlife is aggressively transitioning its brood facilities to produce larger numbers of GR or GR \times HL crosses 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 domestic 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 as the test site 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 that was historically used for rainbow trout spawning operations has also been operated more recently to remove white suckers from the reservoir in the months of May through July during their annual spawning run up the inlet stream. Numbers of suckers and trout captured in the trap vary from year to year, but 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. In 2010, 176 suckers and 153 salmonids were captured in the inlet trap. 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 four suckers and 31 salmonids were captured in the trap due to virtually non-existent runoff conditions. In 2013, 310 suckers and 271 salmonids were captured in the trap, with a large proportion of the salmonids being spawning rainbow-cutthroat crosses.

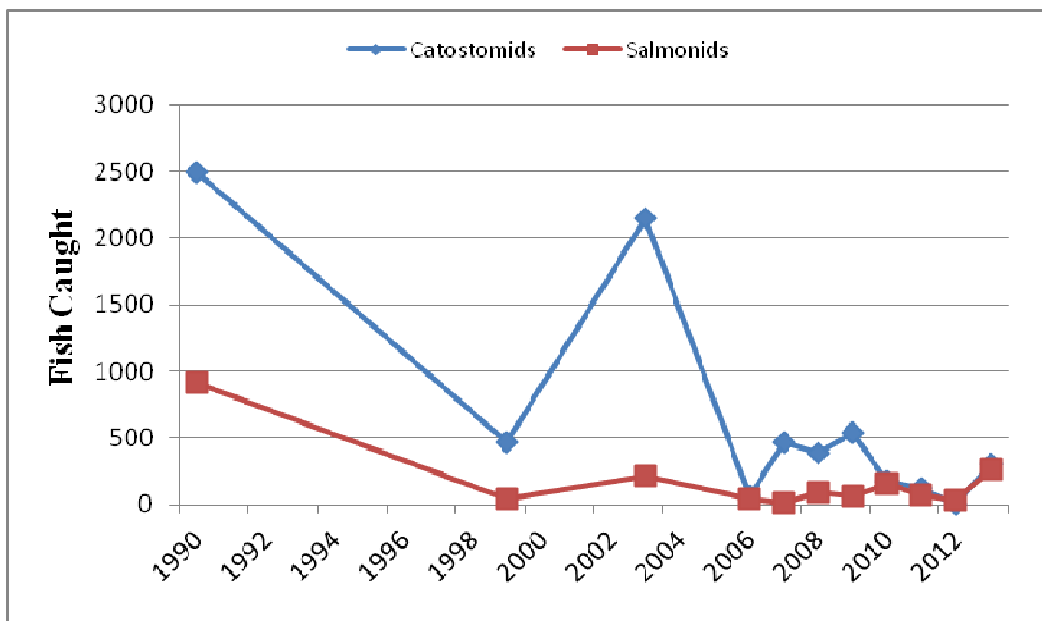


Figure 3.2. Number of catostomids and salmonids captured in the Parvin Lake inlet trap (May-July) in years where data are available.

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, compared with only 14.4% white suckers. In 2010, the proportions were 76.5% rainbow trout and 3.6% white suckers. In 2011, the proportions were 66.1% rainbow trout and 15.2% white suckers. In 2012, the proportions were 58.9% rainbows and 20.3% white suckers. This compares well with the figures from 2006, when over 60% of the total catch was white suckers.

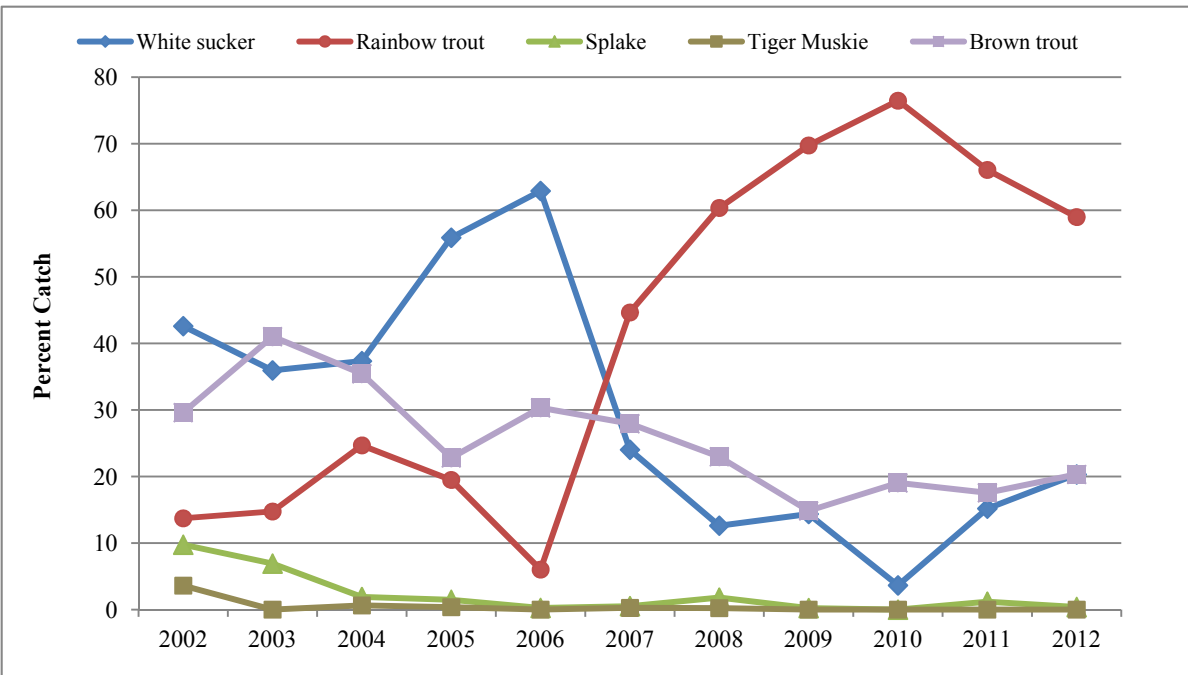


Figure 3.3. Percent of catch by species during fall electroshocking surveys for the years 2002 – 2012.

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, 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).

Fish stocked in 2011 included four varieties of fish, the HHN, RXN, pure GR, and Hofer × Colorado River (GR×CR) cross. In this trial, the HHN (a.k.a., HN2) were created using Snake River cutthroats of the spring spawning variety (SR2), and Hofer-Harrisons as described previously. The RXN were created using Tasmanian rainbows and the spring spawning Snake River cutthroat trout. 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			
	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

In 2012, four lots of fish were stocked, the pure GR, GR×CR cross, and HN2 were stocked as in the previous year. SR2 (pure spring-spawning Snake River cutthroat trout) were also stocked to determine if the pure Snake River cutthroat would perform as well as the HHN (HN2) variety. These fish were stocked on October 29, much later in the year than previous plants, so no fish from that plant were collected during the 2012 sampling events.

Table 3.4. Coded-wire tagged fish stocked in Parvin Lake during 2012.

2012 Plants				
Strain	GR	GR×CR	HHN (HN2)	SR2
Lbs	105.3	68.9	52.1	40.3
Number	2,116	2,116	2,116	2,116
Length (mm)	126.8	110.1	100.3	92.5

Collections of coded-wire tagged fish were made 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 through 2012, all fish were collected by evening boat electroshocking. Marked fish from each year of stocking were subjected to sampling for the first time in August of the year they were stocked. Sample goals (60-90 fish) could typically be accomplished by shocking the entire perimeter of the lake over a three-hour time period. Fish with coded wire tags were identified during the sampling event 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 evaluation. Fish length, weight, tag number and myxospore count for each fish was recorded in a database for each individual sampling event.

RESULTS

During 2012, the samples collected produced a representative cross-section of the fish stocked in previous years. Results for each individual year-class are listed separately below, along with cumulative catch from previous years of sampling to provide a comprehensive overview of each project year results. Fish stocked during 2007 and 2008 became more and more scarce during the 2012 sampling year, so results from those year-classes are limited.

2007 Year Class

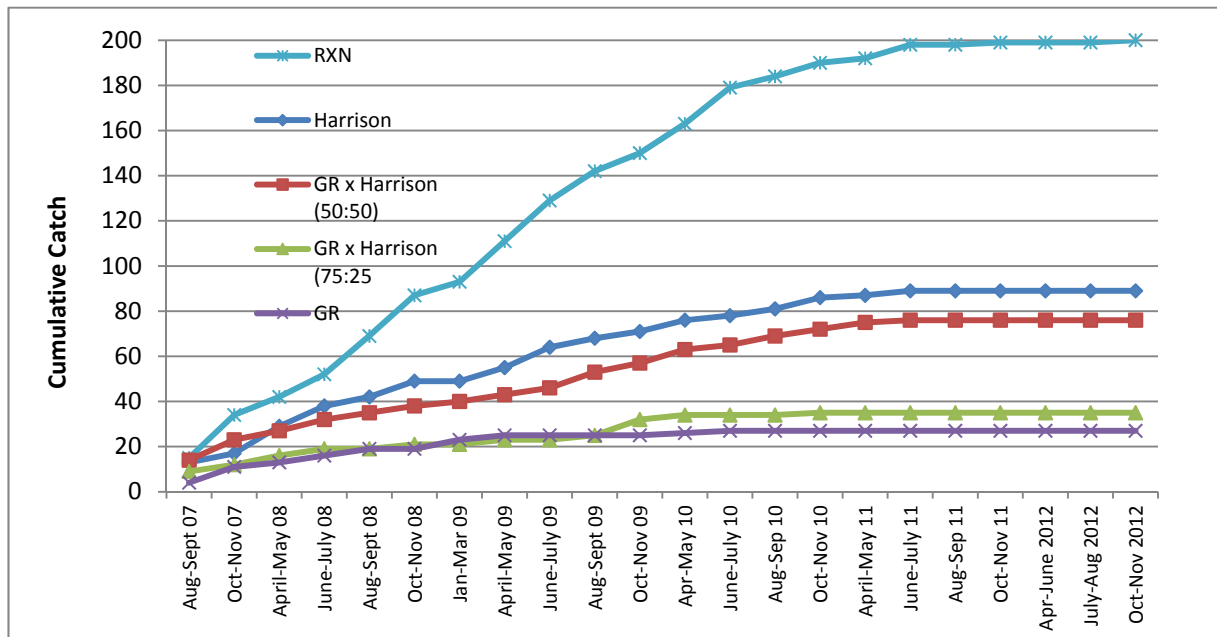


Figure 3.4. Cumulative catch for each of the five varieties of fingerling rainbow trout stocked in Parvin Lake in August 2007.

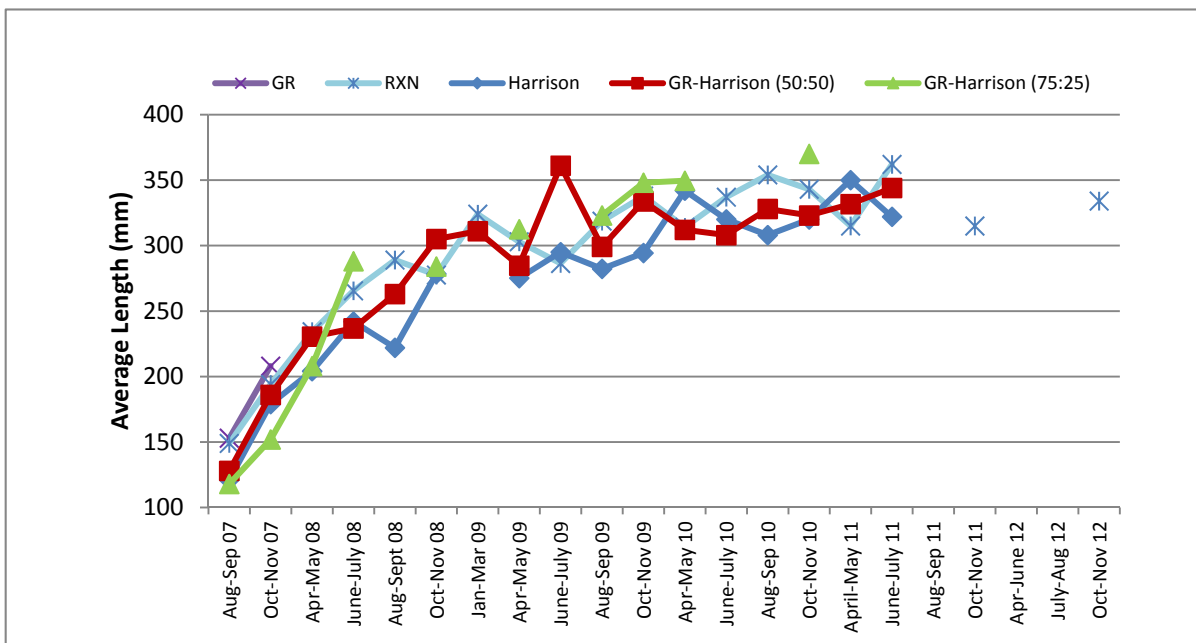


Figure 3.5. Fish length from 2007 through 2012 for each of the five varieties stocked in Parvin Lake in 2007.

Only one fish was found from the 2007 plant in the 2012 sampling. This fish was a 334 mm Bellaire-Snake River cross. All other varieties of fish from this year of stocking had either been caught or died by the end of the 2011 season. Cumulative totals of fish from the 2007 plant (Figure 3.4) resulted in the RXN strain being consistently more abundant in the samples than the other strains, contributing to 46.7% (200 fish) to the overall catch of 427 fish. The Harrison Lake strain contributed to 20.9% (89 fish) to the overall catch. The GR×HL (50:50) cross contributed to 17.8% (76 fish) of the overall catch. The GR×HL (72:25) cross contributed to 8.2% (35 fish) of the overall catch, and the pure GR strain contributed to 6.3% (27 fish) of the overall catch. Growth of the five strains was relatively equal for the 2007 plants (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 strains appear to plateau in growth once reaching 310 mm at about 24 months post-stocking.

2008 Year Class

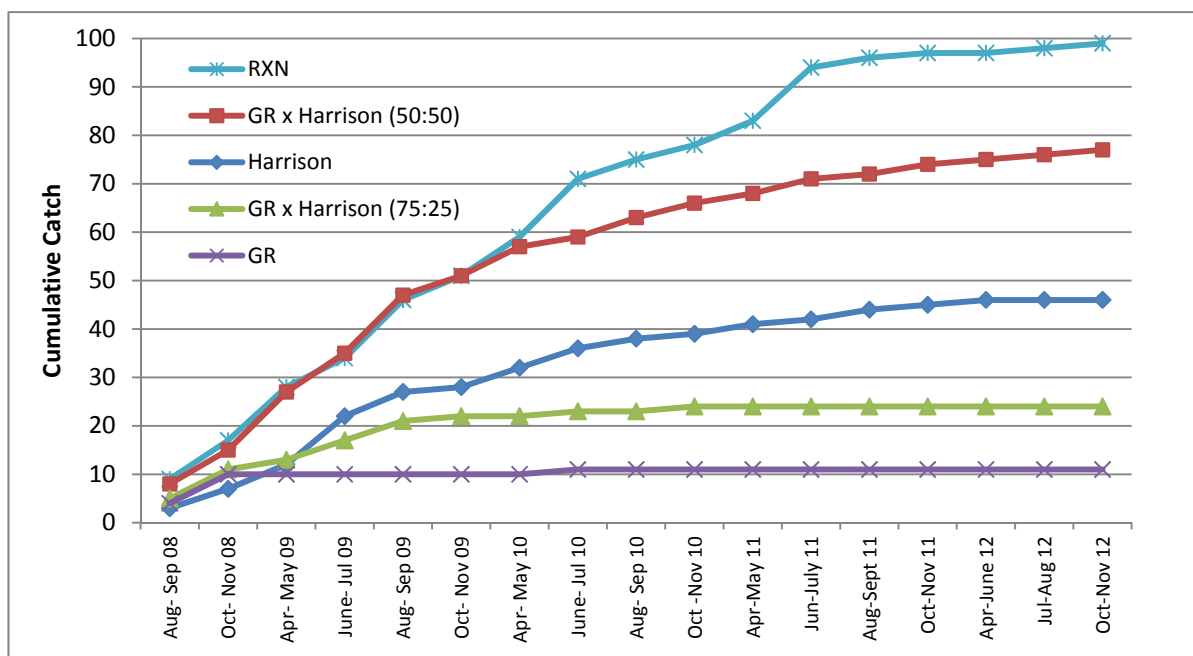


Figure 3.6. Cumulative catch for each of the five varieties of fingerling rainbow trout stocked in Parvin Lake in July 2008.

As with the 2007 year class, fish from the 2008 year-class were limited in number during the 2012 sampling. The collections from this year class were represented by only two Bellaire-Snake River crosses, one Harrison Lake strain fish and three GR×HL (50:50) fish. Cumulative multiple-year collections of fish from the 2008 plant resulted in the RXN and GR×HL (50:50) cross being more abundant in the samples than the other strains (Figure 3.6). The RXN strain contributed to 38.5% (99 fish) of the overall catch of 257 fish. The Harrison Lake strain contributed to 17.9% (46 fish) of the overall catch. The GR×HL (50:50) cross contributed to 29.9% (77 fish) of the overall catch. The GR×HL (72:25) cross contributed to 9.3% (24 fish) of the overall catch, and the pure GR strain contributed to 4.3% (11 fish) of the overall catch. Growth of the five strains was similar to that of the 2007 plants, leveling off at 310 mm at 24

months (Figure 3.7). The exception was the Harrison Lake strain, which grew more slowly than the other varieties, averaging 272 mm over the same time period. Although fish of this strain started out at a smaller size, the rate of growth was slower, and the size of the fish at which growth leveled off (270-280 mm) was also lower than that of the other strains.

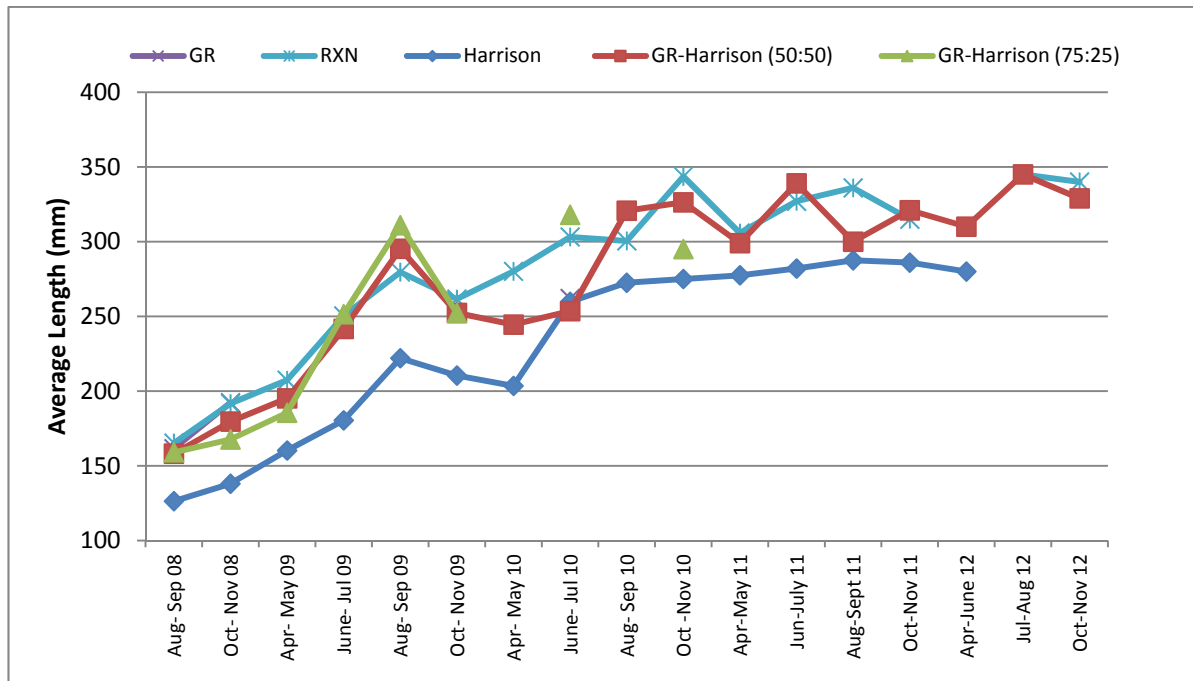


Figure 3.7. Fish length from 2008 through 2011 for each of the five varieties stocked in Parvin Lake in 2008.

2009 Year Class

The 2009 year class consisted of eight different varieties of fish. During the first year of sampling, numbers for each of the strains were relatively equal. However, differentiation of strain abundance began to appear during the 2010 collections. In 2012, the Harrison Lake, GR×HL (50:50 cross), GR×HL (75:25 cross) and Tasmanian strain were all relatively equal, with seven, six, seven, and eight fish captured from each of these groups, respectively. Only one pure GR and two GR×HL (87.5:12.5 cross) were found during the 2011 sampling events. The two strains that were found to be much more abundant than the other six varieties were RXN and HHN, from which 19 and 15 individuals were collected, respectively.

Cumulative collections of fish from the 2009 plant resulted in a total of 375 fish collected by the end of the 2012 field season (Figure 3.8). Harrison Lake were the most abundant at 19.5% of the catch (73 individuals), primarily because of the high catch rate for this variety in the 2010 field season. RXN and HHN were also present in high numbers, with 58 (15.5%) and 60 (16.0%) fish caught, respectively. Catch for the three GR×HL crosses (50:50, 75:25, and 87.5:12.5) was 44 (11.7%), 42 (11.2%), and 30 (8.0%). Catch for the Tasmanian strain was 42 (11.2%), and catch for pure GR strain fish was only 26 individuals (6.9%).

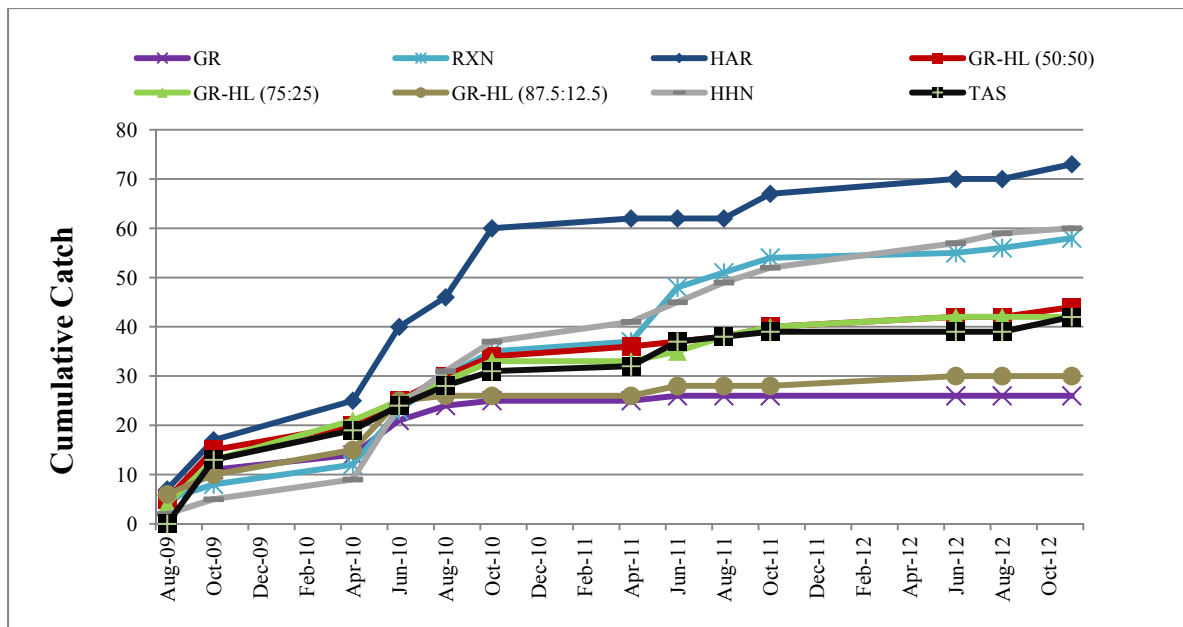


Figure 3.8. Cumulative catch for each of the eight varieties of fingerling rainbow trout stocked in Parvin Lake in July 2009.

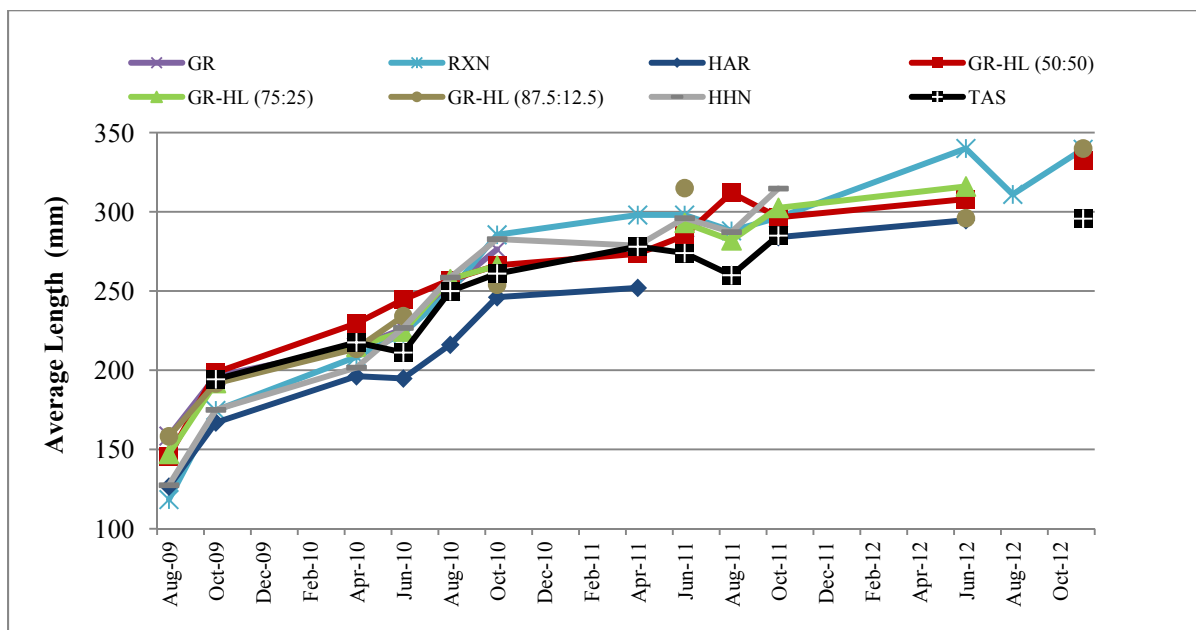


Figure 3.9. Fish length from 2009 through 2012 for each of the five varieties stocked in Parvin Lake in 2009.

As in the 2008 year-class, fish of the Harrison Lake strain appeared to grow more slowly than the other varieties, which were relatively comparable in size throughout all of the sampling occasions (Figure 3.9). However, the average length at 24 months for the Harrison Lake strain (284 mm) was comparable with the average of the other strains (299 mm) over this time period.

2010 Year Class

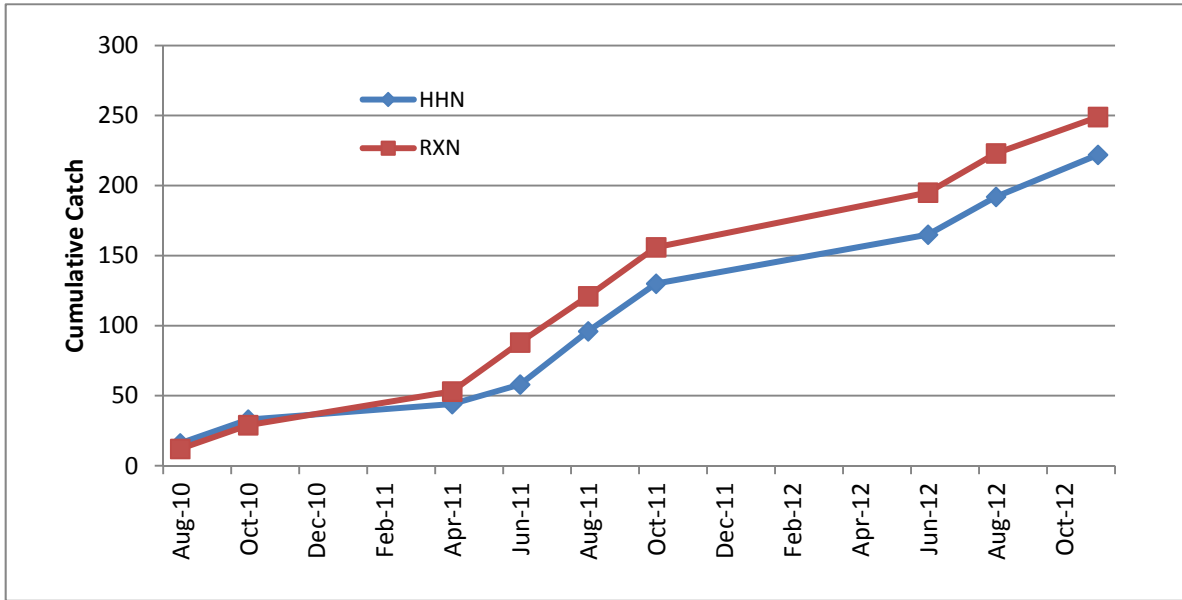


Figure 3.10. Cumulative catch for two varieties of fingerling rainbow trout stocked in Parvin Lake in July 2010.

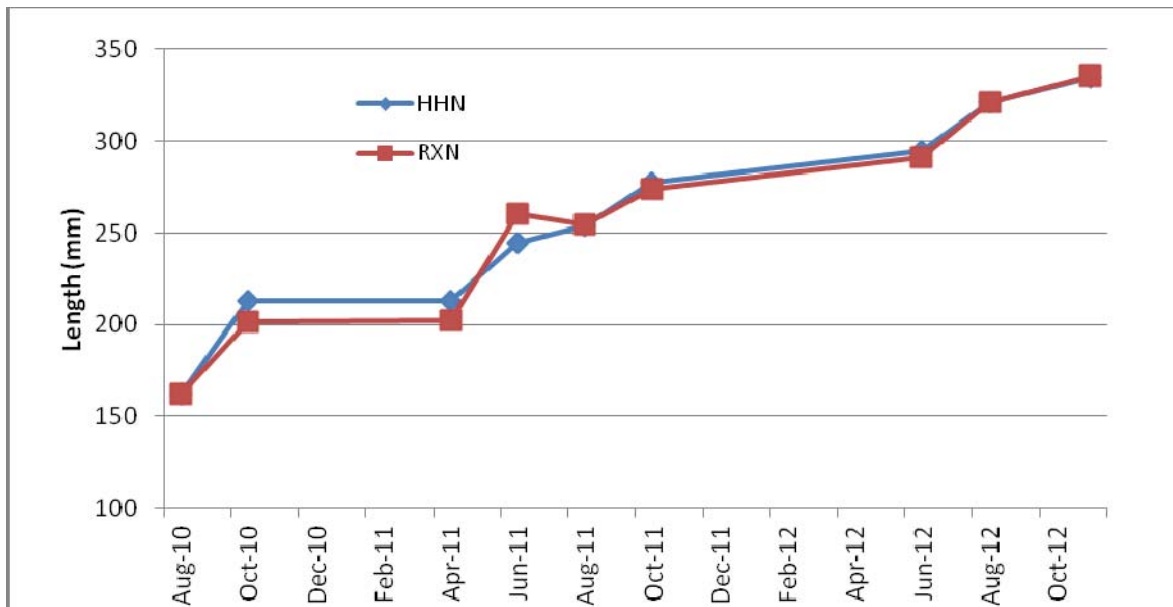


Figure 3.11. Fish length from 2010 to 2012 from the two varieties stocked in Parvin Lake in July 2010.

Sampling of the 2010 year class from 2010 through 2012 resulted in relatively equal numbers for fish of the HHN and RXN varieties. Sample numbers collected in 2010 were nearly identical, consisting of 33 HHN and 29 RXN fish. During 2011, 97 HHN and 127 RXN fish were collected. During 2012, 92 HHN and 93 RXN fish were collected. Collective sums were 222 HHN (47.1%) and 249 RXN (52.9%), for a total of 491 fish (Figure 3.10). Growth was also

nearly identical between the two strains. Average length at the end of the 2012 sampling season was 335 mm for both strains (Figure 3.11).

2011 Year Class

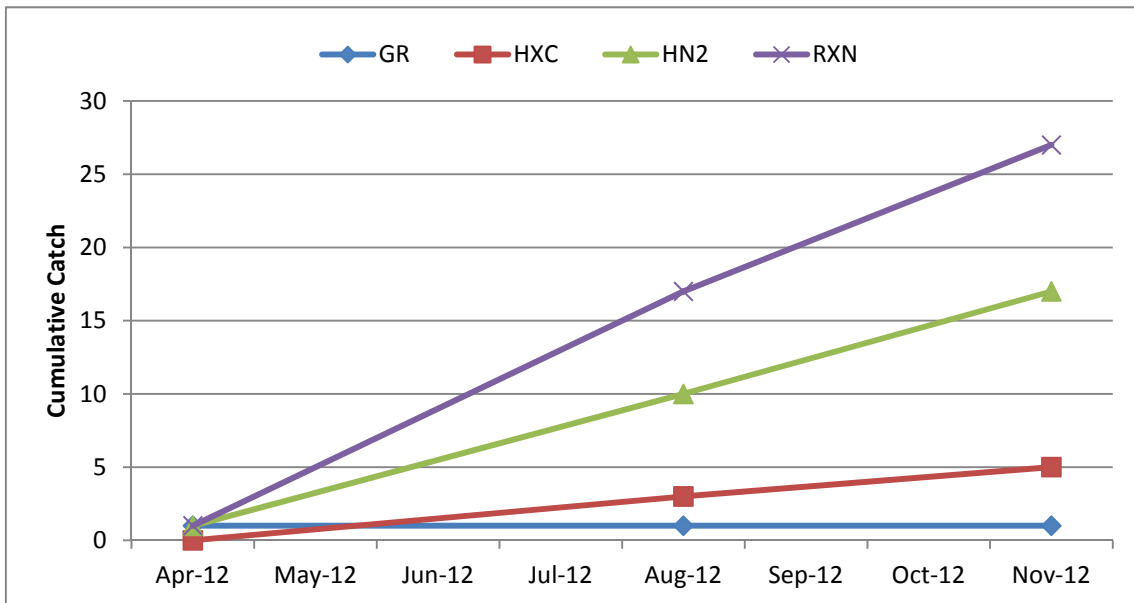


Figure 3.12. Cumulative catch for four varieties of fingerling rainbow trout stocked in Parvin Lake in November 2011.

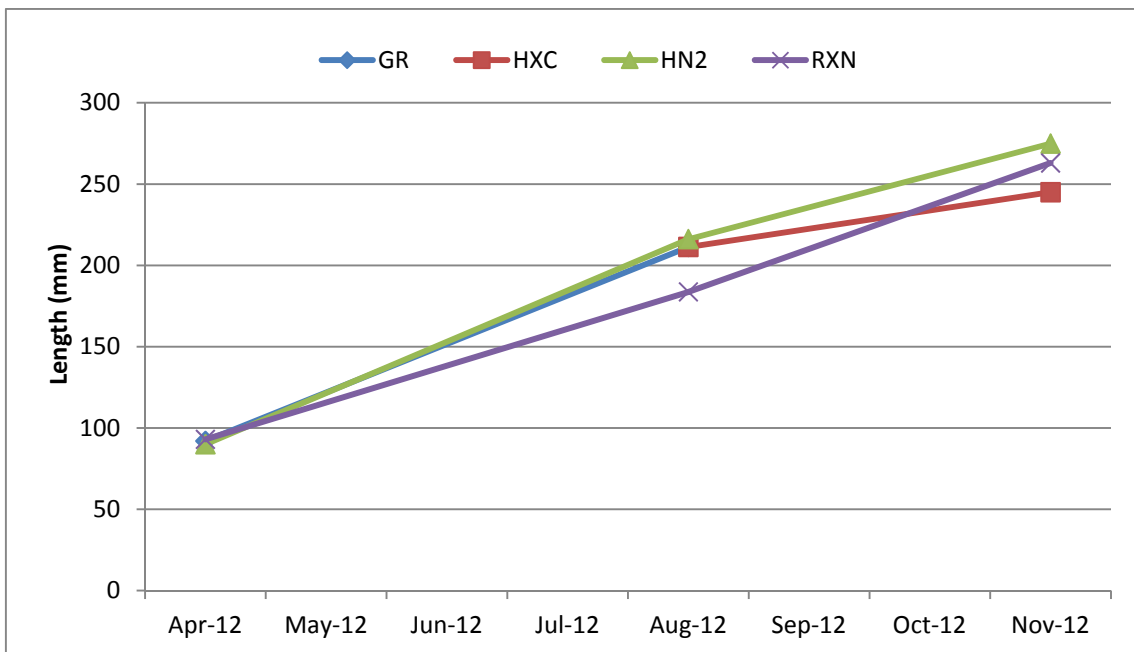


Figure 3.13. Fish length for each of four varieties stocked in Parvin Lake in November 2011. Sampling of the 2011 year class did not begin until the following spring (April 2012) due to the late fall stocking of that year class. Very few fish were found in the initial sampling event

(Figure 3.12). However, divergence of catch rates had already occurred by fall of 2012, with only one pure Hofer, and five HXC fish being found, whereas 17 and 27 HN2 and RXN fish had been collected, respectively. Average lengths of the four strains were very similar, averaging around 250 mm in November 2012 (Figure 3.13).

Myxospore Counts

A sub-set of fish from the 2007 and 2008 plants that were collected during the open-water season in 2009 and 2010 were submitted for *M. cerebralis* testing. In April, 2009, samples were only submitted from the 2007 plant. In the following sampling occasions, fish were collected from both the 2007 and 2008 plants. 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 3.5). Figure 3.14 provides a consolidation of the myxospore data from each of the collection times for both the 2007 and 2008 plants, which consisted of 80 RXN, 38 pure HL, 42 GR×HL (50:50) crosses, 20 GR×HL (75:25) crosses, and two pure GR rainbow trout.

Table 3.5. 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 were collected from each of the year-classes. Those results are broken down by year-class and variety in Figure 3.12. Tasmanian strain rainbow trout had the highest myxospore counts, averaging nearly 150,000 myxospores per fish among those stocked in 2009. The RXN strain consistently had the next highest myxospore counts. Overall myxospore counts among Harrison Lake strain fish were lower than the RXN in the 2011 samples. This is different

from the 2009 and 2010 samples in which the myxospore counts were not significantly different from the RXN strain fish.

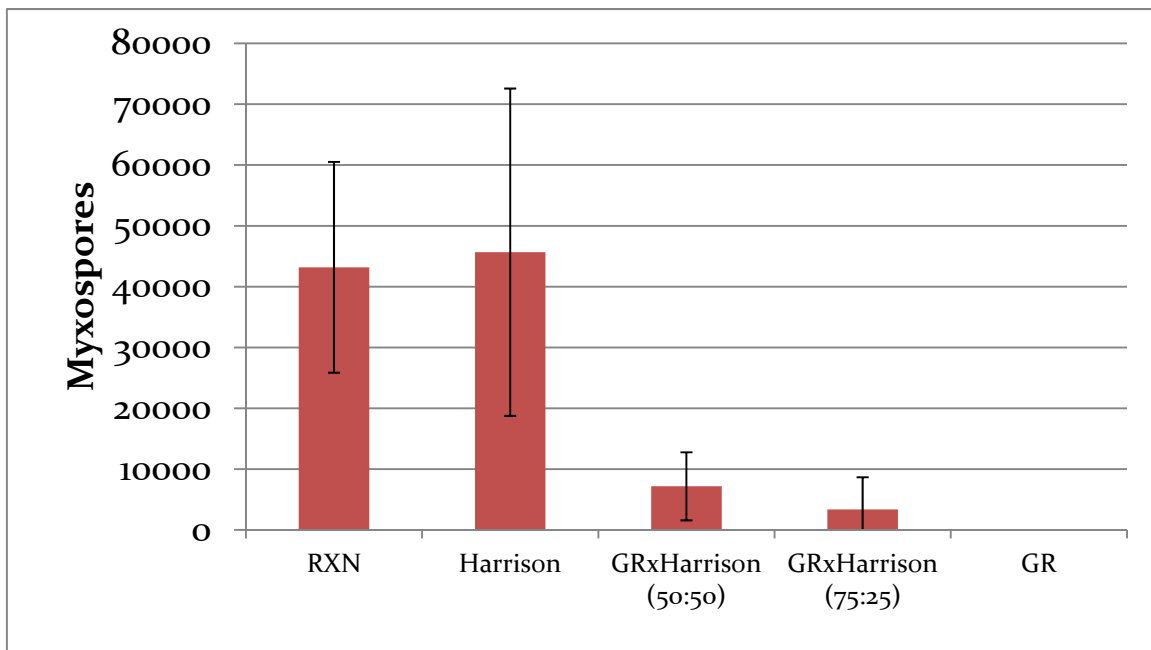


Figure 3.14. Overall averages of myxospore counts for the 2007 and 2008 plants of five strains of rainbow trout during 2009 and 2010.

