Salmonid Disease Studies

Federal Aid Project F-394R-10

George J. Schisler
Principal Investigator

and

Eric R. Fetherman
Brad Neuschwanger

Rick Cables, Director

Federal Aid in Fish and Wildlife Restoration

FINAL Progress Report

Colorado Division of Wildlife

Aquatic Wildlife Research Section

Fort Collins, Colorado

July 2011

STATE OF COLORADO

John W. Hickenlooper, Governor

COLORADO DEPARTMENT OF NATURAL RESOURCES

Mike King, Executive Director

COLORADO DIVISION OF WILDLIFE

Rick Cables, Director

WILDLIFE COMMISSION

Tim Glenn, Chair
Robert G. Streeter, Ph.D., Vice Chair
Kenneth M. Smith, Secretary
David R. Brougham
Dennis G. Buechler
Dorothea Farris
Allan Jones
John W. Singletary
Dean Wingfield

Ex Officio/Non-Voting Members:
Mike King, Department of Natural Resources
John Stulp, Department of Agriculture

AQUATIC RESEARCH STAFF

George J. Schisler, General Professional VI, Aquatic Wildlife Research Leader
Rosemary Black, Program Assistant I
Stephen Brinkman, General Professional IV, Water Pollution Studies
Eric R. Fetherman, General Professions IV, Salmonid Disease Investigations
Ryan Fitzpatrick, General Professional IV, Eastern Plains Native Fishes
Matt Kondratieff, General Professional IV, Stream Habitat Restoration
Jesse M. Lepak, General Professional V, Coldwater Lakes and Reservoirs
R. Barry Nehring, General Professional V, Stream Fisheries Investigations
Brad Neuschwanger, Hatchery Technician IV, Research Hatchery
Kyle Okeson, Technician III, Fish Research Hatchery
Christopher Praamsma, Technician III, Fish Research Hatchery
Kevin B. Rogers, General Professional IV, Colorado Cutthroat Studies
Kevin G. Thompson, General Professional IV, GOCO - Boreal Toad Studies
Harry Vermillion, General Professional III, F-239, Aquatic Data Analysis
Nicole Vieira, Physical Scientist III, Toxicologist

Paula Nichols, Federal Aid Coordinator
Kay Knudsen, Librarian

Prepared by: _____

George J. Schisler, Aquatic Wildlife Research Leader

_____

Eric R. Fetherman, General Professional IV, Aquatic Wildlife Researcher

_____

Brad Neuschwanger, Research Hatchery Technician IV

Date: 7/15/2011

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

Table of Contents

LIST OF TABLES

LIST OF FIGURES

State: Colorado **Project No.** F-394-R10

Project Title: Salmonid Disease Studies/ Whirling Disease-Resistant Rainbow Trout Studies

Period Covered: July 1, 2006 - June 30, 2011

Project Objective: Development of rainbow trout brood stocks resistant to *M. cerebralis* for both hatchery and wild fish management applications.

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 stocks.

Hatchery Production

The whirling disease resistant rainbow trout brood stocks reared at the Fish Research Hatchery, Bellvue, CO (FRH) 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 some unique stocks are individually marked with Passive Integrated Transponder (PIT) 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 2006, FRH personnel checked all of the Hofer[1] (GR) and Harrison Lake brood fish (2, 3, 4 and 5 year-olds) weekly for ripeness. Maturation is indicated by eggs or milt flowing freely with slight pressure applied to the abdomen of the fish. The first females usually maturate two to four weeks after the first group of males. As males are identified, they are moved into a separate section of the raceway to reduce handling and fighting injuries. On November 16, 2006 the fish from 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 or PIT tag). This procedure was repeated each time ripe females were spawned throughout the winter.

The wet spawning method was used, where eggs from the female are stripped into a bowl along with the ovarian fluid. After collecting the eggs, milt from several males is added to the bowl. Water is poured into the bowl to activate the milt. The bowl of eggs and milt is then covered and not disturbed for several minutes while the fertilization

[1] Hofer is used interchangeably with GR throughout this document to describe the resistant strain of rainbow trout obtained in 2003 from facilities in Germany.

process takes place. The eggs are then rinsed with fresh water to expel old sperm, feces, egg shells and dead eggs. The eggs are then poured into an insulated cooler to water-harden for approximately one hour.

The water-hardened fertilized (green eggs) from all the different crosses of the GR and Harrison Lake strains were moved to the FRH main hatchery building. Extreme caution was used to keep each individual cross totally separate from all others. Upon reaching the hatchery the green eggs are tempered and then disinfected (PVP Iodine, Western Chemical Inc., Ferndale, Washington, at 100 ppm for 10 minutes at a pH of 7). Eggs were then put into vertical incubators (Heath Tray, Mari Source, Tacoma, Washington) with 5 gpm of 12.2º C (54º F) of flow-through water. The total number of eggs was calculated using number of eggs per ounce (Von Bayer trough count minus 10%) times total ounces of eggs. Separate 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 minutes) every other day until eye-up.