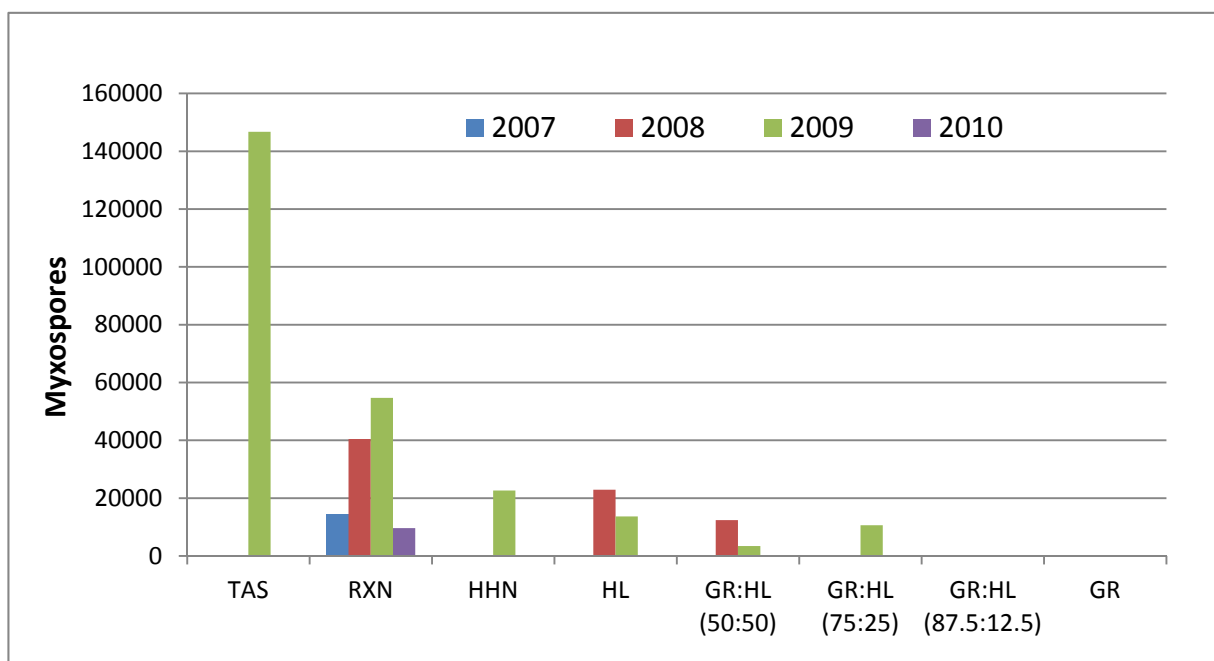


Figure 3.15. Myxospore count results by year class and strain for 2011 samples. The Hofer and Hofer-cross myxospore counts were quite low in the 2011 samples, similar to the 2009 and 2010 samples. This was also true of the HHN variety, which averaged 22,644 myxospores in fish collected from the 2009 year class, and 102 myxospores in fish collected

from the 2010 year class. This compares with an average of 54,716 in RXN collected from the 2009 year class and 9,665 myxospores in fish collected from the 2010 year class (Figure 3.15). Increasing levels of Hofer strain in the crosses resulted in lower myxospore counts across all year-classes.

At the time of this writing, myxospore count results for the 2012 samples had not yet been completed by the Aquatic Animal Health Lab due to staff shortages and workload issues. These results will be provided in the 2014 report.

DISCUSSION

It is important to consider all of the year-classes of fish stocking returns to fully understand the differences in returns and myxospore counts of the various strains of fish. While some specific strains had relatively consistent performance in different years of stocking, some did not. This 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 Hofer (GR) strain fish were present in very low numbers in each year class during the sampling events. Very few pure Hofer strain fish were found in the first year post-stocking, and were essentially absent in the samples after 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 found. Pure GR strain fish stocked again in 2011 were only represented by one fish in the 2012 catch. No *Myxobolus cerebralis* myxospores were found in any of the pure GR fish collected in any of the sampling events. 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. The strain had slightly better survival than the pure GR strain, but was less abundant than all of the other varieties stocked that year. Growth, to the extent that it could be evaluated, was consistent with the other GR-cross varieties. Like the pure GR strain, there were no myxospores found in any of the fish collected of this variety.

The GR×HL (75:25) cross had poor survival compared with most other strains stocked in the same year. Like the pure GR, they were typically found in lower numbers during the collection events, but did survive much better than the pure GR strain. The low survival was likely due to the higher proportion of GR genetics in this variety, resulting in poor predator avoidance. However, the strain was found in much later sampling events than the pure GR strain, and also survived in better numbers than the GR×HL (87.5:12.5) cross when that strain was planted. 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 counts and survival. The strain 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 strain. Growth was consistent with the other strains. In general, it appears that a higher ratio of HL to GR in the crosses is advantageous to post-stocking survival with fingerling plants, albeit increasing the Harrison Lake component results in higher myxospore counts, which could also lead to increased parasite loading in receiving waters.

The Harrison Lake variety was at a distinct disadvantage during the three years in which they were stocked due to their 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. The Harrison Lake strain was the most abundant strain from the 2009 stocking event through 2012. Growth of the Harrison Lake strain, in general, was slower than the other varieties. Myxospore counts in the Harrison Lake strain were relatively high compared to the GR-cross varieties, and not significantly different from the RXN strain fish in the 2007 and 2008 year classes. These results are consistent with laboratory experiments we have conducted in the past, in which Harrison Lake strain rainbow trout developed higher myxospore counts than either pure GR strain or GR crosses (Schisler et al. 2011).

The RXN strain fish survived very well in each year they were stocked. They were much more abundant in the catch from the 2007 plan 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 Harrison Lake, 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, and the Harrison Lake variety surviving better than either of the strains. In the 2010 year class, where only HHN and RXN fish were stocked, the survival was nearly identical between the two strains. Myxospore counts found in each year of collections for the RXN strain were higher than any of the other strains, with the exception of the Tasmanian strain, stocked in 2009, which was much higher than all of the other varieties.

The HHN fish survived as equally well as the RXN fish from both the 2009 and 2010 stocking events. These varieties were out-survived only by the Harrison Lake strain in the 2009 plant. The HHN and RXN had nearly identical growth rates as well. The real difference in the two strains is apparent in the myxospore counts. In both the 2008 and 2009 year classes, the myxospore count was substantially higher in the RXN fish than in the HHN fish. This was expected, due to the GR genetic background of the HHN variety. With the survival of the two varieties being nearly equal, the use of the HHN as a replacement cutbow for recreational stocking is a valuable option. The HN2 variety (essentially the same type of fish as the HHN) stocked in 2011, lagged slightly behind RXN returns in the 2012 sampling.

The preliminary results of the 2011 plant indicate that the GR×CR cross, first stocked in 2011, does not appear to demonstrate good survival as a fingerling plant in a lake environment. Only five fish from the 2011 plant were found in the 2012 samples. However, this entire year-class of fish was not well represented in the 2012 samples, possibly because of the small size of the fish stocked in 2011, so subsequent years of sampling will provide more information on this cross.

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 Harrison Lake and 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 in areas where *M. cerebralis* and high predation pressure exists. However, the RXN variety consistently had better survival than the GR×HL (50:50) cross. The RXN and Harrison Lake varieties survived very well in these experiments, and would likely be preferred lake strains, except for the higher myxospore counts produced by these varieties.

Samples collected on the later year-classes of fish suggest that the HHN variety is a very good option for these types of environments, and will provide a whirling disease-resistant alternative for cutbow stocking that also demonstrates high survival. Because of the resistance to whirling disease, the high survival of the variety, and the general appeal of cutbows overall, the HHN variety seems to be emerging as an optimal variety for lake and reservoir plants. Given that this variety can be easily produced from Hofer-Harrison and pure Snake River cutthroat brood fish in the hatchery system, this variety has the potential for great utility for fingerling plants throughout the state.

Field Performance Evaluations: Poudre Ponds Fingerling Stocking Experiment

INTRODUCTION

The concept of stocking fish reared in a whirling-disease positive facility versus those stocked from a clean facility has been a topic of debate ever since the implementation of the Colorado Division of Wildlife D-9 stocking policy. The argument has been made that fish produced in a clean facility will ultimately become infected and produce myxospores when stocked into an infected environment, so the benefit of producing fish at a clean facility is negated. The goal of this study was to quantify infection levels in fish reared to catchable size in both infected and uninfected environments, and subsequent myxospore production of those fish. Both susceptible and resistant strains of fish were used to determine if using resistant strains would produce a better outcome in either scenario. This long-term experiment was conducted over a period of three years in three separate phases to evaluate overall growth, survival, and infection severity among the various varieties from fingerling to catchable size.

METHODS

The first phase of this experiment began in 2009 with an evaluation of growth, survival, and infection severity of eight varieties of rainbow trout held in two earthen ponds at the Poudre Rearing Unit. This experiment was conducted to determine infection level and growth of the eight varieties reared together in a natural setting known to have high ambient levels of *M. cerebralis*. One thousand fish of pure GR, pure Harrison Lake, pure Tasmanian, RXN, HHN, GR×HL (50:50), GR×HL (75:25), and GR×HL (87.5:12.5) were marked with coded wire tags and stocked as fingerlings (35-70 fish lb⁻¹) into each of the two ponds, for a total of 8,000 fish per pond on June 23, 2009. Samples were collected at eight months and 12 months post-release. All fish collected from the ponds were weighed and measured, and coded wire tags were extracted for variety identification. Fish were then numbered, individually bagged, and a subset was submitted for PTD testing.

The second phase of the experiment involved stocking fish from the first phase that had been grown to catchable size, along with catchable-size fish each of two varieties previously reared in

a *M. cerebralis*-negative environment (Rifle Falls Hatchery) and stocked into an infected pond. The objective of the second phase of the experiment was to determine the level of infection developed by both susceptible and resistant fish reared initially in both infected and non-infected environments, and then exposed to the parasite. The first variety of fish brought to the facility was the susceptible Bellaire rainbow strain (1.97 fish lb⁻¹), which had been created for the experiment in 2005. The second variety of fish was the resistant GR×HL (87.5:12.5) cross (2.12 fish lb⁻¹), which had also been created in 2005 and reared at the same facility. These clean, catchable-size fish were stocked into a third infected pond on the Poudre Rearing Unit, along with all remaining fish from the first phase of the experiment, on October 5, 2010.

Fish from the second phase of the experiment were reared at the Poudre Rearing Unit for another year, after which the third phase of the experiment was initiated. The third phase of the experiment consisted of stocking these fish into a put-and-take fishery to determine final growth, infection level, and return to creel of the ten varieties of fish reared at the Poudre Rearing Unit over the duration of the experiment. The location for this portion of the evaluation was Douglas Reservoir, a typical put-and-take fishery north of Fort Collins, Colorado.

RESULTS

Phase I

Catch results for the eight-month sample are summarized by gear type in Table 3.6. No Harrison Lake rainbow trout were found among the 125 fish collected during the eight-month post-release sample. Only five pure Tasmanian strain fish were found, and six GR×HL (50:50) crosses. The other strains were relatively uniform in catch, ranging from 18 (14.4%) to 26 (20.8%).

Table 3.6. Total catch for the eight month post-release sample at Poudre Ponds.

	Pure HL	Pure TAS	Pure GR	GR×HL 50:50	GR×HL 75:25	GR×HL 87.5:12.5	HHN	RXN
Pond 1								
Hook and Line	0	0	7	2	13	6	2	2
Gill Net	0	1	6	0	3	4	9	7
Pond 2								
Hook and Line	0	3	5	3	4	10	5	8
Gill Net	0	1	6	1	6	6	4	1
TOTAL	0 (0.0%)	5 (4.0%)	24 (19.2%)	6 (4.8%)	26 (20.8%)	26 (20.8%)	20 (16.0%)	18 (14.4%)

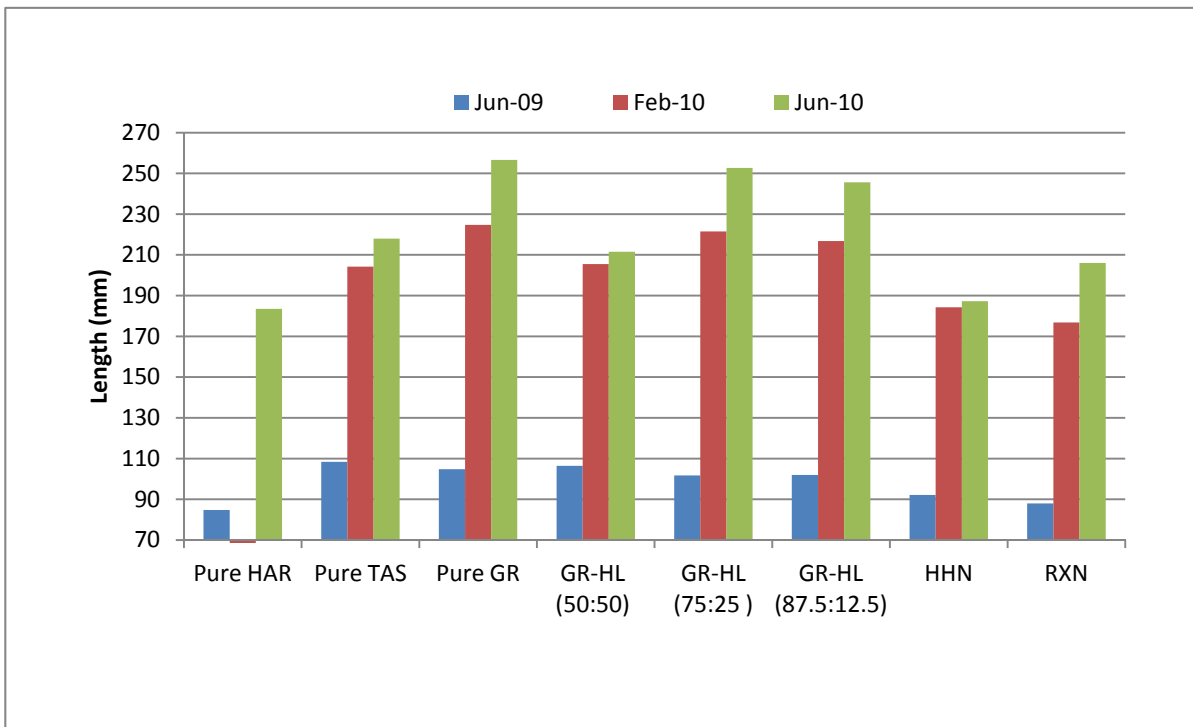


Figure 3.16. Lengths of eight rainbow and rainbow-cutthroat trout cross varieties upon release, eight and 12 months post-release at the Poudre Rearing Ponds.

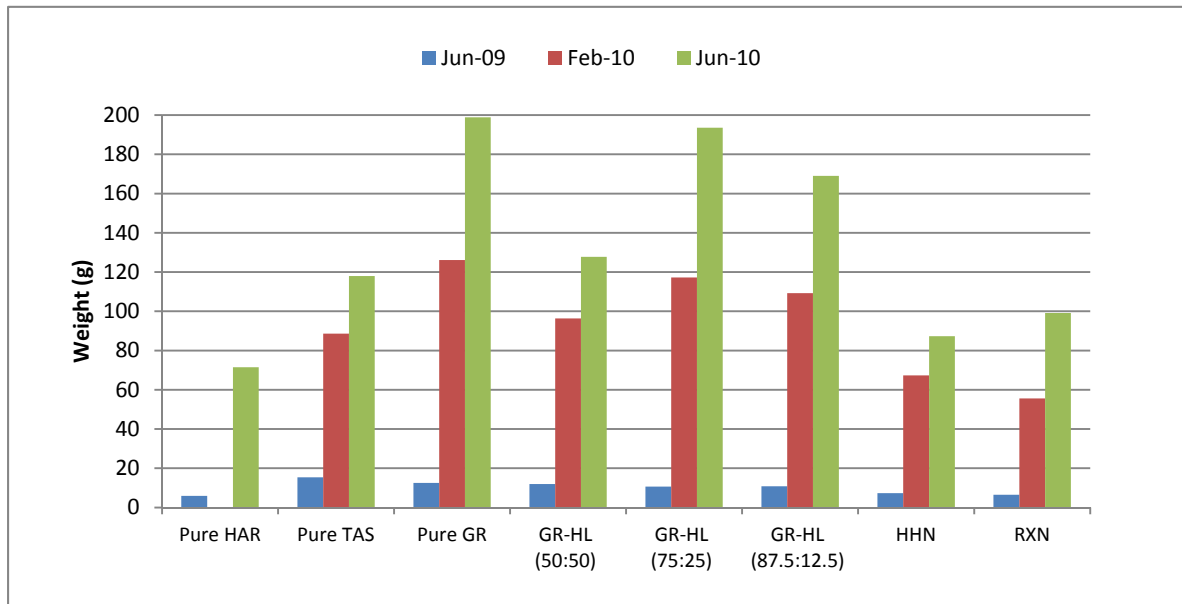


Figure 3.17. Weights of eight rainbow-cutthroat trout cross varieties upon release, eight and 12 months post-release at the Poudre Rearing Ponds.

The eight-month length results suggest that the GR strain and high proportion GR crosses, such as the GR×HL (75:25) and GR×HL (87.5:12.5) had slightly better growth compared to the other varieties (Figure 3.16). Each variety averaged over 210 mm in length at eight months. Weight

measurements demonstrated an even greater advantage for the GR strain and high proportion GR crosses, with all three averaging over 100 grams (Figure 3.17).

Table 3.7. Total catch for the 12 month post-release sample at Poudre Ponds.

	Pure HL	Pure TAS	Pure GR	GR×HL 50:50	GR×HL 75:25	GR×HL 87.5:12.5	HXN	RXN
Pond 1								
Seine	1	4	14	2	8	23	6	7
Pond 2								
Seine	2	3	10	2	12	16	2	3
TOTAL	3 (2.6%)	7 (6.1%)	24 (20.9%)	4 (3.5%)	20 (17.4%)	39 (33.9%)	8 (7.0%)	10 (8.7%)

The 12-month results were very similar, with the GR and high proportion GR crosses exhibiting the best growth as measured by both length and weight. The high proportion GR varieties were also present in the sample at higher rates than the other varieties (Table 3.7). The exception was the HXN variety, in which growth (both length and weight) was more comparable to the RXN variety.

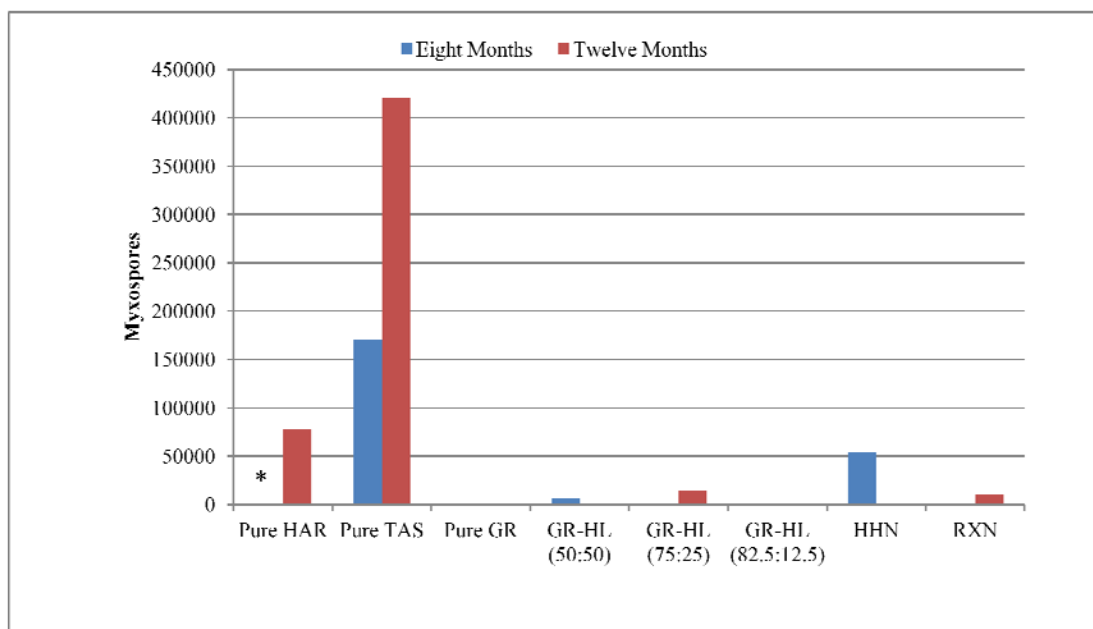


Figure 3.18. Myxospore count by strain at the Poudre Rearing Unit at eight months and 12 months post-release. No Harrison Lake variety fish were found in the eight month collection.

Myxospore count results were very similar to the other experiments in which these varieties were evaluated. At both eight months and twelve months, the Tasmanian strain exhibited much higher parasite loads than the other varieties. Average myxospore count for the Tasmanian strain at twelve months was over 400,000 myxospores per fish (Figure 3.18). This high myxospore level, as observed in a highly infected natural environment, would unquestionably lead to amplification of *M. cerebralis* in waters stocked with this susceptible strain.

Phase II

Myxospore counts in the fish reared from fingerling size on the infected facility doubled between June 2010 and June 2011 among more susceptible groups such as the Tasmanian and GR×HL (50:50) varieties. Pure GR, HHN, HXN, and high proportion GR crosses such as the GR×HL (75:25) and GR×HL (82.5:12.5) maintained relatively low myxospore counts. No Harrison Lake strain fish were found during either of these collections, so myxospore counts and growth are not shown for that strain (Figure 3.19).

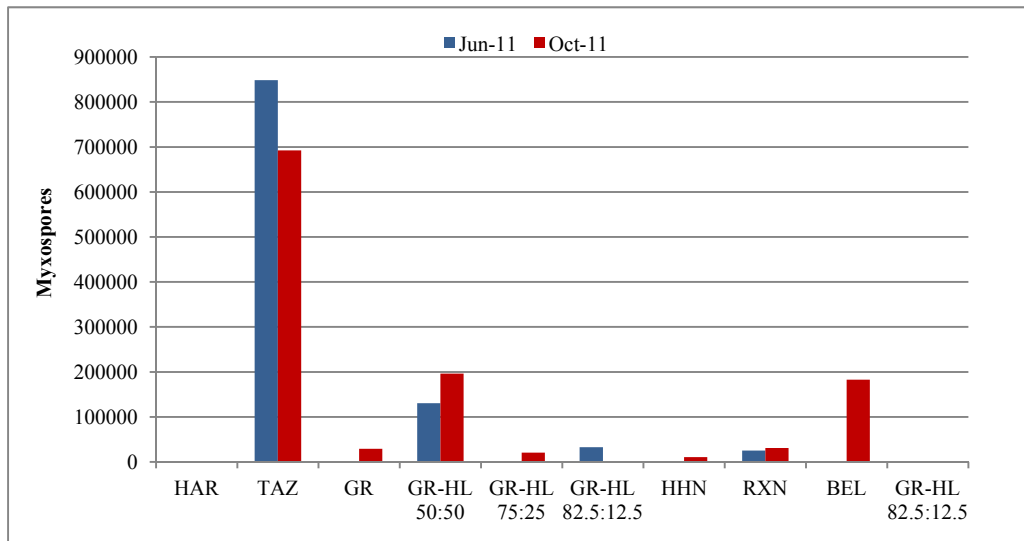


Figure 3.19. Myxospore counts among fish collected at 24 and 28 months post-stocking. The results for the Bellaire (BEL) and GR×HL (82.5:12.5) groups on the far right of the graph are for eight months and 12 months post-stocking into the infected environment as clean catchables. No Harrison Lake variety fish were found in the collections.

For fish that were brought to the facility as clean catchable-sized fish, the Bellaire developed an average myxospore count of 182,908 myxospores per fish in the first year, while the resistant fish (GR×HL (82.5:12.5)) exposed as catchables developed an average myxospore count of only 1,027 myxospores per fish. The myxospore count among the Bellaire exceeded the myxospore count observed in all other varieties reared in the exposed environment, with the exception of the susceptible Tasmanian strain, and the GR×HL (50:50) cross.

Growth in this phase of the experiment followed the same pattern as that in Phase I, with high proportion GR crosses exhibiting the greatest growth as measured by both length and weight. The GR×HL (82.5:12.5) cross brought from the Rifle Falls Hatchery at the beginning of Phase II started out smaller than the Bellaire strain brought over at the same time. By the end of Phase II the GR×HL (82.5:12.5) cross had outgrown the Bellaire strain (Figures 3.20, 3.21).

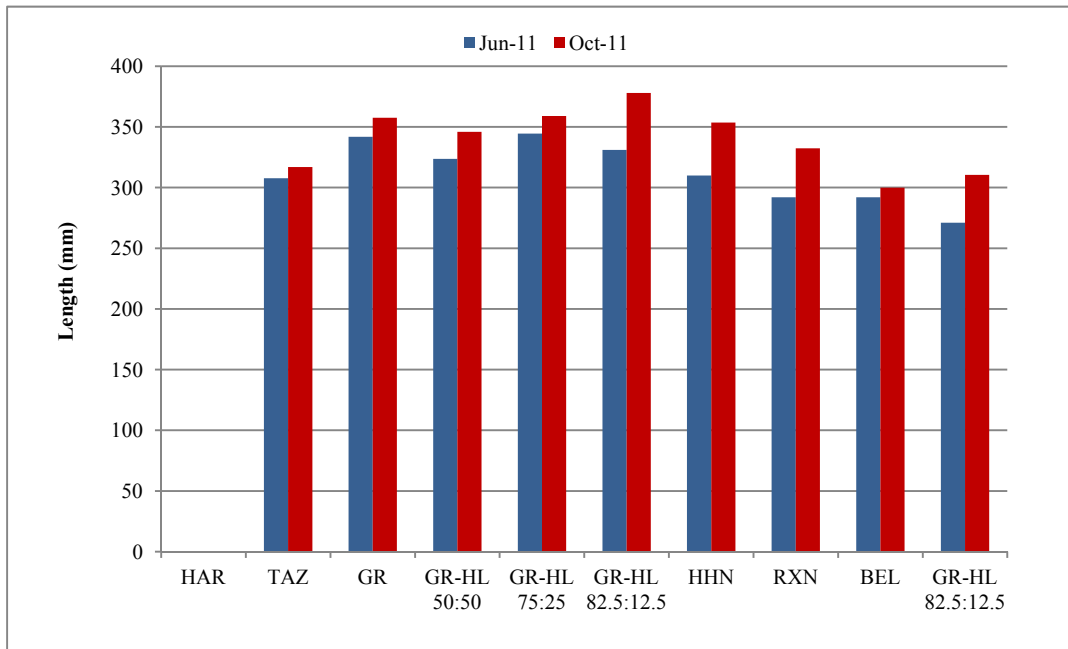


Figure 3.20. Length of fish collected at 24 and 28 months post-stocking. The results for the Bellaire (BEL) and GR×HL (82.5:12.5) groups on the far right of the graph are for eight months and 12 months post-stocking into the infected environment as clean catchables. No Harrison Lake variety fish were found in the collections.

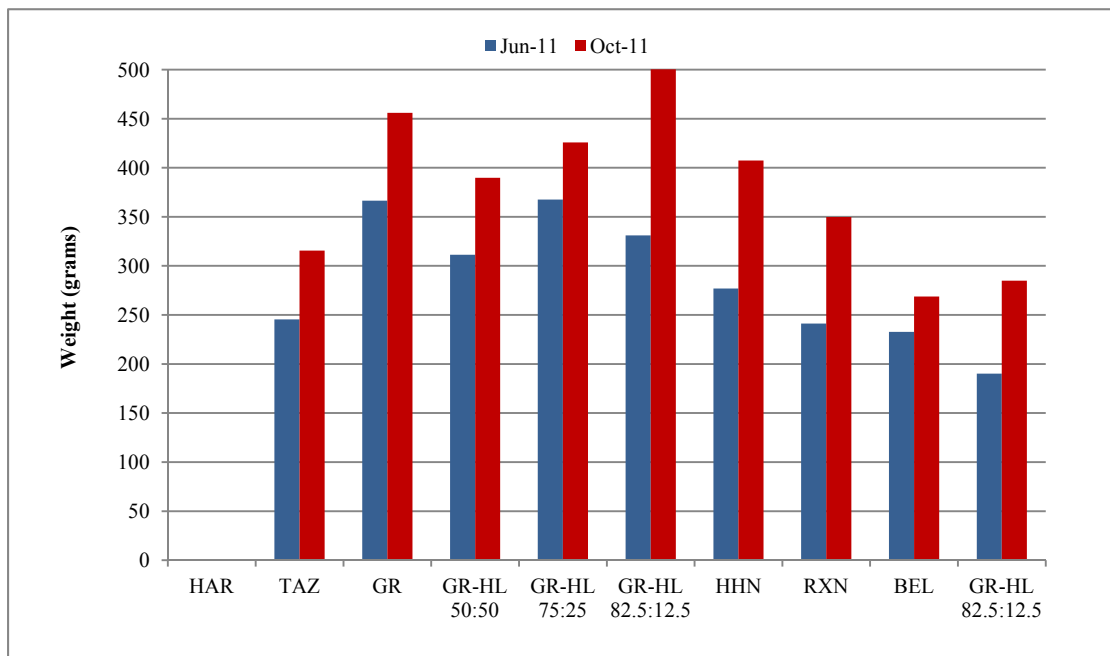


Figure 3.21. Weight of fish collected at 24 and 28 months post-stocking. The results for the Bellaire (BEL) and GR×HL (82.5:12.5) groups on the far right of the graph are for eight months and 12 months post-stocking into the infected environment as clean catchables. No Harrison Lake variety fish were found in the collections.

Phase III

The fish remaining in 'Pond 3', where the fish were reared for one year after reaching catchable size (Phase II), were stocked into Douglas Reservoir on June 4, 2012. Samples were collected immediately prior to stocking for growth and myxospore counts. Total fish stocked, based on average weight of 1.21 per lb, was 13,107 fish. Given that 20,000 fish were stocked into the ponds originally, this was a fairly high rate of survival over the course of the captive rearing period. A creel survey consisting of two weekdays and two weekend days per week was conducted from the beginning of June through the end of August 2012. An estimated 8,890 (SE = 977) rainbow trout were caught during the first three months post-stocking, of which 3,730 (SE = 474) were reported to have been kept, and 5,160 (SE = 735) were reported to have been released. Catch per hour was highest in June, with 0.7109 (SE = 0.1168) being reported on June weekdays, and 0.5632 (SE = 0.0826) being reported on June weekends. July weekdays had a catch rate of 0.1636 (SE = 0.0449), and July weekends had a catch rate of 0.1899 (SE = 0.0623). Catch rates declined dramatically in August, with a catch rate of 0.0041 (SE < 0.001) on weekdays and 0.0071 (SE < 0.001) on weekend days. This was likely due to the much warmer water temperatures in the late summer.

Lengths and heads were collected from fish harvested during the creel surveys through the month of June and the beginning of July. Fish were identified to strain by extracting the coded wire tag from each individual. Five hundred eighty-nine fish were collected with tags. Every third fish collected was submitted for myxospore testing to the Aquatic Animal Health Lab. Final myxospore counts for the third phase of the experiment (pre-release and creel fish) were not complete at the time of this writing, and will be included in the 2014 report.

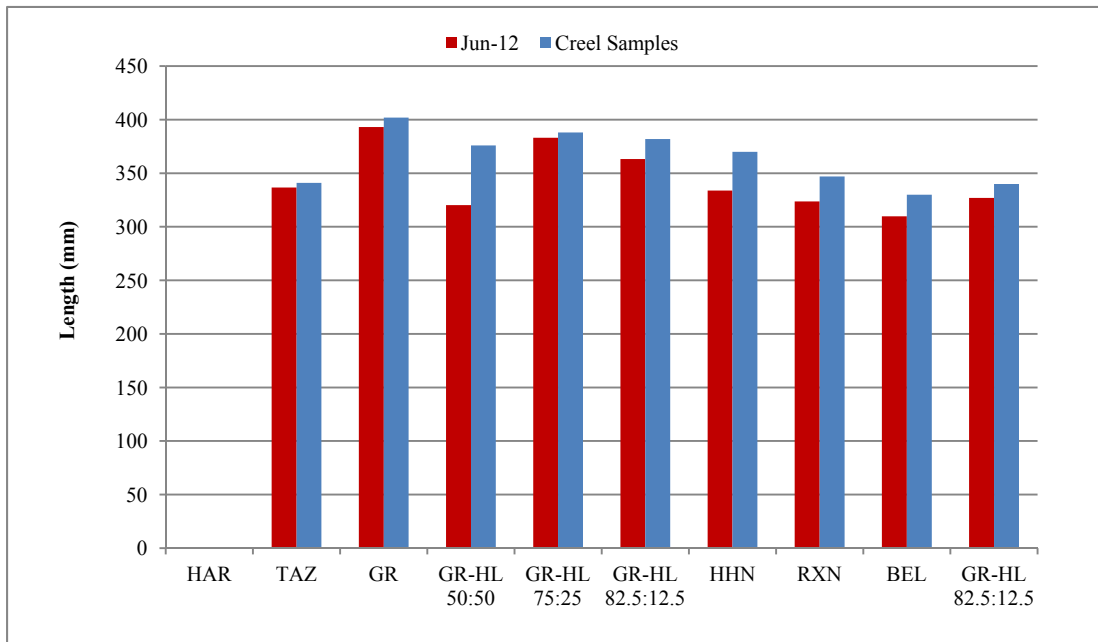


Figure 3.22. Length of fish immediately prior to stocking in Douglas Reservoir, and lengths of fish collected during creel survey efforts in June and July, 2012. No Harrison Lake variety fish were found in the collections.

Lengths of fish of the various varieties immediately prior to stocking were similar to those obtained while the fish were in the pond phase of the experiment (Figure 3.22). Pure GR, GR×HL (82.5:12.5), and GR×HL (75:25) were the three largest varieties. All varieties were larger in the creel samples than immediately prior to stocking. This could be possibly due to some post-release growth, but since the creel samples were from harvested fish, it could also be due to harvest selection for larger fish by anglers.

Overall catch for each of the strains in Phase III at Douglas Reservoir is provided in Figure 3.23. High proportion GR crosses and HHN reared at Poudre Ponds in Phase I and II had the highest return of the varieties evaluated. Further analysis of endpoint survival, myxospore counts, and creel results will be presented in the 2014 report.

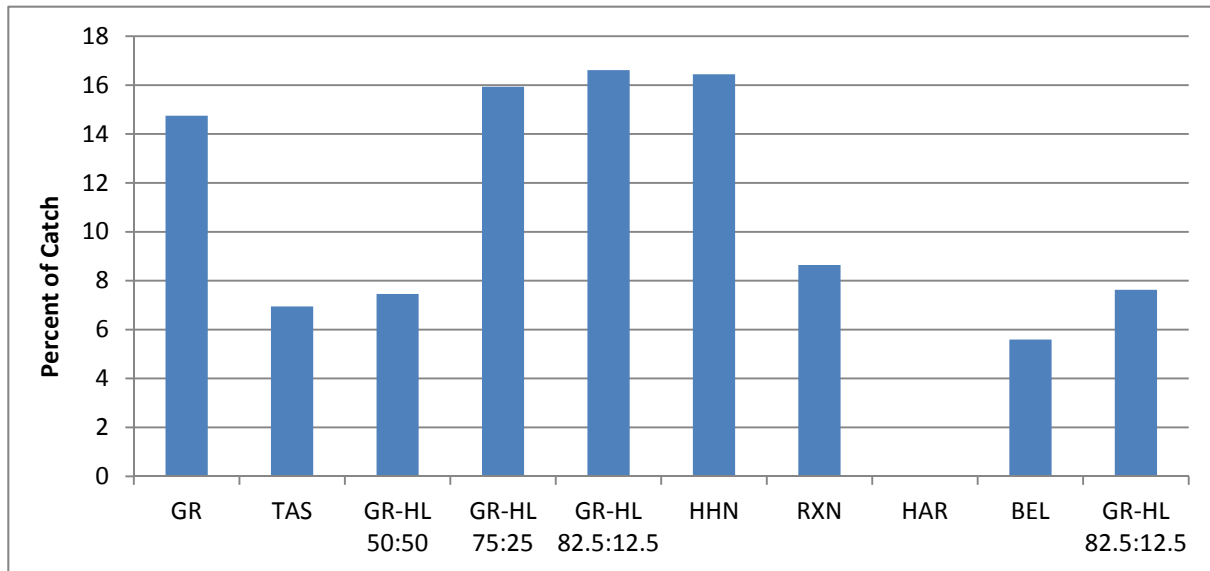


Figure 3.23. Percent of catch in Douglas Reservoir. The Bellaire and GR×HL (82.5:12.5) groups on the right end of the graph are those brought to the Poudre Ponds as clean catchables. No Harrison Lake variety fish were found in the collections.

DISCUSSION

Weight and length gains were superior in the GR and high proportion GR crosses compared to the other strains in every phase of this experiment. This trend has been observed in many of our previous lab experiments, reinforcing the body of evidence for excellent growth rates in the GR crosses. The high proportion GR and HHN varieties raised in an infected environment from the outset of the experiment had the highest returns to creel. The creel results show that high proportion GR crosses are excellent for put-and-take fisheries.

The results of these three experiments also clearly demonstrate the benefits of using varieties with lower susceptibility to whirling disease infection in these controlled rearing conditions. The clean catchable Bellaire strain rainbows developed higher myxospore counts by the end of the Phase II portion of this experiment than all of the resistant varieties reared from fingerling size in an infected environment, with the exception of the GR×HL (50:50) cross. Once myxospore count results are completed for the third phase of the experiment, the additional information will

help determine the magnitude of the benefits of rearing resistant varieties, and at what types of crosses can be definitively reared in infected environments and still produce lower myxospore counts than susceptible fish reared in clean environments.

One other interesting outcome was the low parasite load found in the RXN variety in these experiments. This trend was not found in live-release experiments conducted in Parvin Lake. Identifying the conditions that led to the lower parasite load in this variety under these conditions may be useful, and further experimentation with Hofer-Snake River cutthroat strains may be beneficial to optimize the resistance and long-term survival of fingerling trout in put-grow-and-take fisheries.

References

- Schisler, G. J., and E. R. Fetherman. 2009. Resistant rainbow trout in Colorado: Current status and uses. Colorado Division of Wildlife Aquatic Wildlife Research Section, Fort Collins, CO. 24 pp.
- 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).

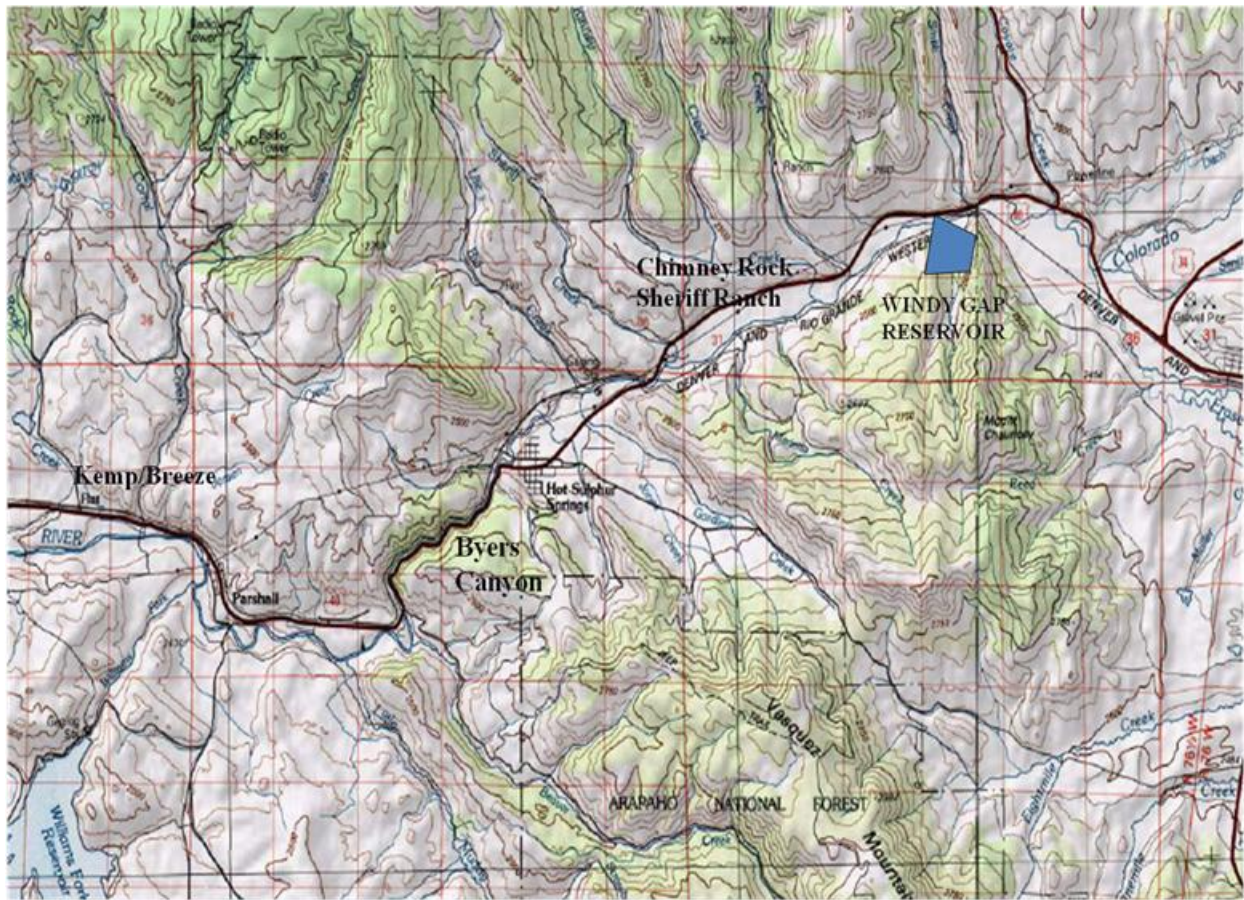


Figure 4.1. Upper Colorado River study area.

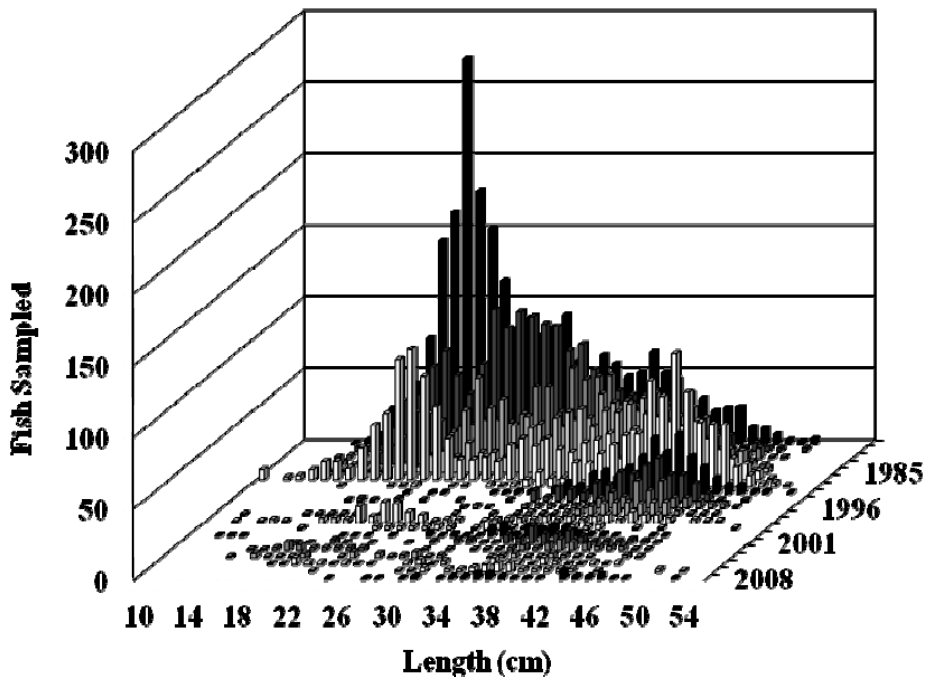


Figure 4.2. Upper Colorado River historic rainbow trout length-frequencies at Kemp-Breeze State Wildlife Area.

ADULT H×C INTRODUCTIONS

Whirling disease resistant rainbow trout introductions (adult Hofer × Colorado River Rainbow [H×C]; > 150 mm) first occurred in the upper Colorado River in June of 2006, with a second introduction occurring in January of 2009, and a third introduction occurring in June of 2010. Following these introductions, the population in the upper Colorado River, specifically within the Chimney Rock/Sheriff Ranch study area, was monitored on a yearly basis. Adult population estimates were conducted in the spring to determine the abundance and survival rate of the stocked H×Cs. In addition, fry shocking was used to evaluate the rainbow trout and brown trout fry populations in the upper Colorado River, and to determine if rainbow trout offspring were being produced by the stocked adult H×Cs. The majority of this work was conducted as part of a Ph.D. project through Colorado State University (CSU) and has since been published in chapter two of the dissertation entitled *Introduction and Management of Myxobolus cerebralis-Resistant Rainbow Trout in Colorado*, which was submitted to CSU in Summer 2013 (Job 4, Appendix 1).

H×C FRY INTRODUCTIONS

Although reproduction is occurring, and the fry being produced are better able to survive exposure to whirling disease in the upper Colorado River, the numbers of fry surviving through the fall are still fairly low. This will result in fairly low recruitment to the adult population, and could take a number of years for the adult rainbow trout population to begin to show an increase due to this recruitment. Additionally, declines in the adult rainbow trout population are likely to occur during this period. Therefore, we have initiated a project introducing whirling disease resistant rainbow trout (H×C) as fry into the Chimney Rock/Sheriff Ranch section of the river.

This approach has shown promising results, both in terms of fry survival and recruitment to the adult population, in the Colorado River below Byers Canyon.

JUSTIFICATION

HxC have been introduced to the upper Colorado River as fry below Byers Canyon, from the Paul Gilbert State Wildlife Area downstream to below the Kemp-Breeze State Wildlife Area. In 2010, 2011, and 2012, up to 200,000 rainbow trout fry were stocked in this section of the river in late July or early August. As a result, the rainbow trout fry population has exceeded the brown trout fry population in the months following their introduction; although abundance is reduced in the fall, similar numbers of rainbow trout and brown trout fry have been encountered in these lower study sections in October of each of these years. In addition, the number of rainbow trout fry remaining in October is up to five times higher than the numbers of naturally produced fry remaining in the Chimney Rock Ranch section of the river.

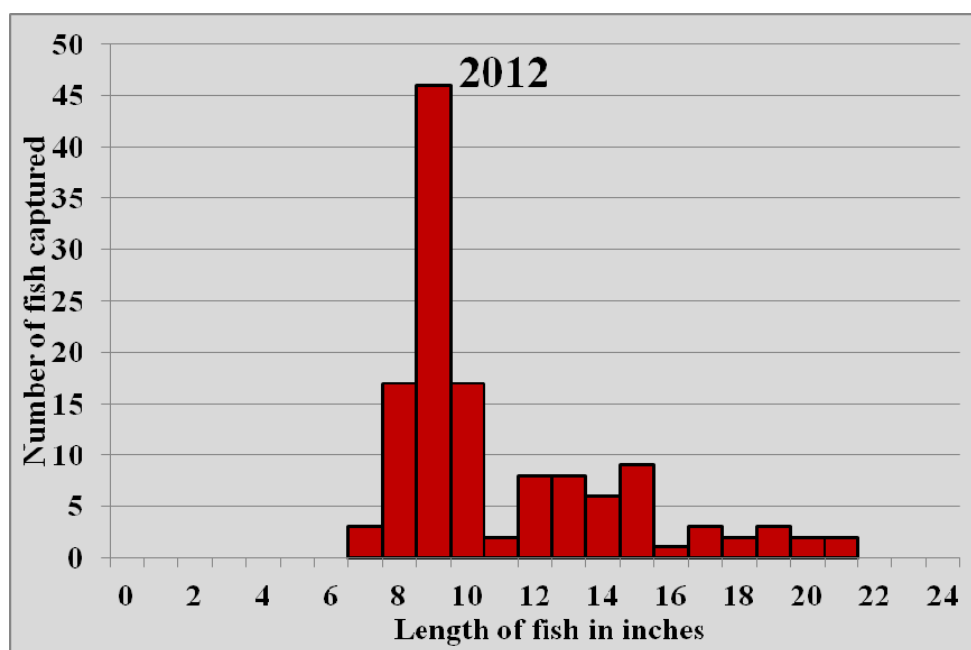


Figure 4.3. Number of rainbow trout captured in each length class in the Parshall-Sunset reach of the upper Colorado River in 2012.

As a result of these fry introductions, and the increased survival rates of the introduced fry, these fish have begun recruiting to the adult (6+ inches) population, with an increase from 71 adult rainbow trout per mile in 2010 to 306 in 2012. Additionally, results from this section suggest that the HxC stocked as fry exhibit extraordinary growth rates, gaining an average of up to six inches each year. For example, during the September 2012 population estimates in the Parshall-Sunset reach of the Colorado River, a large number of the fish stocked in 2011 appeared in the population estimate as average 9” in length, with the fish stocked in 2010 appearing in the population sample between 12 and 14” in length (Figure 4.3).

METHODS and TIMELINES

An initial adult population estimate was conducted in the Chimney Rock Ranch study section using raft-mounted electrofishing units in the spring of 2013 to provide baseline data on the status of the adult salmonid populations in this section of the river prior to the introduction of H×C fry. Adult population estimates will be conducted every spring following the introduction of the H×C fry to determine recruitment and spawning status of the fry recruiting to the adult population. The final adult population estimate, used to evaluate the overall success of the fry introductions will occur in the spring of 2016.

H×C fry introductions will take place in July of the years 2013, 2014, and 2015. The production of 200,000 H×C fry was requested to the hatchery system for the first introduction to the Chimney Rock Ranch section of the upper Colorado River in 2013. Fry will be stocked using two rafts with live wells containing the fry. The rafts will travel down each bank of the river and distribute the fry evenly throughout the section. Half of the fry will be stocked between Hitching Post Bridge and Corral Creek at the Red Barn location. At Red Barn, the hatchery truck will help load the second half of the fish onto the rafts, with these fish being stocked between Red Barn and the Sheriff Ranch pullout.

Fry population sampling will occur once a month, June through October, in 2013, 2014, and 2015. The fry population estimates will allow us to track the survival of the introduced fry over time and gauge the success of the introduction in each of the three years. Four historical sites, one on the Sheriff Ranch, two in the Red Barn area, and one at Hitching Post Bridge will be used to monitor the fry population in each of these months. The data obtained from both the adult and fry population sampling will appear in future reports, starting in 2014.

EXPECTED RESULTS

The fry introduction experiment conducted on the Chimney Rock Ranch 2013-2016 is expected to help determine the survival rate of the introduced fry, the recruitment rate from the fry to adult population, the time at which fish introduced as fry become sexually mature, and the growth performance of fish introduced as fry. The overall goal is to help create a self-sustaining rainbow trout population in the upper Colorado River. The results of the fry introductions Below Byers canyon suggest that significant increases in the adult rainbow trout population could be observed on the Chimney Rock Ranch within two years of the first fry introduction. In addition, the rainbow trout population would consist of whirling disease resistant rainbow trout, maintaining lower infection rates in this part of the river.

Cache la Poudre River

INTRODUCTION AND OVERVIEW

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 4.4). 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 not

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.

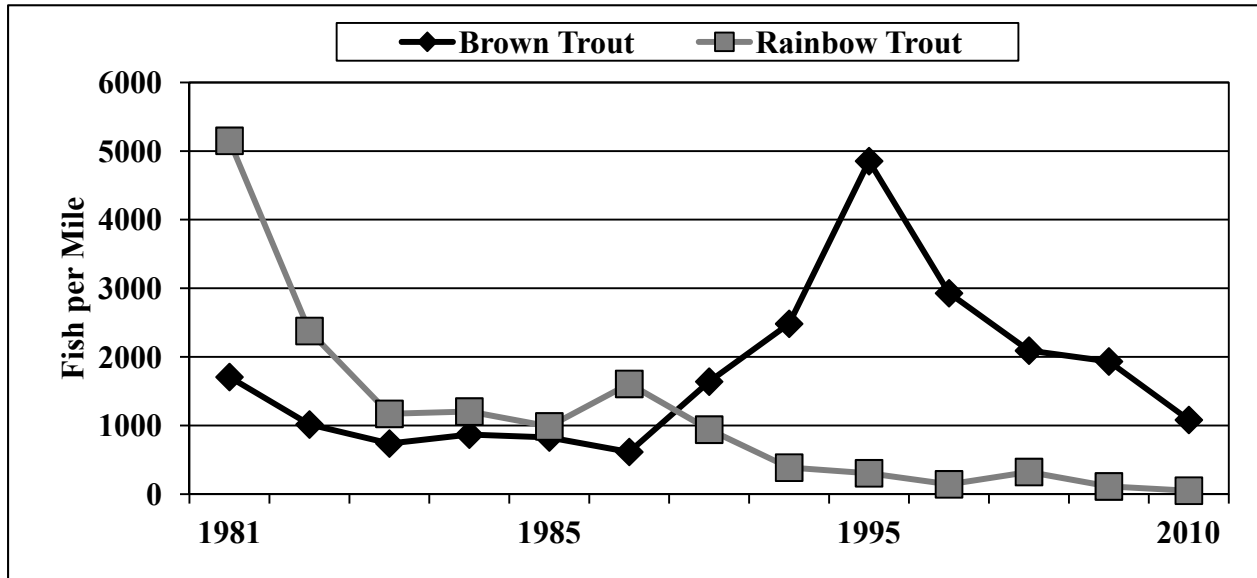


Figure 4.4. Estimated number of rainbow trout and brown trout per mile in the upper Colorado River between 1981 and 2010. Notice the large increase in the brown trout population in the early 1990s as the rainbow trout population declines due to the introduction of whirling disease.

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 (H×C and H×H) as an evaluation of which strain is better for use in reintroductions in rivers and streams.

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 H×C and H×H 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?

The majority of this work was conducted as part of a Ph.D. project through Colorado State University (CSU) and has since been published in chapter four of the dissertation entitled *Introduction and Management of Myxobolus cerebralis-Resistant Rainbow Trout in Colorado*, which was submitted to CSU in Summer 2013 (Job 4, Appendix 2).

East Portal of the Gunnison River H×C Brood Stock

INTRODUCTION

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 electrofishing 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.

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 4.5).

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, was postponed until further research could be conducted on the genetic and resistance characteristics of the East Portal rainbow trout.

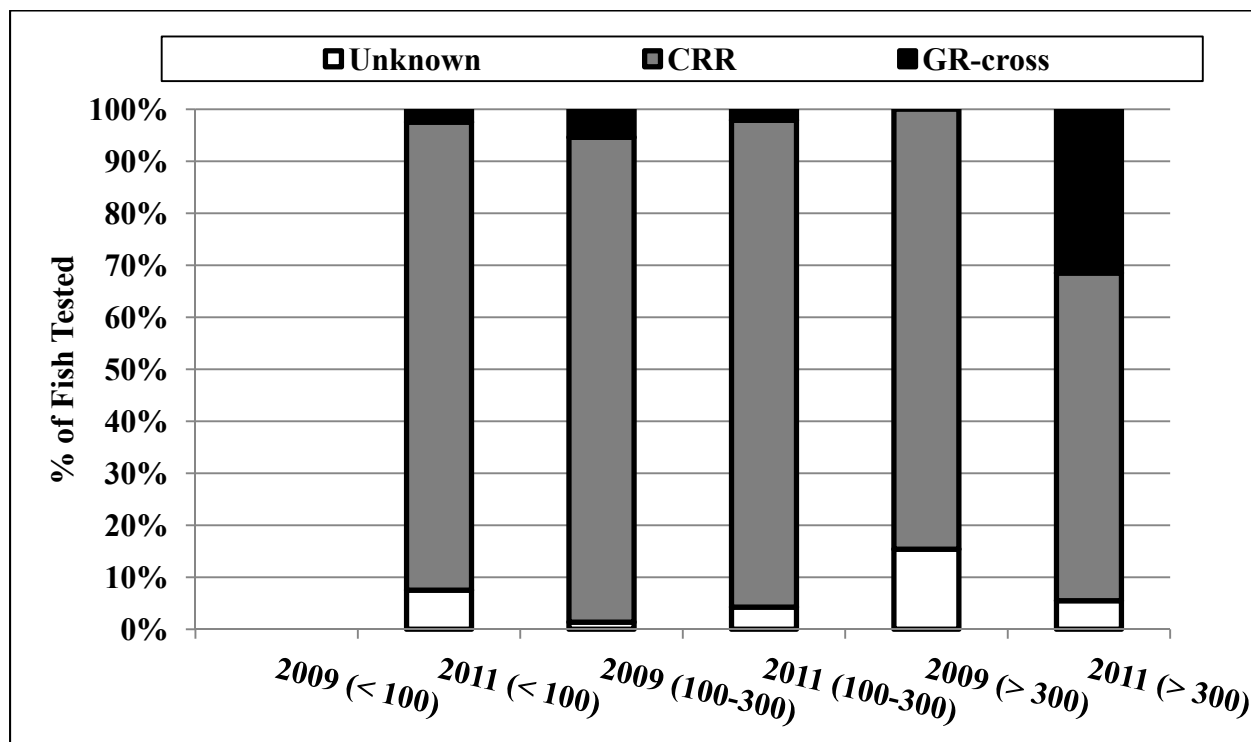


Figure 4.5. Percent of fry (< 100 mm), juvenile, and adult (100-300 and > 300 mm) rainbow trout, encountered during the East Portal of the Gunnison River population estimate in 2009 and disease inspection in 2011, categorized as unknown, pure CRR, and GR-cross fish.

In 2012, eggs were collected from the East Portal rainbow trout during the spring spawning season. The objectives of this experiment were to determine which strains of rainbow trout were spawning in the East Portal of the Gunnison River, and to determine if offspring produced by these fish exhibited increased resistance characteristics when exposed to *Myxobolus cerebralis* in the laboratory.

SPAWNING AND REARING

Rainbow trout in the East Portal were captured via boat electrofishing unit at three time points within the spawning period: 1) April 17, 2012, 2) May 1, 2012, and 3) May 15, 2012. Eggs were collected over these three time periods to obtain a range of families over the course of the spawning period in case CRR or GR-cross fish attained spawn-ready status at different times. On each spawning occasion, fish were captured the day prior to the spawn, separated by gender, and held in two live cages overnight. Fish were spawned in the morning of the dates listed above. Following spawn, eggs were water hardened in five gallon water coolers for one hour; eggs were also disinfected using iodine during water hardening. Once eggs had water hardened, the iodine was rinsed out of the coolers, and clean water was added to the coolers for transport to the CPW Aquatic Toxicology Lab in Fort Collins, Colorado. In the Aquatic Toxicology Lab,

eggs were held at different temperatures so that eggs collected at each of the time points would hatch at the same time. Fish were reared in the Aquatic Toxicology Lab until swim-up.

One hundred and twenty rainbow trout were captured via electrofishing on April 16 and 17 for spawning and genetic sample collection. Of these, 102 were green or spent females (69), or immature fish (33); genetic samples were collected from all of these fish to determine whether they were pure CRR or GR-cross fish. Of the 69 females, 65 were green and only four were spent. These fish averaged 408 mm in length, ranging from 297 to 557 mm. Four fish were used to create "Group 1" for the exposure experiment. Group 1 consisted of two male-female pairs. The first pair was a 496 mm female spawned a 527 mm male. The second pair was a 416 mm female spawned with a 415 mm male. Genetic samples were taken from each of the fish for comparison to offspring genetics following the exposure experiment. The remaining eight fish captured were ripe males. These fish were not used during the spawning operations, and no genetic samples were collected from these fish. Upon arriving at the Aquatic Toxicology Lab, eggs were held at a temperature of 6.9°C to prolong hatching, so that these fish and fish collected later in the spawning period would hatch at the same time.

Fifty eight rainbow trout were captured via electrofishing on April 30 and held in net pens overnight for spawning. Of these, 44 (76%) were females and 14 (24%) were males. Of the females, ten (23%) were ripe, 24 (55%) were green, and ten (23%) were spent. All of the males were ripe. Four groups were created using the ripe males and females. All groups consisted of two male-female pairs. Group 2 consisted of a 437 mm female spawned with a 495 mm male, and a 425 mm female spawned with 415 mm male. The 425 mm female was a previously green female that had been captured and from which genetic information had been collected on April 17 (evidenced by the fin clip on the upper caudal fin). Group 3 consisted of a 445 mm female spawned with 378 mm male, and a 507 mm female (recapture; previously green) spawned with a 430 mm male. Group 4 consisted of a 468 mm female spawned with a 440 mm male, and a 411 mm female spawned with a 483 mm male. Group 5 consisted of a 507 mm female (recapture; previously green) spawned with a 373 mm male, and a 435 mm female spawned with a 362 mm male. The first female used to create this group was mostly spent, containing only a few eggs. The eggs were discarded and not included in the group; however, a genetic sample was collected from this fish. Similarly, the first male used to create this group did not produce enough milt for fertilization. The milt was discarded and not included in the group; however, a genetic sample was collected from this fish. Five ripe males were remaining following the spawning operations. A genetic sample was collected from each, and the fish were returned to the river. Upon arriving at the Aquatic Toxicology Lab, eggs were held at a temperature of 9.2°C.

In addition to the spawn, 60 rainbow trout and 60 brown trout were collected for PTD sampling on April 30. Genetic samples were collected from the rainbow trout, and genetic sample number and head number were paired to facilitate matching of myxospore count to strain.

Two hundred five rainbow trout were captured via electrofishing on May 15. Of these, 102 (50%) were females, 20 (10%) were males, and 83 (40%) were immature. Of the 102 females, 30 (29%) were green, 11 (11%) were ripe, and 61 (60%) were spent. Of the 20 males, 18 (90%) were ripe, and two (10%) were spent. All ripe females and males were kept in separate net pens for spawning. All green, spent, and immature fish were returned to the river. Two groups were created using ripe males and females. Group 6 consisted of a 426 mm female spawned with a

397 mm male, and a 378 mm female spawned with a 286 mm male. The first female spawned was mostly spent, and the few remaining eggs were overripe. The eggs were discarded and not included in the group; however, a genetic sample was collected from this fish. A third pair of fish was then spawned to create eggs for this group: a 440 mm female spawned with a 248 mm male. Only a small number of eggs were produced by this female, and though they looked good, it was decided that another female should be used to obtain more eggs. The next female/male combination produced a high number of quality eggs, which were retained to make up the remainder of group 6. Group 7 consisted of a 516 mm female spawned with a 292 mm male, and a 437 mm female spawned with a 349 mm male. Eight ripe males were remaining following the spawning operations. A genetic sample was collected from each, and the fish were returned to the river. Upon arriving at the Aquatic Toxicology Lab, eggs were held at a temperature of 15.5°C.

Eggs from all groups began to hatch on June 4. By June 9, all groups had finished hatching. All groups were maintained in the Aquatic Toxicology Lab through swim-up; fish were transported from the Aquatic Toxicology Lab to the Parvin Lake Research Station on July 16 for the *Myxobolus cerebralis* exposure experiment. No mortalities occurred during transport.

MYXOBOLUS CEREBRALIS EXPOSURE EXPERIMENT

The seven groups were maintained in separate 76-L flow through tanks within the Parvin Lake Research Station Lab. One week prior to exposure to *Myxobolus cerebralis*, family groups were split into control tanks and exposure tanks; numbers of fish were reduced to 25 fish per tank. Tanks containing control fish were maintained in a separate row from the exposure tanks so that no cross contamination could occur during the exposure experiment.

Unfortunately, the *Tubifex tubifex* worm cultures maintained at the Parvin Lake Research Station did not produce any triactinomyxons for the exposure experiment. As a result, exposure fish were transported from their tanks at the Parvin Lake Research Station to the CPW Poudre Rearing Unit for exposure. Fish were put in 3-in diameter PVC cages, designed to allow water to flow in through a grate in the top of the cages and out of the bottom of the tube, which was covered with fine mesh netting to prevent fish escape. Cages were placed in the inlet of Pond 5, which receives water from the Cache la Poudre River, known to be a *Myxobolus cerebralis*-infested water source. Fish remained in the cages in Pond 5 for one month prior to being transported back to the Parvin Lake Research Station. Control and exposure fish were held at the Parvin Lake Research Station through May 2013 to allow full development of myxospores within the exposed fish.

On May 9, 2013, all remaining rainbow trout within the control and exposure tanks were sacrificed using an overdose of MS-222. Lengths, weights, and signs of infection (cranial, spinal, lower jaw, and opercular deformities, and blacktail) were recorded from each individual. Heads were removed, placed in individually labeled bags, and sent to the Brush Fish Health Lab for myxospore enumeration using the Pepsin-Trypsin Digest method. Fin clips were also taken from each individual to determine genetic background relating to the parents spawned in the East Portal in the spring of 2012.

RESULTS AND DISCUSSION

At the time of this report, myxospore enumeration had not been completed for any of the family groups from the *Myxobolus cerebralis* exposure experiment. Results regarding myxospore counts and resistance will be available in the next reporting cycle.

All rainbow trout spawned to create the family groups used in the *Myxobolus cerebralis* exposure experiment were found to be pure CRR individuals. As such, all offspring contained within the exposure experiment were also found to be pure CRR. This was unexpected as the East Portal of the Gunnison River has only been stocked with GR-cross fish since 2006. The results of this experiment, and the genetic testing that occurred in 2011, suggest that the GR-cross fish are not surviving well in the East Portal, and are not contributing to the offspring being naturally produced in the river. As such, we are suggesting that this location not be considered as a wild GR-cross brood stock location at this time.

The genetic test suggested that there is some amount of differentiation between the pure CRR individuals encountered in the East Portal, and hatchery CRR stocks that had been used in 2008-2010 to develop the GR versus CRR differentiation test. The CRR in the Gunnison River have maintained a self-sustaining rainbow trout population despite the presence of *Myxobolus cerebralis*, although, infection levels in the East Portal are lower than many other rivers in Colorado, and were never high enough to result in a collapse in the East Portal rainbow trout population. The combination of low infection levels and natural recruitment in this location created conditions that may be leading to the development of *Myxobolus cerebralis*-resistance in the East Portal CRR population. Myxospore counts from fish in the *Myxobolus cerebralis* exposure experiment will be useful in determining if natural resistance has begun to develop in this population.

The low survival and lack of recruitment observed in the GR-cross fish stocked into the East Portal of the Gunnison River is puzzling. It is suspected that these hatchery-derived fish may not be well suited for competing for resources with the naturally produced rainbow trout, and may also be more susceptible to brown trout predation in this section of the river. Another potential explanation is that the GR-cross fish are moving downstream, going over the diversion structure at the lower end of the East Portal, and are unable to return to the section to spawn. To try and determine the fate of stocked GR-cross fish in the East Portal, 21,000 GR-cross fish were tagged with coded wire tags at the Rifle Falls Hatchery in June 2013, prior to being stocked. Fry shocking in 2013 will be used to assess the fry survival of the GR-cross fish stocked in the East Portal of the Gunnison River, and determine the ratio of wild fish to stocked hatchery fish at multiple time points throughout the year. In addition, an adult population estimate will be conducted in the East Portal in 2014 to determine if the GR-cross fish have recruited to the age-1 rainbow trout population. Finally, electrofishing will occur below the lower diversion structure in both 2013 and 2014 to determine if GR-cross fish moved downstream out of the East Portal.

Lake Catamount H×H Brood Stock

Hofer × Harrison Lake (H×H) rainbow trout crosses have been stocked into Lake Catamount and the Yampa River near Steamboat Springs since 2007 with the objectives of reducing infection levels within the Yampa River and establishing a wild H×H brood stock in Lake Catamount.

Previous exposure experiments have shown a reduction in infection severity in the rainbow trout in the Yampa River and its tributaries between 2002 (no H×H present in the system) and 2010 (three years post-introduction of H×H to the system). In addition, H×H stocked into Harrison Creek, a tributary to Lake Catamount, have exhibited a fidelity to Harrison Creek during the spawning period, suggesting that a wild egg take from the fish returning to Harrison Creek could be used to replace hatchery brood stocks of H×H in Colorado hatcheries.

An exposure experiment, similar to that conducted on the East Portal of the Gunnison River H×C brood stock, is being used to assess the resistance characteristics of the offspring produced by fish returning to Harrison Creek to spawn. In May 2013, rainbow trout were captured in Harrison Creek via electrofishing to obtain eggs for an exposure experiment. Five family groups were created from the fish in Harrison Creek, each consisting of two male-female pairs. In addition, three families groups were created using rainbow trout (presumed to be H×Hs) captured via trap nets in Lake Catamount that had not run up Harrison Creek. All eight family groups were spawned on the same day and transported back to the Aquatic Toxicology Lab in Fort Collins for rearing. Eggs were maintained at 12°C and held until they eyed up. Upon eye up, eggs were transported to the Parvin Lake Research Station where they hatched.

The fish will be reared until they have reached 650 degree-days post-hatch. At that time, the family groups will be split into control and exposure tanks. Fish within the exposure tanks will be exposed to a dose of 2,000 triactinomyxons per fish. Triactinomyxons will be obtained from worm cultures maintained at the Parvin Lake Research Station. Following exposure, fish will be held for up to eight months to allow full development of myxospores. Similar to the East Portal exposure experiment, fish will be euthanized at the end of the experiment with an overdose of MS-222, heads will be sent to the Brush Fish Health Lab for myxospore enumeration, and genetic samples will be used to compare the genetic backgrounds of the offspring to the parental brood stock in Lake Catamount. Results should be available within the next reporting cycle.

Genetic Techniques

HOFER VERSUS HARRISON DIFFERENTIATION TEST

Use of the 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 to identify H×H fry and adults. A Hofer versus Harrison Lake differentiation test was developed by Melinda Baerwald at the University of California Davis using Single Nucleotide Polymorphism (SNP) chips.

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 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 spanned a range of possible crosses between the Hofer and the Harrison Lake. Offspring 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. A large amount of differentiation between the pure GR and Pure Harrison Lake strains allowed the test to quickly and correctly differentiate the two pure strains and their crosses.

To evaluate the accuracy of the test, 96 samples of the pure strains and their crosses (GR, F1, F2, B2 GR, B2 HAR, and HAR) collected from the Bellvue Fish Research Hatchery were run through the SNP probability test as blind samples. Only 4 samples were incorrectly identified: an F2 identified as a B2 GR, a B2 HAR identified as a pure HAR, a GR identified as a B2 HAR, and F1 identified as a B2 HAR. These results suggested that the differentiation test had a 96% accuracy rate. In addition to identification, the SNP test provides a comparison of the amount of GR and HAR genes found in each of the pure strains and their crosses. Examination of the percentage of GR and HAR genes in each of the pure strains and individual crosses showed that the test is accurately identifying and differentiating these genes, as the percentage of genes identified as GR or HAR were similar to what would be predicted using a genetic Punnett's square for each strain or cross (Figure 4.6).

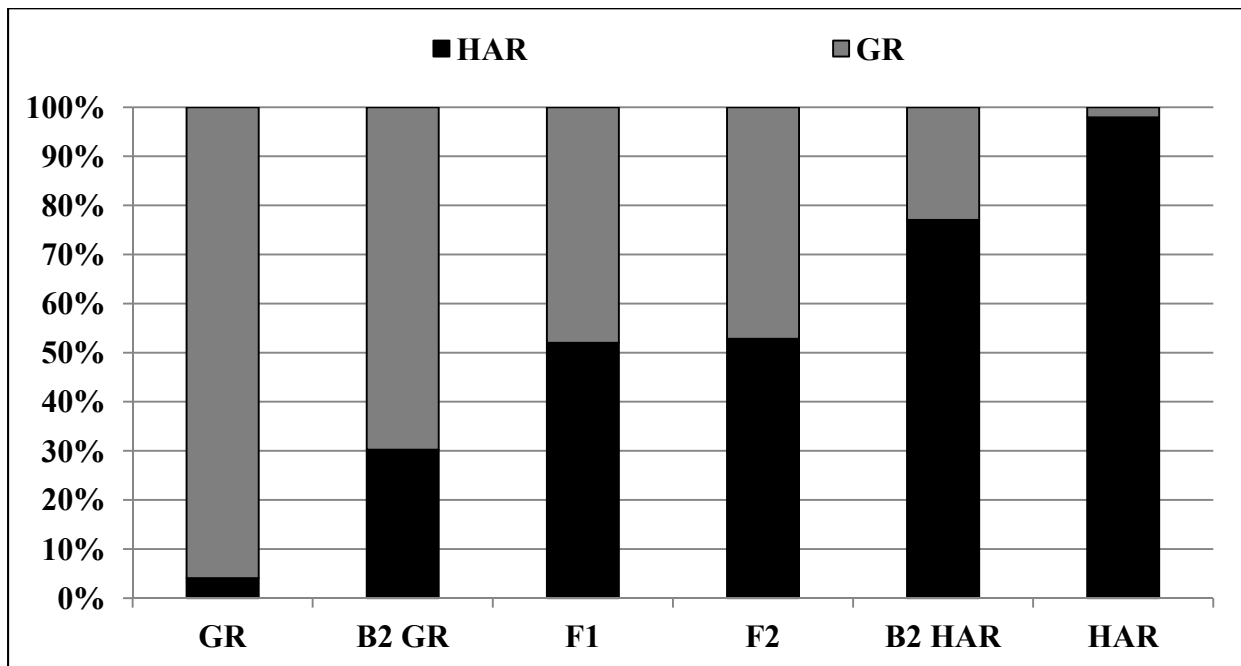


Figure 4.6. Percentage of GR and HAR genes found in each of the pure strains and their crosses, identified using a SNP chip.

The H×H SNP differentiation test can accurately identify the two pure strains and their crosses. The SNP chips developed in this test have been used to help identify GR and HAR fish in the Yampa River and Lake Catamount. In the future, this test will be used to determine the genetic composition of H×H wild and hatchery brood stocks. In addition, families can be created and reared separately during wild egg takes, and the H×H differentiation test can be used to determine which families have the desired genetic characteristics and should be retained to enhance hatchery brood stocks and wild fish plants in other location across the state.

References

- Hedrick, R. P., T. S. McDowell, M. Gay, G. D. Marty, M. P. Georgiadis, and E. MacConnell.
1999. Comparative susceptibility of rainbow trout *Oncorhynchus mykiss* and brown trout *Salmo trutta* to *Myxobolus cerebralis*, the cause of salmonid whirling disease. *Diseases of Aquatic Organisms* 37(3):173-183.

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., and G. J. Schisler. 2012. Whirling disease resistant rainbow trout: Recovering Colorado's rainbow trout populations. 2012 Annual Meeting of the American Fisheries Society Fish Health Section. La Crosse, Wisconsin. August 2, 2012.
2. Fetherman, E. R., and G. J. Schisler. 2012. Whirling disease resistant rainbow trout strain development and uses in Colorado. 2012 Annual Meeting of the U.S. Trout Farmers Association. Denver, Colorado. September 7, 2012.
3. Fetherman, E. R. 2012. Whirling disease resistant rainbow trout introductions and management. Departmental Seminar for the Department of Fish, Wildlife and Conservation Biology, Colorado State University. Fort Collins, Colorado. December 7, 2012.
4. Fetherman, E. R. 2013. Whirling disease resistant rainbow trout introductions: What have we learned? Colorado Parks and Wildlife Aquatic Biologist Meeting. Steamboat Springs, Colorado. January 24, 2013.
5. Fetherman, E. R. and G. J. Schisler. 2013. Hofer rainbow trout: Uses and management in Colorado. Colorado Parks and Wildlife 2013 Northeast Region Biology Days. Denver, Colorado. February 1, 2013.
6. Fetherman, E. R., D. L. Winkelman, K. Davies, and B. W. Avila. 2013. Survival and movement rates of brown trout and reintroduced, whirling disease-resistant rainbow trout in the Cache la Poudre River. 2013 Annual Meeting of the Colorado/Wyoming Chapter of the American Fisheries Society. Fort Collins, Colorado. February 25-27, 2013.
7. Fetherman, E. R. and G. J. Schisler. 2013. Hofer rainbow trout: Uses and management in Colorado. Colorado Parks and Wildlife 2013 Southeast Region Biology Days. Pueblo, Colorado. April 18, 2013.
8. Fetherman, E. R., D. L. Winkelman, K. Davies, and B. W. Avila. 2013. Estimating survival and movement rates of PIT-tagged salmonids in the Cache la Poudre River, Colorado. 2013 Annual Meeting of the Western Division of the American Fisheries Society. Boise, Idaho. April 18, 2013.

9. Fetherman, E. R. 2013. Whirling disease resistant rainbow trout introductions. 2013 Annual Meeting of the Colorado Cooperative Fish and Wildlife Research Unit Coordinating Committee Meeting. Fort Collins, Colorado. April 23, 2013.

Technical assistance milestones included the peer review of four manuscripts:

- Anonymous. 2012. Comparative pattern of reproductive traits in *Labeo rohita* (Hamilton 1822) from six tropical rivers of Ganga Basin: a new insight. Submitted to the Proceedings of the National Academy of Sciences, India Section B: Biological Sciences.
- Anonymous. 2012. Length-weight relationships and condition factor of snow trout, *Scizothorax richardsonii* (Gray, 1832) from three diverse Himalayan rivers in India. Submitted to the Proceedings of the National Academy of Sciences, India Section B: Biological Sciences.
- Lü, A., X. Hu, X. Shen, X. Li, Y. Lan, Y. Wang, and Q. Ming. 2013. Comparative analysis of the acute response of zebrafish skin to two different bacterial infections. Submitted to the Journal of Aquatic Animal Health.
- Harbicht, A. M. AlShamli, C. Wilson, and D. Fraser. 2013. Environmental and anthropogenic correlates of hybridization between hatchery and wild trout. Submitted to the Canadian Journal of Fisheries and Aquatic Sciences.

Technical assistance milestones also included the publication of two peer-reviewed journal articles:

- Lepak, J. M., E. R. Fetherman, W. M. Pate, C. Craft, and E. I. Gardunio. 2012. An experimental approach to determine esocid prey preference in replicated pond systems. *Lake and Reservoir Management* 28:224-231.
- Fetherman, E. R., D. L. Winkelman, G. J. Schisler, and M. F. Antolin. 2012. Genetic basis of differences in myxospore count between whirling disease-resistant and -susceptible strains of rainbow trout. *Diseases of Aquatic Organisms* 102:97-106.

In addition to those manuscripts published in peer-reviewed journals, one other manuscript was submitted, and was accepted for publication in June 2013:

- Fetherman, E. R., and J. M. Lepak. In press. Addressing depletion failure and estimating gear efficiency using back-calculation of capture probabilities. *Fisheries Research*.

Lastly, the Ph.D. dissertation entitled *Introduction and Management of Myxobolus cerebralis-Resistant Rainbow Trout in Colorado* was completed and submitted to Colorado State University for publication. Chapters from this dissertation are included as appendices in this report.

Job No. 4 Appendix 1

Reintroduction of Rainbow Trout to the Upper Colorado River using Whirling Disease-Resistant Rainbow Trout Strains (Chapter Two from the Ph.D. dissertation entitled *Introduction and Management of Myxobolus cerebralis-Resistant Rainbow Trout in Colorado* by Eric R. Fetherman; submitted to Colorado State University, Summer 2013)

INTRODUCTION

Extirpations of wild salmonid populations have been caused by a variety of factors and have led to a focus on captive breeding (i.e., hatcheries) to sustain or reintroduce populations (Hesthagen and Larsen 2003; Flagg et al. 2004; Bosch et al. 2007; Carmona-Catot et al. 2012). However, successful reintroduction attempts using captive-reared salmonids usually involve mitigating or removing the factors responsible for the original extirpation (Fraser 2008). For instance, artificial liming has been used to reduce river acidification and has aided in successful reintroduction of Atlantic salmon (*Salmo salar*; Hesthagen and Larsen 2003). Greenback cutthroat trout have also been successfully reintroduced in streams with suitable habitat that are protected from reinvasion by other invasive trout species (Harig and Fausch 2000). However, when factors causing extirpations have not been fully mitigated prior to reintroduction, stocking has generally been unsuccessful (Fraser 2008).

In Colorado, introduction of *Myxobolus cerebralis*, the parasite responsible for salmonid whirling disease, caused the extirpation of wild rainbow trout (*Oncorhynchus mykiss*) populations from many of the state's rivers (Nehring and Thompson 2001). Unlike extirpations caused by factors that could potentially be mitigated or reversed, pathogens such as *M. cerebralis* cannot be removed once introduced into an ecosystem. However, disruption of the parasite's life cycle has been attempted either through habitat manipulation to reduce populations of the intermediate oligochaete host (*Tubifex tubifex*) or through introduction of resistant lineages of *T. tubifex*. Neither approach has been completely successful (Thompson 2011). One promising approach for the recovery of Colorado's rainbow trout populations has been the production of rainbow trout that are genetically resistant to the parasite. To this end, management and research in Colorado have focused on using crosses between resistant, hatchery-derived rainbow trout and wild rainbow trout strains (Schisler et al. 2006).

The German Rainbow (GR) is a hatchery-derived rainbow trout strain that was exposed to *M. cerebralis* for decades in a Bavarian hatchery in Germany (Hedrick et al. 2003). Although the GR strain can be infected with *M. cerebralis*, parasite burdens are usually low (Hedrick et al. 2003; Schisler et al. 2006; Fetherman et al. 2012) and the GR strain is known to survive and reproduce in the presence of and infected with *M. cerebralis*. Low parasite burdens and the strain's ability to persist when exposed to *M. cerebralis* have been termed "resistance," and this resistance is presumed to be a result of long-term exposure to the parasite over multiple generations (Hedrick et al. 2003). Despite the resistance seen in the GR strain, its survival and viability in the wild was uncertain due to the strain's history of domestication (Schisler et al. 2006). Therefore, the GR strain was experimentally crossed with the Colorado River Rainbow (CRR; Schisler et al. 2006; Fetherman et al. 2011; Fetherman et al. 2012), a wild rainbow trout strain that had been widely stocked in Colorado and comprised many naturally reproducing wild rainbow trout fisheries prior to the introduction of *M. cerebralis* (Walker and Nehring 1995).

Intermediate crosses of the two strains have been rigorously evaluated. Laboratory experiments showed that the first filial generational cross between the two strains (termed the H×C) exhibited resistance characteristics similar to those of the GR strain (Schisler et al. 2006; Fetherman et al. 2012), and was capable of attaining critical swimming velocities similar to those of the CRR strain (Fetherman et al. 2011). It was suggested that the H×C cross may be the best candidate for reintroducing rainbow trout populations; however, its utility needed to be evaluated in a natural setting (Fetherman et al. 2012). Overall, I wanted to evaluate the performance of H×C that were stocked into the upper Colorado River in an attempt to reintroduce a self-sustaining population in the presence of *M. cerebralis*. The objectives of this study were to examine the survival, abundance and growth of the stocked H×C population. Additionally, if offspring were produced, indicating that reproduction had occurred, I wanted to evaluate the genetic composition of the age-0 individuals and whether they displayed increased resistance and survival characteristics compared to their wild CRR counterparts.

METHODS

Site Description

The 4.2 km (3.9 mi) upper Colorado River study site is situated approximately 1.6 km downstream of Windy Gap Reservoir and 3.2 km upstream of the town of Hot Sulphur Springs in Grand County, Colorado. Flows in this section are partially regulated by Windy Gap dam, with a mean annual discharge of 7.2 cubic meters per second (cms), ranging from a mean of 2.2 cms in the winter to 22.5 cms during peak flows; temperatures range from 3.4°C in the winter to 16.2°C in the summer, with an mean annual temperature of 10.7°C (USGS 2009). The study section is on private land, primarily managed for cattle grazing; however, land owners also allow private fishing access.

Prior to the introduction of *M. cerebralis* in the upper Colorado River, adult CRR had an average abundance of 428 fish km⁻¹ (687 fish mi⁻¹) and adult brown trout averaged 239 fish km⁻¹ (384 fish mi⁻¹; Nehring and Thompson 2001), resulting in a ratio of rainbow trout to brown trout of 2:1. Rainbow trout fry abundance ranged from 5,600 to 8,400 fry km⁻¹ of stream bank and brown trout fry ranged from 2,600 to 5,700 fry km⁻¹ (Walker and Nehring 1995). Traditionally, eggs were harvested from this wild CRR brood stock, reared in state hatcheries, and used to stock many rivers across the state.

M. cerebralis was unintentionally introduced to the upper Colorado River in the 1980s when privately-reared rainbow trout previously exposed to *M. cerebralis* were stocked into three private water bodies located upstream of Windy Gap Reservoir. Fish below Windy Gap Reservoir tested positive for *M. cerebralis* in 1988, and a subsequent decline in the younger age classes of rainbow trout was observed in the early 1990s (Nehring 2006). While several reasons for the declines were investigated (Schisler et al. 1999a,b; Schisler et al. 2000), exposure to *M. cerebralis* was determined to be the primary cause for the disappearance of the younger age classes (Nehring and Thompson 2001). In an effort to restore the rainbow trout fishery, tens of thousands of CRR were stocked annually between 1994 and 2008. Despite these repeated stocking efforts, the CRR exhibited low survival and little recruitment success, resulting in rainbow trout abundances that were approximately 90% lower than those observed prior to the establishment of *M. cerebralis* (Nehring 2006). Into the 2000s, the upper Colorado River below

Windy Gap Reservoir continued to be one of the rivers with the highest prevalence of *M. cerebralis* infection in the state.