On the 14th day in the incubator at 12.2º C (54º F), the eggs reach the eyed stage of development. The eyed eggs are removed from the trays and physically shocked to detect dead eggs, which turn white when disturbed. Dead eggs were removed (both by hand and with a Van Gaalen fish egg sorter, VMG Industries, Grand Junction, Colorado) on the 15th day. The total number of good eyed eggs was calculated using the number of eggs per ounce times total ounces. On the 16th day the eyed eggs were shipped via insulated coolers to other state agency hatcheries. The whole process was repeated throughout the spawning season with separate crosses of GR and Harrison Lake rainbow trout.

The GR and Harrison Lake rainbow trout production on-site spawn started on November 16, 2006 with ripe GR females. The last group of Harrison Lake females was spawned on February 1, 2007. With a goal in the fall to produce @ 200,000 eyed eggs, the egg take far exceeded the production needs with over 442,500 eyed eggs produced (Table 1.1). With the availability of both ripe males and females of several year classes and combinations of previous years crosses (F1 and B2) of GR and Harrison Lake strains, FRH personnel produced over 20 different lots during the spawn take. Surprisingly the overall egg quality remained quite good with 1st egg pick-off of only 26%. FRH personnel were able to fill all GR egg requests for Colorado, California, and Utah for both production and research directed projects in 2006-2007.

Table 1.1. Fish Research Hatchery on-site spawning information for GR and Harrison Lake rainbow trout strains during the winter 2006-2007 spawning season.

STRAIN (CROSSES)	DATE SPAWNED	# OF SPAWNED FEMALES	# OF GREEN EGGS	# OF EYED EGGS	SHIPPED TO
100% GR	11/29/06-1/11/07	101	299,250	212,400	CO and CA State Hatcheries/Research
75% GR 25% Harrison Lake	11/16/06-1/30/07	92	266,600	202,300	CO and UT State Hatcheries/Research
50% GR 50% Harrison Lake	1/04/07-1/19/07	15	21,350	16,350	CO Hatcheries
100% Harrison Lake	1/04/07-2/01/07	12	15,300	11,800	CO Hatcheries
Total	11/16/06-2/01/07	220	602,500	442,850	

Table 1.2. Fish Research Hatchery on-site spawning information for GR and Harrison Lake rainbow trout strains during the winter 2007-2008 spawning season.

STRAIN (CROSSES)	DATE SPAWNED	# OF SPAWNED FEMALES	# OF GREEN EGGS	# OF EYED EGGS	SHIPPED TO
100% GR	11/16/07-12/14/07	32	98,600	78,400	CO Hatcheries/ Research
GR x Harrison Lake	10/24/07-1/24/08	316	972,800	870,100	CO, CA and UT State Hatcheries/Research
100% Harrison Lake	1/04/08-1/24/08	11	20,800	13,900	CO Hatcheries/ Research
Total	10/24/07-1/24/08	359	1,092,200	962,400	

Table 1.3. Fish Research Hatchery on-site spawning information for GR, HL, GRxHL, and GRxCRR rainbow trout strains during the winter 2008-2009 spawning season.

STRAIN (CROSSES)	DATE SPAWNED	# OF SPAWNED FEMALES	# OF GREEN EGGS	# OF EYED EGGS	SHIPPED TO
100% GR	11/28/07-12/24/08	48	122,700	121,200	CO Hatcheries/ Research
100% Harrison Lake	12/24/08	5	12,100	10,600	CO Hatcheries/ Research
GRxHL	11/20/08-1/10/09	263	466,200	359,700	CO, CA State Hatcheries/Research
GRxHL*	12/6/08	47	141,000		CO, NV State Hatcheries
GRxCRR*	12/5/08	54	194,500		CO State Hatcheries
GRxCRR	11/13/08-1/9/09	141	389,200	362,200	CO State/USFWS Hatcheries/Research
Total	11/13/08-1/10/09	558	1,325,700	853,700	86% Good Eggs to Eye-up

*Green eggs shipped to Poudre Hatchery, Poudre Canyon, CO.