Rainbow Trout Stocking

The first introduction of *M. cerebralis*-resistant rainbow trout to the upper Colorado River occurred on June 2, 2006, with an introduction of 3,000 H×Cs. Prior to being stocked, each fish was tagged with an individually numbered fine-filament Floy tag, secondarily adipose clipped for identification in the event of tag loss, and measured to the nearest mm; fish averaged (\pm SD) 238 (\pm 23) mm in total length (TL). Larger rainbow trout were used in the introduction because they were 1) less susceptible to *M. cerebralis* infection (Ryce et al. 2005), and 2) less susceptible to brown trout predation. Fish were distributed throughout the study section, with approximately 1,250 fish stocked at the upstream end of the section, 1,100 stocked in the middle of the section, and 650 stocked at the downstream end of the section.

Additional stocking attempts occurred in January 2009, with an introduction of 5,000 H×Cs averaging 209 (\pm 23) mm TL, and June 2010, with an introduction of 2,000 H×Cs averaging 172 (\pm 18) mm TL; these fish were similarly tagged with an individually numbered Floy tags and measured to the nearest mm prior to stocking. Hatchery space constraints required the 2009 introduction to occur in winter, and fish were stocked through a hole drilled in the ice cover. As a result, the 2009 introduction was unsuccessful; no H×Cs from the 2009 introduction have been encountered in subsequent sampling events, and so these fish will not be discussed in the remainder of this chapter. In addition, only one sampling occasion occurred following the introduction of H×Cs to the upper Colorado River in 2010, and as a result, survival was not estimated for these fish; however, these fish contributed to adult fish population abundance estimates in 2011 and potentially contributed offspring produced during the study. Therefore, survival and growth analyses regarding the adult rainbow trout population are performed using only data collected from the group of H×Cs introduced to the upper Colorado River in 2006, but abundance estimates include fish introduced in 2006 and 2010.

Adult Rainbow Trout Population

Population Sampling

Adult rainbow trout abundance and survival were estimated during recapture occasions occurring in the fall of 2006 and 2007, and the spring of 2008, 2009, 2010, and 2011. Efforts in the fall of 2006 and 2007 consisted of two-pass removal estimates (Temple and Pearsons 2007) conducted in a 305-m stretch of the upper Colorado River located at the upstream end of the study section, and were used to estimate abundance on a local habitat scale and recapture fish for survival estimation. Estimates were completed using a four-electrode bank shocking unit and removal passes were conducted subsequently within the same day. Floy tag numbers, lengths, and weights were recorded for all H×Cs encountered during the sampling. As the 2006 and 2007 abundance estimates were conducted on a smaller geographical scale and during a different season (fall) than those conducted in 2008 through 2011 (spring), recapture information from the 2006 and 2007 sampling events was used only in the adult H×C survival analyses.

A two-pass, mark-recapture electrofishing effort, with a minimum of one day between passes to allow for the redistribution of marked fish, was used to sample the adult rainbow trout population in the upper Colorado River in the spring of 2008, 2009, 2010, and 2011. Two raft-mounted

electrofishing units were used to complete the sampling, with one raft covering each half of the river. Fish encountered on both the mark and recapture passes were processed approximately every 0.8 km and returned to the river following processing. On the mark pass, fish were given a caudal fin punch for identification on the recapture pass. Floy tag presence/absence and number, TL (mm), and weight (g) were recorded for all rainbow trout captured on both passes.

Floy-tagged fish were identified as H×Cs and were therefore included in the survival, growth, and abundance analyses; however, Floy tag loss occasionally prevented individual identification of H×Cs. Rainbow trout missing a Floy tag but retaining an adipose clip were identified as H×Cs for the purpose of abundance estimation, but were not included as part of the survival or growth analyses. Rainbow trout from which a Floy tag and adipose clip were absent were identified as CRR, which were presumed to be remaining in the section from previous stocking events, allowing CRR abundance to be estimated separately from H×C abundance during the 2008, 2009, 2010, and 2011 sampling occasions.

Statistical Analyses

A Lincoln-Peterson estimator with a Bailey (1951) modification, which accounted for fish being returned to the population following examination of marks on the recapture pass (Van Den Avyle and Hayward 1999), was used to obtain H×C and CRR abundance estimates (\hat{N}) for each year of the study. Estimates were calculated for the entire study reach and divided by 4.2 (km sampled) to obtain an estimate of adult H×C and CRR km⁻¹ of river. Variance in abundance estimates was calculated using the equation presented in Van Den Avyle and Hayward (1999), and 95% confidence intervals (CIs) calculated from the variance estimates were used to compare differences in abundance between the H×C and CRR within and across years.

Apparent survival probability (ϕ), the probability that fish survived and were retained within the study section, was estimated for the H×C on a monthly basis, accounting for varying time intervals between primary sampling occasions, using the Cormack-Jolly-Seber (CJS) open capture-recapture estimator in Program MARK (White and Burnham 1999). If tagged fish were encountered during either secondary sampling occasion (i.e., pass), the associated recapture data were used to create the encounter histories for the primary sampling occasions (fall 2006 and 2007, and spring 2008, 2009, 2010, and 2011). The model set included models in which detection probability (p) was constant (\cdot), varied with discharge at time of sampling (cms), or varied by effort (effort; bank electrofishing in the fall versus raft electrofishing in the spring), or the additive combination of cms and effort. For survival estimation, the model set included models in which ϕ was constant (\cdot), varied by length at release (length; included as an individual covariate), with minimum discharge between primary sampling occasions (min), maximum discharge (max) between primary sampling occasions, or followed a trend with time (T). Although length was allowed to appear additively with min, max, or T, these three covariates never appeared in the same model. Models were ranked using Akaike's Information Criterion corrected for small sample sizes (AICc; Burnham and Anderson 2002). Model averaging was used to incorporate model selection uncertainty into the parameter estimates, and unconditional standard errors (SE) were reported for the model averaged parameter estimates (Anderson 2008).

Absolute growth (TL) and absolute growth rate (TL year⁻¹) of the H×C were calculated using equations presented in Busacker et al. (1990). Repeated measures of TL from individuals stocked in 2006 and recaptured between 2008 and 2011 were used to fit a von Bertalanffy

growth curve by means of the Fabens (1965) method, where time at large (days), TL at release, and TL upon recapture were known. Time at large was converted from days to years prior to analysis, and parameters for the growth curve were estimated iteratively using a nonlinear regression approach (Isely and Grabowski 2007) implemented in SAS (Proc NLIN; SAS Institute, Inc. 2010). Age at recapture was calculated based on the knowledge that H×Cs were approximately 1.6 years of age at stocking. The von Bertalanffy model is a predictive model of growth, where growth rate declines with age, becoming zero as fish near a maximum possible size. The model is represented as $l_t = L_\infty(1 - e^{-K(t-t_0)})$, where l_t is length at time t , L_∞ is the asymptotic length, K is a growth coefficient, and t_0 is a time coefficient at which length would theoretically be zero (von Bertalanffy 1938).

Age-0 Trout Population

Population Sampling

The age-0 (fry) population was sampled in September 2007 and October 2008 to determine the baseline genetic composition of the rainbow trout fry population produced in the upper Colorado River in these years. From 2009 to 2012, the salmonid fry population was sampled once a month, June through October, to determine fry abundance, as well as to determine if shifts in genetic composition of the rainbow trout fry population changed over time. Three pass removal estimates were conducted using two LR-24 Smith-Root backpack electrofishing units run side-by-side to include all available fry habitat at four, 15.2 m-long sites, one located at the downstream end of the study section, two in the middle of the study section, and one at the upstream end of the study section.

All fry encountered during the sampling were identified to species, measured (TL; mm), and examined for signs of *M. cerebralis* infection. A fin clip was taken from all rainbow trout fry encountered during this sampling for genetic analysis. Additional electrofishing efforts outside of the population estimation sites were used to increase the number of the rainbow trout fry used in the genetic and disease (myxospore enumeration) analyses.

Genetic Assignment of Rainbow Trout Fry

The Genomic Variation Laboratory at the University of California at Davis identified a suite of microsatellite markers capable of distinguishing pure GR and GR-cross fish, including H×C (F1), second generation H×C (F2), and backcross generations (B2C: F1 × CRR; B2H: F1 × GR), from pure CRR fish. Over 300 microsatellite markers were specifically identified for the purpose of genetically screening wild rainbow trout fry to detect and differentiate offspring produced by GR-cross fish from those produced by residual CRR fish. Known samples of pure GR, pure CRR, and their crosses, were used to identify microsatellite markers that were most effective for differentiation based on the frequency of appearance in the pure strains; the ability of this microsatellite array to differentiate known samples was assessed prior to use on unknown samples from the wild.

The software program NewHybrids (Anderson and Thompson 2002) was used to differentiate the parentage of individuals based on microsatellite differences. The NewHybrids program uses the framework of Bayesian model-based clustering to compute, by Markov Chain Monte Carlo, the posterior probability that an individual belongs to each of a distinct set of defined hybrid classes. The posterior probability reflects the level of certainty that an individual belongs to a

hybrid category (Anderson and Thompson 2002); an individual was positively identified as a specific strain or hybrid if the posterior probability for the given category was $\geq 80\%$ for that individual. If none of the hybrid categories met this criterion, the individual was classified as unknown. Using the NewHybrids software program, unclassified rainbow trout fry collected from the upper Colorado River were identified to strain (pure GR, pure CRR) or cross (F1, F2, B2C, and B2H). The proportion of the rainbow trout fry population assigned to the pure CRR or GR-cross hybrid categories, as well as classified as unknown, was ascertained on a per year basis, and trends across years were examined to determine if the H×C had successfully reproduced in the upper Colorado River.

Quantification of M. cerebralis Infection

Signs of infection as a result of exposure to *M. cerebralis*, including cranial, spinal, opercular, and lower jaw deformities, and blacktail, were recorded for each salmonid fry encountered between 2009 and 2012. In October of 2009 and 2011, five brown trout fry and up to five rainbow trout fry were collected from each of the four sites to quantify myxospores, a measure of the severity of infection following exposure to *M. cerebralis*. Myxospores were enumerated (O'Grodnick 1975) using the pepsin-trypsin digest (PTD) method (Markiw and Wolf 1974) by the Colorado Parks and Wildlife (CPW) Fish Health Laboratory (Brush, Colorado).

Statistical Analyses

A three pass removal estimator (Seber and Whale 1970) was used to obtain rainbow trout fry population abundance estimates (\hat{N}) at each of the sampling sites. Estimates were converted to $\hat{N} \text{ km}^{-1}$ of river bank by multiplying the estimate by 65.8; estimates from the four sampling sites were averaged within a month, providing an estimate of fry km^{-1} of river bank for the entire study section. Confidence intervals (Seber and Whale 1970) were used to compare differences in rainbow trout fry abundance both within and across years.

To evaluate the difference in myxospore counts of rainbow trout fry collected in 2009 and 2011, I used a general linear model (GLM) as implemented in SAS ProcGLM; two models were included in the models set, an intercept-only model and a model including year as a categorical variable to capture inter-annual variation. The genetic assignment test was then used to associate myxospore count with rainbow trout fry determined to have CRR or GR-cross origins. A second GLM was run to examine if genotype conferred resistance to *M. cerebralis*, and if CRR and GR-cross fry differed from brown trout fry in average myxospore count. Two models were included in the model set, an intercept-only model, and a model including species as a categorical variable to capture inter-species variation. Logistic regression (SAS ProcLOGISTIC) was used to assess the factors that influenced the probability that an individual fry would exhibit signs of *M. cerebralis* infection (cranial, spinal, opercular, and lower jaw deformities, and blacktail); disease sign was treated as a binary response variable (response was 'yes' or 'no'). For the logistic regression analysis, I considered an intercept-only model, as well as models that included effects of species only, year only (2009, 2010, and 2011), and models with additive and interactive effects between species and year. Model weights and delta AICc ranking were used to determine support for each of the models included in the model sets, and parameter estimates were reported from the candidate model with the lowest AICc value (Burnham and Anderson 2002).

RESULTS

Adult Rainbow Trout Population

Adult H×C abundance (\hat{N} km⁻¹; fish stocked in 2006 only) did not differ from adult CRR abundance in the upper Colorado River in any year. Both populations exhibited significant decreases in abundance between 2008 and 2011, declining from an estimated (\pm SD) 57 (\pm 8) H×C and 68 (\pm 15) CRR km⁻¹ in 2008, to only 4 (\pm 1) H×C and 6 (\pm 1) CRR km⁻¹ in 2011 (Figure A1.1). Floy tag loss likely caused the annual estimates of H×C abundance to be biased low. Interestingly, the adult brown trout population also exhibited a significant decrease in abundance between 2009 and 2011, declining from an estimated 1,201 (\pm 78) km⁻¹ in 2009 to 525 (\pm 47) km⁻¹ in 2011.

Apparent survival (ϕ) was more affected by discharge than a general trend with time. Models that allowed survival to vary as a function of minimum flow (top two models) between primary sampling occasions had twice as much support as those that modeled survival as a function of maximum flows (models ranked three and four; Table A1.1). Discharge had a positive effect on survival ($\hat{\beta} = 0.033 \pm 0.007$), with survival increasing with an increase in minimum flow. Survival was also positively affected by length at release ($\hat{\beta} = 0.006 \pm 0.002$), with length at release appearing in all six of the models with a Δ AICc value < 4.0 . In general, model-averaged monthly apparent survival was lower in 2006 and 2007 than it was in later years of the study (2008 through 2011; Figure A1.2), primarily due to minimum flows between primary sampling occasions that were nearly twice as low, on average, in 2006 and 2007 (1.21 ± 0.13 cms) than in 2008 through 2011 (2.06 ± 0.06 cms). Apparent survival for the entire study period (June 2006 to May 2011) was estimated to be 0.007 (SE < 0.001). Detection probability differed with effort (bank electrofishing $p = 0.05$ [SE ± 0.008]; raft electrofishing $p = 0.22$ [SE ± 0.06]), with effort appearing in all six models with a Δ AICc < 4.0 (Table A1.1), and was likely due to the amount of stream length covered by the two sampling methods and the season in which sampling occurred. Discharge had a weak negative effect on p (associated 95% confidence intervals overlapped zero), and appeared in only three of the models with a Δ AICc value < 4.0 , and not in the top model.

Average absolute increase in TL (\pm SE) of the H×C was 111 (± 3.5) mm, with an average absolute annual rate of increase in TL of 45 (± 1.3) mm. Parameter estimates for the von Bertalanffy equation were $\hat{L}_{\infty} = 424.5$, $\hat{K} = 0.37$, and $\hat{t}_0 = -0.16$ (Figure A1.3).

Age-0 Trout Population

Wild rainbow trout fry abundance exhibited a declining trend in 2009 and 2010, and no rainbow trout fry were detected in any of the sampling sites in October of either year. Rainbow trout fry abundance patterns differed in 2011 and 2012 in that a decreasing trend in abundance was not apparent. Potentially indicative of an increase in resistance and survival, rainbow trout fry were still detected within the study sites in October of both 2011 and 2012 (Figure A1.4).

Genetic assignments revealed a shift in the genetic composition of the rainbow trout fry population over time. In 2007, CRR and unknown fish comprised the entirety of the population (Figure A1.5). GR-cross fish first appeared in the fry population in 2008, comprising about 35%

of the population. The proportion of GR-cross fish in the fry population increased over time, with GR-cross fish comprising nearly 80% of the fry population in 2011 (Figure A1.5).

Model selection results for differences in average myxospore count in rainbow trout indicated that the model that included year was more supported by the data than the intercept model (AICc weight = 0.98). Fry collected in October of 2009 averaged (\pm SE) 47,708 (\pm 8,650) myxospores fish⁻¹, whereas fry collected in October of 2011 averaged 2,672 (\pm 4,379) myxospores fish⁻¹. When brown trout were included in the analysis and myxospore count was assigned to specific CRR or GR-cross rainbow trout individuals using the genetic assignment test, model selection results indicated that a model containing species/cross differences in myxospore count was most supported by the data (AICc weight = 0.93). CRR fry exhibited a higher myxospore count than either the GR-cross or brown trout fry (Figure A1.6).

A species by year interaction had the largest influence on the probability that an individual fry would exhibit signs of *M. cerebralis* infection (AICc weight = 0.99; Table A1.2). A higher proportion of rainbow trout than brown trout fry exhibited signs of infection in 2009; however, no differences in the proportion of fish exhibiting signs of infection was observed between the two species in 2010 or 2011. The proportion of rainbow trout fry exhibiting signs of infection decreased between 2009 and 2011 (Figure A1.7), concurrent with the increase in the proportion of GR-cross fish in the fry population and decrease in infection severity (myxospores fish⁻¹).

DISCUSSION

The objectives of this study were to examine abundance, survival, growth, and reproduction of a stocked H×C population in the upper Colorado River, and determine if the offspring produced had parental genotypes and displayed increased resistance characteristics compared to their wild counterparts. Stocked adult rainbow trout exhibited low survival following stocking; however, they did reproduce. Age-0 rainbow trout exhibited lower infection severity over time as a result. Genetic results suggest that infection severity decreased with a shift in genetic composition of the rainbow trout fry population from susceptible to resistant genotypes over time. In addition, GR-cross fry exhibited significantly lower myxospore counts relative to their wild CRR counterparts.

In 2006, H×C rainbow trout were stocked into my study section and they began to reproduce in 2008. Initially, CRR individuals comprised the entire fry population due to ongoing stocking of this strain in the upper Colorado River. Subsequent age-0 sampling indicates that GR-cross genotypes are increasing in prevalence, relative the CRR strain. Interestingly, I observed the first age-0 recruitment into October in 2010 and 2011. I believe that the 2009 stocking was a failure due to stocking in the winter and that fish stocked in 2010 probably did not begin to reproduce until 2012; therefore, neither group is responsible for the observed changes in the genetic composition of the population.

As resistant genotypes increased, average infection severity (myxospores fish⁻¹) and percentage of age-0 exhibiting signs of exposure to *M. cerebralis* decreased. The myxospore counts of age-0 fish collected in 2009 were similar to those obtained from age-0 rainbow trout collected in the upper Colorado River from about 1990 to 2000 and were indicative of infection levels that caused the original decline (Nehring and Thompson 2001; Nehring 2006). Myxospore counts of fish collected in 2011 were significantly lower than most myxospore counts observed in earlier

studies (Thompson et al. 1999; Ryce et al. 2001) and as low as those observed for brown trout. *M. cerebralis* is endemic in brown trout from central Europe to southeastern Asia and does not cause disease in these populations (Granath et al. 2007). Similarly, GR strain fish were artificially selected for resistance to *M. cerebralis* in a German fish hatchery (Hedrick et al. 2003). In the upper Colorado River, age-0 GR-cross did not differ in infection severity from the age-0 brown trout, suggesting that they were just as resistant to infection and development of clinical signs as the brown trout.

Age-0 CRR had significantly higher myxospore levels than both the GR-cross and brown trout and this is consistent with other studies showing that CRR are highly susceptible to *M. cerebralis* infection (Ryce et al. 2001; Schisler et al. 2006; Fetherman et al. 2012). Myxospore levels in CRR individuals indicate that the parasite is still prevalent in the upper Colorado River and that the low myxospore levels in the GR strain are not a result of reduced parasite numbers. Although differences in myxospore count were previously observed during laboratory experiments (Schisler et al. 2006; Fetherman et al. 2012), my field observations are the first to document such differences in wild populations. Reduced myxospore burdens in age-0 GR-cross trout indicate that stocking this cross may ultimately lead to an overall reduction in infection prevalence and severity in the salmonid populations of the upper Colorado River.

Recruitment of age-0 fish into October, observed in 2011 and 2012, was associated with the shift in genetic composition and decrease in infection severity. Prior to 2011, age-0 rainbow trout quickly developed clinical signs and were not observed in the river by October (Nehring and Thompson 2001; Nehring 2006). I attribute the lack of recruitment to low survival in the younger age classes following exposure to *M. cerebralis* and this is supported by *in situ* studies conducted in the same area (Nehring and Thompson 2001). Survival of rainbow trout fry into October of 2011 and 2012 suggests that GR-cross rainbow trout fry produced in the river may be better able to survive exposure to *M. cerebralis* than their wild CRR counterparts, and that natural recruitment may soon start to aid in the recovery of the wild rainbow trout population in the upper Colorado River.

Fetherman et al. (2012) suggest that resistance to *M. cerebralis* is a heritable trait that should respond to natural selection in the wild. Therefore, continued exposure to *M. cerebralis* in the wild should favor retention of resistance traits, increasing the probability of their persistence. Resistance to *M. cerebralis* in a similar rainbow trout population from Harrison Lake, Montana has increased with continued exposure to the parasite (Miller and Vincent 2008). Miller and Vincent (2008) suggest that as more resistant young from the population mature and reproduce, it may be possible for the population to return to abundance levels observed prior to parasite establishment. Although recovery of wild rainbow trout populations in Colorado was expected to be relatively slow given the low survival of *M. cerebralis* infected fish in wild CRR populations (Nehring and Thompson 2003), the introduction of resistant GR-crosses may facilitate quicker recovery of these populations (Fetherman et al. 2012).

Apparent survival was low in stocked H×C rainbow trout. The hatchery derived origin and history of domestication selection for growth and resistance in the GR strain may have contributed to the low survival rates observed in the reintroduced H×C population; the GR strain is also known to exhibit low heterozygosity (El-Matbouli et al. 2006) which may be an issue with stocked H×C populations. In addition, research has shown that the GR-strain and high proportion GR-crosses (≥ 0.75) exhibit lower survival and increased predation susceptibility

when introduced to natural systems with many terrestrial predators and piscivorous fish species (Fetherman and Schisler 2012). Despite potential drawbacks associated with the resistant, domestic GR strain, laboratory experiments confirmed that H×C exhibited a higher resistance to *M. cerebralis* relative to the susceptible, wild CRR strain, and that critical swimming velocities did not differ from that of the CRR strain (Fetherman et al. 2011). Therefore, the H×C was expected to be better suited for survival in the upper Colorado River than either parental strain.

Survival was also influenced by environmental factors, particularly flow. Both H×C and wild brown trout populations exhibited similar population declines over the study period suggesting that environmental conditions may have influence H×C survival, and results suggest that minimum discharge had a large negative effect on H×C survival. Lower flows result in higher summer water temperatures and lower dissolved oxygen levels (Williams et al. 2009), both of which can directly affect salmonid survival (Hicks et al. 1991). Increased stress due to low flow may have also intensified the effects of *M. cerebralis* infection. Ectoparasite infestation peaks during periods of low flow and high mean water temperatures in the upper Colorado River and could significantly increase mortality in these populations (Schisler et al. 1999b). Low flows also reduce suitable habitat and can lead to high densities and overcrowding, increased predation, and increased competition (Arismendi et al. 2012). Brown trout competition with rainbow trout results in exclusion of rainbow trout from preferred feeding and resting habitats, possibly resulting in population level effects with respect to abundance and survival (Gatz et al 1987).

Food resources may be another environmental factor that will influence reintroduction efforts. The upper Colorado River below Windy Gap Reservoir has undergone significant changes to aquatic invertebrate diversity and abundance; in particular the abundance of the giant stonefly (*Pteronarcys californica*) has significantly decreased in recent years (Nehring et al. 2011). I believe that differences in prey diversity, abundance and size may explain current adult rainbow trout size and differences with historic rainbow trout size. My von Bertalanffy modeling and parameter estimates provide the first description of growth for *M. cerebralis*-resistant rainbow trout in a natural system. Maximum asymptotic length (424.5 mm) is similar to maximum lengths observed in brown trout during the study (CPW, unpublished data). However, prior to the introduction of *M. cerebralis*, rainbow trout (CRR) and brown trout greater than 425 mm were consistently observed during annual population estimates (Nehring and Thompson 2001). Laboratory experiments indicate that H×C fish grew faster and were significantly larger than CRR fish of the same age (Fetherman et al. 2011) and I would expect that H×C fish would attain larger sizes than those observed in the pre-*M. cerebralis* CRR population. I believe that differences in fish length pre- and post-*M. cerebralis* introduction are, at least in part, due to changes in food resources rather than *M. cerebralis* infection or strain performance differences.

CONCLUSIONS

Reintroduction of a self-sustaining population of rainbow trout in the upper Colorado River will be influenced by environmental conditions as well as disease resistance. It has been suggested that successful reintroduction of salmonids may take 15 to 20 years or longer (Fraser 2008). Success will likely depend on favorable environmental conditions as well as increased resistance to *M. cerebralis*. Although the rainbow trout population in the upper Colorado River is showing signs of recovery, it has not yet become a self-sustaining population (Fraser 2008). My results

suggest that supplemental stocking will be needed for continued persistence in the upper Colorado River; however, age-0 results clearly show that resistant fish reproduced, and that their offspring survived at least until the fall in the upper Colorado River. The survival of age-0 fish to the fall suggests that recruitment may be forthcoming. However, lack of recruitment continues to contribute to the decline in the adult rainbow trout population in the upper Colorado River. Recruitment may have occurred in 2012 as age-0 rainbow trout were still present in October 2011; low water prevented population evaluation in the spring of 2012.

I suggest that artificial supplementation and annual monitoring of the rainbow trout population should continue to evaluate whether my observed survival of age-0 fish is followed by subsequent recruitment to the adult reproductive population. Future management should focus on increasing adult rainbow trout survival and retention in locations where H×C are reintroduced. Such management strategies may include brown trout removal or habitat modifications. Additional introduction strategies should be evaluated, such as introducing large numbers of smaller H×C. I believe that the introduction of *M. cerebralis* resistant rainbow trout remains a promising management strategy for the reintroduction of rainbow trout fisheries in Colorado and elsewhere.

Table A1.1. Model selection results for factors influencing apparent survival (ϕ) and detection probability (p) of the Floy tagged H×C fish introduced to the upper Colorado River in June 2006. Models for which there was weight are shown.

Model	log(L)	K	AICc	Δ_i	w_i
$\phi(L,MIN) p(E)$	-873.51	5	1757.04	0.00	0.35
$\phi(L,MIN) p(E,CMS)$	-872.82	6	1757.67	0.64	0.25
$\phi(L,MAX) p(E, CMS)$	-873.27	6	1758.57	1.53	0.16
$\phi(L,MAX) p(E)$	-874.63	5	1759.28	2.25	0.11
$\phi(L,T) p(E)$	-875.14	5	1760.31	3.27	0.07
$\phi(L,T) p(E,CMS)$	-874.27	6	1760.56	3.52	0.06
$\phi(MIN) p(E)$	-881.02	4	1770.05	13.01	< 0.01
$\phi(MIN) p(E,CMS)$	-880.58	5	1771.18	14.14	< 0.01
$\phi(L) p(E)$	-881.72	4	1771.45	14.41	< 0.01
$\phi(L) p(E,CMS)$	-880.85	5	1771.73	14.69	< 0.01
$\phi(MAX) p(E, CMS)$	-881.00	5	1772.03	15.00	< 0.01
$\phi(MAX) p(E)$	-882.06	4	1772.13	15.09	< 0.01
$\phi(T) p(E)$	-882.51	4	1773.03	15.99	< 0.01
$\phi(T) p(E,CMS)$	-881.89	5	1773.81	16.77	< 0.01
$\phi(L,MIN) p(CMS)$	-882.29	5	1774.60	17.56	< 0.01
$\phi(L) p(CMS)$	-884.09	4	1776.20	19.16	< 0.01
$\phi(L,T) p(CMS)$	-883.40	5	1776.83	19.80	< 0.01
$\phi(L,MAX) p(CMS)$	-884.00	5	1778.03	20.99	< 0.01

The maximized log-likelihood ($\log(L)$), the number of parameters (K) in each model, and the small sample size-corrected AICc values (AICc) are shown. Models are ranked by their AICc differences (Δ_i) relative to the best model in the set and Akaike weights (w_i) quantify the probability that a particular model is the best model in the set given the data and the model set.

NOTE: L = length, E = effort, CMS = discharge, MIN = minimum discharge between primary sampling occasions, MAX = maximum discharge between primary sampling occasions, and T = trend over time.

Table A1.2. Model selection results for factors influencing the probability that a fish exhibits signs of *M. cerebralis* infection in the upper Colorado River in the years 2009 through 2011.

Model	R^2	$\log(L)$	K	AICc	Δ_i	w_i
Species*Year	0.15	-214.06	6	445.06	0.00	0.99
Species+Year	0.10	-222.27	4	454.65	9.58	0.01
Species	0.08	-226.23	2	457.03	11.97	0.00
Year	0.06	-230.44	3	468.09	23.02	0.00
Intercept-only	0.00	-239.75	1	481.68	36.62	0.00

R^2 values are maximum rescaled R^2 values. The maximized log-likelihood ($\log(L)$), the number of parameters (K) in each model, and the small sample size-corrected AICc values (AICc) are shown. Models are ranked by their AICc differences (Δ_i) relative to the best model in the set and Akaike weights (w_i) quantify the probability that a particular model is the best model in the set given the data and the model set.

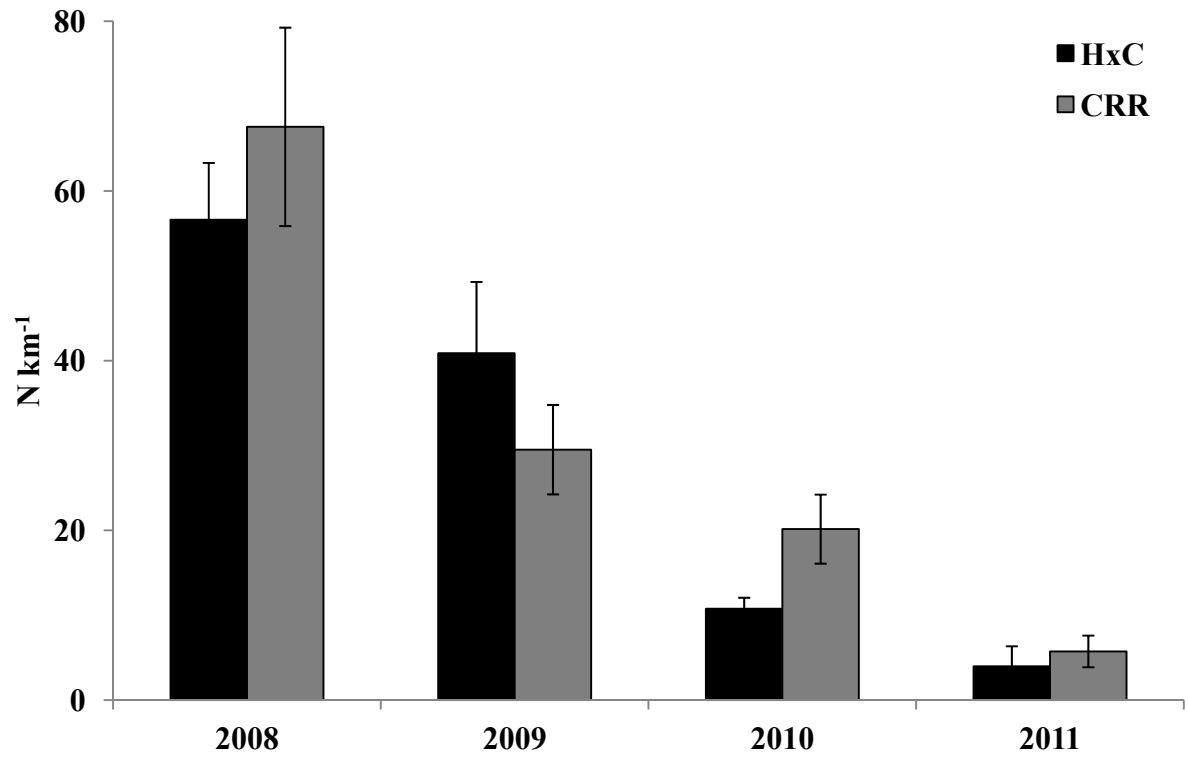


Figure A1.1. Adult H×C and CRR abundance (N km⁻¹; SE bars) in the upper Colorado River study section for the years 2008 to 2011.

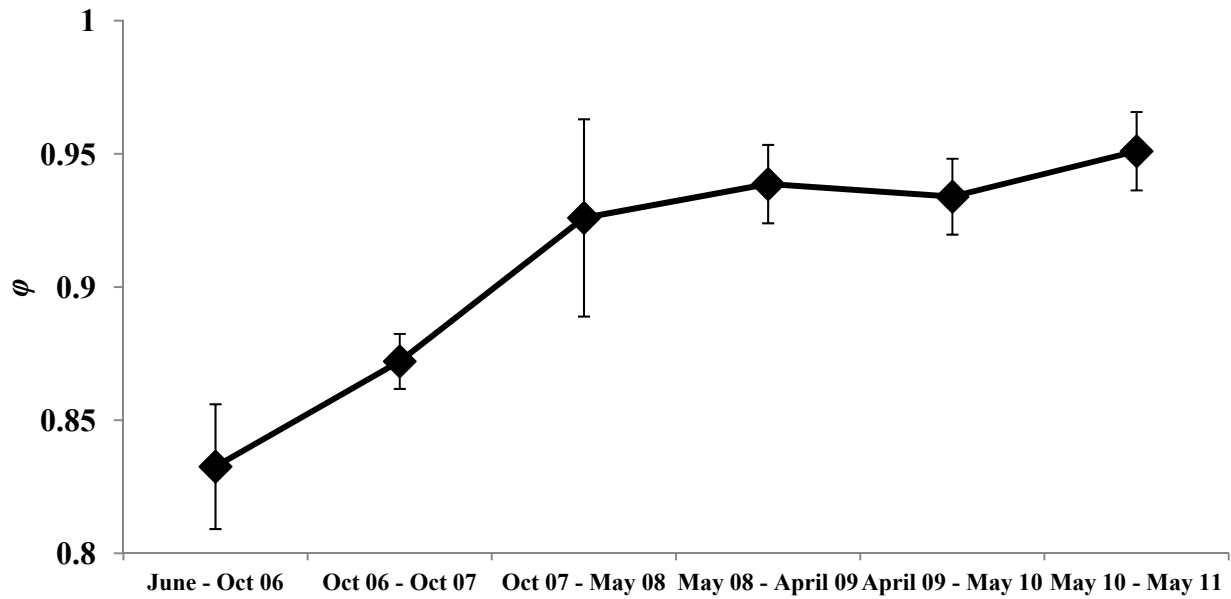


Figure A1.2. Model-averaged monthly apparent survival rate (ϕ ; SE bars) for the H×C stocked in the upper Colorado in June 2006. Date ranges (x-axis) represent the periods between primary sampling occasions for the adult rainbow trout population.

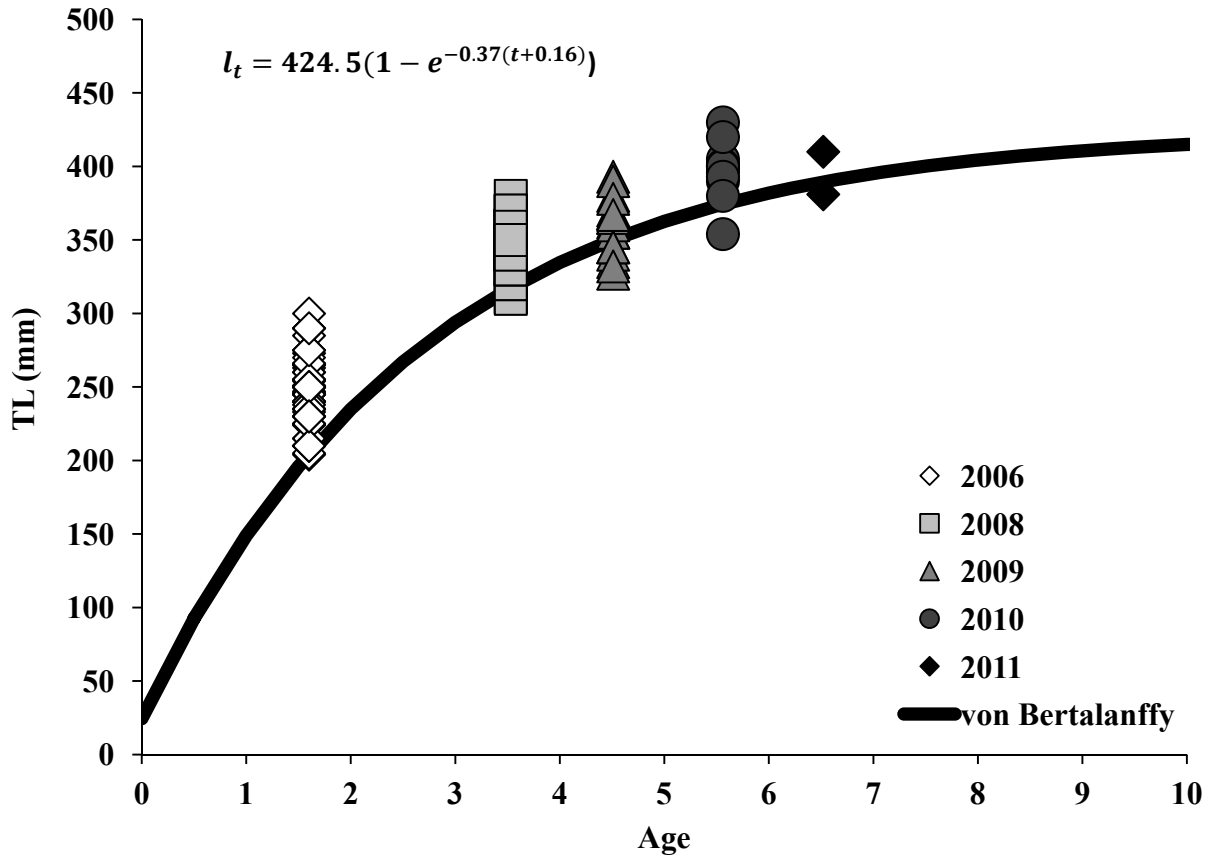


Figure A1.3. Predictive model of growth (TL; mm) trends of the H×C stocked in the upper Colorado River in 2006. The von Bertalanffy growth function was determined using repeated measures of length from fish stocked in 2006 (1.6 years of age) and recaptured in 2008, 2009, 2010, or 2011.

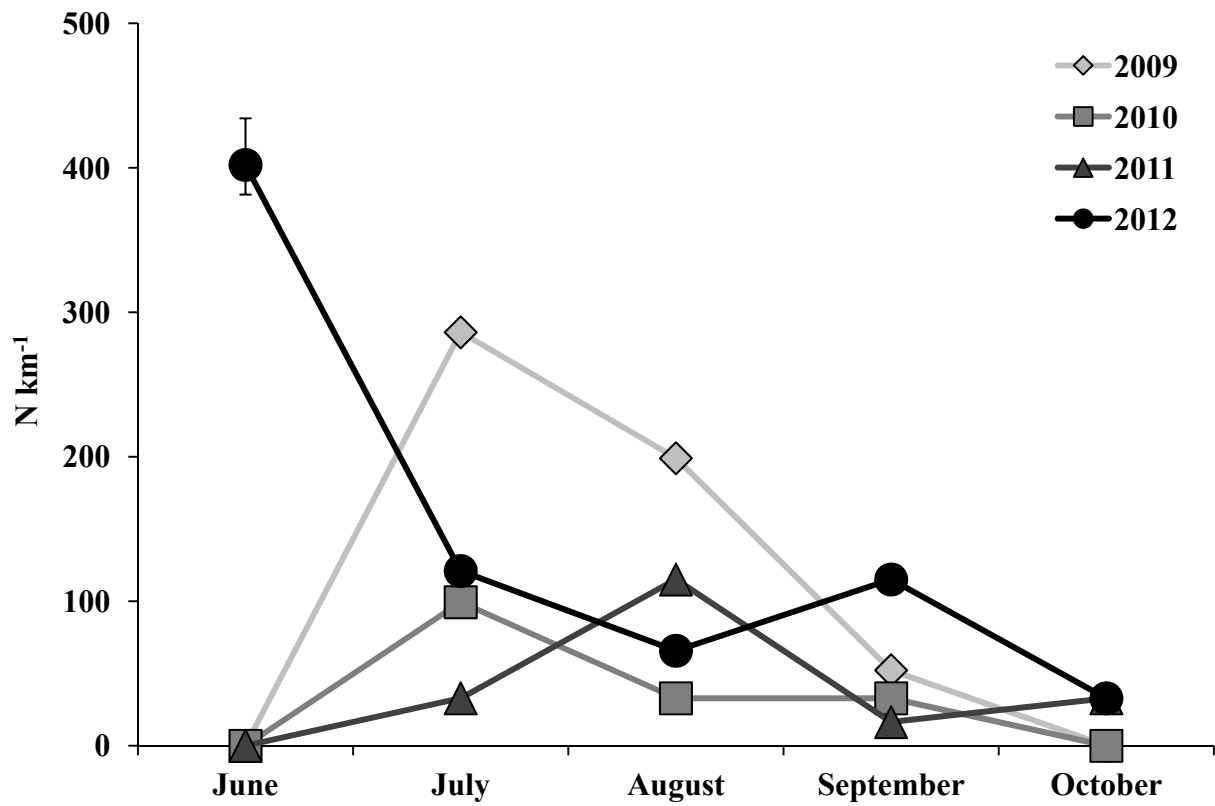


Figure A1.4. Rainbow trout fry abundance ($N\ km^{-1}$; SE bars) in June, July, August, September, and October of 2009, 2010, 2011, and 2012.

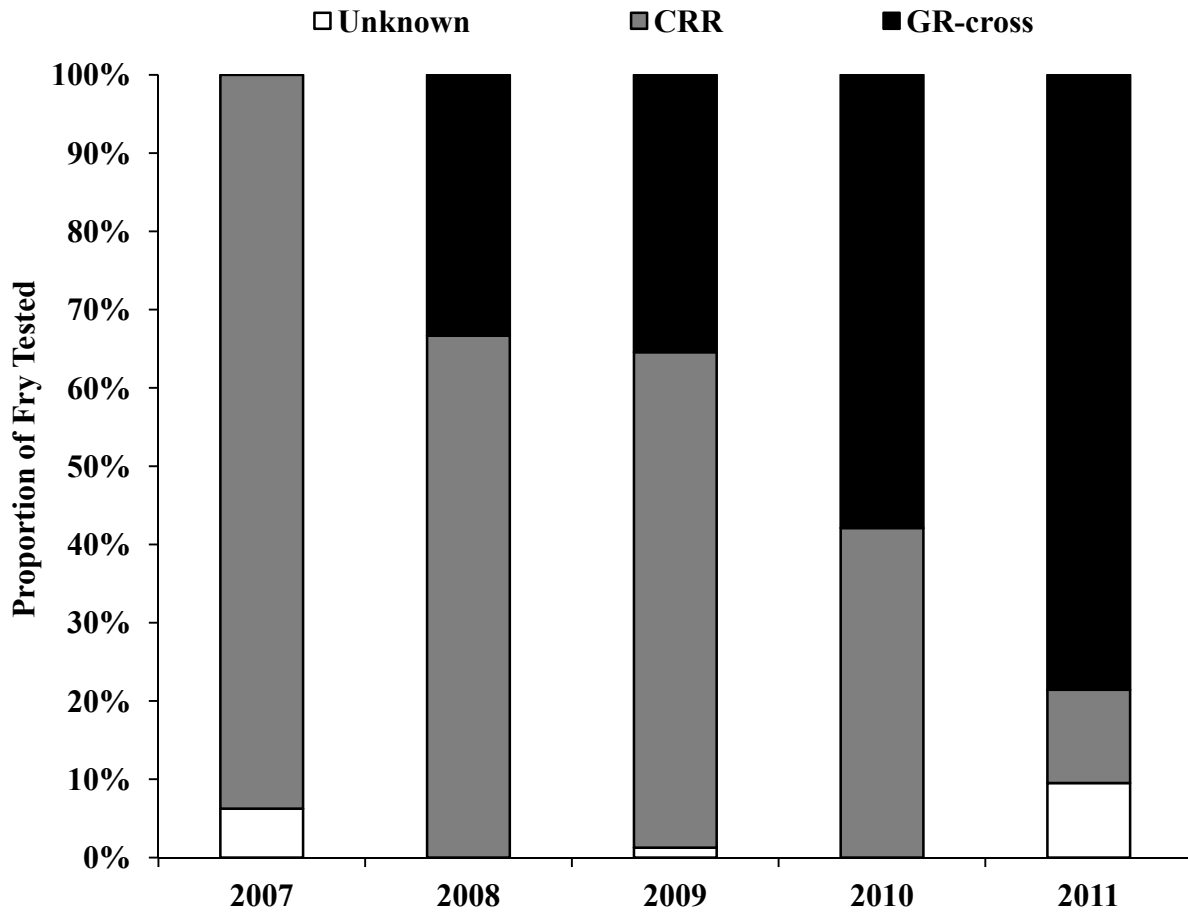


Figure A1.5. Proportion of the wild rainbow trout fry population collected from the upper Colorado River in 2007 ($N = 16$), 2008 ($N = 21$), 2009 ($N = 79$), 2010 ($N = 57$), and 2011 ($N = 42$) that were assigned as CRR, GR-cross, or unknown (posterior probability < 0.80) using the microsatellite marker genetic differentiation test.

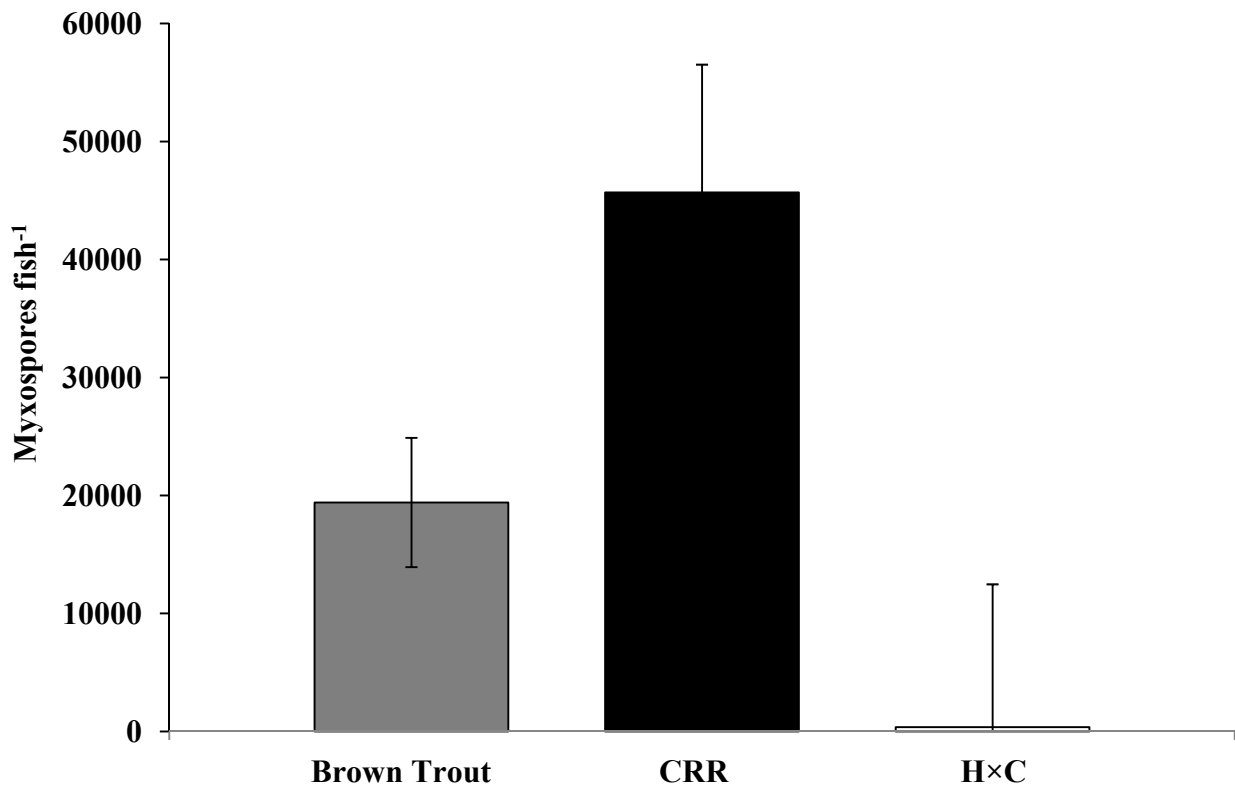


Figure A1.6. Average myxospore count (myxospores fish⁻¹; SE bars) of the brown trout ($N = 60$), CRR ($N = 13$), and H×C ($N = 11$) fry collected in October of 2009 and 2011 from the upper Colorado River.

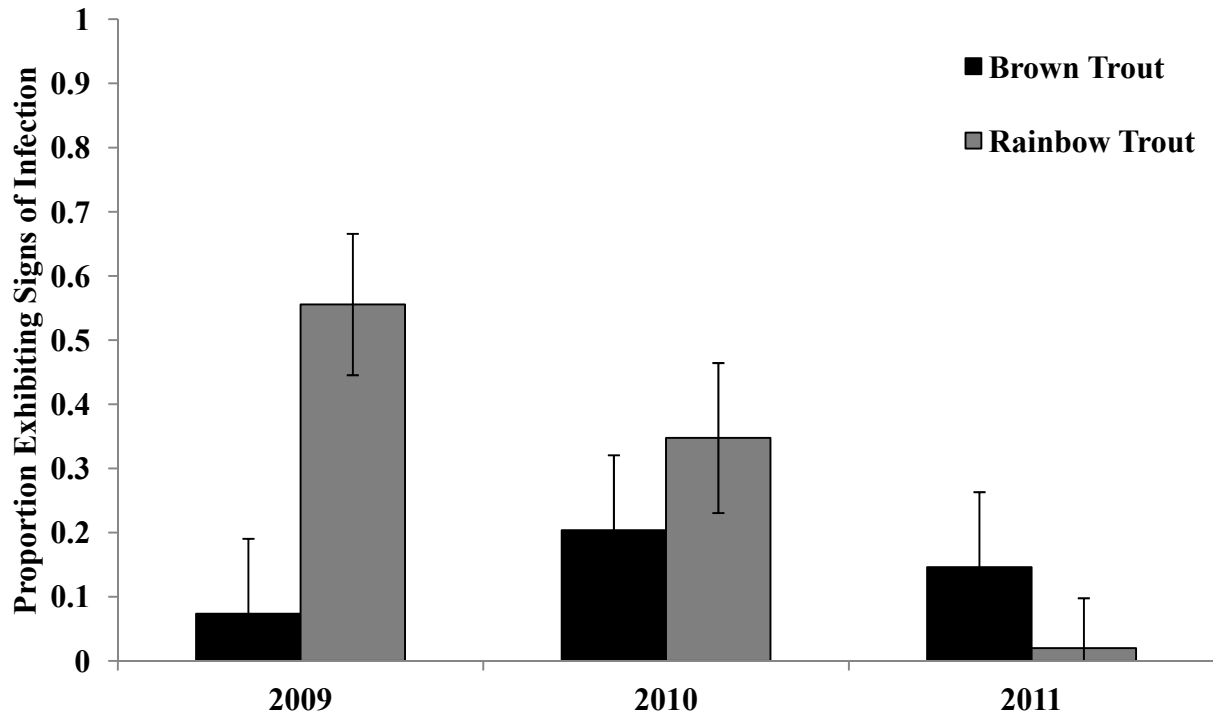


Figure A1.7. Proportion (SE bars) of the brown trout and rainbow trout fry populations in 2009 (brown trout: $N = 277$; rainbow trout: $N = 29$), 2010 (brown trout: $N = 64$; rainbow trout: $N = 41$), and 2011 (brown trout: $N = 138$; rainbow trout: $N = 19$) exhibiting signs of *M. cerebralis* infection.

LITERATURE CITED

- Anderson, D. R. 2008. Model based inference in the life sciences: A primer on evidence. Springer, LLC, New York, New York.
- Anderson, E. C., and E. A. Thompson. 2002. A model-based method for identifying species hybrids using multilocus genetic data. *Genetics* 160:1217-1229.
- Arismendi, I., M. Safeeq, S. L. Johnson, J. B. Dunham, and R. Haggerty. 2012. Increasing synchrony of high temperature and low flow in western North America streams: double trouble for coldwater biota? *Hydrobiologia* (2012):1-10.
- Bailey, N. J. J. 1951. On estimating the size of mobile populations from recapture data. *Biometrika* 38:293-306.
- Bosch, W. J., T. H. Newsome, J. L. Dunnigan, J. D. Hubble, and D. Neeley. 2007. Evaluating the feasibility of reestablishing a Coho salmon population in the Yakima River, Washington. *North American Journal of Fisheries Management* 27:198-214.
- Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: A Practical information-theoretic approach (second ed.). Springer-Verlag, New York, New York.
- Busacker, G. P., I. R. Adelman, and E. M. Goolish. 1990. Growth. Pages 363-387 *in* Schreck, C. B., and P. B. Moyle, editors. *Methods for Fish Biology*. American Fisheries Society, Bethesda, Maryland.
- Carmona-Catot, G., P. B. Moyle, and R. E. Simmons. 2012. Long-term captive breeding does not necessarily prevent reestablishment: lessons learned from Eagle Lake rainbow trout. *Reviews in Fish Biology and Fisheries* 22:325-342.
- El-Matbouli, M., A. Oucible, V. Severin, U. Meyer, D. Grabner, and R. P. Hedrick. 2006. Data on the mechanisms associated with the resistance of Hofer- and wild rainbow trout strains to whirling disease. Pages 22-23 *in* War of the Whirls. Proceedings of the 12th Annual Whirling Disease Symposium. Whirling Disease Initiative, Bozeman, MT
- Fabens, A. J. 1965. Properties and fitting of the von Bertalanffy growth curve. *Growth* 29: 265-289.
- Fetherman, E. R. and G. J. Schisler. 2012. Sport Fish Research Studies. Federal Aid in Fish and Wildlife Restoration, Job Progress Report. Colorado Parks and Wildlife, Aquatic Wildlife Research Section. Fort Collins, Colorado.
- Fetherman, E. R., D. L. Winkelman, G. J. Schisler, and C. A. Myrick. 2011. The effects of *Myxobolus cerebralis* on the physiological performance of whirling disease resistant and susceptible strains of rainbow trout. *Journal of Aquatic Animal Health* 23:169-177.

- Fetherman, E. R., D. L. Winkelman, G. J. Schisler, and M. F. Antolin. 2012. Genetic basis of differences in myxospore count between whirling disease-resistant and –susceptible strains of rainbow trout. *Diseases of Aquatic Organisms* 102:97-106.
- Flagg, T. A., M. C. McAuley, P. A. Kline, M. S. Powell, D. Taki, and J. C. Gislason. 2004. Application of captive broodstocks to preservation of ESA-listed stocks of Pacific salmon: Redfish Lake Sockeye Salmon case example. *American Fisheries Society Symposium* 44:387-400.
- Fraser, D. J. 2008. How well can captive breeding programs conserve biodiversity? A review of salmonids. *Evolutionary Applications* 1:535-586.
- Gatz, A. J., M. J. Sale, and J. M. Loar. 1987. Habitat shifts in rainbow trout: competitive influences of brown trout. *Oecologia (Berlin)* 74:7-19.
- Granath, W. O., Jr., M. A. Gilbert, E. J. Wyatt-Pescador, and E. R. Vincent. 2007. Epizootiology of *Myxobolus cerebralis*, the causative agent of salmonid whirling disease in the Rock Creek drainage of west-central Montana. *Journal of Parasitology* 93(1):104-119.
- Harig, A. L., and K. D. Fausch. 2002. Minimum habitat requirements for establishing translocated cutthroat trout populations. *Ecological Applications* 12:535-551.
- Hedrick R. P., T. S. McDowell, G. D. Marty, G. T. Fosgate, K. Mukkatira, K. Myklebust, and M. El-Matbouli. 2003. Susceptibility of two strains of rainbow trout (one with suspected resistance to whirling disease) to *Myxobolus cerebralis* infection. *Disease of Aquatic Organisms* 55:37-44.
- Hesthagen, T., and B. M. Larsen. 2003. Recovery and re-establishment of Atlantic salmon, *Salmo salar*, in limed Norwegian rivers. *Fisheries Management and Ecology* 10:87-95.
- Hicks, B. J., R. L. Beschta, and R. D. Harr. 1991. Long-term changes in streamflow following logging in western Oregon and associated fisheries implications. *Water Resources Bulletin* 27(2):217-226.
- Hoffman, A. A., and J. Merilä. 1999. Heritable variation and evolution under favourable and unfavourable conditions. *Trends in Ecology and Evolution* 14:96-101.
- Isely, J. J., and T. B. Grabowski. 2007. Age and growth. Pages 187-228 in Guy, C. S., and M. L. Brown, editors. *Analysis and Interpretation of Freshwater Fisheries Data*. American Fisheries Society, Bethesda, Maryland.
- Markiw, M. E., and K. Wolf. 1974. *Myxosoma cerebralis*: isolation and centrifugation from fish skeletal elements – sequential enzymatic digestions and purification by differential centrifugation. *Journal of Fisheries Research Board Canada* 31:15-20.
- Miller, M. P., and E. R. Vincent. 2008. Rapid natural selection for resistance to an introduced parasite of rainbow trout. *Evolutionary Applications* 1:336-341.

- Nehring, R. B. 2006. Colorado's cold water fisheries: whirling disease case histories and insights for risk management. Colorado Division of Wildlife. Aquatic Wildlife Research Special Report Number 79. February 2006.
- Nehring, R. B., and K. G. Thompson. 2001. Impact assessment of some physical and biological factors in the whirling disease epizootic among wild trout in Colorado. Colorado Division of Wildlife. Aquatic Research Special Report Number 76. March 2001.
- Nehring, R. B., and K. G. Thompson. 2003. Whirling disease risk assessment: the Colorado perspective. Pages 31-32 in *Managing the risk*. Proceedings of the 9th Annual Whirling Disease Symposium. Whirling Disease Initiative, Bozeman, MT
- Nehring, R. B., B. Heinold, and J. Pomeranz. 2011. Colorado River Aquatic Resources Investigations. Federal Aid in Fish and Wildlife Restoration, Job Progress Report. Colorado Division of Wildlife, Aquatic Wildlife Research Section. Fort Collins, Colorado.
- O'Grodnick, J. J. 1975. Whirling disease *Myxosoma cerebralis* spore concentration using the continuous plankton centrifuge. *Journal of Wildlife Diseases* 11:54-57.
- Ryce, E. K. N., A. V. Zale, E. MacConnell, and M. Nelson. 2005. Effects of fish age versus size on the development of whirling disease in rainbow trout. *Diseases of Aquatic Organisms* 63:69-76.
- Ryce, E. K. N., A. V. Zale, and R. B. Nehring. 2001. Lack of selection for resistance to whirling disease among progeny of Colorado River rainbow trout. *Journal of Aquatic Animal Health* 12:63-68.
- SAS Institute, Inc. 2010. SAS system software, Release 9.2. Cary, North Carolina.
- Schisler, G. J., E. P. Bergersen, and P. G. Walker. 1999a. Evaluation of chronic gas supersaturation on growth, morbidity, and mortality of fingerling rainbow trout infected with *Myxobolus cerebralis*. *North American Journal of Aquaculture* 61:175-183.
- Schisler, G. J., P. G. Walker, L. A. Chittum, and E. P. Bergersen. 1999b. Gill ectoparasites of juvenile rainbow trout and brown trout in the upper Colorado River. *Journal of Aquatic Animal Health* 11:170-174.
- Schisler, G. J., E. P. Bergersen, and P. G. Walker. 2000. Effects of multiple stressors on morbidity and mortality of fingerling rainbow trout infected with *Myxobolus cerebralis*. *Transactions of the American Fisheries Society* 129:859-865.
- Schisler, G. J., K. A. Myklebust, and R. P. Hedrick. 2006. Inheritance of *Myxobolus cerebralis* resistance among F1-generation crosses of whirling disease resistant and susceptible rainbow trout strains. *Journal of Aquatic Animal Health* 18:109-115.

- Seber, G. A. F., and J. F. Whale. 1970. The removal method for two and three samples. *Biometrics* 26(3):393-400.
- Temple, G. M., and T. N. Pearsons. 2007. Electrofishing: backpack and drift boat. Pages 95-132 in D. H. Johnson, B. M. Schrier, J. S. O'Neal, J. A. Knutzen, X. Augerot, T. A. O'Neal, and T. N. Pearson, editors. *Salmonid field protocols handbook: techniques for assessing status and trends in salmon and trout populations*. American Fisheries Society, Bethesda, Maryland.
- Thompson, K. G. 2011. Evaluation of small-scale habitat manipulation to reduce the impact of the whirling disease parasite in streams. *Aquatic Ecosystem Health and Management* 14(3):305-317.
- Thompson, K. G., R. B. Nehring, D. C. Bowden, and T. Wygant. 1999. Field exposure of seven species of subspecies of salmonids to *Myxobolus cerebralis* in the Colorado River, Middle Park, Colorado. *Journal of Aquatic Animal Health* 11:312-329.
- USGS. 2009. USGS Real Time Water Data for the Nation. Retrieved July 17, 2009, from <<http://nwis.waterdata.usgs.gov/co/nwis/uv/?>>.
- Van Den Avyle, M. J., and R. S. Hayward. 1999. Dynamics of exploited fish populations. Pages 127-166 in Kohler, C. G., and W. A. Hubert, editors. *Inland Fisheries Management in North America* (second ed.). American Fisheries Society, Bethesda, Maryland.
- von Bertalanffy, L. 1938. A quantitative theory of organic growth. *Human Biology* 10:181-213.
- Walker, P. G., and R. B. Nehring. 1995. An investigation to determine the cause(s) of the disappearance of young wild rainbow trout in the Upper Colorado River, in Middle Park, Colorado. Colorado Division of Wildlife Report. Denver, CO.
- White, G. C., and K. P. Burnham. 1999. Program MARK: Survival estimation from populations of marked animals. *Bird Study* 46 Supplement, 120-138.
- Williams, J. E., A. L. Haak, H. M. Neville, and W. T. Colyer. 2009. Potential consequences of climate change to persistence of cutthroat trout populations. *North American Journal of Fisheries Management* 29(3):533-548.

Job No. 4
Appendix 2

Brown Trout Removal Effects on Short-term Survival and Movement of Reintroduced Whirling Disease-Resistant Rainbow Trout (Chapter Four from the Ph.D. dissertation entitled *Introduction and Management of Myxobolus cerebralis-Resistant Rainbow Trout in Colorado* by Eric R. Fetherman; submitted to Colorado State University, Summer 2013)

INTRODUCTION

Following its introduction to Colorado, *Myxobolus cerebralis*, the parasite responsible for salmonid whirling disease, caused a significant decline in wild rainbow trout (*Oncorhynchus mykiss*) populations across the state. Brown trout (*Salmo trutta*), however, are more resistant to *M. cerebralis* than rainbow trout because they evolved with *M. cerebralis* in their native, European home ranges (Hoffman 1970; Hedrick et al. 1999; Hedrick et al. 2003) and therefore did not experience similar population level declines (Nehring and Thompson 2001; Nehring 2006). Consequently, brown trout population densities have increased in many of Colorado's rivers following the loss of the rainbow trout populations (Nehring and Thompson 2001). Similar brown trout population increases were observed in several drainages in Montana following rainbow trout population declines from exposure to *M. cerebralis* (Baldwin et al. 1998; Granath et al. 2007).

Competition with and predation by brown trout can cause significant declines in salmonid populations living in sympatry, including brook trout (*Salvelinus fontinalis*; Fausch and White 1981; Alexander 1977), cutthroat trout (*Oncorhynchus clarki*; Wang and White 1994), and rainbow trout populations (Gatz et al. 1987). Competition between brown trout and rainbow trout results in exclusion of rainbow trout from preferred feeding and resting habitats, possibly causing population-level effects (Gatz et al. 1987). High densities of large brown trout exert heavy predation pressure on stocked rainbow trout fingerlings (Nehring 2006) as well as compete with sub-catchable- and catchable-sized *M. cerebralis*-resistant rainbow trout being reintroduced to Colorado waters. Brown trout switch to piscivory after reaching three years of age (> 175 mm total length [TL]; Jonsson et al. 1999), at which time energy intake and growth tend to increase markedly (Elliott and Hurley 2000). Piscivorous brown trout can significantly alter both sympatric salmonid and other prey species' population structure and dynamics. Large brown trout are known to consume considerable numbers of small trout and are a significant source of fry and fingerling mortality in sympatric salmonid populations (Alexander 1977). In addition, brown trout prey largely on other salmonid species rather than consuming juveniles of their own species, and the number consumed increases with an increase in brown trout length (Jensen et al. 2004). Jensen et al. (2006) calculated that a brown trout population (8,445 individuals > 25 cm TL) consumed about 1.5 million vendace (*Coregonus albula*) and 400,000 whitefish (*Coregonus clupeaformis*) annually, illustrating the catastrophic effects large piscivorous brown trout can have on other fish populations.

Control and eradication of brown trout are potential management options for reducing competition and predation effects and increasing the survival of other salmonid and prey fish species in rivers (Gatz et al. 1987). Considerable removal efforts may be needed to attain a desired effect on the target populations. For example, removal of 66% of the brown trout

population in the Au Sable River in Michigan did not result in population or size at age increases in the target sympatric brook trout population (Shetter and Alexander 1970). Therefore, predatory brown trout numbers may need to be reduced by considerably more than 60% to attain a significant increase in survival or change in other population characteristics of the target species (Alexander 1977).

The objective of my study was to determine if brown trout removal increased the short-term survival and retention probabilities of reintroduced, *M. cerebralis*-resistant rainbow trout. I used Radio Frequency Identification (RFID) passive integrated transponder (PIT) tags and antennas to passively estimate survival and to track movements made by brown trout and rainbow trout in reaches where brown trout had or had not been removed. Additionally, survival and movement probabilities were estimated for two crosses of rainbow trout introduced to the river following brown trout removal to determine which cross is best for use in river reintroductions.

METHODS

Site Description

The Cache la Poudre River is a high-gradient freestone river that originates in Rocky Mountain National Park and flows north and east until joining the South Platte River on the eastern plains of Colorado (Sipher and Bergersen 2005). Maximum summer temperatures of the upper reaches of the Cache la Poudre River range from 5°C to 12°C annually and rarely exceed 13°C (Nehring and Thompson 2001). Rainbow trout and brown trout are the principle game fish in the Cache la Poudre River, but brook trout, native cutthroat trout, and mountain whitefish (*Prosopium williamsoni*) are also present in low numbers (Klein 1963; Allen and Bergersen 2002). Prior to the introduction of *M. cerebralis* to the Cache la Poudre River, \geq age-1 rainbow trout were found in higher than average densities (170 fish ha⁻¹) than \geq age-1 brown trout (103 fish ha⁻¹; Nehring and Thompson 2001), and were historically present in an average ratio of 60 rainbow trout to 40 brown trout (Klein 1963).

Myxobolus cerebralis was first detected in the Cache la Poudre River drainage at the Colorado Parks and Wildlife (CPW) Poudre Rearing Unit (PRU) in 1988. PRU is a large rainbow trout production facility with six earthen ponds located on the upper reaches of the river, approximately 117.5 km west of Fort Collins (Nehring 2006). Allen and Bergersen (2002) showed that the earthen ponds at the unit supported dense populations of *Tubifex tubifex* worms, a necessary intermediate host for the parasite life cycle. Subsequent testing revealed that *T. tubifex* in the ponds produced high densities of *M. cerebralis* triactinomyxons (TAMs) that were discharged into the river (Nehring and Thompson 2001). Infection prevalence of rainbow trout held in the ponds was often as high as 100% with average myxospore counts greater than 470,000 myxospores fish⁻¹, ranging as high as 1.63 million for individual trout (Nehring and Thompson 2003). In addition to TAM releases from PRU, Schisler (2001) reported that more than one million trout from infected hatcheries and rearing units, a large majority of which originated from PRU, were stocked into the Cache la Poudre River, as well as into lakes, reservoirs, and tributaries within the Cache la Poudre River drainage between 1990 and 2001. However, Nehring (2006) suggests that despite the number of infected fish stocked in the drainage, TAM densities discharged to the river from PRU ponds alone were sufficient to cause a complete loss of rainbow trout fry downriver of the unit. Following introduction of *M.*

cerebralis, severe declines were experienced by the rainbow trout population; by 1995, no \geq age-1 rainbow trout were detected in population estimates. Brown trout did not suffer significant population level declines in the river following *M. cerebralis* introduction (Nehring and Thompson 2001), and brown trout biomass compensated for the loss of rainbow trout biomass to some degree (Allen and Bergersen 2002).

Two reaches of the Cache la Poudre River were designated for this experiment, a control reach (no removal) and a removal reach (brown trout removal). The moderate-gradient, 1.3-km control reach was located just downstream of the town of Rustic, Colorado, in a section of the Cache la Poudre Canyon known as Indian Meadows, and the higher-gradient, 1.0-km removal reach was located eight km upstream of the control reach in a narrower section of the canyon known as Black Hollow (Figure A2.1, A2.2). Both study reaches were located downstream of the CPW PRU and were part of special regulation catch-and-release sections of the river; the study sites were placed here in part to prevent angler removal of PIT-tagged fish.

All brown trout taken out of the removal reach were relocated approximately 24.1 km downstream, released below a section of the river known as the Narrows (Figure A2.1); fish were relocated rather than sacrificed to maintain public support for the experiment. The Narrows is a high-gradient, high velocity section of the Cache la Poudre River, suspected to be at least a partial barrier to upstream movement. A potential barrier to upstream movement was desired as brown trout are known to exhibit directed and rapid homing to locations from which they have been displaced (Armstrong and Herbert 1997).

***Myxobolus cerebralis*-Resistant Rainbow Trout**

The German Rainbow (GR; Hofer) is a hatchery-derived rainbow trout strain that was exposed to *M. cerebralis* for decades in a Bavarian hatchery in Germany where it was reared as a food fish for human consumption (Hedrick et al. 2003). Although the GR strain can be infected with *M. cerebralis*, parasite burdens are usually low (Hedrick et al. 2003; Schisler et al. 2006; Fetherman et al. 2012) and the GR strain can survive and reproduce in the presence of *M. cerebralis*. Low parasite burdens and the strain's ability to persist following exposure to *M. cerebralis* have been termed "resistance," and this resistance is presumed to be a result of long-term exposure to the parasite (Hedrick et al. 2003). Despite the resistance of the GR strain, its survival and viability in the wild was uncertain due to its history of domestication (Schisler et al. 2006). Therefore, the GR strain was experimentally crossed with the Colorado River Rainbow (CRR; Schisler et al. 2006; Fetherman et al. 2011; Fetherman et al. 2012), a wild rainbow trout strain that had been widely stocked in Colorado and comprised many of the naturally reproducing wild rainbow trout fisheries prior to the introduction of *M. cerebralis* (Walker and Nehring 1995). However, the CRR strain exhibits high susceptibility to infection by *M. cerebralis* (Ryce et al. 2001; Sipher and Bergersen 2005; Schisler et al. 2006; Fetherman et al. 2012), and experienced widespread population declines following its introduction (Nehring and Thompson 2001).

Intermediate crosses of the two strains have been rigorously evaluated. Laboratory experiments showed that the first filial generational cross between the two strains (termed the H \times C) exhibited resistance characteristics similar to that of the GR strain (Schisler et al. 2006; Fetherman et al. 2012), and critical swimming velocities similar to those of the CRR strain (Fetherman et al. 2011). As such, it was suggested that this cross may be the best candidate for reintroducing

rainbow trout populations; however, its utility needed to be evaluated in a natural setting (Fetherman et al. 2012). The H×C has been experimentally introduced to other systems within the state (e.g., the Colorado River); however, it has exhibited low apparent survival in high density, brown trout-dominated systems. Therefore the effect of brown trout removal on the survival and retention of this cross was evaluated in this experiment. H×C fish for this experiment were spawned and reared at the CPW Glenwood Springs Hatchery, Glenwood Springs, Colorado.

The GR has also been experimentally crossed with the Harrison Lake rainbow trout strain (origin: Harrison Lake, Montana), a cross termed the H×H. The Harrison Lake strain of rainbow trout has exhibited enhanced resistance to *M. cerebralis* relative to other rainbow trout strains (Vincent 2002; Wagner et al. 2006). Resistance was suspected to be partially a result of the common ancestry of the Harrison Lake and Wounded Man Lake strains, with both exhibiting resistance despite no previous exposure to the parasite (Wagner et al. 2006). The Harrison Lake strain has also exhibited rapid development of resistance to *M. cerebralis* in the presence of the parasite through natural selection (Miller and Vincent 2008). Although marginally resistant itself, resistance to *M. cerebralis* was increased significantly when Harrison Lake fish were crossed with GR strain fish (Schisler 2006). However, due to its history as a lake strain (Wagner et al. 2006), its survival and retention following introduction to a river was unknown, and was therefore evaluated in this experiment. H×H fish for this experiment were spawned and reared at the CPW Bellvue Fish Research Hatchery in Bellvue, Colorado.

Fish Marking Procedures

Brown trout and rainbow trout were tagged with 32 × 3.85 mm half-duplex (HDX) PIT tags, inserted posterior of the pectoral fin through the midventral body wall into the peritoneal cavity using a hypodermic needle (Prentice et al. 1990; Acolas et al. 2007); the insertion opening was not closed (e.g., with stitching or glue) following tag insertion. Four thousand rainbow trout, 2,000 of each cross, were tagged at the CPW Glenwood Springs Hatchery (H×C) and Bellvue Fish Research Hatchery (H×H) 1.5 months prior to their introduction to the Cache la Poudre River. Total length (TL; mm), weight (g), and PIT tag number were recorded for each fish. Crosses were also differentially fin clipped (H×C: adipose; H×H: adipose and right pelvic) so that cross identification would be possible during population estimates in the event of tag loss. During tagging, H×Cs and H×Hs were randomly separated into two groups of 1,000 fish each, with known tag numbers in each group, designated for introduction to either the control or removal reaches of the Cache la Poudre River.

Tagging fish 1.5 months prior to their introduction to the Cache la Poudre River provided an opportunity to monitor tag retention and mortality as a result of the tagging procedure. One month post-tagging, 100 fish from each group of 1,000 were scanned for tags using a handheld, portable PIT tag reader. Tag retention was calculated by averaging the proportion of the 100 scanned fish missing a tag, and subtracting from one. Mortality was calculated based on the number of dead fish removed from the raceway by CPW staff.

Wild brown trout and rainbow trout above, within, and below the control reach were captured using two raft-mounted electrofishing units (one fixed-boom and one throw electrode) and were PIT-tagged one week prior to the introduction of rainbow trout. Three passes, made on consecutive days, were used to capture and tag approximately equal numbers of brown trout

within the 1.3-km control reach and in two 0.8-km sections above and below the control reach. All fish encountered on the first pass were PIT-tagged, measured (TL; mm) and weighed (g). On subsequent passes, untagged fish were similarly tagged, measured, and weighed. Tag number was also recorded from all previously tagged fish captured on subsequent passes. PIT-tagging fish within the control reach, as well as in the sections directly upstream and downstream of the reach, allowed us to estimate the survival and directional movement probabilities of brown trout following rainbow trout introduction.

Wild brown trout and rainbow trout located above and below the removal reach were PIT-tagged during the brown trout removal. Two passes were made through the 0.8-km sections upstream and downstream of the removal reach to collect brown trout for tagging; fish were tagged using the same methods described above and returned to the section from which they had been caught. PIT tagging brown trout above and below the removal reach allowed us to monitor movement back into the reach following the removal. In addition, a subsample of 200 brown trout captured within the removal reach were PIT tagged prior to being relocated below the Narrows to determine if brown trout could navigate the Narrows and return to the removal reach in the months following relocation.

Statistical Analyses

To evaluate if there were differences in length or weight among the rainbow trout crosses (H×C and H×H) stocked into the control or removal reaches, I used a general linear model (GLM) as implemented in SAS ProcGLM (SAS Institute, Inc. 2010). I considered an intercept-only model, as well as models that included effects of cross only, reach only, and models with additive and interactive effects between cross and reach. Model weights and delta AICc were used to determine support for each of the models included in the model set, and parameter estimates were reported from the candidate model with the lowest AICc value (Burnham and Anderson 2002).

Brown trout and wild rainbow trout abundance was estimated above, within, and below the control reach, and above and below the removal reach to provide a baseline estimate of the wild salmonid population prior to the introduction of rainbow trout to the Cache la Poudre River. Three-pass mark-recapture population estimates for the brown trout and wild rainbow trout were obtained using the Huggins closed capture-recapture estimator in program MARK (White and Burnham 1999). The Huggins form of the closed capture-recapture estimator differs from the traditional closed capture-recapture estimator in that only two types of parameters (initial capture, p , and recapture, c , probabilities) are included in the likelihood; abundance, N , is conditioned out of the likelihood and estimated as a derived parameter using capture probability estimates (Huggins 1989). Encounter histories were constructed by denoting the pass or passes in which a fish was captured or recaptured (denoted by a '1') and the pass or passes in which a fish was not encountered (denoted by a '0'). For example, an encounter history of '011' represents a fish that was captured and tagged on the second pass and recaptured on the third pass. Brown trout and wild rainbow trout were included as groups in the analysis. Models in which detection probability (p) and recapture probability (c) were independently estimable or equal with regards to each other (i.e., same probability of capture and recapture) were included in the model set. Group, fish length, and pass were included as covariates affecting the estimation of p and c (20 models total). Models were ranked using Akaike's Information Criterion

corrected for small sample sizes (AICc; Burnham and Anderson 2002). Model averaging was used to incorporate model selection uncertainty into the parameter estimates, and unconditional standard errors (SE) were reported for the model averaged parameter estimates (Anderson 2008).

Brown Trout Removal

Brown trout removal occurred August 16-18, 2010, one week following the wild salmonid PIT tagging operations in the control reach and antenna installation in both reaches. Prior to the removal, block fences, constructed of chicken wire fencing attached to t-bar posts pounded into the riverbed, were erected across the river at the upstream and downstream ends of the removal reach to prevent fish from moving into the section during the removal. Fences were monitored continuously throughout the removal to prevent build-up of debris; fencing did not fail at any point during the removal. The removal was accomplished using 14 Smith-Root LR-24 backpack electrofishing units, four raft-mounted, fixed-boom electrofishing units, and one three electrode cat-raft; over 100 CPW biologists, researchers, and volunteers assisted with the removal. Backpack and cat-raft crews formed one continuous line across the width of the Cache la Poudre River and worked upstream from the bottom of the reach. These crews were able to make five passes total through the section over the three day removal, one pass on the first day, and two passes on each of the subsequent days. Raft electrofishing crews made several passes through the section daily, following the thalweg of the river on each pass. Fish collected by the raft electrofishing crews were combined with the fish collected by the backpack and cat-raft crews; therefore, brown trout removal was accomplished using five removal passes.

All fish removed from the reach were measured (mm) and weighed (g) before being relocated. Brown trout captured throughout the day were kept in well oxygenated tanks on hatchery trucks. At the end of each day, fish were taken 15 miles downstream to the relocation section below the Narrows. All other species of fish encountered during the removal were returned to the river below the downstream block fence. Other species encountered included rainbow trout, mountain whitefish, longnose sucker (*Catostomus catostomus*), and white sucker (*Catostomus comersonii*).

Statistical Analyses

Five pass removal population estimates for the number of brown trout and wild rainbow trout present in the removal reach prior to removal were obtained using a Huggins closed-capture recapture estimator in program MARK. Although both p and c are included in the likelihood, c was fixed to zero since individuals removed on any given pass were not available for recapture (Hense et al. 2010; Saunders et al. 2011). Encounter histories were constructed by denoting the pass in which a fish was removed from the reach by a '1' and all other passes by a '0' (e.g., an encounter history of '00100' represents a fish that was removed on the third pass). Group was used as a categorical covariate, and four groups were included in the analysis: 1) adult brown trout (> 150 mm), 2) fry and juvenile brown trout (\leq 150 mm), 3) adult rainbow trout (> 150 mm), and 4) fry and juvenile rainbow trout (\leq 150 mm). Models in which p was constant or varied by group, pass, fish length (continuous, individual covariate), and all additive combinations were included in the set (eight models). Models were ranked using AICc; model averaging was used to incorporate model selection uncertainty into the parameter estimates, and unconditional standard errors (SE) were reported for the model averaged parameter estimates.

Rainbow Trout Introduction

Rainbow trout were introduced to the control and removal reaches the day following brown trout removal. In the removal reach, block fences remained in place until after the rainbow trout were introduced. The removal reach runs parallel and adjacent to Highway 14, allowing easy access for stocking. Rainbow trout were stocked in this section at three locations, one about a third of a mile downstream of the upper end of the reach, one in the middle of the reach, and one at the lower end of the reach. In each of these locations, fish were evenly distributed throughout the reach using buckets. Block fences were removed immediately following rainbow trout introduction.

The control reach at Indian Meadows is located about 0.5 km from Highway 14 and can only be accessed by foot. Therefore, rainbow trout were exchanged from the hatchery truck into coolers containing a mix of hatchery and river water, and loaded onto rafts about 0.5 miles above the upstream end of the reach. Rafts were used to transport the rainbow trout down to the control reach. Stocking commenced upon entering the control reach, and rainbow trout were evenly distributed throughout the reach.

RFID PIT Tag Antennas

The use of PIT tag technology has increased in fisheries within the past decade as a result of easy application, high retention, infinite life, and minimal effects on growth and survival (Gries and Letcher 2002; Zydlewski et al. 2006). In addition, stationary antennas have been used in conjunction with PIT tagging to study fish behavior, specifically habitat selection and migration processes (Nunnallee et al. 1998; Zydlewski et al. 2006; Bond et al. 2007; Compton et al. 2008; Connolly et al. 2008; Aymes and Rives 2009). In my study, RFID HDX PIT tag antennas were deployed prior to brown trout removal to detect movements of PIT-tagged brown trout and rainbow trout in the Cache la Poudre River. Pass-over antenna loops were constructed of eight-gauge, multi-strand copper speaker wire and were anchored to the bottom of the river using duckbill anchors jack-hammered into the substrate. The speaker wire was connected to a tuner box, used to tune the antenna for optimal detection distance, and tuner boxes were connected to a reader using twin-ax cable. In addition, antenna loops were paired at both the upstream and downstream ends of the control (upper and lower control, respectively) and removal (upper and lower removal, respectively) reaches to determine directionality of movement (Figure A2.2). Paired antennas at each location were run off a multiplexer reader to prevent proximity detection errors (Aymes and Rivas 2009). Readers were powered by two 12-volt marine, deep cycle batteries (120 Ah) connected in parallel. Solar panel arrays were used to charge the batteries, increasing battery life and preventing more frequent battery changes, especially during the winter months.

Antennas spanned the width of the river, ranging from 60 to 80.5 feet in length, and averaging 3 feet in width. Optimal antenna placement in the river was chosen based on hatchery detection experiments that showed that antenna detection was greater than 0.89 when fish passed over the array within two vertical feet of the antenna coil and when velocity did not exceed 0.50 m sec^{-1} . Antennas were placed at the tail end of pools that satisfied these conditions; average depth at the antennas during the highest discharge period (September 3-9, 2010) did not exceed 1.37 ft. In addition, antennas were placed such that velocity refuges were not contained within or between

the antenna loops to reduce the possibility of multiple tags being present within the detection field, resulting in no tags being detected (tag collision; Axel et al. 2005; O'Donnell et al. 2010).

Antennas were run continuously from August 15, 2010 to April 14, 2011. Antenna efficiency (Zydlewski et al. 2006) was monitored on a weekly basis during the primary study period (August 15 – November 3, 2010) and on a monthly basis during the winter study period (November 4, 2010 – April 14, 2011; Table A2.1), and was assessed using the stick test methods of Nunnallee et al. (1998) and Compton et al. (2008). Continuous operation of the antenna system was monitored using marker tags, and weekly efficiencies (i.e., the probability that a tag is detected at both antennas within an array) were adjusted based on the proportion of the week an antenna system was operational (Table A2.1). Adjusted efficiencies were used to fix weekly detection probability, p , for each antenna system within the multistate capture-recapture analyses (below). Velocity measurements were also collected on a weekly basis during the primary study period; discharge (cms) was calculated from these velocity estimates and included as a variable affecting transition probability in the primary study period multistate capture-recapture analyses. Velocity measurements were not collected over the winter study period due to ice formation.