Table 1.4. Fish Research Hatchery on-site spawning information for GR, HL, GRxHL, and GRxCRR rainbow trout strains during the winter 2009-2010 spawning season**.**

STRAIN (CROSSES)	DATE SPAWNED	# OF SPAWNED FEMALES	# OF GREEN EGGS	# OF EYED EGGS	SHIPPED TO
100% GR	12/24/09-29/09	35	111,000	96,800	CO Hatcheries/ Research
100% Harrison Lake	1/6/10-1/18/10	53	37,300	29,700	CO Research Hatchery
GRxHL	11/18/09-12/29/09	141	183,400	170,900	CO Hatcheries/ Research
GRxCRR*	11/18/09-12/29/09	134	393,000		CO State Hatcheries
GRxCRR	11/18/09-1/6/10	140	425,400	331,700	CO State/USFWS Hatcheries/Research
Total	11/18/09-1/18/10	503	1,150,100	629,100	83% Good Eggs to Eye-up

*Green eggs shipped to Poudre Hatchery, Poudre Canyon, CO.

Table 1.5. Fish Research Hatchery on-site spawning information for GR, HL, GRxHL, and GRxCRR rainbow trout strains during the winter 2010-2011 spawning season**.**

STRAIN (CROSSES)	DATE SPAWNED	# OF SPAWNED FEMALES	# OF GREEN EGGS	# OF EYED EGGS	SHIPPED TO
100% GR	11/17/10-23/10	102	186,846	145,231	CO Hatcheries/ Research
100% Harrison Lake	1/20/11-2/14/11	31	28,882	24,796	CO Research Hatchery/USFWS Hatcheries
GRxHL	12/04/10-12/16/10	26	68,155	49,719	CO Hatcheries/ Research
GRxCRR	11/29/10-12/13/10	56	254,412	182,850	CO Hatcheries/USFWS Hatcheries
Total	11/17/10-2/14/11	215	538,295	402,776	75% Good Eggs to Eye-up

Research Projects

Eggs produced specifically for research projects comprise a large proportion of the total production from the FRH. Specific details of those individual crosses and families created for the laboratory and field experiments are described in their respective sections of this report. The bulk of these family group descriptions appear in Job 2: Whirling Disease Resistance Laboratory Experiments and Job 3: Whirling Disease Resistant Domestic Brood Stock Development and Evaluation.

Job No. 2: Whirling Disease Resistance Laboratory Experiments

Job Objective: Evaluate the inheritability and stability of whirling disease resistance in selected strains of rainbow trout.

HOFER-CCR CROSSES

Experiment 1: Inheritance of *Myxobolus cerebralis* resistance among second generation crosses of the Hofer (GR) and Colorado River (CRR) rainbow trout strains

Introduction

The Hofer (GR) rainbow trout strain has been identified as more resistant to whirling disease than other rainbow trout strains when exposed to *Myxobolus cerebralis* in laboratory conditions (Hedrick et al. 2003). However, the survival and viability of the strain in the wild is questionable and the consequences of stocking the strain directly into wild trout waters is unknown (Schisler et al. 2006). In 2004, a study was conducted in which GR strain rainbow trout and Colorado River rainbow (CRR) strain rainbow trout were crossed. The principle aim of that project was to incorporate whirling disease resistance from the GR into the CRR strain, a strain that is typically used to establish wild rainbow trout populations in Colorado (Schisler et al. 2006). Results of exposure experiments with the GR-CRR (50:50) cross (F1 generation) showed that spore counts per fish were reduced significantly from those found in the pure CRR strain. While average infection severity in the first generation cross was much lower than the pure CRR strain, it was not reduced to the spore count levels of the pure GR strain. However, some families, created from individual male-female pairs, were more resistant than others. In addition, many individual fish from those crosses appeared to inherit a similar level of resistance as observed in the pure GR strain. A second exposure experiment was initiated to evaluate the performance of the pure GR, pure CRR, F1 generation, and a second generation GR-CRR (25:75) backcross (defined as the B2 generation) in the presence of the whirling disease parasite. This experiment would provide insight to the continued inheritability of resistance to *M. cerebralis*, particularly in F1 generation fish back-crossed with the wild CRR strain.

Methods

Spawning of all families occurred at the Colorado Division of Wildlife Fish Research Hatchery (FRH) and Colorado Cooperative Fish and Wildlife Unit (COOP) wet lab from mid-November 2005 through the end of December 2006 (Tables 2.1 and 2.2). Both male and female pure GR and F1 fish are held on site. F1 individuals had been tagged with Passive Integrated Transponder (PIT) tags to identify them by family group.