Multistate Capture-Recapture Models

Multistate capture-recapture models (Hestbeck et al. 1991; Brownie et al. 1993; Lebreton and Pradel 2002) provide a useful approach to interpreting highly structured tagging data collected during complex studies of fish movement and migration patterns (Buchanan and Skalski 2010; Horton et al. 2011; Frank et al. 2012). These models allow estimation of apparent survival probabilities (ϕ), detection probability (p), and transition probabilities (ψ ; Lebreton and Pradel 2002) between and among states. States can be defined in variety of ways including spatial or geographical location and physiological status (Buchanan and Skalski 2010). In my study, states were defined by spatial location (control and removal reach) and transition location (representing directional movement past an antenna station). Primary assumptions of multistate models include that 1) marks are not lost, 2) individuals act independently, and 3) all marked individuals assigned to a state have the same probabilities of survival, movement, and capture (Hestbeck 1995).

In a traditional multistate model, apparent survival is conditional on the departure state, and movement is conditional on survival (Lebreton and Pradel 2002); therefore, apparent survival in the departure state is estimated first, and movement between the departure state and a new state is estimated second. Because I did not physically capture or recapture individual fish, with the exception of when they were tagged at the outset of the study, I used antenna detections as recaptures when estimating the parameters of the multistate capture-recapture models (O'Donnell et al. 2010). Using the paired antenna array, fish were recaptured at the stationary antenna stations as they were moving between states. I assumed that if a tag was detected at an antenna station, the tag was 1) in a live fish, and 2) in the original fish that had been given that tag. Therefore, survival prior to the movement was known (1.0) and survival following movement was unknown. A paired record was included in the encounter history for each week, with the first value in the pair representing observed movement (transition state letter or '0' for fish that did not move). The second value used was a dummy variable (always '0') that allowed me to reverse the usual order of events in the model, and estimate movement (transitions, ψ) before apparent survival (ϕ ; Figure A2.3).

Encounter histories were developed for each tagged individual. Each encounter history began with a release state (Figure A2.3). For instance, rainbow trout were either released into the removal reach (release state R) or into the control reach (state C; Figure A2.4). Brown trout had five release states depending on their location at tagging (Figure A2.5). Release states appeared only once in the encounter history because fish were not detectable by the antennas within the release area (i.e., $p = 0$ for the release state). The remainder of the encounter history consisted of transition states when fish were detected moving over an antenna station (Figure A2.4, A2.5, A2.6). Unique states were used to represent both the direction of movement and antenna location at which movement occurred (Figure A2.3). Known movement occurred if two conditions were met: 1) the fish was detected by both antennas within the array (i.e., directionality of movement was *known*), and 2) there was no return movement within the same week (i.e., a fish did not begin and end the week in the same location). Lack of movement was indicated by including a '0' in the encounter history. For example, the three week encounter history CA000B0 represents a rainbow trout that was initially released in the control reach (state C; Figure A2.3). In the first week, the fish moved downstream out of the control reach and was detected at both antennas of the lower control antenna array (state A; Figure A2.3, A2.4). The zero following the A is the dummy variable described above. The fish was not detected in week two of the study, so both paired entries for week two were '0' (Figure A2.3). In week three, the fish made an upstream movement returning to the control section and was detected by both antennas at the lower array (state B; Figure A2.3, A2.4). Encounter histories were constructed in this way using the detection data from the antennas for every PIT-tagged brown trout and rainbow trout in the Cache la Poudre River.

Multistate models were constructed to estimate apparent survival (ϕ) and movement (ψ) probabilities for brown trout and rainbow trout ($H \times C$ and $H \times H$) in both the control and removal reaches; weekly estimates of ϕ and ψ were obtained during both the primary (11 weeks; August 15 – November 3, 2010) and winter (23 weeks; November 4, 2010 – April 14, 2011) study periods. The primary study period was used to determine the short-term retention and survival of rainbow trout within the two reaches following introduction and brown trout removal. In addition, the primary study period was used to determine how quickly brown trout moved back into the removal reach and if the addition of rainbow trout resulted in movement out of the control reach by resident brown trout. Three model sets were used to separately estimate apparent survival and movement, one each for the brown trout, $H \times C$ s, and $H \times H$ s during the primary study period; although desired, model set size and parameter number limited the ability to include both crosses as groups in a single rainbow trout analysis. The brown trout model set included 13 states, five release states and eight additional states representing upstream and downstream movement (Figure A2.5), whereas the rainbow trout model sets included 10 states, two release states and eight movement states (Figure A2.4). Brown trout were tagged and released upstream (state L), within (state C), and downstream (state K) of the control reach, and upstream (state O) and downstream (state M) of the removal reach. Rainbow trout ($H \times C$ and $H \times H$) were introduced within both the control (state C) and removal (state R) reaches. The eight movement states remained the same among the model sets, with each representing directional movement obtained via detections at each antenna location (Figures 4.4, 4.5).

I estimated movement between all species-specific states for each weekly time interval; however, because of the distance between the two study reaches, there was very little movement between

the reaches (only 4 brown trout and 2 rainbow trout were observed making movements between the two reaches during the primary study period). Therefore, all movements (transitions; ψ 's) between the two reaches (e.g., movement from state C to state G) were fixed to zero to reduce the number of parameters to estimate; all other movements were considered estimable (Table A2.2). In all three model sets, detection probability (p) for each release state was fixed to zero because individuals were never recaptured within a release state. Detection probabilities for each movement state was fixed to the adjusted efficiencies measured weekly at each antenna array (Table A2.1).

Movement past an antenna array was required for the estimation of transition probability (ψ). Therefore, initial transitions (ψ 's) represented the first movement made by tagged fish from their initial release sites (states). Initial movement probabilities for rainbow trout were compared between removal and control reaches and among the two genetic strains. I expected that rainbow trout released into the removal reach may exhibit lower movement out of the study reach compared to the control. I also expected the H×H individuals may be more likely to move than H×C individuals. Likewise, I compared initial brown trout movement probabilities among sections to determine if movement into the removal reach was higher than into the control reach, representing a desire to fill open habitat despite the presence of the stocked rainbow trout population. Subsequent movement probabilities are estimated for fish that moved out of their original release state (Table A2.2). This allowed me to differentiate initial movements of fish that may be elevated as a result of capture, marking, and introduction, from subsequent weekly movement probabilities of fish into or out of the study reaches after the fish had acclimated.

Brown trout, H×C, and H×H model sets included models in which apparent survival (ϕ) was constant, varied by section (above, within, or below the control and removal reaches; six survival parameters), and varied by fish length or fish weight (included as individual covariates). Fish length was included to test whether apparent survival was size specific, potentially a result of competition. Fish weight was included to test whether apparent survival was affected by the PIT tag in relation to fish size. All additive combinations of apparent survival covariates were included in the model set, except length and weight were never included in the same model because they were correlated. Models also included variation in movement probability (ψ) structures. Specifically, I considered models in which the probability of movement was: constant over time and states, varied by state (estimable transitions only; Table A2.2), varied with discharge (categorical covariate), varied with fish length, or varied within the first two weeks (FTW). Fish length was included to test whether the probability of movement was size specific, again addressing the idea of competition among size classes. The FTW variable was used to examine whether the probability of movement was higher during the first two weeks because I thought that the stocking of rainbow trout into a novel environment might influence movement patterns. The brown trout model set also included models with an interaction between state and spawn because the study occurred during the brown trout spawning season and I wanted to test whether brown trout movement probabilities varied during the pre-spawn (August 15 – September 3) versus spawning period (September 24 – November 3). Similar to survival, all additive combinations of movement probability covariates were included in the brown trout, H×C, and H×H model sets.

I conducted similar analyses to estimate weekly apparent survival and movement probabilities over the winter. The winter study period was used to determine the survival and retention of

rainbow trout and brown trout within the two study reaches over the winter months, specifically during periods with ice cover and no ice cover as competition for resources under the ice was expected to cause higher movement and lower survival during periods of ice cover. Three model sets were used to estimate apparent survival and movement for brown trout, H×C, and H×H over the winter study period. The model sets included 14 states, six starting states, and the same eight movement states included in the primary study period model sets (Figure A2.6). Starting states for the winter study period, lettered similar to the release states from the brown trout and rainbow trout primary study period model sets, were defined as the last known location of an individual upon conclusion of the primary study period. Like the primary study period models, only certain transitions were considered estimable (Table A2.2). The number of estimable transitions was reduced from those of the primary study period because movement generally occurred on a smaller scale. In all three model sets, detection probability (p) for the starting states was fixed to zero; p for the movement states was fixed to the adjusted efficiencies (Table A2.1).

Apparent survival (ϕ) in all three model sets was either constant or varied by section. Length and weight were not included as covariates in the winter model sets because size was unknown during this time period. Movement probabilities (ψ) were either constant, varied by state only (Table A2.2), varied by ice cover only, or varied by the additive and interactive effects between state and ice cover. Ice cover consisted of three separately estimated time periods, a pre-ice period (November 4 – December 16, 2010), an ice cover period (December 17, 2010 – March 17, 2011), and a post-ice period (March 18 – April 14, 2011), and was included to determine variability in ψ during periods where ice cover was present (ice cover period) or absent (pre-ice and post-ice periods).

I fit all models to the data using program MARK (White and Burnham 1999) and used model selection procedures to determine relative support for each candidate model (Burnham and Anderson 2002). I report the difference in AICc values (ΔAICc) and model weights for supported models (Burnham and Anderson 2002). Model averaged estimates and unconditional 95% confidence intervals were used to incorporate model selection uncertainty in the parameter estimates of apparent survival and movement.

RESULTS

Fish Marking

Model selection results for differences in average total length (TL) of the stocked rainbow trout indicated that the model that included an interaction between cross and reach was most supported by the data (AICc weight = 0.99; Table A2.3). H×Cs stocked in both reaches were longer than the stocked H×Hs, but the difference was slightly larger in the control reach (H×C average TL (\pm SE) = 199.5 (\pm 0.8) mm; H×H average TL = 156.9 (\pm 0.8) mm) compared to the removal reach (H×C average TL (\pm SE) = 195.6 (\pm 0.8) mm; H×H average TL = 157.7 (\pm 0.5) mm). Similarly, model selection results for differences in average weight of the stocked rainbow trout indicated that the model that included an interaction between cross and reach was most supported by the data (AICc weight = 0.99; Table A2.3). Again, H×Cs stocked in both reaches were heavier than the stocked H×Hs, but the differences were slightly larger in the control reach (H×C average weight (\pm SE) = 92.8 (\pm 1.0) g; H×H average weight = 41.2 (\pm 1.0) g) compared to the removal

reach (H×C average weight = 86.8 (± 1.0) g; H×H average weight = 40.3 (± 0.7) g). Differences in total length and weight within a cross was considered biologically negligible, suggesting that apparent survival and movement differences between the reaches within a cross were not due to differences in fish size.

Tagging mortality was estimated to be 2.95% (59 mortalities) for the H×C and 0.55% (11 mortalities) for the H×H. The 32 × 3.85 mm PIT tags weighed 0.8 g (0.9% and 2.0% of the average H×C and H×H weight, respectively) and it is unlikely that mortality was associated with PIT tag weight (Zale et al. 2005). Based on scanning 100 fish from each group of 1,000, estimated tag retention was 98.5% for the H×C and 99% for the H×H and was similar to that observed in other studies (Roussel et al. 2000; Zydlewski et al. 2001; Compton et al. 2008). Therefore, differences in apparent survival and movement were not due to differential tag loss.

A total of 676 brown trout were PIT-tagged throughout the control reach, 222 upstream of the reach, 270 within the reach, and 184 downstream of the reach. Model-averaged abundance estimates (± SE) indicated that 1,028 (± 387) brown trout were present upstream of the reach, and 1,354 (± 784) brown trout were present downstream of the reach; therefore, approximately 21% and 13% of the brown trout population was tagged in these two sections, respectively. Within the control reach, model-averaged abundance estimates (± SE) indicated that 1,679 (± 451) brown trout were present; therefore approximately 16% of the brown trout population was tagged within the reach. Average length (± SD) of the brown trout tagged throughout the control reach was 275 (± 9) mm and average weight was 221 (± 17) g. Model-averaged abundance estimates (± SE) of wild rainbow trout upstream of, within, and downstream of the control reach indicated that there were 38 (± 25), 59 (± 42), and 20 (± 19) fish section⁻¹, respectively.

One hundred eighty two brown trout were PIT-tagged upstream of the removal reach, and 216 brown trout were PIT-tagged downstream of the reach. Average length (± SD) of the brown trout PIT-tagged around the removal reach was 270 (± 17) mm and average weight was 203 (± 30) g. Average length (± SD) of the 200 brown trout taken out of the removal reach, PIT-tagged, and relocated below the Narrows was 276 (± 47) mm and average weight was 217 (± 90) g.

Brown Trout Removal

A total of 1,399 brown trout were removed from the removal reach, 726 on the first day, 429 on the second day, and 263 on the third day. Model-averaged removal estimates indicated that 1,975 (1,184-2,765; 95% CI) brown trout were present in the reach prior to the removal; therefore, 71% of the brown trout population was removed. Seven hundred and forty-four of the estimated (± SE) 834 (± 49) adult brown trout were removed, equating to about 89% of the adult population. In contrast, 655 of the estimated (± SE) 1,141 (± 354) fry and juvenile brown trout were removed, equating 57% of the fry or juvenile population. Fewer rainbow trout were estimated to be present in the removal reach, with an estimated 26 (± 2) adult rainbow trout and 4 (± 2) fry or juvenile rainbow trout present in the reach prior to the removal.

Detection probability during the removal was most affected by fish length and pass (Table A2.4). Group (species/size class) had less of an effect on detection probability, included only in the second best model of the set ($\Delta\text{AICc} = 4.88$, $\text{AICc weight} = 0.08$). For all fish, estimates of

detection probability were higher during the first passes compared to the subsequent passes (Figure A2.7).

Apparent Survival and Movement

Antenna Performance

Average antenna efficiency (i.e., the probability of detection by both antennas within an array) was 0.90 for the lower control antenna station, 0.54 for the upper control antenna station, 0.88 for the lower removal antenna station, and 0.86 for the upper removal antenna station during the primary study period; antenna efficiencies during the primary study period were similar to those reported in other studies (Zydlewski et al. 2006; Compton et al. 2008). All antenna stations were functioning 100% of the time during the primary study period. Antenna efficiencies were higher during the winter study period, with an average antenna efficiency of 0.99 for the lower control antenna station, 0.74 for the upper control antenna station, 0.93 for the lower removal antenna station, and 0.98 for the upper removal antenna station; antenna efficiencies during the winter study period were similar to those reported in other studies (Nunnallee et al. 1998; Connelly et al. 2008). The percentage of time during which the antennas were functioning properly was lower during the winter study period, ranging from 84% for the upper control station to 94% for the lower removal antenna station (Table A2.1).

Apparent Survival

Rainbow trout apparent survival during the primary study period was affected by section (above, within, or below the control or removal reaches), fish length, and to a lesser extent, fish weight (Table A2.5). Apparent survival for both rainbow trout crosses was most affected by section, which appeared in all supported models within the H×C and H×H model sets. Fish length and fish weight had less of an effect on apparent survival for both crosses, appearing in fewer supported models than section; total length affected survival more in the H×Cs than the H×Hs, appearing in the top model of the H×C model set. Estimates for the effect of length and weight on apparent survival were both positive (taken from the top model in which they appeared), but these estimates suggested a weak relationship, and the associated 95% confidence intervals overlapped zero (H×C: $\hat{\beta}_{length} = 0.003$ [-0.0009, 0.007] and $\hat{\beta}_{weight} = 0.001$ [-0.002, 0.004]; H×H: $\hat{\beta}_{length} = 0.004$ [-0.002, 0.011] and $\hat{\beta}_{weight} = 0.005$ [-0.003, 0.013]).

The H×C did not exhibit differences in apparent survival between fish within the control and removal reaches during the primary study period (Figure A2.8A). For the H×H, apparent survival was higher for fish in the control reach than in the removal reach (Figure A2.8B). Comparing longitudinally for both rainbow trout crosses, apparent survival was higher within the control and removal reaches than in the 0.8-km sections above or below the reaches; however, estimates of apparent survival in the sections above and below the study reaches likely reflect permanent emigration from the study areas, which cannot be differentiated from survival in my study. Survival did not differ in the sections above or below the reaches for either cross (Figure A2.8).

Apparent survival probabilities of brown trout during the primary study period were affected by section, fish length, and fish weight, all of which appeared in the top models (Table A2.6). Survival was most affected by section, appearing in all six of the top models within the set; but fish length and fish weight also had some influence on apparent survival probabilities. Estimates of the effect size and associated 95% CIs from the top models including length or weight suggested a positive, but small relationship between fish length or weight on apparent survival ($\hat{\beta}_{length} = 0.002$ [0.0004, 0.005] and $\hat{\beta}_{weight} = 0.001$ [0.0003, 0.002]).

Comparing removal and control reaches, brown trout survival was lower for fish within the removal reach than fish within the control reach during the primary study period (Figure A2.9). Apparent survival probabilities for brown trout in the 0.8-km sections above the removal and control reaches were lower than those in the sections below the two study reaches. Comparing longitudinally in the removal reach, survival of fish within the reach did not differ from that of fish upstream; however, survival of fish downstream was higher than those of fish either within or upstream of the reach. Comparing longitudinally in the control reach, survival of fish within the reach did not differ from that of fish downstream, although survival of fish upstream was lower than that of fish within or downstream of the reach (Figure A2.9).

Winter weekly apparent survival probabilities of both the H×C and H×H fish were affected by section (Table A2.7). During the winter study period, model-averaged H×C apparent survival did not differ among fish within the control or removal reaches; however, the H×H fish exhibited lower apparent survival in the control reach than within the removal reach (Figure A2.10). Comparing longitudinally, apparent survival of H×C fish did not differ among fish within the control reach compared to those in the 0.8-km sections above or below the reach (Figure A2.10A). For the H×H fish, apparent survival was extremely low for fish in the 0.8-km section downstream of the control reach, suggesting that fish in this section were seen only once prior to permanently emigrating from the study area; apparent survival probabilities increased for fish within the control reach and in the 0.8-km section above the reach (Figure A2.10B). Apparent survival probabilities of H×C fish and H×H fish within the removal reach did not differ from those of H×C and H×H fish in the 0.8-km section upstream of the reach; however, both were higher than those of H×C and H×H fish in the 0.8-km section downstream of the reach (Figure A2.10).

Brown trout exhibited differences in apparent survival among sections during the winter study period (Table A2.8). Brown trout survival did not differ for fish within the control and removal reaches during the winter study period (Figure A2.11). Comparing longitudinally, model-averaged apparent survival probabilities for fish within the removal reach did not differ from that of fish in the 0.8-km section upstream of the reach; however, survival of fish in the 0.8-km section downstream of the reach was lower than that of fish within or upstream of the reach. Apparent survival did not differ between fish within the control reach compared to fish in the 0.8-km sections upstream or downstream of the reach (Figure A2.11).

Movement

Movement probabilities for both the H×C and H×H during the primary study period were most affected by state (estimable transitions) and discharge, both of which appeared in the top models for both crosses (Table A2.5). Model selection results also suggested that movement

probabilities were lower in the first two weeks of the study period compared to subsequent weeks (H×C: $\hat{\beta}_{treatment} = -0.40$ [-0.46, -0.35]; H×H: $\hat{\beta}_{treatment} = -0.54$ [-0.76, -0.33]). Fish length had less of an effect on movement probabilities in both crosses, though length did appear in the top model for both crosses; estimates of the effect size suggest that there was a positive, but small relationship between length and movement probabilities in the H×H ($\hat{\beta}_{length} = 0.009$ [0.001, 0.016]), and a negative, but small relationship between length and movement probabilities in the H×C ($\hat{\beta}_{length} = -0.007$ [-0.008, -0.007]). Weekly model-averaged movement out of the control and removal reaches was similar for the H×C (Figure A2.12A); however, weekly movement out of the control reach was higher than out of the removal reach for the H×H (Figure A2.12B). For both crosses, movement was lower for the weeks in which discharge was high (> 1.98 cms; 8/19-9/23); movement did not differ among weeks during which discharge was low (< 1.98 cms; 9/24-11/4). Patterns from secondary movements suggest that movement back into both the control and removal reaches was higher than movement out of the reaches for both the H×C and H×H on a weekly basis. Average net secondary movement (difference in the average of secondary movements into and out of a reach \pm SE) into the removal reach was higher than into the control reach for both the H×C and H×H (H×C: control = 0.67 ± 0.09 and removal = 0.92 ± 0.02 ; H×H: control = 0.51 ± 0.30 and removal = 0.95 ± 0.01), suggesting that both crosses were more likely to return to the reach in which brown trout were absent following initial movement out of the reaches.

Rainbow trout estimates of movement during the winter study period were extremely low and highly variable. Initial movement estimates for both crosses were low (< 0.015) and showed little difference among the pre-ice, ice, and post-ice periods for either cross. As a result of low initial movement, the effects of secondary movements are not applicable for either cross.

Movement probabilities for brown trout during the primary study period were most affected by discharge (CMS), differences in the first two weeks (FTW), and the interaction between state and spawn, all of which appeared in the top models of the set (Table A2.4). Brown trout moved into both the control and removal reaches during the primary study period. Movement into the removal reach was slightly higher than into the control reach, especially during the first and third weeks of the study. Discharge negatively affected movement ($\hat{\beta}_{CFS} = 0.0278$ [0.0276, 0.0279]), with more movement occurring during low rather than high discharge periods. Movement probabilities for all states (estimable transitions) were also higher during the brown trout spawning period than the pre-spawning period (Figure A2.13). Directional movements were similar in both the control and removal reach. Additionally, directionality of movement into or out of the control or removal reaches was similar for secondary movements, suggesting that brown trout were in a state of equilibrium in both reaches after initial movement past the antenna stations.

Movement probabilities for brown trout during the winter study period were most affected by state (estimable transitions), with ice cover having a smaller effect; there was no evidence of a state by ice cover interaction (Table A2.8). Within the control reach, movement was lowest during the pre-ice period (Figure A2.14). Movement was higher during the ice cover and post-ice periods in the control reach; however, there was no difference in directionality of movement (in or out of the reach) during these three periods in the control reach. Within the removal reach, movement during the ice cover period was higher than during the pre-ice period; no differences

in directionality of movement were evident for these two periods. In the post-ice period, movement into the removal reach was similar to that which occurred during the ice cover period, and was higher than the movement out of the reach. There was no difference in model-averaged movement between the control and removal reaches during the pre-ice, ice, or post-ice periods (Figure A2.14). Directionality of movement into or out of the control or removal reaches did not differ for secondary movements made during the pre-ice, ice, or post-ice periods, suggesting that brown trout were in a state of equilibrium in both reaches after initial movement past an antenna station. Brown trout movement was higher than rainbow trout movement during the ice and post-ice periods.

Seven of the 200 brown trout relocated from the removal reach to below the Narrows were observed entering the control reach (a 16.1-km upstream movement; Table A2.9). Upstream movement from the relocation section occurred relatively quickly for two of these fish, entering the control reach only two and ten days after being relocated, and slower for others, entering the control reach 2.5 months after being relocated. Six of the seven fish remained in or around the control reach. Only one brown trout successfully returned to the removal reach, with return to the reach occurring 2.5 months after being relocated (Table A2.9).

DISCUSSION

Recovery of wild rainbow trout populations in Colorado is dependent on the development of rainbow trout that are resistant to *Myxobolus cerebralis*, and the ability of these fish to survive and reproduce in the presence of abundant brown trout populations. Through an intensive selective breeding program and subsequent laboratory experiments, crosses of rainbow trout have been developed that both exhibit resistance to *M. cerebralis* (Schisler et al. 2006; Fetherman et al. 2012) and may have the wild characteristics necessary to produce self-sustaining rainbow trout populations in Colorado's rivers (Fetherman et al. 2011). However, evaluations of these populations following introduction suggested that apparent survival for the reintroduced populations was low (Chapter 2) and it was suspected that low survival might be due to abundant brown trout populations (Nehring and Thompson 2001). My primary goal was to evaluate whether the removal of brown trout would increase the retention and survival of reintroduced, *M. cerebralis*-resistant rainbow trout. Overall, brown trout removal did not appear to affect H×C apparent survival, and H×H apparent survival was initially lower in the removal section than the control section. These observations suggest that brown trout removal may not be necessary for increasing initial survival of stocked rainbow trout.

Analogous to the establishment of an invasive species, reintroduced rainbow trout are subject to the three basic phases of the invasion process: arrival or introduction, establishment, and integration (Vermeij 1996). Introduction in this case was facilitated by the stocking of rainbow trout into locations from which they had been eliminated by whirling disease, and introduction success was partially dependent upon the characteristics of the rainbow trout (Townsend 1996). For example, the H×C was developed using the Colorado River Rainbow trout strain, a wild rainbow trout strain that had been widely stocked in Colorado and comprised many of the naturally reproducing wild rainbow trout fisheries prior to the introduction of *M. cerebralis* (Walker and Nehring 1995).

Brown trout presence or absence did not have a large effect on the H×C in the Cache la Poudre River and H×C movement and survival were similar in reaches in which brown trout were present or absent. In addition, survival probabilities were similar between the control and removal reaches during the winter study period. The lack of effects on H×C survival and movement due to brown trout removal is consistent with historic observations regarding the wild parental CRR background of the H×C. Historical ratios of rainbow trout to brown trout in the Cache la Poudre River (60:40; Klein 1963) suggest that the CRR strain was able to survive and reproduce in the wild despite the presence of brown trout. Overall, brown trout removal did not appear to influence survival or movement of H×C, suggesting that, like the parental CRR strain, the H×C was well suited for river reintroductions.

The H×H exhibited similar responses to brown trout removal as the H×C but may have shown greater preference for areas in which brown trout had been removed. For example, initial movements out of the control reach were higher compared to the reach where brown trout had been removed. In addition, secondary movement by H×H fish back into the removal reach was higher than that of H×H fish into the control section, suggesting that H×H fish were more likely to return to the reach where brown trout abundance was lower. Although there was evidence of movement back into the removal reach by brown trout, survival by the H×H within the removal reach was higher during the winter study period, presumably because of the lower brown trout abundance within the reach due to the removal. Taken together, these results suggest that brown trout removal had a positive effect on retention of reintroduced H×H populations; however retention rates were higher than expected in both experimental reaches, regardless of removal status. Higher retention occurred despite the Harrison Lake rainbow trout's reputation as a lake strain (Wagner et al. 2006) and low apparent survivals in other river stockings in Colorado. Since the H×H exhibits lower mortality and myxospore development following exposure to *M. cerebralis* compared to other rainbow trout strains (Fetherman and Schisler 2012; Wagner et al. 2012) it may warrant further consideration in river reintroductions, particularly because the H×H and H×C performed similarly in regards to both survival and retention within the removal reach.

Successful introduction and establishment of a species is also dependent upon the characteristics of the receiving community (Townsend 1996). Newly arriving or introduced species may experience ecological resistance (Elton 1958), consisting of three interacting elements, environmental, biotic, and demographic resistance (Moyle and Light 1996; Vermeij 1996). Reduction of biotic resistance through brown trout removal was the primary focus of this study. The increase in brown trout densities following the introduction of *M. cerebralis* (Baldwin et al. 1998; Nehring and Thompson 2001) suggests that brown trout may have expanded to fill the biological niche vacated by the lost rainbow trout (Baldwin et al. 1998). The introduction of rainbow trout to rivers in which these populations are established could result in changes in the frequency of competitive interactions, levels of food availability, or a functional response to predators, and influence the growth and survival of the wild fish (Einum and Fleming 2001). The addition of large numbers of fish into limited habitat also inevitably affects population density (Einum and Fleming 2001), affecting any density-dependent characteristics of the environment or the fish themselves (Elliot 1989). Although we did not observe low brown trout survival rates in the control section following rainbow trout stocking, this effect could account for the lower survival rates for brown trout returning to the removal reach during the primary study period, where the competitive interactions likely changed due to rainbow trout establishment in the absence of brown trout.

Competitive interactions in the control reach likely favored the better established and relatively undisturbed brown trout population. Rainbow trout exhibit niche shifts away from preferred brown trout habitat when the two species occur in sympatry, and as a result, rainbow trout are forced into areas with deficiencies such as higher water velocities, greater distance from cover, or lower food availability (Gatz et al. 1987). As such, it was expected that the rainbow trout would have a harder time competing with the expanded brown trout populations in the control reach, and this competition is one likely explanation for the higher movement rates observed in the control reach for the H×H.

The timing of the removal and the behavior of the brown trout population itself may have also increased the biotic resistance of the system to rainbow trout establishment, especially during the primary study period. Brown trout typically occupy the same core area and exhibit little movement except during the spawning season (Solomon and Templeton 1976; Burrell et al. 2000), during which time they exhibit increased activity and extensive movements associated with spawning (Burrell et al. 2000; Bettinger and Bettoli 2004; James et al. 2007). We observed an increase in movement into both the control and removal sections during periods of low discharge and during the brown trout spawning period, and this was associated with higher rates of movement out of the sections by both strains of rainbow trout. In addition, brown trout have been shown to return to their home ranges following artificial displacement (Halvorsen and Stabell 1990). Although only one tagged brown trout returned to the removal section, while six others arrived in the control section, these movements suggest that untagged relocated brown trout also moved back to both of the reaches, potentially further increasing the competitive interactions between brown trout and rainbow trout in these reaches. As a result, the brown trout removal did not appear to change survival or movement rates to the extent we expected.

Mechanical removals of piscivorous fish species have been used to promote the survival of target species in other systems across the United States with varying degrees of success. In West Long Lake, Nebraska, a three year removal of northern pike was successful in altering the size structure of the yellow perch (*Perca flavescens*) and increasing the relative abundance and size structure of the bluegill (*Lepomis macrochirus*; Jolley et al. 2008). The relative abundance of six native littoral species increased within two years as a result of a six-year smallmouth bass (*Micropterus dolomieu*) removal in Little Moose Lake in the Adirondacks (Weidel et al. 2007). Additionally, repeated yearly removals in the Colorado River have resulted in declines in large non-native predators (McAda 1997; Brooks et al. 2000; Modde and Fuller 2002). These studies suggest that mechanical removal can be utilized to obtain desired changes in predator and prey dynamics in wild systems.

Several factors must be considered when determining whether mechanical removal is necessary and has the potential to be successful. The first consideration is whether the removal is necessary for the reintroduction and establishment of the target species. In my case, the data suggest that brown trout removal did not dramatically effect apparent survival or emigration from the study site. The long-term goal of the resistant rainbow trout reintroduction program is to produce and maintain self-sustaining whirling disease resistant rainbow trout populations in Colorado waters in which there is a high prevalence of *M. cerebralis* infection (Schisler et al. 2006; Fetherman et al. 2011; Fetherman et al. 2012). Models examining the interactions between rainbow trout introduction size (propagule pressure [Townsend 1996]; demographic

resistance [Moyle and Light 1996]), environmentally stochastic *M. cerebralis* exposure rates, and brown trout population size (biotic resistance; Moyle and Light 1996) suggest that a single introduction of rainbow trout will not result in a self-sustaining rainbow trout population in rivers like the Cache la Poudre River. Therefore, multiple reintroductions, with or without brown trout removal, will likely be needed to overcome ecological resistance factors and to see long-term positive effects of brown trout removal in Colorado's rivers.

The second consideration is whether the removal will be successful after one removal effort, or if multiple removal efforts are needed to overcome biotic resistance and see an effect. For example, a single removal of 66% of the brown trout population in the Au Sable River in Michigan did not result in population or size at age increases in the sympatric brook trout population (Shetter and Alexander 1970). Movement probabilities of brown trout moving back into the removal section in my study suggest that brown trout returned to the removal section fairly quickly. Therefore, the observed benefits of the removal on the short term may not necessarily translate to a continued positive response in reintroduced rainbow trout populations over the long term.

Exposure to *M. cerebralis* also contributes to biotic resistance (Moyle and Light 1996) and could result in low survival in reintroduced rainbow trout populations as disease can interact with predation to have an even larger effect on survival. Exposure to disease has been shown to increase susceptibility to predation (Seppälä et al. 2004), and diseased prey are often eaten in higher than expected proportions due to increased prey vulnerability or active predator selection (Mesa and Warren 1997). Parasites also lower the energy reserves of their host (Poulin 1993), and parasitized fish often take more risks to feed in the presence of a predator than unparasitized fish (Milinski 1985; Godin and Sproul 1988). Therefore, compounding effects of disease exposure and increased susceptibility to predation may lead to lower survival in locations where *M. cerebralis* and predator abundance (aquatic or terrestrial) is high.

A third consideration is whether environmental resistance factors (temperature, flow, abiotic resources; Moyle and Light 1996) may prevent the removal from being a success. Reintroductions in Colorado occur in rivers that have large annual fluctuations in water flow and temperature. Rivers like the Colorado and Cache la Poudre Rivers can experience extensive low flow periods during the summer months (USGS 2009), and minimum discharge has been shown to have a large effect on the survival of reintroduced rainbow trout (Chapter 2). Lower flows result in higher summer water temperatures and lower dissolved oxygen levels (Williams et al. 2009), both of which can directly affect salmonid survival (Hicks et al. 1991). Biotic resistance may also be increased as a result of low flows and high temperatures. Increased stress due to low flow may intensify the effects of *M. cerebralis* infection, and ectoparasite infestation has been shown to peak during periods of low flow and high mean water temperature, potentially significantly increasing mortality in these rivers (Schisler et al. 1999b). Low flows also reduce suitable habitat and can lead to high densities and overcrowding, increased predation, and increased competition (Arismendi et al. 2012).

Finally, the cost of the removal and the benefits received from such a cost must be considered. For example, nearly \$4.4 million has been spent to mechanically remove > 1.5 million non-native predatory fish from the Colorado River; however, 86% of published reports (as of 2005) suggested that native species did not benefit from the removal efforts (Mueller 2005).

Additionally, the logistic constraints associated with large removal efforts may be limiting. In this study, over 100 volunteers were utilized to remove 89% of the brown trout population from a 1.0-km reach of the Cache la Poudre River. Assembling and maintaining this large of a volunteer base for removals of the same size in multiple locations, or a removal effort over longer distances, would not be an easy feat.

Although the results of this study suggest that brown trout removal did have a positive effect on the retention of the H×Hs, the overall benefit of the removal is questionable. Due to the logistical constraints of conducting removals in other large river systems in Colorado, the return of brown trout to the removal reach, and the fact that removal did not appear to have an effect on the survival of either cross or the retention of the H×Cs, I conclude that adult brown trout removal is not a viable management option to pursue in future *M. cerebralis*-resistant rainbow trout introductions in Colorado. The stocked rainbow trout appeared to be well suited for introduction, and seem to be capable of overcoming many of the ecological resistance factors encountered, potentially becoming established in both reaches of the Cache la Poudre River. Further study is needed to determine if rainbow trout have become established and integrated into the Cache la Poudre River ecosystem. Additional research should also focus on rainbow trout reintroduction strategies, with regard to fish size, reintroduction size, and the number of reintroductions needed to produce a self-sustaining rainbow trout population in Colorado.

Table A2.1. Antenna efficiencies (E ; the probability of being detected at both antennas within an array) estimated on a weekly basis at each antenna location during the primary study period, and on a monthly basis during the winter study period. Efficiencies were adjusted based on the proportion of the week a reader was functioning (Op), and adjusted efficiencies were used to fix detection probability (p) for each location in the multistate capture-recapture analyses.

Week	Lower Control			Upper Control			Lower Removal			Upper Removal		
	E	Op	p	E	Op	p	E	Op	p	E	Op	p
Primary Study Period												
8/19-8/26	0.91	1.00	0.91	0.54	1.00	0.54	0.73	1.00	0.73	0.77	1.00	0.77
8/27-9/2	0.90	1.00	0.90	0.65	1.00	0.65	0.88	1.00	0.88	0.88	1.00	0.88
9/3-9/9	0.71	1.00	0.71	0.29	1.00	0.29	0.66	1.00	0.66	0.76	1.00	0.76
9/10-9/16	0.85	1.00	0.85	0.38	1.00	0.38	0.78	1.00	0.78	0.82	1.00	0.82
9/17-9/23	0.91	1.00	0.91	0.44	1.00	0.44	0.91	1.00	0.91	0.82	1.00	0.82
9/24-9/30	0.96	1.00	0.96	0.67	1.00	0.67	1.00	1.00	1.00	0.89	1.00	0.89
10/1-10/7	0.92	1.00	0.92	0.54	1.00	0.54	0.96	1.00	0.96	0.94	1.00	0.94
10/8-10/14	0.90	1.00	0.90	0.63	1.00	0.63	0.91	1.00	0.91	0.89	1.00	0.89
10/15-10/21	0.94	1.00	0.94	0.60	1.00	0.60	0.96	1.00	0.96	0.88	1.00	0.88
10/22-10/28	0.92	1.00	0.92	0.58	1.00	0.58	0.93	1.00	0.93	0.90	1.00	0.90
10/29-11/4	0.92	1.00	0.92	0.58	1.00	0.58	0.93	1.00	0.93	0.90	1.00	0.90
Winter Study Period												
11/5-11/11	0.92	1.00	0.92	0.58	1.00	0.58	0.93	1.00	0.93	0.90	1.00	0.90
11/12-11/18	0.92	1.00	0.92	0.58	1.00	0.58	0.93	0.86	0.80	0.90	0.42	0.38
11/19-11/25	1.00	1.00	1.00	0.55	1.00	0.55	1.00	1.00	1.00	0.95	1.00	0.95
11/26-12/2	1.00	1.00	1.00	0.55	1.00	0.55	1.00	0.42	0.42	0.95	0.71	0.68
12/3-12/9	1.00	1.00	1.00	0.55	1.00	0.55	1.00	1.00	1.00	0.95	1.00	0.95
12/10-12/16	1.00	1.00	1.00	0.55	1.00	0.55	1.00	1.00	1.00	0.95	1.00	0.95
12/17-12/23	1.00	1.00	1.00	0.63	1.00	0.63	1.00	1.00	1.00	1.00	1.00	1.00
12/24-12/30	1.00	1.00	1.00	0.63	1.00	0.63	1.00	1.00	1.00	1.00	1.00	1.00
12/31-1/6	1.00	0.29	0.29	0.63	1.00	0.63	1.00	1.00	1.00	1.00	1.00	1.00
1/7-1/13	1.00	0.42	0.42	0.63	1.00	0.63	1.00	1.00	1.00	1.00	1.00	1.00
1/14-1/20	1.00	1.00	1.00	0.63	1.00	0.63	1.00	1.00	1.00	1.00	1.00	1.00
1/21-1/27	1.00	0.42	0.42	0.91	1.00	0.91	1.00	0.71	0.71	1.00	1.00	1.00
1/28-2/3	1.00	1.00	1.00	0.91	0.57	0.52	1.00	0.71	0.71	1.00	1.00	1.00
2/4-2/10	1.00	1.00	1.00	0.91	1.00	0.91	1.00	1.00	1.00	1.00	1.00	1.00
2/11-2/17	1.00	1.00	1.00	0.91	0.86	0.78	1.00	1.00	1.00	1.00	0.14	0.14
2/18-2/24	1.00	1.00	1.00	0.91	1.00	0.91	1.00	1.00	1.00	1.00	1.00	1.00
2/25-3/3	1.00	1.00	1.00	0.91	1.00	0.91	1.00	1.00	1.00	1.00	0.29	0.29
3/4-3/10	1.00	1.00	1.00	0.91	1.00	0.91	1.00	1.00	1.00	1.00	1.00	1.00
3/11-3/17	1.00	1.00	1.00	0.91	0.86	0.78	1.00	1.00	1.00	1.00	1.00	1.00
3/18-3/24	1.00	1.00	1.00	0.91	0.00	0.00	1.00	1.00	1.00	1.00	0.71	0.71
3/25-3/31	1.00	1.00	1.00	0.78	1.00	0.78	0.52	1.00	0.52	0.96	0.42	0.41
4/1-4/7	1.00	0.71	0.71	0.78	1.00	0.78	0.52	1.00	0.52	0.96	0.71	0.67
4/8-4/14	1.00	0.71	0.71	0.78	1.00	0.78	0.52	1.00	0.52	0.96	1.00	0.96

Table A2.2. Estimated transitions (ψ 's) included in the brown trout and rainbow trout model sets for both the primary and winter study periods. Initial ψ represent the first movement made by tagged fish from their release site (state). Secondary ψ were only estimated for fish that moved out of their release state, representing weekly movement into and out of the study reaches.

Species	Study Period	Study Reach	Initial ψ	Secondary ψ	
Brown Trout	Primary	Control	C → A	A → B	
			C → D	A → D	
			K → B	B → A	
			K → D	B → D	
			L → A	D → A	
			L → E	D → E	
				E → A	
			E → D		
			Removal	M → G	F → G
				M → H	F → H
				O → F	G → F
				O → I	G → H
					H → F
					H → I
		I → F			
		I → H			
H×C H×H	Primary	Control	C → A	A → B	
			C → D	A → D	
				B → A	
				B → D	
				D → A	
				D → E	
				E → A	
			E → D		
			Removal	R → F	F → G
				R → H	F → H
					G → F
					G → H
					H → F
					H → I
		I → F			
		I → H			
Brown Trout H×C H×H	Winter	Control	C → A	A → B	
			C → D	B → A	
			K → B	D → E	
			L → E	E → D	
			Removal	R → F	F → G
				R → H	G → F
				M → G	H → I
				O → I	I → H

Table A2.3. Model selection results for differences in rainbow trout length and weight at stocking in the Cache la Poudre River in August 2010.

Model	R^2	$\log(L)$	K	AICc	Δ_i	w_i
Length						
Cross*Reach	0.58	-11181.40	4	22372.86	0.00	0.99
Cross+Reach	0.58	-11190.30	3	22387.80	14.94	0.01
Cross	0.58	-11194.20	2	22393.04	20.18	0.00
Reach	0.00	-12895.50	2	25795.59	3422.73	0.00
Intercept-only	0.00	-12897.10	1	25796.37	3423.51	0.00
Weight						
Cross*Reach	0.57	-12032.40	4	24074.88	0.00	0.99
Cross+Reach	0.57	-12039.30	3	24085.88	11.00	0.01
Cross	0.57	-12052.00	2	24108.65	33.77	0.00
Reach	0.00	-13706.10	2	27416.84	3341.96	0.00
Intercept-only	0.00	-13711.50	1	27425.12	3350.24	0.00

The maximized log-likelihood ($\log(L)$), the number of parameters (K) in each model, and the small sample size-corrected AICc values (AICc) are shown. Models are ranked within the length or weight model sets by their AICc differences (Δ_i) relative to the best model in the set and Akaike weights (w_i) quantify the probability that a particular model is the best model in the set given the data and the model set.

Table A2.4. Model selection results for Huggins closed-population models containing covariates thought to influence estimates of detection probability during the brown trout removal conducted August 14-16, 2010 in the Cache la Poudre River.

Model	$\log(L)$	K	AICc	Δ_i	w_i
p (P,TL)	-1849.64	6	3711.33	0.00	0.92
p (G,P,TL)	-1849.04	9	3716.21	4.88	0.08
p (TL)	-1887.73	2	3779.46	68.13	0.00
p (G,TL)	-1886.26	5	3782.57	71.24	0.00
p (P)	-1889.04	4	3786.10	74.78	0.00
p (G)	-1903.71	4	3815.44	104.12	0.00
p (•)	-1933.09	1	3868.18	156.85	0.00

The maximized log-likelihood ($\log(L)$), the number of parameters (K) in each model, and the small sample size-corrected AICc values (AICc) are shown. Models are ranked by their AICc differences (Δ_i) relative to the best model in the set and Akaike weights (w_i) quantify the probability that a particular model is the best model in the set given the data and the model set. NOTE: P = pass, TL = total length, G = group (brown trout > 150 mm, brown trout \leq 150 mm, rainbow trout > 150 mm, rainbow trout \leq 150 mm), and • = intercept model.

Table A2.5. Model selection results for multistate models fit to stocked rainbow trout data during the primary study period. The candidate model sets included over 150 models with various structures for apparent survival (ϕ) and movement (ψ); models for which there were weight are shown for both the H×C and H×H crosses.

Model	log(L)	K	AICc	Δ_i	w_i
H×C					
$\phi(S, TL) \psi(ST, CMS, TL, FTW)$	-5510.79	30	11082.54	0.00	0.27
$\phi(S) \psi(ST, CMS, FTW)$	-5512.85	28	11082.55	0.01	0.27
$\phi(S, W) \psi(ST, CMS, TL, FTW)$	-5511.44	30	11083.84	1.30	0.14
$\phi(S, TL) \psi(ST, CMS, FTW)$	-5512.83	29	11084.56	2.02	0.10
$\phi(S, W) \psi(ST, CMS, FTW)$	-5512.84	29	11084.59	2.05	0.10
$\phi(S) \psi(ST, CMS, TL, FTW)$	-5512.85	29	11084.60	2.06	0.10
$\phi(S, TL) \psi(ST, CMS, TL)$	-5514.49	29	11087.89	5.36	0.02
$\phi(S, TL) \psi(ST, CMS)$	-5516.54	28	11089.93	7.39	0.01
$\phi(S) \psi(ST, CMS, TL)$	-5520.07	28	11096.98	14.45	< 0.01
$\phi(S) \psi(ST, CMS)$	-5521.23	27	11097.24	14.70	< 0.01
$\phi(S, W) \psi(ST, CMS, TL)$	-5519.64	29	11098.18	15.64	< 0.01
$\phi(S, W) \psi(ST, CMS)$	-5521.22	28	11099.28	16.74	< 0.01
H×H					
$\phi(S) \psi(ST, CMS, TL, FTW)$	-3969.38	29	7997.64	0.00	0.28
$\phi(S, TL) \psi(ST, CMS, TL, FTW)$	-3968.45	30	7997.86	0.23	0.25
$\phi(S, W) \psi(ST, CMS, TL, FTW)$	-3968.53	30	7998.02	0.38	0.23
$\phi(S, TL) \psi(ST, CMS, FTW)$	-3970.28	29	7999.45	1.80	0.11
$\phi(S, W) \psi(ST, CMS, FTW)$	-3970.42	29	7999.73	2.09	0.10
$\phi(S) \psi(ST, CMS, FTW)$	-3972.27	28	8001.37	3.73	0.04
$\phi(S) \psi(ST, CMS, TL)$	-3981.30	28	8019.43	21.79	< 0.01

The maximized log-likelihood ($\log(L)$), the number of parameters (K) in each model, and the small sample size-corrected AICc values (AICc) are shown. Models are ranked within the H×C or H×H model sets by their AICc differences (Δ_i) relative to the best model in the set and Akaike weights (w_i) quantify the probability that a particular model is the best model in the set given the data and the model set. NOTE: S = section (above, within, or below the control or removal reaches), TL = length, W = weight, ST = state (estimable transitions), CMS = discharge, FTW = first two weeks.

Table A2.6. Model selection results for multistate models fit to wild PIT-tagged brown trout data during the primary study period. The model set included over 300 models with various structures for apparent survival (ϕ) and movement (ψ); models for which there was weight are shown.

Model	log(L)	K	AICc	Δ_i	w_i
$\phi(S, W) \psi(ST*SP, CMS, FTW)$	-3056.20	61	6241.89	0.00	0.52
$\phi(S, W) \psi(ST*SP, CMS, TL, FTW)$	-3056.03	62	6243.80	1.90	0.20
$\phi(S, L) \psi(ST*SP, CMS, FTW)$	-3057.32	61	6244.14	2.25	0.17
$\phi(S, L) \psi(ST*SP, CMS, TL, FTW)$	-3057.22	62	6246.18	4.29	0.06
$\phi(S) \psi(ST*SP, CMS, FTW)$	-3060.19	60	6247.62	5.72	0.03
$\phi(S) \psi(ST*SP, CMS, TL, FTW)$	-3059.57	61	6248.64	6.75	0.02

The maximized log-likelihood ($\log(L)$), the number of parameters (K) in each model, and the small sample size-corrected AICc values (AICc) are shown. Models are ranked by their AICc differences (Δ_i) relative to the best model in the set and Akaike weights (w_i) quantify the probability that a particular model is the best model in the set given the data and the model set. NOTE: S = section (above, within, or below the control or removal reaches), TL = length, W = weight, ST = state (estimable transitions), SP = spawn, CMS = discharge, FTW = first two weeks, and * = interaction.

Table A2.7. Model selection results for multistate models fit to stocked rainbow trout data during the winter study period. The candidate model sets had 10 models each with various structures for apparent survival (ϕ) and movement (ψ); models for which there were weight are shown for both the H×C and H×H crosses.

Model	log(L)	K	AICc	Δ_i	w_i
H×C					
$\phi(S) \psi(ST)$	-3937.85	22	7920.22	0.00	0.83
$\phi(\bullet) \psi(ST)$	-3944.96	17	7924.23	4.01	0.11
$\phi(\bullet) \psi(ST,IC)$	-3943.77	19	7925.93	5.71	0.05
$\phi(S) \psi(ST,IC)$	-3940.43	24	7929.49	9.27	0.01
H×H					
$\phi(S) \psi(ST)$	-1777.23	22	3598.97	0	0.54
$\phi(S) \psi(ST,IC)$	-1775.33	24	3599.28	0.31	0.46
$\phi(\bullet) \psi(ST)$	-1789.80	17	3613.92	14.95	< 0.01
$\phi(\bullet) \psi(ST,IC)$	-1788.92	19	3616.23	17.26	< 0.01

The maximized log-likelihood ($\log(L)$), the number of parameters (K) in each model, and the small sample size-corrected AICc values (AICc) are shown. Models are ranked within the H×C or H×H model sets by their AICc differences (Δ_i) relative to the best model in the set and Akaike weights (w_i) quantify the probability that a particular model is the best model in the set given the data and the model set. NOTE: S = section (above, within, or below the control or removal reaches), ST = state (estimable transitions), IC = ice cover, and \bullet = intercept model.

Table A2.8. Model selection results for multistate models fit to wild PIT-tagged brown trout data during the winter study period. The candidate model set had 10 models with various structures for apparent survival (ϕ) and movement (ψ); models for which there was weight are shown.

Model	$\log(L)$	K	AICc	Δ_i	w_i
$\phi(S) \psi(ST)$	-7272.71	22	14590.38	0	0.48
$\phi(S) \psi(ST,IC)$	-7272.26	24	14591.57	1.19	0.26
$\phi(\bullet) \psi(ST,IC)$	-7276.85	19	14592.43	2.05	0.17
$\phi(\bullet) \psi(ST)$	-7279.57	17	14593.71	3.33	0.09
$\phi(\bullet) \psi(IC)$	-7297.72	4	14603.48	13.10	< 0.01
$\phi(S) \psi(IC)$	-7293.50	9	14605.16	14.79	< 0.01
$\phi(\bullet) \psi(\bullet)$	-7300.83	2	14605.68	15.30	< 0.01
$\phi(S) \psi(\bullet)$	-7295.88	7	14605.87	15.49	< 0.01

The maximized log-likelihood ($\log(L)$), the number of parameters (K) in each model, and the small sample size-corrected AICc values (AICc) are shown. Models are ranked by their AICc differences (Δ_i) relative to the best model in the set and Akaike weights (w_i) quantify the probability that a particular model is the best model in the set given the data and the model set. NOTE: S = section (above, within, or below the control or removal reaches), ST = state (estimable transitions), IC = ice cover, and \bullet = intercept model.

Table A2.9. Movement of relocated brown trout within the control and removal reaches. The dates at which brown trout entered and exited each reach, direction of movement upon exit from a reach, and the last known location is shown for each of the relocated brown trout detected within the control and removal reaches.

Tag #	Control Reach			Removal Reach			Last Known Location
	Enter	Exit	Direction	Enter	Exit	Direction	
173863414	9/18	---	---	---	---	---	Control
173863424	9/22	9/24	Upstream	11/1	11/5	Downstream	Below Removal
173863427	8/28	---	---	---	---	---	Control
173863486	10/4	10/8	Downstream	---	---	---	Below Control
173863525	11/5	11/6	Upstream	---	---	---	Above Control
173863546	8/20	10/4	Upstream	---	---	---	Above Control
173863571	10/21	10/24	Upstream	---	---	---	Above Control

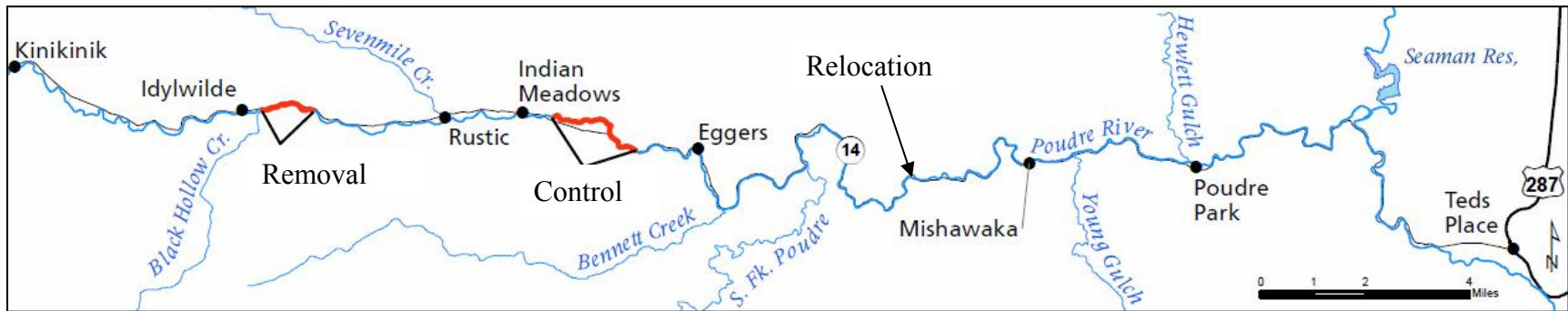


Figure A2.1. Location of the control, removal, and relocation reaches within the Cache la Poudre River, Colorado.

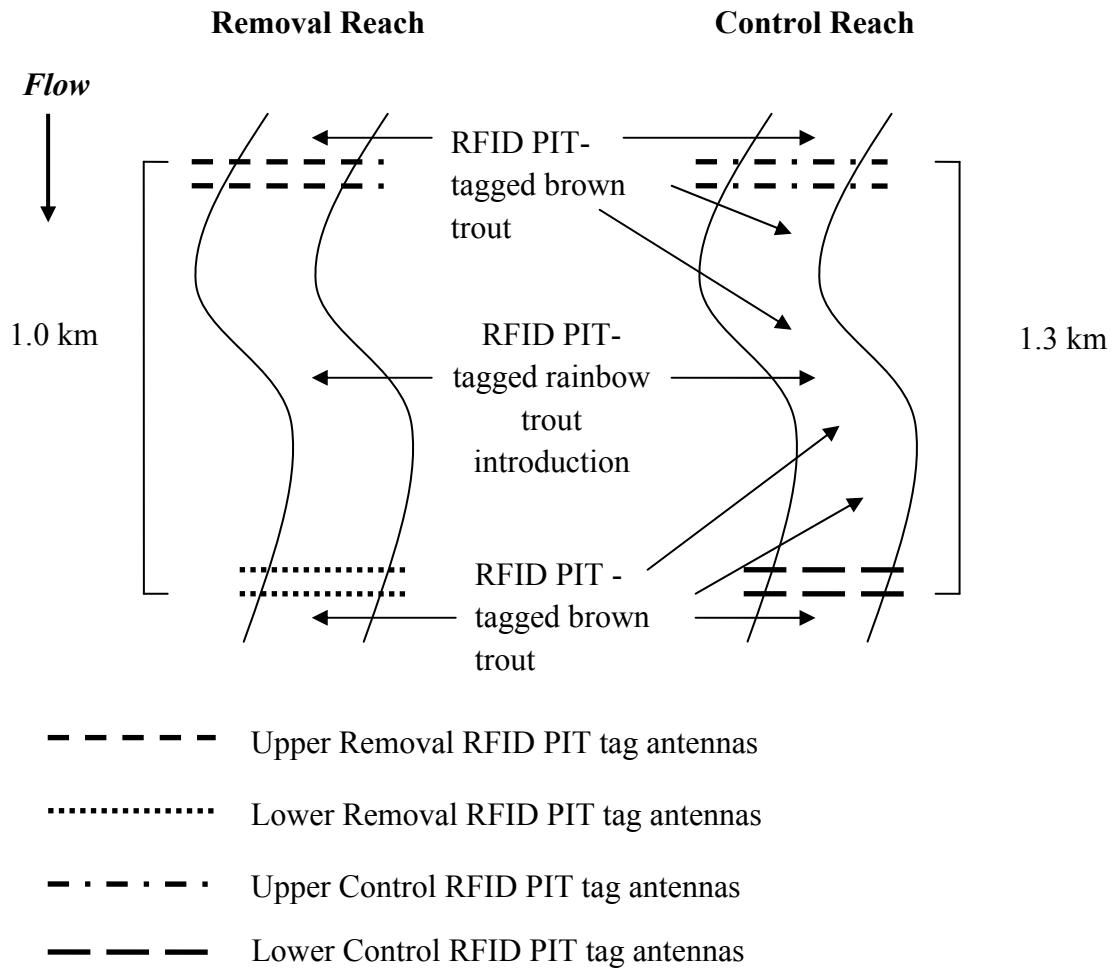


Figure A2.2. Experimental design of the brown trout removal experiment conducted in the Cache la Poudre River. The experiment consisted of a 1.3-km control reach (no removal) and a 1.0-km removal reach (brown trout removal). Both reaches were bordered by paired RFID PIT tag antennas used to determine directionality of movement of PIT-tagged brown trout and rainbow trout into and out of the reaches.

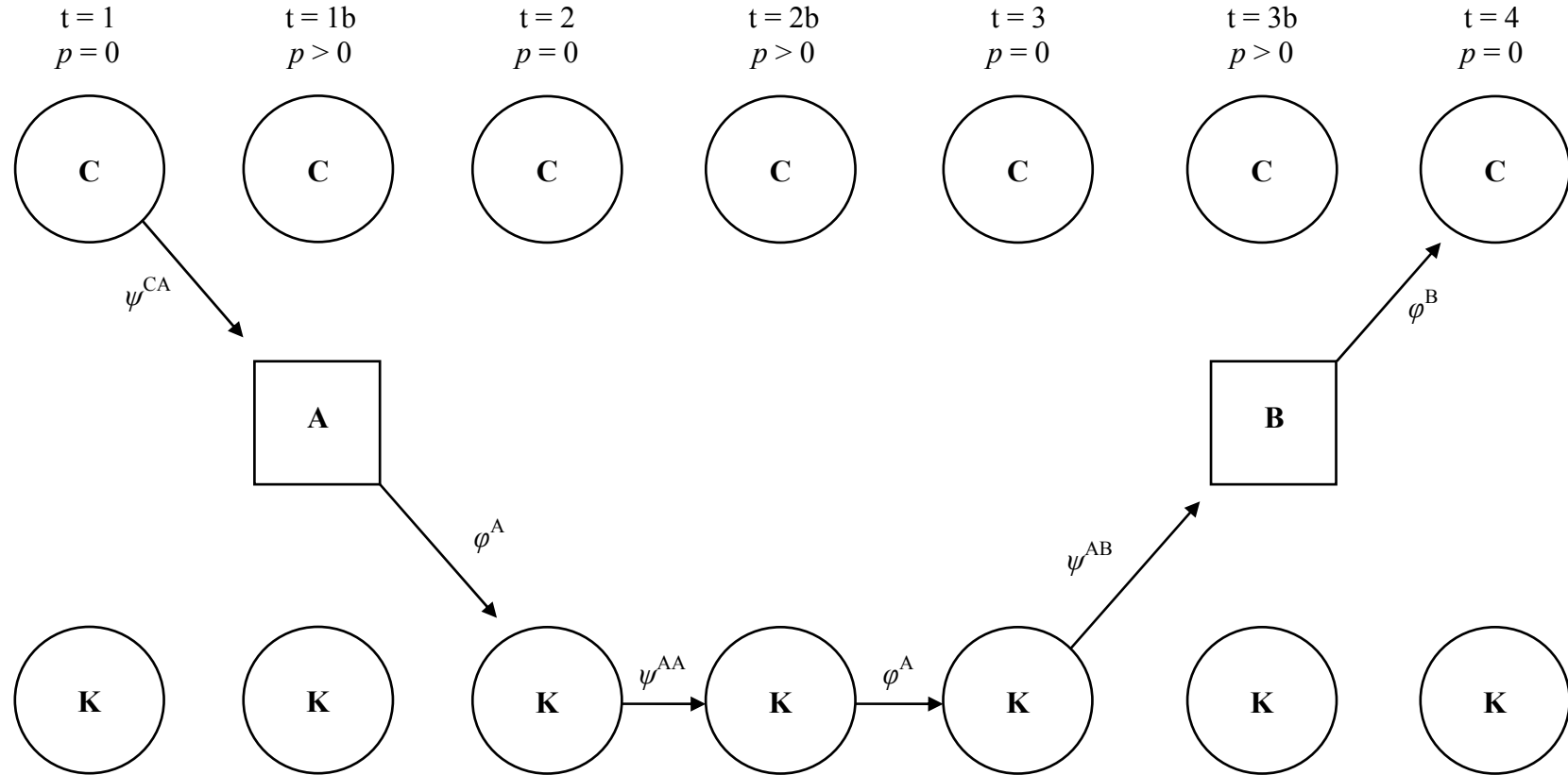


Figure A2.3. Example of the multistate model used to estimate transition (ψ), survival (ϕ), and detection probability (p) for a fish with the encounter history of CA000B0. This fish was released in the control reach (release state C) at time 1. Because the fish is undetectable (circles) in C and the downstream state (K), p is zero. Between time 1 and 2, the fish was recaptured (squares) by the reader making a downstream movement past the lower control antenna station (transition state A) and the transition probability (ψ^{CA}) was estimated between time periods 1 and 1b. The fish was assumed to be alive while making the transition; therefore, survival (ϕ^A) was estimated between time periods 1b and 2 once the transition had been made. Between time periods 2 and 3, the fish remained in the downstream section, and the probability of retention (ψ^{AA}) and ϕ^A were estimated. Between time periods 3 and 4, the fish was observed making an upstream movement (transition state B); ψ^{AB} was estimated between time periods 3 and 3b, and ϕ^B was estimated

between time periods 3b and 4. At time periods 1b, 2b, and 3b, p was fixed to the adjusted efficiency for the lower control antenna station (Table A2.1).

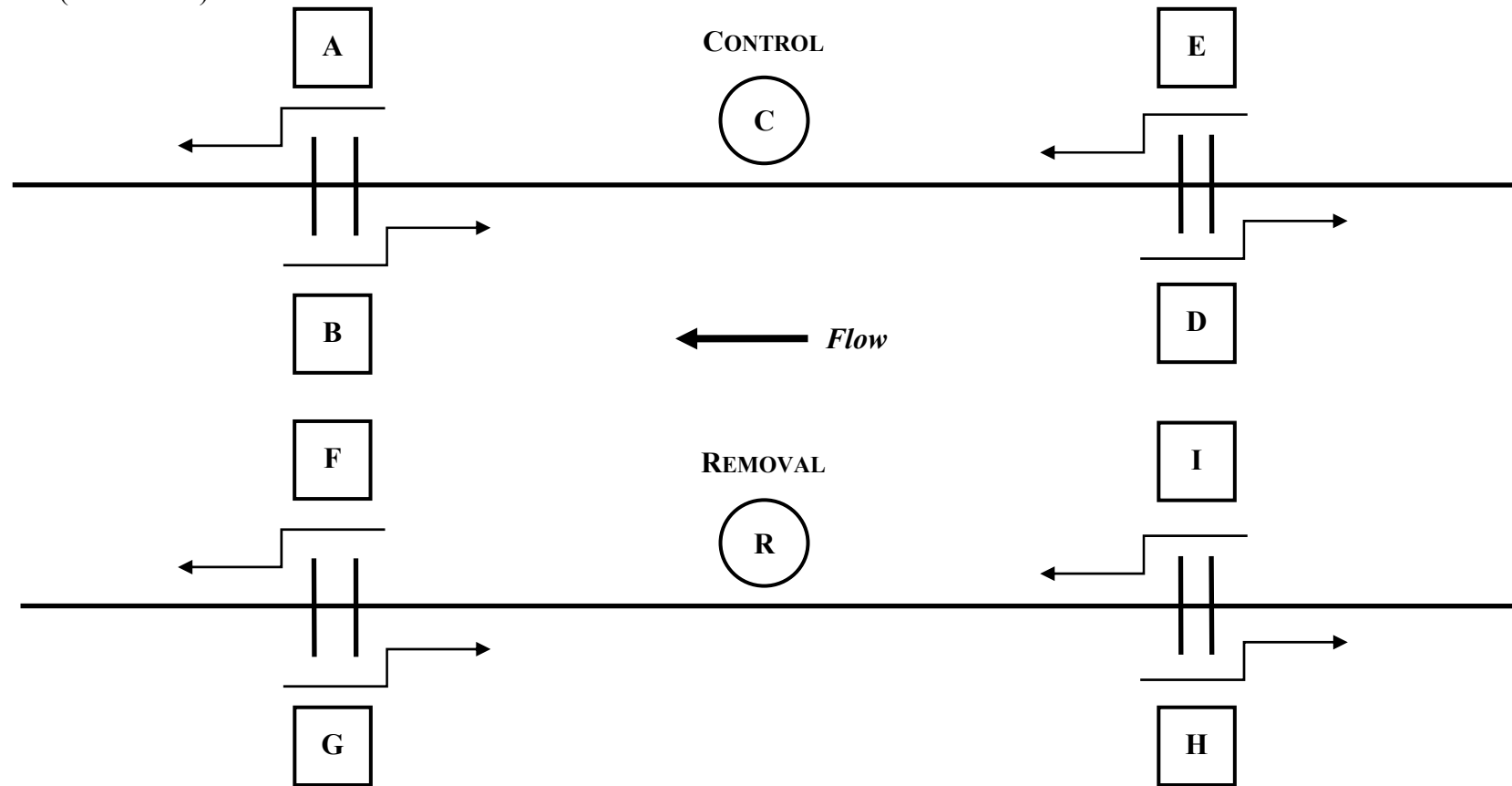


Figure A2.4. Release (circle) and transition (square) states used in the multistate models estimating weekly apparent survival (ϕ) and movement (ψ) probabilities for rainbow trout (H×C and H×H) during the primary study period (August 15 – November 3, 2010).

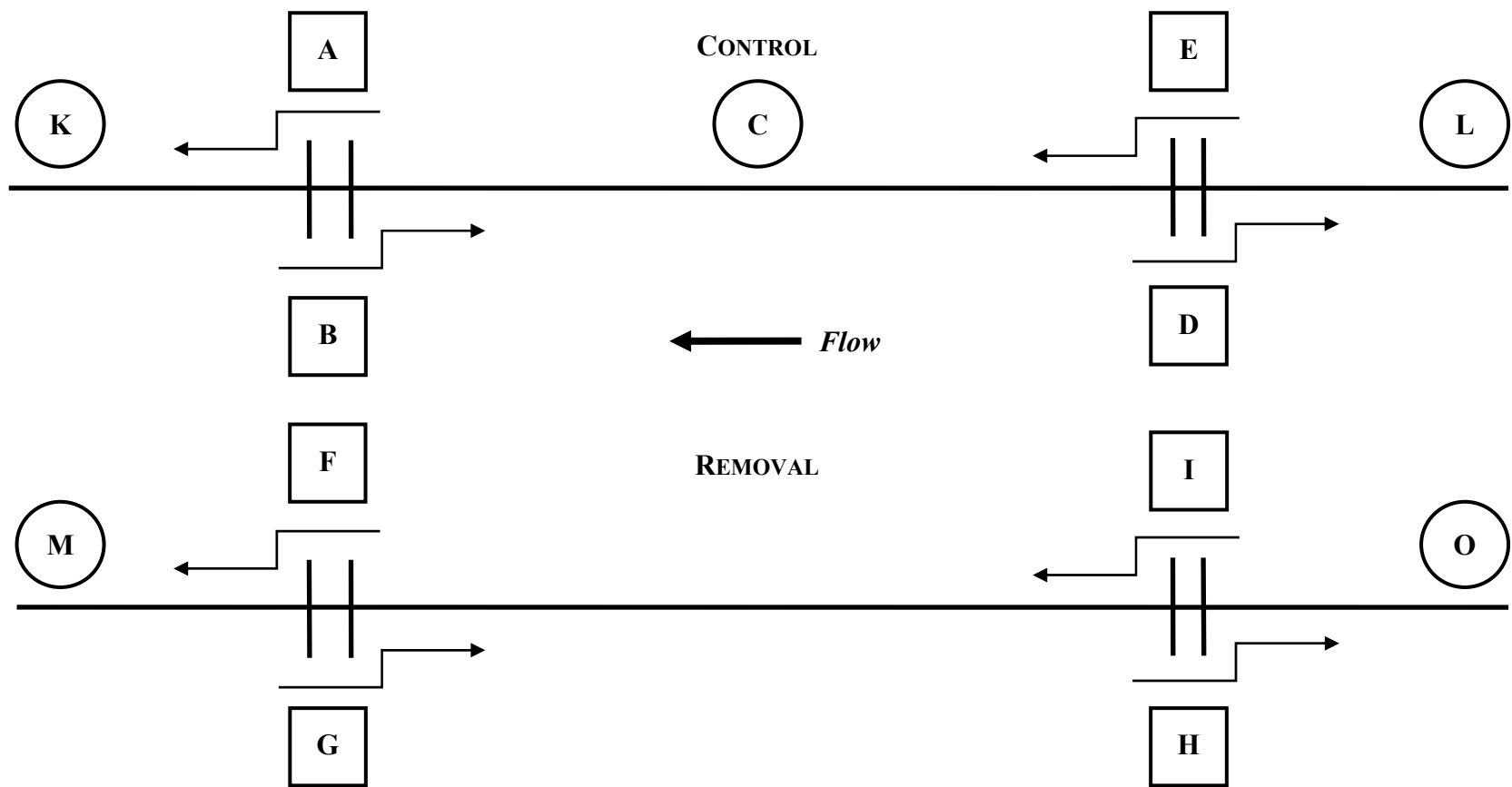


Figure A2.5. Release (circle) and transition (square) states used in the multistate model estimating weekly apparent survival (ϕ) and movement (ψ) brown trout during the primary study period (August 15 – November 3, 2010).

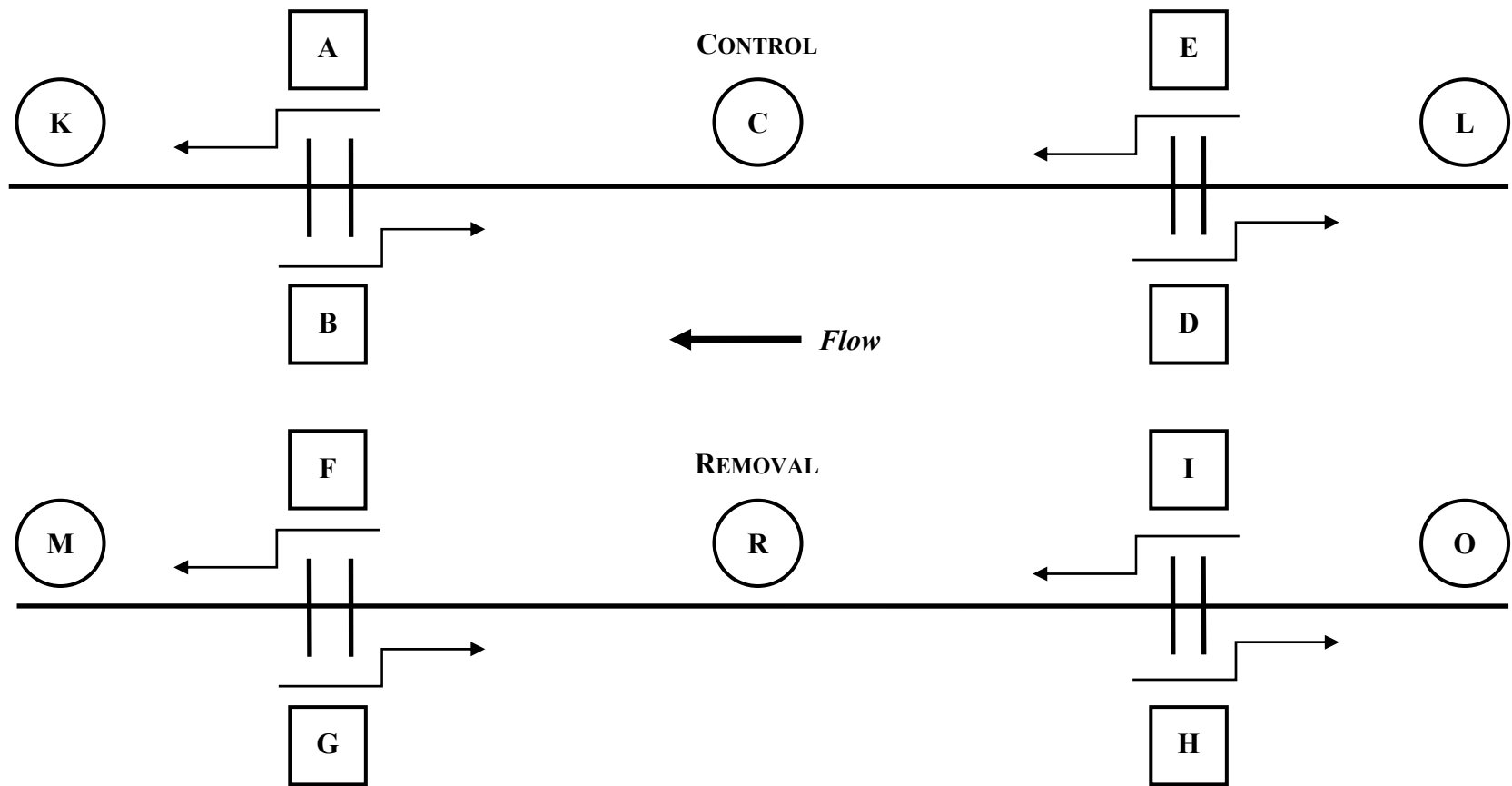


Figure A2.6. Release (circle) and transition (square) states used in the multistate models estimating weekly apparent survival (ϕ) and movement (ψ) probabilities for brown trout and rainbow trout (H×C and H×H) during the winter study period (November 4, 2010 – April 14, 2011).

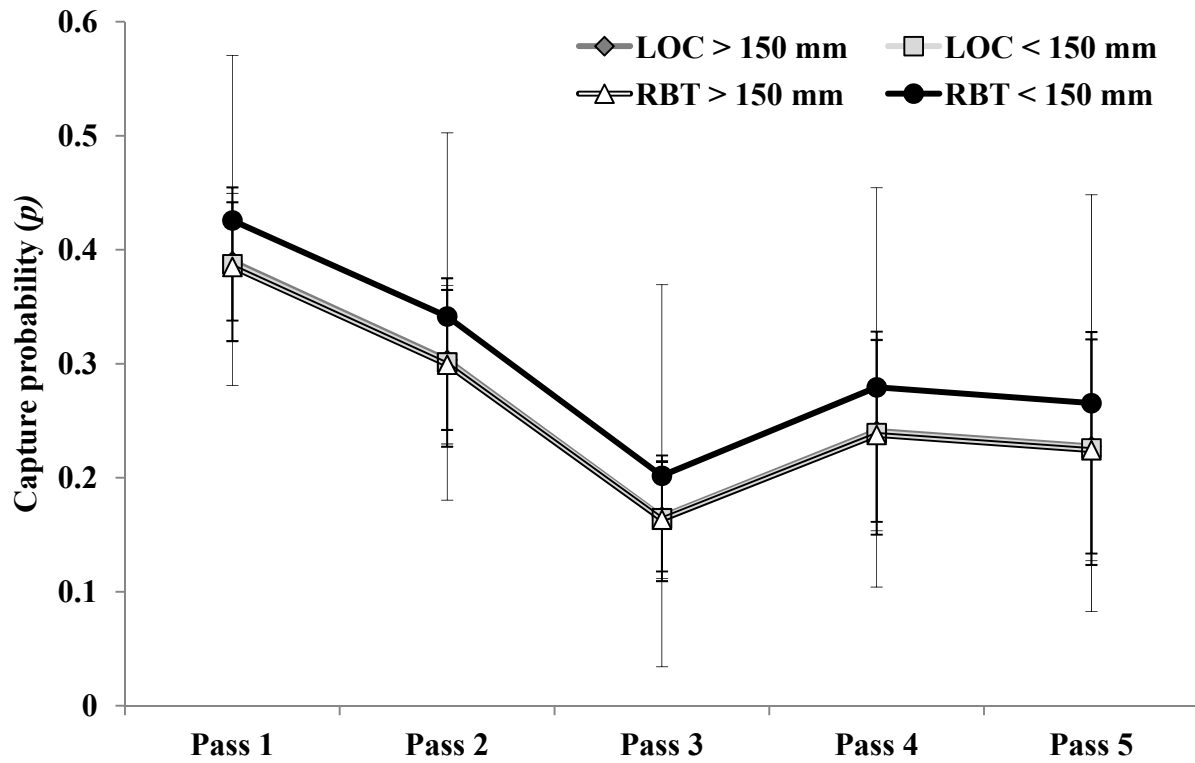


Figure A2.7. Model-averaged estimates of pass-specific capture probability for two size classes (> 150 mm, ≤ 150 mm) of brown trout and rainbow trout during the removal (August 16-18, 2010).

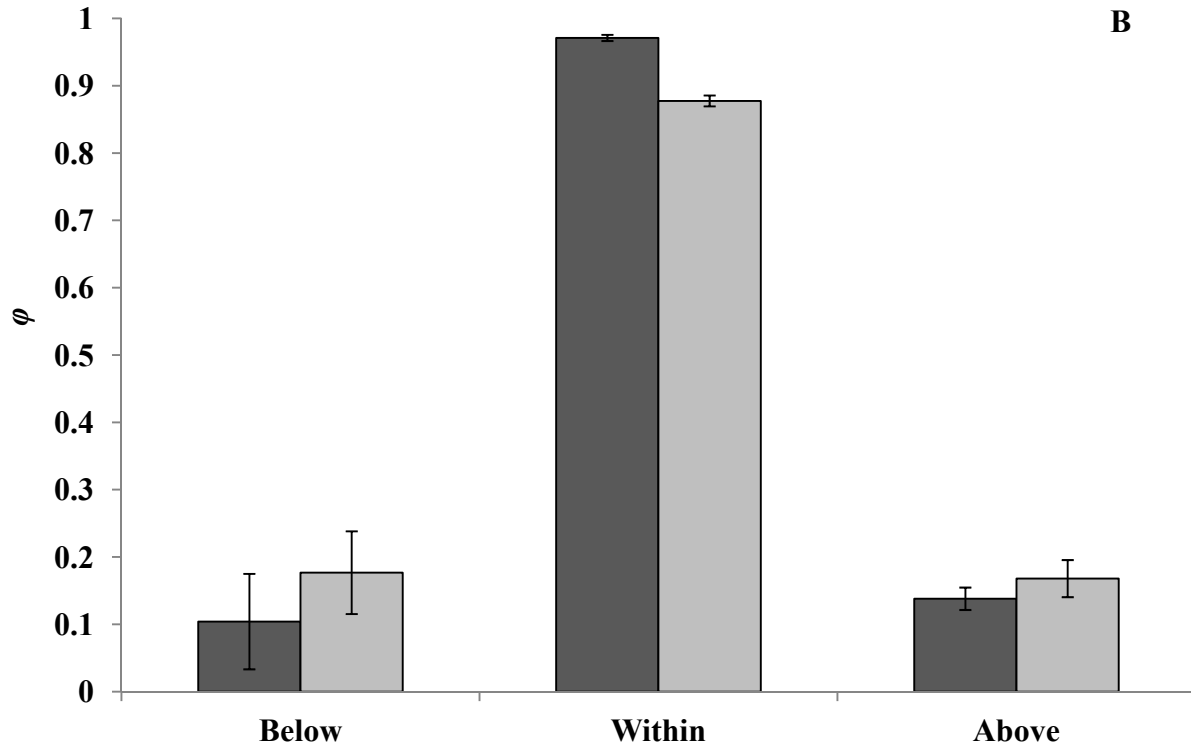
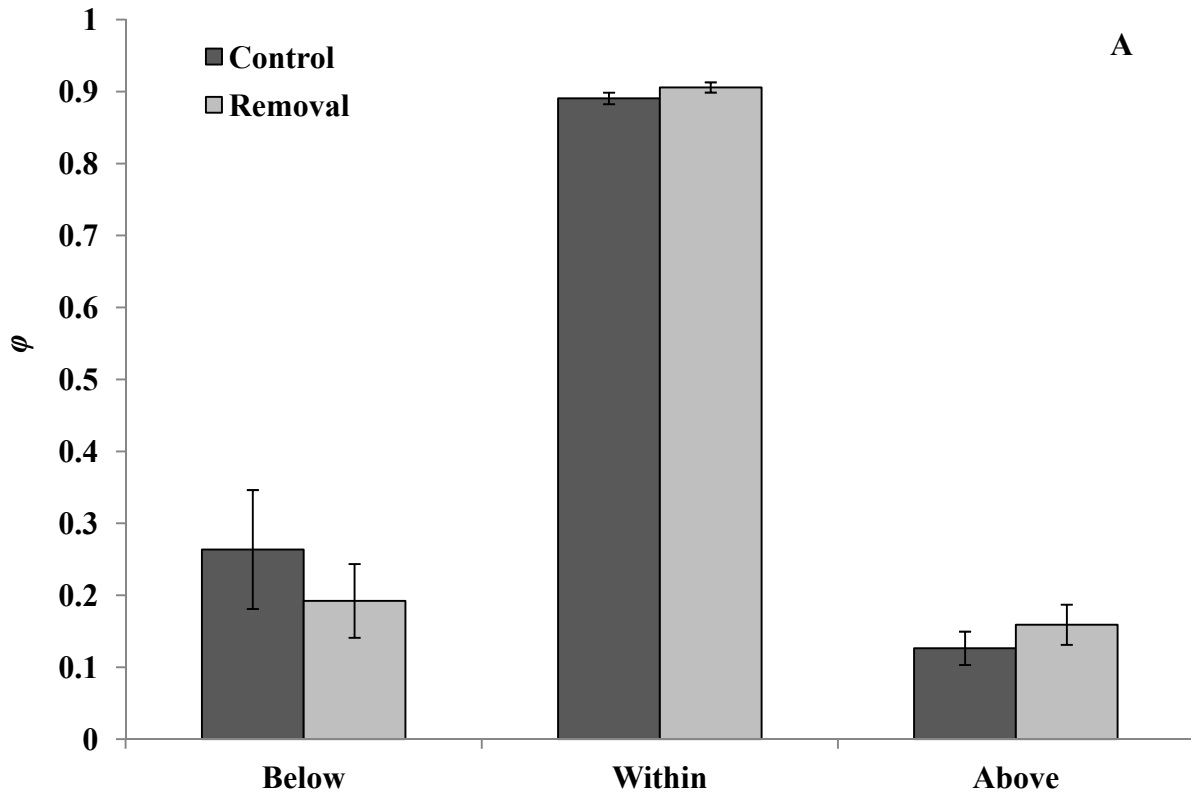


Figure A2.8. Model-averaged apparent primary study period weekly survival probabilities (φ ; SE bars) for H×C (A) and H×H (B) below, within, and above the control and removal reaches.