Only the lowest spore count families of the F1 variety were retained for this second generation of crosses. These fish were identified by their 10 digit alpha numeric code prior to spawning. All tagged or untagged individuals were also numbered in the order that they were spawned. Pure CRR individuals were held at the Colorado Division of Wildlife Glenwood Springs Hatchery (GWSH). Males were spawned at the GWSH and their sperm was transported in individual, numbered containers back to the FRH for fertilization of the GR and F1 eggs. In addition, live male and female CRR rainbow trout were transported back to the FRH and spawned with each other as well as GR and F1 males. An anal fin clip was taken from each spawned individual and stored in 70% ETOH for later genetic analysis. Eggs were placed in incubators at the FRH or COOP wet lab and held until they were eyed. Once eyed, eggs were placed in 76 liter (20 gallon) tanks containing short (7 cm) standpipes for a greater amount of water turnover at the COOP wet lab, where they were hatched.

Individual families (single male/female matings) were used as replicates in this experiment. Three pure GR families, three pure CRR families, 10 F1 families, and 16 B2 families were used in this evaluation. In some cases, up to 2,000 fertilized eggs are produced with each paired cross. For the purposes of this exposure experiment, fish were culled down to approximately 50 per family until immediately before exposure. At that time the families were then reduced to 30 fish each.

Fish from each group were exposed to an average of 2,000 triactinomyxons per fish as 2-month old fry. The fish were reared for five months post-exposure. Fish were fed a maintenance diet (Rangen trout feed, Rangen Inc., Buhl, Idaho) of roughly 2% body weight per day. Mortalities were removed and recorded daily. At the conclusion of the experiment, 10 fish were randomly selected from each family. The fish were measured and weighed, physical deformities were recorded, and heads were processed to enumerate myxospores per fish with the PTD (pepsin-trypsin digest) method.
Length, weight, and myxospore results were compared between strains using Proc GLM in SAS system software. If significant differences were observed, Tukey's Studentized Range (HSD) test was used to determine which strains differed from each other. Alpha was set at 0.05 for all tests.

Results

The GR rainbow trout developed the lowest spore counts of the groups tested, averaging 1,482 spores per fish (Figure 2.1). The CRR families developed the highest spore counts, averaging 232,973 spores per fish. The F1 families averaged 47,128 spores per fish. These results were similar to those found in the prior experiment. The B2 families developed higher spore counts, averaging 125,168 spores per fish. The statistical tests indicated that the CRR strain had significantly higher spore counts than the GR, F1 and B2 strains. The B2 strain had significantly higher spore counts than the GR strain, but not significantly higher than the F1 strain. The spore counts in the GR and F1 strains were not significantly different from each other. The GR, B2 and F1 strains averaged 15.3, 12.7, and 10.9 grams, respectively, at the end of the experiment. The pure

CRR strain weighed significantly less than the GR strain at 7.7 grams. The pure CRR strain grew to an average of 87.3 mm, which was significantly shorter than the pure GR, B2, and F1 strains at 113.5, 108.4, and 105.5 mm, respectively.

Discussion

In both the 2004 exposure experiment (Schisler et al. 2006) and this experiment, the F1 generation exhibited noticeable variation in spore counts and physical deformities between families. Within family variation in infection severity was relatively low. In this experiment, the B2 generation exhibited much more within family variation in infection severity (Figure 2.2). This is due to the re-assortment of genes and loss of resistance in some individual offspring of the B2 generation, but not of others. Only individuals inheriting resistance to whirling disease will be successful with regard to survival and reproductive potential in areas where the parasite has eliminated pure CRR populations. The rapid loss of resistance to *M. cerebralis* in subsequent generations of back-crosses in a hatchery setting could result in selection pressures that do not attain the goal of wild-strain fish with resistance to the parasite. Space constraints also limit the scope of this type of intensive selection in an artificial setting. An alternative to selecting families in a fish culture facility is to allow the selection among first generation crosses to occur in the wild. The selection pressure for individuals with both wild characteristics and resistance to *M. cerebralis* is immediate in locations where the parasite is endemic. Relatively good survival has been observed in first generation crosses in the wild (See Job 4, Whirling Disease Resistant Wild Strain Brood Stock Development and Evaluation). Therefore, it may be unnecessary to continue backcrossing F1 or B2 strains with pure CRR to ensure survival in the wild.

Figure 2.1. Average spore counts for the three Hofer (GR), three Colorado River rainbow (CRR), ten F1 [GR-CRR (50:50)] and 16 B2 [GR-CRR (25:75)] strains. Each point represents average spore counts for each individual family.

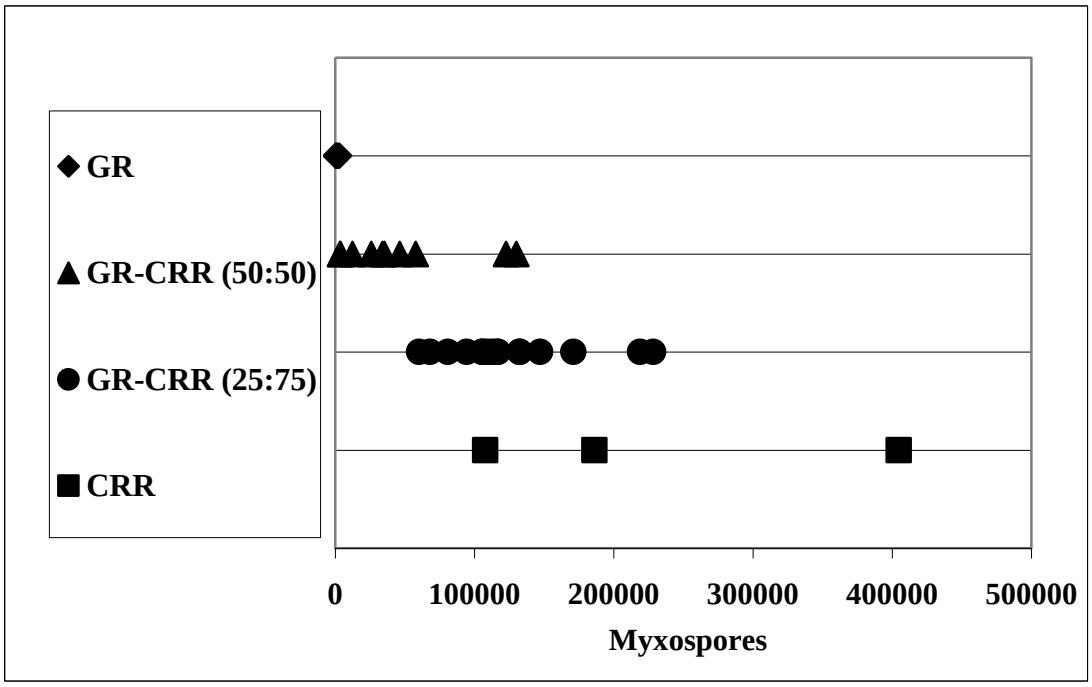
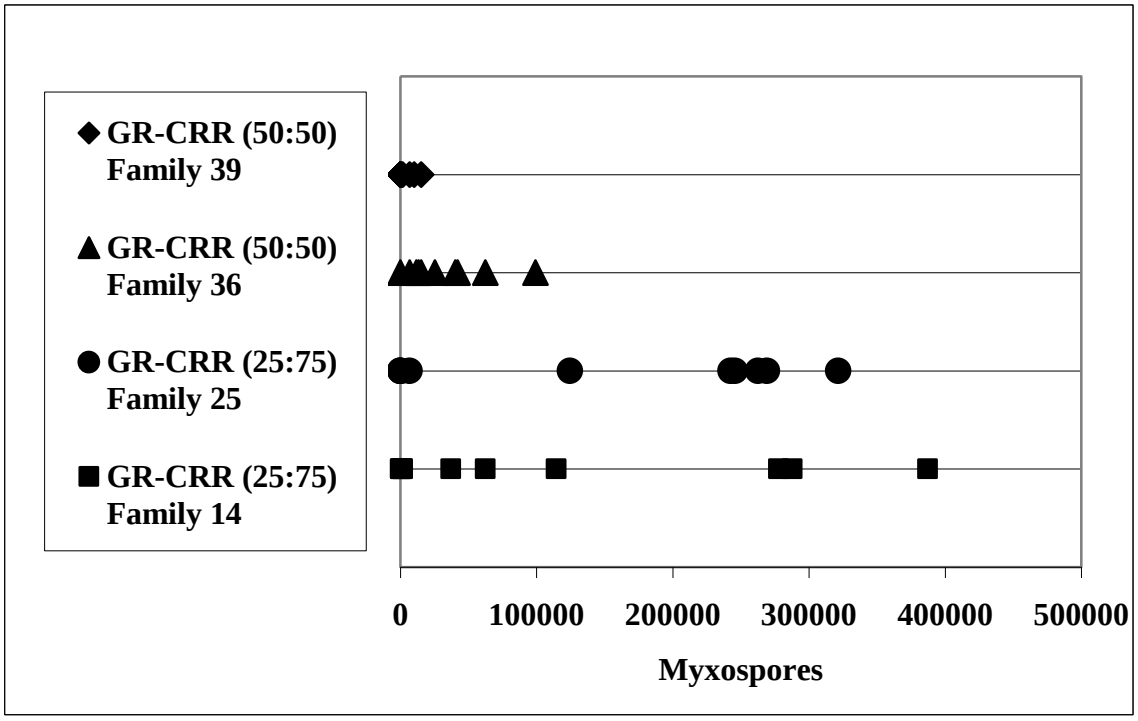


Figure 2.2. Example of inter-family variability in infection severity. Spore counts for two F1 [GR-CRR (50:50)] families and two B2 [GR-CRR (25:75)] families are shown. Ten fish per family were sampled. In this graph each point represents spore counts for each individual fish. Note that the B2 families show a large range of variation, from 0 to almost 400,000 spores, whereas the F1 families show a smaller range of variation, from 0 to only about 100,000 spores.