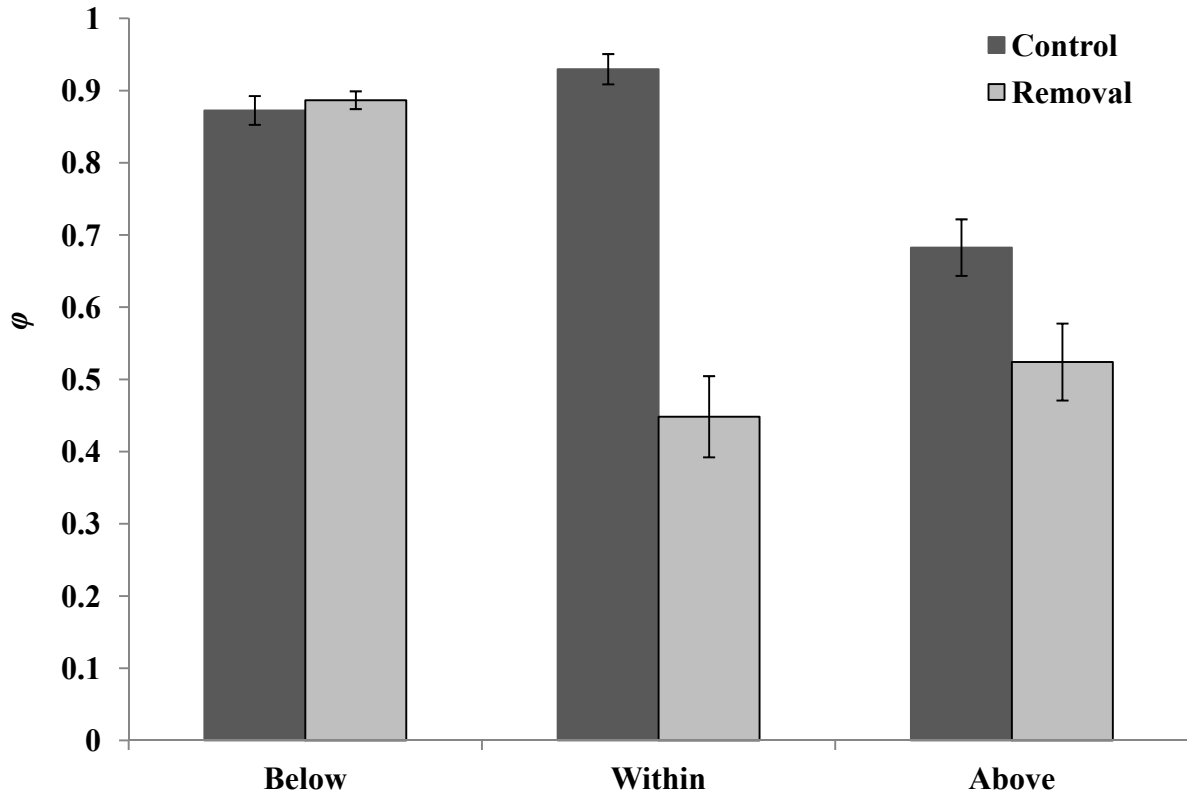


Figure A2.9. Model-averaged apparent survival probabilities (φ ; SE bars) for brown trout below, within, and above the control and removal reaches during the primary study period.

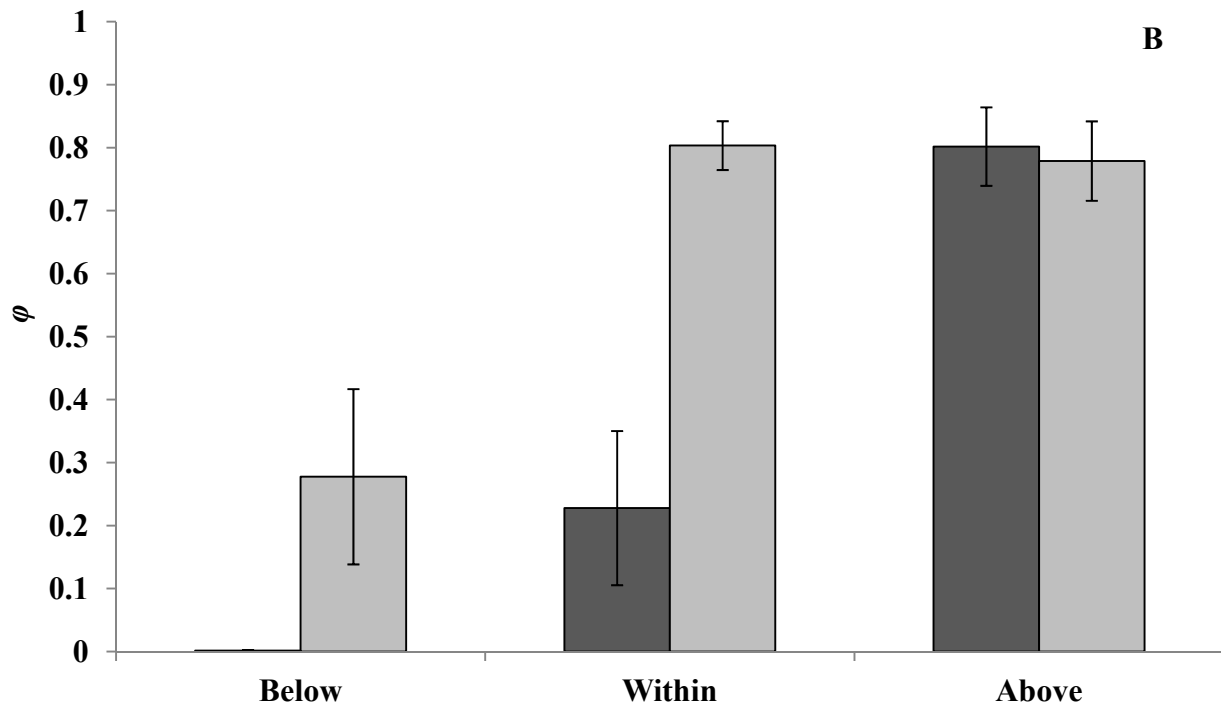
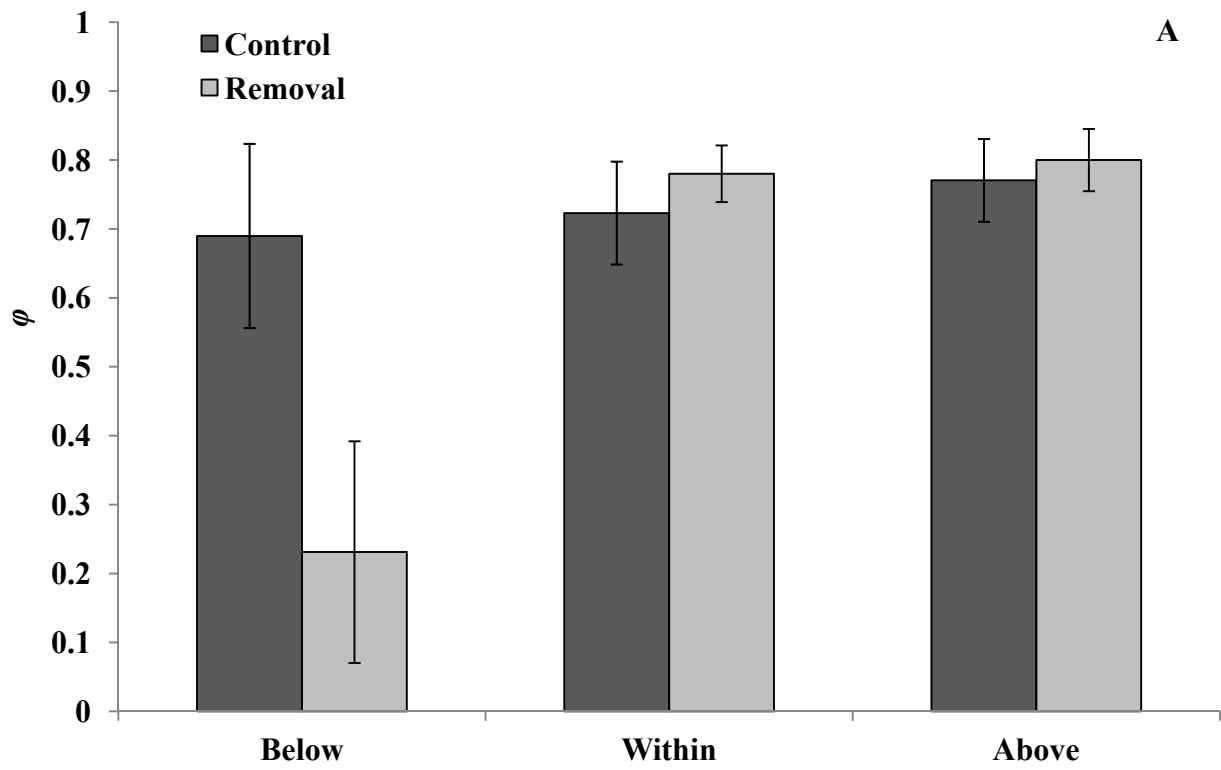


Figure A2.10. Model-averaged apparent winter weekly survival probabilities (ϕ ; SE bars) for H×C (A) and H×H (B) fish below, within, and above the control and removal reaches.

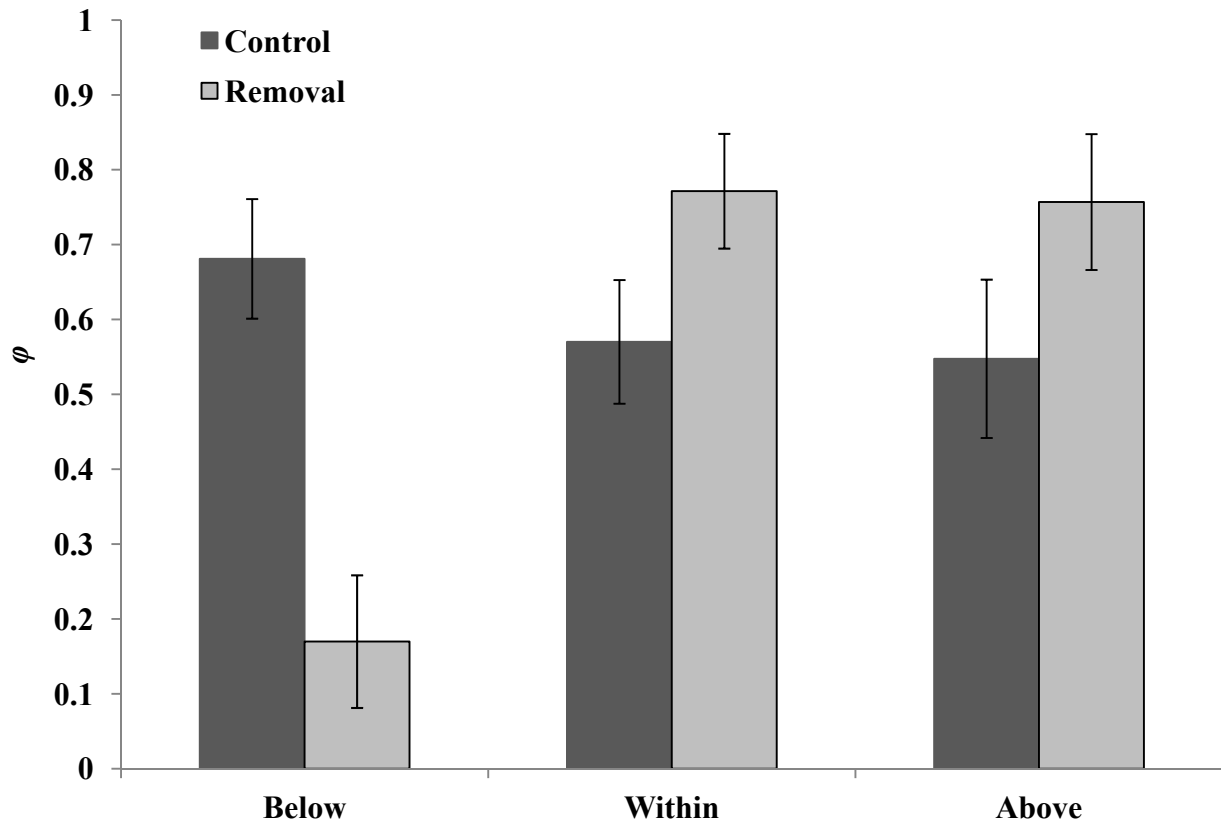


Figure A2.11. Model-averaged apparent survival probabilities (ϕ ; SE bars) for brown trout below, within, and above the control and removal reaches during the winter study period.

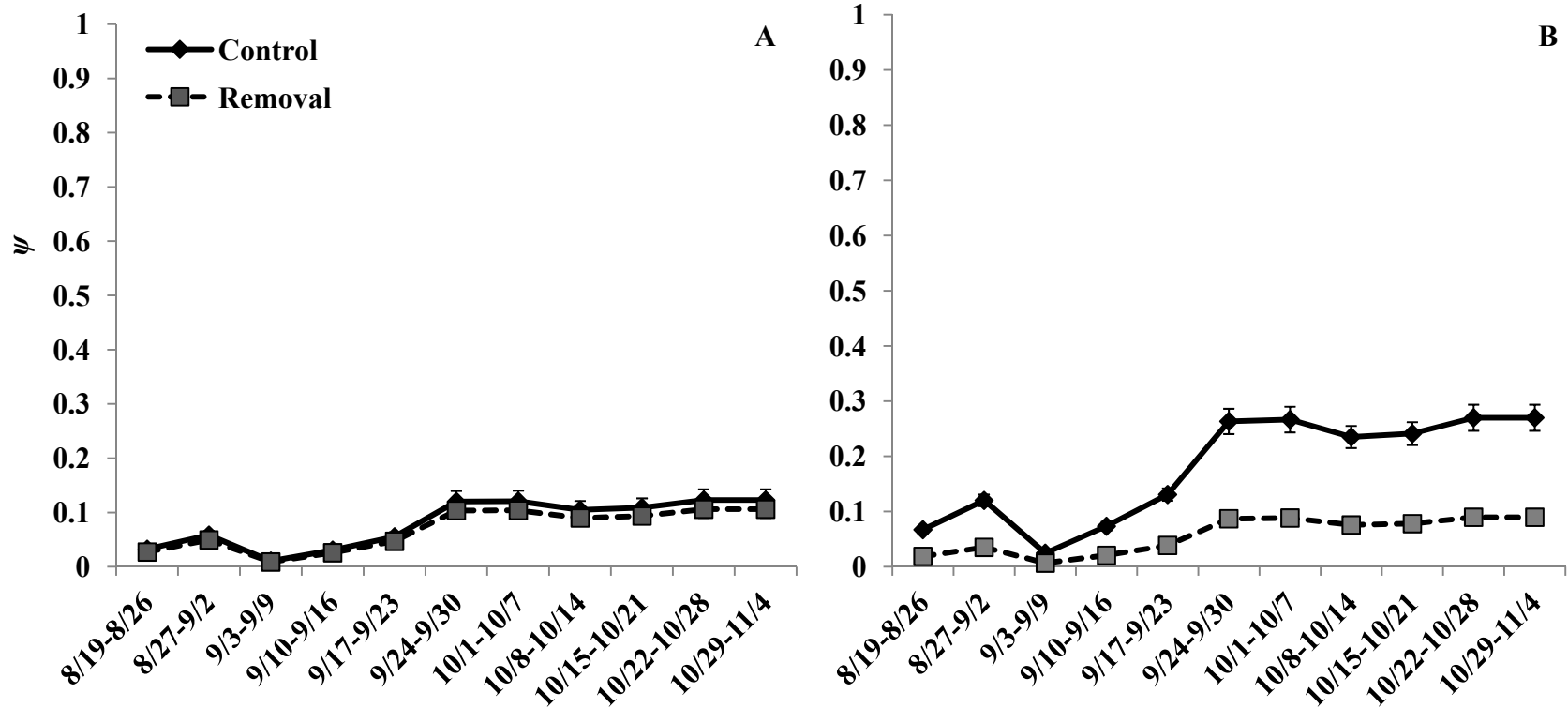


Figure A2.12. H×C (A) and H×H (B) initial movement probabilities (ψ ; SE bars), the sum of movements downstream and upstream out of the control (C→A and C→D, respectively) and removal (R→F and R→H, respectively) reaches during the primary study period.

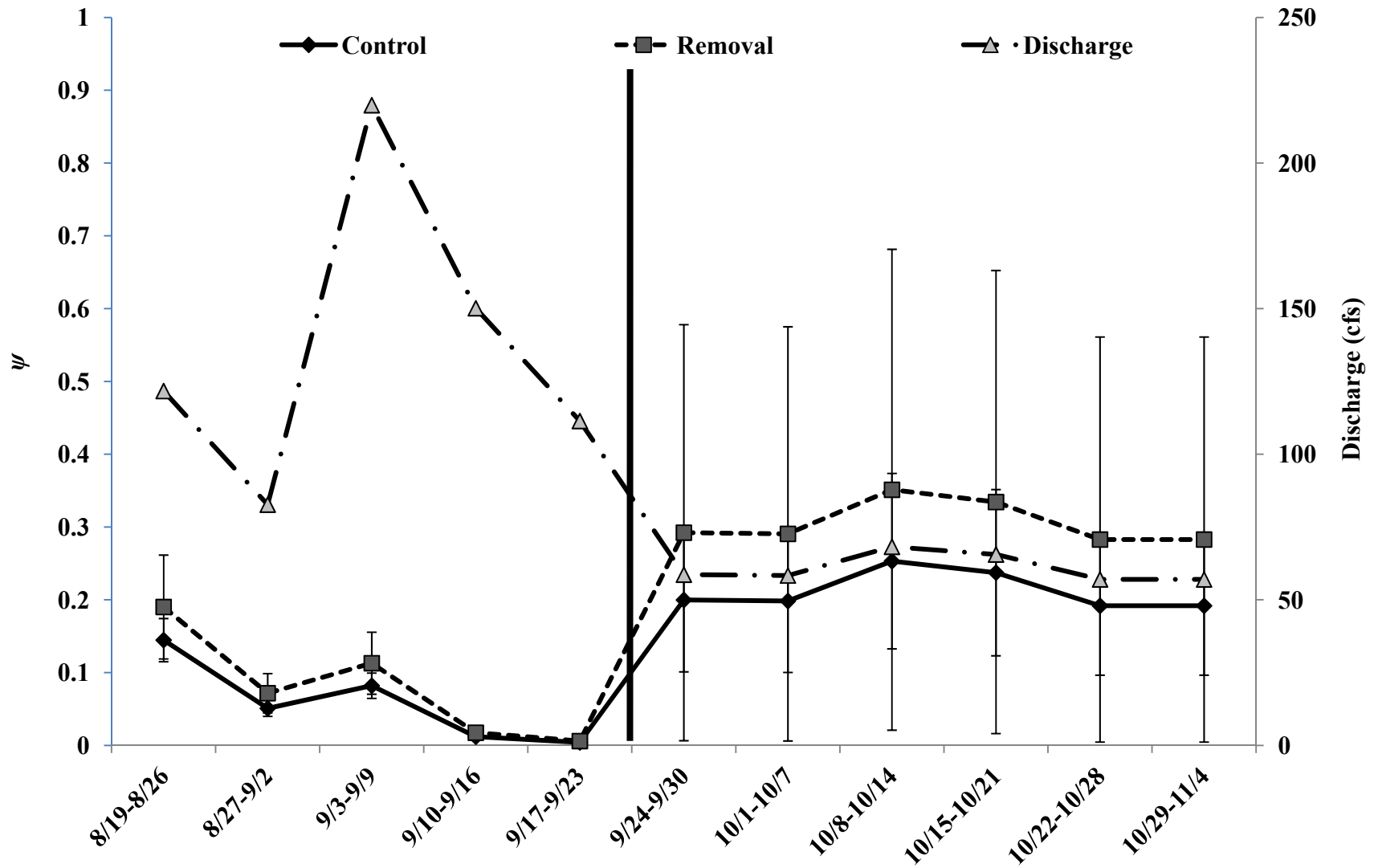


Figure A2.13. Brown trout net initial movement probabilities (ψ ; SE bars) into the control and removal reaches (difference in the sum of movement into and out of the reaches) during the primary study period. Discharge and spawn (solid black line; indicates transition from pre-spawn to spawning period) had a large effect on movement probabilities within the primary study period.

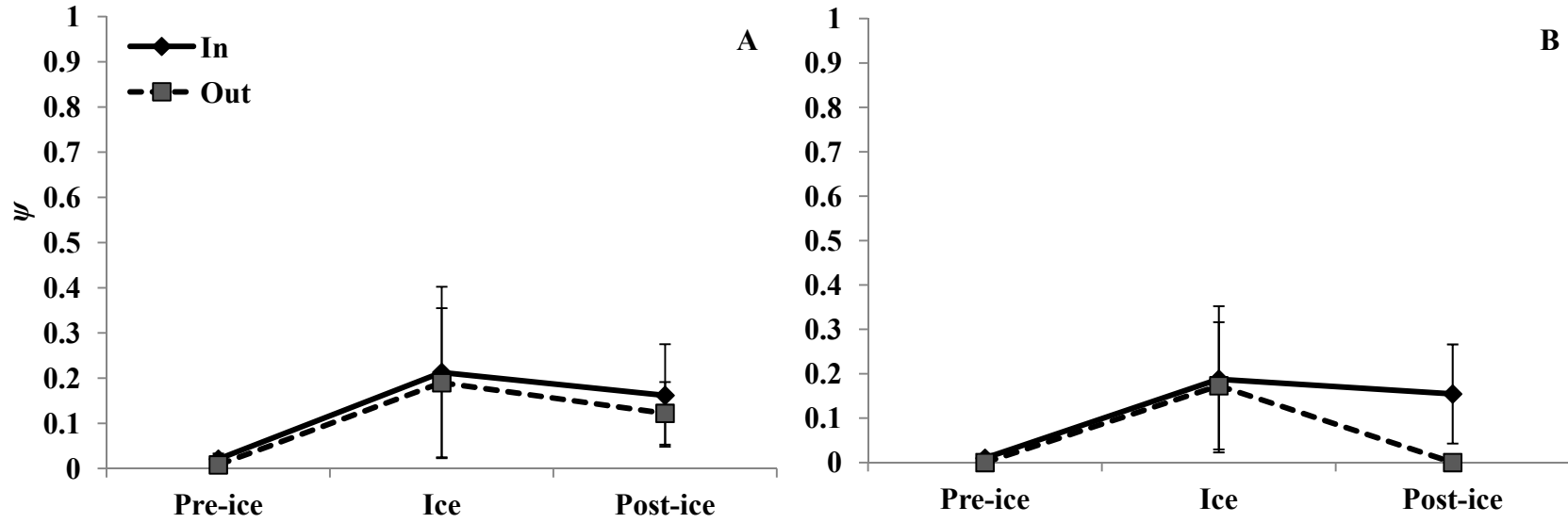


Figure A2.14. Brown trout initial pre-ice (11/5-12/16), ice (12/17-3/17), and post-ice (3/18-4/14) movement probabilities (ψ ; SE bars) into and out of the control (A) and removal (B) reaches during the winter study period.

LITERATURE CITED

- Acolas, M. L., J.-M. Roussel, J. M. Lebel, and J. L. Baglinière. 2007. Laboratory experiment on survival, growth and tag retention following PIT injection into the body cavity of juvenile brown trout (*Salmo trutta*). *Fisheries Research* 86:280-284.
- Alexander, G.R. 1977. Consumption of small trout by large predatory brown trout in the north branch of the Au Sable River, Michigan. Michigan Department of Natural Resources, Fisheries Research Report Number 1855.
- Allen, M. B., and E. P. Bergersen. 2002. Factors influencing the distribution of *Myxobolus cerebralis*, the causative agent of whirling disease, in the Cache la Poudre River, Colorado. *Diseases of Aquatic Organisms* 49:51-60.
- Anderson, D. R. 2008. Model based inference in the life sciences: A primer on evidence. Springer, LLC, New York, New York.
- Armstrong, J. D., and N. A. Herbert. 1997. Homing movements of displaced stream-dwelling brown trout. *Journal of Fish Biology* 50:445-449.
- Axel, G. A., E. F. Prentice, and B. P. Sandford. 2005. PIT-tag detection system for larger-diameter juvenile fish bypass pipes at Columbia River basin hydroelectric dams. *North American Journal of Fisheries Management* 25:646-651.
- Aymes, J. C., and J. Rives. 2009. Detection efficiency of multiplexed passive integrated transponder antennas is influenced by environmental conditions and fish swimming behavior. *Ecology of Freshwater Fish* 18:507-513.
- Baldwin, T. J., J. E. Peterson, G. C. McGhee, K. D. Staigmilller, E. S. Motteram, C. C. Downs, and D. R. Stanek. 1998. Distribution of *Myxobolus cerebralis* in salmonid fishes in Montana. *Journal of Aquatic Animal Health* 10:361-371.
- Bettinger, J. M., and P. W. Bettoli. 2004. Seasonal movement of brown trout in the Clinch River, Tennessee. *North American Journal of Fisheries Management* 24:1480-1485.
- Bond, M. H., C. V. Hanson, R. Baertsch, S. A. Hayes, and R. B. MacFarlane. 2007. A new low cost instream antenna system for tracking passive integrated transponder (PIT)-tagged fish in small streams. *Transactions of the American Fisheries Society* 136:562-566.
- Brooks, J. E., M. J. Buntjer, and J. R. Smith. 2000. Non-native species interactions: Management implications to aid in recovery of the Colorado pikeminnow *Ptychocheilus lucius* and razorback sucker *Xyrauchen texanus* in the San Juan River, CO-NM-UT. San Juan River Basin Recovery Implementation Program, U.S. Fish and Wildlife Service, Albuquerque, New Mexico.

- Brownie, C., J. E. Hines, J. D. Nichols, K. H. Pollock, and J. B. Hestbeck. 1993. Capture recapture studies for multiple strata including non-Markovian transitions. *Biometrics* 49:1173-1187.
- Buchanan, R. A., and J. R. Skalski. 2010. Using multistate mark-recapture methods to model adult salmonid migration in an industrialized river. *Ecological Modeling* 221:582-589.
- Burnham, K. P., and D. R. Anderson. 2002. *Model selection and multimodel inference: A practical information-theoretic approach*. Second edition. Springer-Verlag, New York, New York.
- Burrell, K. H., J. J. Isely, D. B. Bunneell, D. H. Van Lear, and C. A. Dolloff. 2000. Seasonal movement of brown trout in a southern Appalachian river. *Transactions of the American Fisheries Society* 129:1373-1379.
- Compton, R. I., W. A. Hubert, F. J. Rahel, M. C. Quist, and M. R. Bower. 2008. Influences of fragmentation on three species of native warmwater fish in a Colorado River Basin headwater stream system, Wyoming. *North American Journal of Fisheries Management* 28: 1733-1743.
- Connolly, P. J., I. G. Jezorek, K. D. Martens, and E. F. Prentice. 2008. Measuring the performance of two stationary interrogation systems for detecting downstream and upstream movement of PIT-tagged salmonids. *North American Journal of Fisheries Management* 28:402-417.
- Elliott, J. M., and M. A. Hurley. 2000. Daily energy intake and growth of piscivorous brown trout, *Salmo trutta*. *Freshwater Biology* 44:237-245.
- Elton, C. S. 1958. *The ecology of invasive plants and animals*. Methuen, London.
- Fausch, K. D., and R. J. White. 1981. Competition between brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) for positions in a Michigan stream. *Canadian Journal of Fisheries and Aquatic Sciences* 38:1220-1227.
- Fetherman, E. R. 2009. Heritability of myxospore count and the effects of *Myxobolus cerebralis* exposure on the physiological performance of whirling disease resistant and susceptible strains of rainbow trout. M.S. thesis, Department of Fish, Wildlife and Conservation Biology, Colorado State University, Fort Collins, CO.
- Fetherman, E. R., and G. J. Schisler. 2012. Sport Fish Research Studies. Federal Aid in Fish and Wildlife Restoration, Job Progress Report. Colorado Parks and Wildlife, Aquatic Wildlife Research Section. Fort Collins, Colorado.

- Fetherman, E. R., D. L. Winkelman, G. J. Schisler, and C. A. Myrick. 2011. The effects of *Myxobolus cerebralis* on the physiological performance of whirling disease resistant and susceptible strains of rainbow trout. *Journal of Aquatic Animal Health* 23:169-177.
- Fetherman, E. R., D. L. Winkelman, G. J. Schisler, and M. F. Antolin. 2012. Genetic basis of differences in myxospore count between whirling disease-resistant and –susceptible strains of rainbow trout. *Diseases of Aquatic Organisms* 102:97-106.
- Frank, B. M., O. Gimenez, and P. V. Baret. 2012. Assessing brown trout (*Salmo trutta*) spawning movements with multistate capture-recapture models: a case study in a fully controlled Belgian brook. *Canadian Journal of Fisheries and Aquatic Sciences* 69:1091-1104.
- Fraser, D. J. 2008. How well can captive breeding programs conserve biodiversity? A review of salmonids. *Evolutionary Applications* 1:535-586.
- Gatz, A. J., M. J. Sale, and J. M. Loar. 1987. Habitat shifts in rainbow trout: competitive influences of brown trout. *Oecologia (Berlin)* 74:7-19.
- Godin, J.-G. J., and C. D. Sproul. 1988. Risk taking in parasitized sticklebacks under threat of predation: effects of energetic need and food availability. *Canadian Journal of Zoology* 66:2360-2367.
- Granath, W. O., Jr., M. A. Gilbert, E. J. Wyatt-Pescador, and E. R. Vincent. 2007. Epizootiology of *Myxobolus cerebralis*, the causative agent of salmonid whirling disease in the Rock Creek drainage of west-central Montana. *Journal of Parasitology* 93(1):104-119.
- Gries, G., and B. H. Letcher. 2002. Tag retention and survival of age-0 Atlantic salmon following surgical implantation with passive integrated transponder tags. *North American Journal of Fisheries Management* 22:219-222.
- Halvorsen, M., and O. B. Stabell. 1990. Homing behaviour of displaced stream-dwelling brown trout. *Animal Behavior* 39:1089-1097.
- Hedrick, R. P., and M. El-Matbouli. 2002. Review: Recent advances with taxonomy, life cycle , and development of *Myxobolus cerebralis* in the fish and oligochaete hosts. Pages 45-54 in Bartholomew, J. L., and J. C. Wilson, editors. *Whirling disease: Reviews and current topics*. American Fisheries Society Symposium 29, Bethesda, Maryland.
- Hedrick, R. P., T. S. McDowell, G. D. Marty, G. T. Fosgate, K. Mukkatira, K. Myklebust, and M. El-Matbouli. 2003. Susceptibility of two strains of rainbow trout (one with suspected resistance to whirling disease) to *Myxobolus cerebralis* infection. *Disease of Aquatic Organisms* 55:37-44.

- Hense, Z., R. W. Martin, and J. T. Petty. 2010. Electrofishing capture efficiencies for common stream fish species to support watershed-scale studies in the central Appalachians. *North American Journal of Fisheries Management* 30:1041-1050.
- Hestbeck, J. B. 1995. Bias in transition-specific survival and movement probabilities estimated using capture-recapture data. *Journal of Applied Statistics* 22:737-750.
- Hestbeck, J. B., J. D. Nichols, and R. A. Malecki. 1991. Estimates of movement and site fidelity using mark resight data of wintering Canada geese. *Ecology* 72:523-533.
- Hoffmann, G. L. 1970. Intercontinental and transcontinental dissemination and transfaunation of fish parasites with emphasis on whirling disease (*Myxosoma cerebralis*) and its effects on fish. Symposium on Diseases of fisheries and Shellfishes. Edited by S. F. Snieszko. American Fisheries Society, Special Publication Number 5:69-81.
- Horton, G. E., B. H. Letcher, and W. L. Kendall. 2011. A multistate capture-recapture modeling strategy to separate true survival from permanent emigration for a passive integrated transponder tagged population of stream fish. *Transactions of the American Fisheries Society* 140(2):320-333.
- Huggins, R. M. 1989. On the statistical analysis of capture experiments. *Biometrika* 76:133-140.
- James, D. A., J. W. Erickson, and B. A. Barton. 2007. Brown trout seasonal movement patterns and habitat use in an urbanized South Dakota stream. *North American Journal of Fisheries Management* 27:978-985.
- Jensen, H., P. -A. Amundsen, J. M. Elliott, T. Bøhn, and P. E. Aspholm. 2006. Prey consumption rates and growth of piscivorous brown trout in a subarctic watercourse. *Journal of Fish Biology* 68:838-848.
- Jolley, J. C., D. W. Willis, T. J. DeBates, and D. D. Graham. 2008. The effects of mechanically reducing northern pike density on the sport fish community of West Long Lake, Nebraska, USA. *Fisheries Management and Ecology* 15:251-258.
- Jonsson, N., T. F. Næsje, B. Jonsson, R. Saksgård, and O. T. Sandlund. 1999. The influence of piscivory on life history traits of brown trout. *Journal of Fish Biology* 55:1129-1141.
- Klein, W. D. 1963. Quality fishing on the Poudre River. Colorado Department of Game, Fish and Parks. Fisheries Research Division Report Number 1.
- Lebreton, J. D., and R. Pradel. 2002. Multistate recapture models: Modeling incomplete individual histories. *Journal of Applied statistics* 29:353-369.
- Letcher, B. H., and G. E. Horton. 2008. Seasonal variation in size-dependent survival of juvenile Atlantic salmon (*Salmo salar*): performance of multistate capture-mark-recapture models. *Canadian Journal of Fisheries and Aquatic Sciences* 65:1649-1666.

- McAda, C. W. 1997. Mechanical removal of northern pike from the Gunnison River, 1995-1996. Colorado River Recovery Implementation Program, project 58. U.S. Fish and Wildlife Service, Grand Junction, Colorado.
- Mesa, M. G., and J. J. Warren. 1997. Predator avoidance ability of juvenile Chinook salmon (*Oncorhynchus tshawytscha*) subjected to sublethal exposures of gas supersaturated water. Canadian Journal of Fisheries and Aquatic Sciences 54:757-764.
- Milinski, M. 1985. Risk of predation of parasitized sticklebacks (*Gasterosteus aculeatus* L.) under competition for food. Behavior 93:203-216.
- Miller, M. P., and E. R. Vincent. 2008. Rapid natural selection for resistance to an introduced parasite of rainbow trout. Evolutionary Applications 1:336-341.
- Modde, T. and M. Fuller. 2002. Feasibility of channel catfish reduction in the lower Yampa River. Upper Colorado River Basin Recovery Implementation Program Project 88. Colorado River Fish Project, Vernal, Utah.
- Moyle, P. B., and T. Light. 1996. Biological invasions of fresh water: empirical rules and assembly theory. Biological Conservation 78:149-161.
- Mueller, G. A. 2005. Predatory fish removal and native fish recovery in the Colorado River mainstem: what have we learned? Fisheries 30(9):10-19.
- Nehring, R. B. 2006. Colorado's cold water fisheries: whirling disease case histories and insights for risk management. Colorado Division of Wildlife. Aquatic Wildlife Research Special Report Number 79. February 2006.
- Nehring, R. B., and K. G. Thompson. 2001. Impact assessment of some physical and biological factors in the whirling disease epizootic among wild trout in Colorado. Colorado Division of Wildlife. Aquatic Research Special Report Number 76. March 2001.
- Nehring, R. B., and K. G. Thompson. 2003. Whirling disease risk assessment: the Colorado perspective. Pages 31-32 in 9th Annual Whirling Disease Symposium: Managing the Risk. February 6-7, Seattle, Washington.
- Nunnallee, E. P., E. F. Prentice, B. F. Jonasson, and W. Patten. 1998. Evaluation of a flat-plate PIT tag interrogation system at Bonneville Dam. Aquaculture Engineering 17:261-272.
- O'Donnell, M. J., G. E. Horton, and B. H. Letcher. 2010. Use of portable antennas to estimate abundance of PIT-tagged fish in small streams: Factors affecting detection probability. North American Journal of Fisheries Management 30(2):323-336.

- Piper, R. G., I. B. McElwain, L. E. Orme, J. P. McCraren, L. G. Fowler, and J. R. Leonard. Fish Hatchery Management. U. S. Department of the Interior, Fish and Wildlife Service, Washington, D. C., 1982.
- Poulin, R. 1993. Age-dependent effects of parasites on anti-predator responses in two New Zealand freshwater fish. *Oecologia* 96:431-438.
- Prentice, E. F., T. A. Flagg, C. S. McCutcheon, D. F. Brastow, and D. C. Cross. 1990. Equipment, methods, and an automated data-entry station for PIT tagging. Fish Marking Techniques. Edited by N. C. Parker, A. E. Giorgi, R. C. Heidinger, D. B. Jester, Jr., E. D. Prince, and G. A. Winans. American Fisheries Society, Symposium 7, Bethesda, Maryland, pgs. 335-340.
- Roussel, J.-M., A. Haro, and R. A. Cunjak. 2000. Field test of a new method for tracking small fishes in shallow rivers using passive integrated transponder (PIT) technology. *Canadian Journal of Fisheries and Aquatic Sciences* 57:1326-1329.
- Ryce, E. K. N., A. V. Zale, and R. B. Nehring. 2001. Lack of selection for resistance to whirling disease among progeny of Colorado River rainbow trout. *Journal of Aquatic Animal Health* 12:63-68.
- SAS Institute, Inc. 2010. SAS system software, Release 9.2. Cary, North Carolina.
- Saunders, W. C., K. D. Fausch, and G. C. White. 2011. Accurate estimation of salmonid abundance in small streams using nighttime removal electrofishing: an evaluation using marked fish. *North American Journal of Fisheries Management* 31:403-415.
- Schisler, G. J. 2001. Whirling Disease Investigations. Federal Aid in Fish and Wildlife Restoration Job Progress Report. Colorado Division of Wildlife, Fish Research Section. Fort Collins, Colorado.
- Schisler, G. J. 2006. Salmonid Disease Studies. Federal Aid in Fish and Wildlife Restoration Job Progress Report. Colorado Division of Wildlife, Fish Research Section. Fort Collins, Colorado.
- Schisler, G. J., K. A. Myklebust, and R. P. Hedrick. 2006. Inheritance of *Myxobolus cerebralis* resistance among F1-generation crosses of whirling disease resistant and susceptible rainbow trout strains. *Journal of Aquatic Animal Health* 18:109–115.
- Schisler, G. J., P. J. Schler, and E. R. Fetherman. 2007. Salmonid Disease Studies. Federal Aid in Fish and Wildlife Restoration, Job Progress Report. Colorado Division of Wildlife, Fish Research Section. Fort Collins, Colorado.

- Schisler, G. J., E. R. Fetherman, and P. J. Schler. 2008. Salmonid Disease Studies. Federal Aid in Fish and Wildlife Restoration, Job Progress Report. Colorado Division of Wildlife, Fish Research section. Fort Collins, Colorado.
- Seppälä, O., A. Karvonen and E. T. Valtonen. 2004. Parasite-induced change in host behavior and susceptibility to predation in an eye fluke-fish interaction. *Animal Behavior* 68(2):257-263.
- Shetter, D. S., and G. R. Alexander. 1970. Results of predator reduction on brook trout and brown trout in 4.2 miles (6.76 km) of the north branch of the Au Sable River. *Transactions of the American Fisheries Society* 99:312-319.
- Sipher, C. R., and E. P. Bergersen. 2005. The effects of whirling disease on growth and survival of Snake River cutthroat and Colorado River rainbow trout fingerlings. *Journal of Aquatic Animal Health* 17(4):353-364.
- Solomon, D. J., and R. E. Templeton. 1976. Movements of brown trout *Salmo trutta* L. in a chalk stream. *Journal of Fish Biology* 9:411-423.
- Townsend, C. R. 1996. Invasion biology and ecological impacts of brown trout *Salmo trutta* in New Zealand. *Biological Conservation* 78:13-22.
- USGS. 2009. USGS Real Time Water Data for the Nation. Retrieved July 17, 2009, from <<http://nwis.waterdata.usgs.gov/co/nwis/uv/?>>.
- Vermeij, G. J. 1996. An agenda for invasion biology. *Biological Conservation* 78:3-9.
- Vincent, E. R. 2002. Relative susceptibility of various salmonids to whirling disease with emphasis on rainbow and cutthroat trout. *American Fisheries Society Symposium* 29:109-115.
- Wagner, E. J., C. Wilson, R. Arndt, P. Goddard, M. Miller, A. Hodgson, R. Vincent, and K. Mock. 2006. Evaluation of disease resistance of the fish Lake-DeSmet, Wounded Man, and Harrison Lake strains of rainbow trout exposed to *Myxobolus cerebralis*. *Journal of Aquatic Animal Health* 18:128-135.
- 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.
- Walker, P. G., and R. B. Nehring. 1995. An investigation to determine the cause(s) of the disappearance of young wild rainbow trout in the Upper Colorado River, in Middle Park, Colorado. Colorado Division of Wildlife Report. Denver.

- Wang, L., and R. J. White. 1994. Competition between wild brown trout and hatchery Greenback cutthroat trout of largely wild parentage. *North American Journal of Fisheries Management* 14:475-487.
- Weidel, B. C., D. C. Josephson, and C. E. Kraft. 2007. Littoral fish community response to smallmouth bass removal from and Adirondack lake. *Transactions of the American Fisheries Society* 136:778-789.
- White, G. C., and K. P. Burnham. 1999. Program MARK: Survival estimation from populations of marked animals. *Bird Study* 46 Supplement, 120-138.
- White, G. C., D. R. Anderson, K. P. Burnham, and D. L. Otis. 1982. Capture-recapture and removal methods for sampling closed populations. Los Alamos National Laboratory, LA-8787-NERP, Los Alamos, New Mexico.
- Zale, A. V., C. Brooke, and W. C. Fraser. 2005. Effects of surgically implanted transmitter weights on growth and swimming stamina of small adult west slope cutthroat trout. *Transactions of the American Fisheries Society* 136:778-789.
- Zydlewski, G. B., A. Haro, K. G. Whalen, and S. D. McCormick. 2001. Performance of stationary and portable passive transponder detection systems for monitoring of fish movements. *Journal of Fish Biology* 58:1471-1475.
- Zydlewski, G. B., G. Horton, T. Dubreuil, B. Letcher, S. Casey, and J. Zydelewski. 2006. Remote monitoring of fish in small streams: a unified approach using PIT tags. *Fisheries* 31(10):492-502.