References

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. Diseases of Aquatic Organisms 55:37-44.

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.

Experiment 2: Physiological characteristics and inheritance of *Myxobolus cerebralis* resistance among multiple generational crosses of the Hofer (GR) and Colorado River (CRR) rainbow trout strains

Introduction

A laboratory experiment was conducted through 2007 and into 2008, at the Colorado Cooperative Fish and Wildlife Unit wet lab (or Quonset hut) in Fort Collins, Colorado to test the resistance of the German "Hofer" rainbow (GR) and Colorado River rainbow (CRR) trout strains, and crosses of these strains, to whirling disease. CRRs have historically been used for stocking in Colorado and they retain many of the desired wild rainbow trout characteristics needed to survive in Colorado's waterways. However, CRRs are highly susceptible to whirling disease and their populations have experienced dramatic declines over the past decade. The GR strain has demonstrated very strong resistance to whirling disease in past exposure experiments. However, because the GRs are a highly domesticated food fish, their survival and viability in the wild is uncertain. Also, the consequences of stocking this strain directly into the wild are unknown. In 2003, a breeding program was established to examine various crosses between the GR and CRR trout strains, with the ultimate goal of identifying those crosses that have the correct combination of resistant rainbow trout characteristics and wild rainbow trout characteristics to survive and reproduce in the wild in areas where heavy *Myxobolus cerebralis* infection exists.

The resistance of two of these crosses, F1s and B2s, has been examined in previous exposure experiments. F1s are the first filial generation cross between a pure GR individual and a pure CRR individual. Genetically, an F1 individual is heterozygous across all of their genotypes. Effectively, they are 50% GR and 50% CRR at any given locus, expressing characteristics of both, assuming a lack of dominance for either allele. B2s are the second generation backcross between an F1 individual and a pure CRR individual. Genetically, a B2 individual is effectively one-quarter GR and three-quarters CRR, with any individual genotype at a given locus having a 50% chance of being homozygous CRR, expressing only CRR characteristics, or a 50% chance of being heterozygous GR and CRR, expressing characteristics of both. These crosses have been included in this exposure experiment to gain more knowledge about their inherited resistance to whirling disease. In addition, a third cross has been included to gain a better understanding of how resistant trout characteristics and wild trout characteristics are inherited in subsequent generations. This third cross is termed the F2s, which are the second filial generation cross between two F1 individuals. Genetically, an F2 individual is effectively 50% GR and 50% CRR, with any individual genotype at a given locus having a 25% chance of being homozygous CRR, expressing only CRR characteristics, a 50% chance of being heterozygous GR and CRR, expressing characteristics of both, or a 25% chance of being homozygous GR, expressing only GR characteristics. The large amount of genetic variability within these crosses leads to a lot of individual variation in resistance and physiological characteristics.

In addition, reciprocal families of the F1 and B2 crosses were also evaluated for their resistance to whirling disease. A F1 reciprocal family is created by spawning a GR male with a CRR female, or a CRR male with a GR female. A B2 reciprocal family is created by spawning an F1 male with a CRR female, or a CRR male with an F1 male. Reciprocal crosses were not possible to create for the F2 strain because these are created by spawning two F1 individuals. These reciprocal crosses were included in the experiment to determine if the direction of spawning leads to differences in inheritance of resistance to whirling disease, or differences in performance in their physiological characteristics.

The ultimate goal of this laboratory experiment was to further evaluate the resistance of the GR and CRR trout strains, and their crosses, to whirling disease, and to evaluate other characteristics that may play an important role in their survival in the wild including growth, swimming performance, and predator avoidance. Growth and swimming performance were evaluated both to test the difference in the growth and swimming potential of each of the crosses and their pure ancestors, and to determine if there is a cost of resistance that affects other physiological functions. Predator avoidance was evaluated to determine which of the crosses can recognize and avoid piscine and other forms of predation, as well as to determine whether there is a difference in an infected and non-infected individual's ability to avoid predation.

Methods

Infection Experiment

Spawning of all families occurred at the Colorado Division of Wildlife Bellevue Fish Research Hatchery (BFRH) from mid-November 2006 through the end of January 2007. Both male and female pure GR and F1 fish are held on site. F1 individuals had been tagged with Passive Integrated Transponder (PIT) tags prior to spawning, and were identified by their 10 digit alpha numeric code. All spawned individuals, tagged or untagged, were also numbered in the order that they were spawned for fin clip and parental identification. Pure CRR individuals were held at the Colorado Division of Wildlife Glenwood Springs Hatchery (GWSH). Males were spawned at the GWSH and their milt was transported in individual, numbered container back to the BFRH for mixing with GR and F1 eggs. In addition, live males and females were transported back to the BFRH and spawned with each other as well as GR and F1 males. An anal fin clip was taken from each spawned individual for later genetic analysis. Eggs were placed in incubators at the BFRH or Quonset hut and held until they were eyed. Once eyed, eggs were placed in 20 gallon (76 liter) tanks, containing short standpipes for a greater amount of water turnover, at the Quonset hut, where they were hatched.

One hundred tanks total were used in the exposure and growth experiments, 80 tanks containing infected families, and 20 tanks containing uninfected control families. The GR and CRR strains were represented by 10 tanks, each containing an individual family, and the F1, F2 and B2 crosses were each represented by 20 tanks, each containing

an individual family. The 20 F1 families and 20 B2 families were made up of two sets of reciprocal families, ten of each, to test whether there was a difference in performance of the fish when exposed to whirling disease. All five strains were represented by four tanks of uninfected controls, each containing an individual family, and were split out from one of the pre-existing families pre-exposure to whirling disease.

Tanks were reduced to 25 fish per tank, and the fish were infected at an average of 678 degree days (ºC) post-hatch. Triactinomyxons (TAMs) for infections beginning on February 15, 2007 and continuing through April 20, 2007 came from Dr. Ron Hedrick's lab at U.C. Davis. TAMs for infections after April 20, 2007 came from R. Barry Nehring with the Colorado Division of Wildlife in Montrose, Colorado. Cultures of TAMs in both cases were produced from Mt. Whitney *Tubifex tubifex* worms. TAMs were counted by mixing 1,000 µl of filtrate containing the TAMs and 60 µl of crystal violet used to dye the TAMs to make them easier to see; 84.6 µl of this mixture was then placed on a slide and TAMs per slide were counted. Ten counts were conducted in this fashion to get a good mix of TAM concentrations in the filtrate. An average of the counts was taken, and this number was used to calculate the number of TAMs per ml. Fish were infected with 2,000 TAMs per individual, a total of 50,000 TAMs per tank. Before exposure, the water flow to each aquarium was stopped and each aquarium received aeration with an air stone to ensure full mixing of the TAMs and equal exposure of all fish. The approximate ml of filtrate to deliver 2,000 TAMs per fish was measured out, placed in a 1,000 ml beaker, and evenly distributed throughout each aquarium. This was done in two passes to ensure equal distribution of TAMs in the tank and to account for a possible unequal distribution of TAMs within the filtrate. Water remained stopped for one hour to ensure complete infection of all fish. Twenty tanks, four tanks of each of the five crosses, were used as controls and with the exception of not being infected with whirling disease, were treated in the same manner as the infection tanks used in the experiment.

The exposure experiment concluded once the fish reached approximately 2,000 degree-days (ºC) post-exposure, or approximately five months post-exposure. During this time, developing signs of disease and mortalities were recorded daily. The first individuals sacrificed for exposure evaluation were sacrificed on August 8th, 2007, and the evaluations concluded with the last sacrifice on October 24th, 2007. At the time of evaluation, 15 individuals from each tank were removed and sacrificed. Ten individuals were used for spore count enumeration using the pepsin-trypsin digest (PTD) method and five individuals were kept for histological analysis if necessary. The heads were removed by severing the head from the body just behind the operculum and pectoral fins. Each head was placed into an individually labeled bag that allowed for later identification of each individual. The bodies were also placed into similarly labeled bags to be used for later protein and lipid analyses. Heads to be used for spore count enumeration were sent to the Colorado Division of Wildlife Brush Fish Health Laboratory in Brush, Colorado.

The lengths, weights, and disease signs were recorded for each individual at the time of evaluation. Lengths were measured to the nearest millimeter, and weights were measured to the nearest gram. Disease signs recorded included cranial, spinal, lower jaw

and opercular deformities, exopthalmia, cleft peduncles, and black tail. Cranial deformities were defined as sunken in facial features and indentations in the cranium. Spinal deformities were defined as unusual bends or curves of the spine. Lower jaw deformities were defined by shortened lower jaws, or lower jaws that were extended to one side or the other. Opercular deformities were defined by the operculum being indented or pulled back exposing the gills. Exopthalmia is defined by the eyes being inflated in their sockets, extending past the orbitals. This condition is commonly known as pop-eye. Cleft peduncles were defined by a larger than 45 degree bend in the ventral direction of the spine around or just beyond the location of the adipose fin. Blacktail is a condition commonly displayed when many other deformities are present. It is caused by pressure being placed on the caudal nerves that control pigmentation and is defined by the posterior quarter of the fish turning black. This condition was identified pre-mortem in the sampled individuals because it disappears upon death and a loss of circulation in the fish. Disease signs were recorded as 0 if absent and 1 if present.

The fish that remained in each tank after the conclusion of the exposure experiment were kept alive for later use in the predator avoidance experiment conducted in spring 2008.

Spore count data was analyzed using a Duncan's Multiple Range Test in SAS Proc GLM. Percent mortalities was calculated using the equation, $m = 1 - (S/N)$ for each of the crosses, where m is the percent mortality experienced by a cross, S is the number of fish surviving at the conclusion of the exposure experiment in a given cross, and N is the number of fish at the beginning of the exposure experiment, starting on the day of exposure, in a given cross. Total percent deformities was calculated for each cross by adding up the number of individuals showing any sign of disease, and dividing this by the total number of individuals in a cross. The percentage of fish showing a given deformity within a cross was calculated by adding up the number of individuals showing that deformity, and dividing it by the number of individuals showing any kind of deformity, giving the percentage of individuals with a given deformity of those deformed individuals within a cross. Length and weight analysis was also conducted using a Duncan's Multiple Range Test in SAS Proc GLM.

Growth Experiment

Once hatched, small standpipes were left in the tanks until the first individuals began to swim-up. Upon swim-up, tall standpipes were placed in the tanks and the fish were started on size 0 trout diet. After approximately 335 degree days (ºC), fish were started on size-1 trout diet. At this time, families were reduced to 50 fish per family. This was considered the beginning of the growth experiment. Each family was batch weighed and fed four percent of the total batch weight. Families were held at 50 fish until the day before infection in order to account for any mortality that may occur as a result of being fed a larger feed size. In addition, an additional 50 fish from four of the families from each strain were split out and placed in uninfected control tanks. The day before infection, families were reduced to 25 fish. Again, fish were batch weighed and

fed four percent of the total batch weight. Control families were also reduced to 25 fish at this time.

Fish were reweighed every two weeks and feed amount was changed accordingly. Fish started on size 2 trout diet at a batch weight of 75 grams, size 3 trout diet at a batch weight of 162.5 grams, and size 4 trout diet at a batch weight of 500 grams, according to hatchery trout feed guidelines. Changing a given tank to a different feed size at these batch weights helped to avoid any feeding related mortalities due to fish being too small for the next feed size. If any mortality occurred in a tank, the fish were reweighed so that the four percent batch weight feed amount remained constant for every tank over the course of the experiment. On July 9th, 2007, one of the tanks included in the experiment experienced an almost complete die-off. This was exactly four months from the start of the growth experiment. The die-off occurred because of low flow conditions creating lowered water quality in the tank. The fish had reached fairly large sizes for the tanks, and were more susceptible to subtle changes in water quality because of the large proportion of the tank the fish occupied. To avoid more losses from increasing fish sizes, the growth experiment was concluded at four months post-exposure. When a tank reached the four month post-exposure point, the tank was batch weighed, and this was the final weight used for analysis. After the conclusion of the growth experiment, fish continued to be batch weighed every two weeks and were fed a maintenance diet of two percent of their batch weight for the remainder of the exposure experiment. If a batch weight exceeded 1,875 grams, the tank was put on size 5 trout diet. This batch weight was only exceeded during the maintenance feeding stage and not during the actual growth experiment.

The growth analysis was conducted using a Duncan's Multiple Range Test in SAS Proc GLM. In addition, a feed conversion ratio and feed efficiency was calculated for each of the crosses. The feed conversion ratio was calculated by summing up the total grams of feed fed over the course of the growth experiment for a given individual, and dividing this by the total weight that an individual gained over the course of the growth experiment. The individuals within a given cross were then averaged for a feed conversion ratio for a given cross. Feed efficiency is the reciprocal of the feed conversion ratio and is calculated by the equation $FE = 1/FCR$. The feed conversion ratio shows how many grams of feed is required by an individual to gain one gram of weight, and feed efficiency shows how efficient an individual is at converting feed into body mass.

Swimming Experiment

The swimming experiment was begun on April 9, 2007 and was conducted using the same fish included in the exposure and growth experiments described above. Five fish from four tanks of each strain (20 fish/strain), both infected and control, were swam during each of four time periods: 14 days post-exposure, 30 days post-exposure, 74 days post-exposure and 134 days post-exposure. All four control tanks for each strain were swum, and four infected tanks for each strain were chosen at random to be used in the

swimming experiment. A total of 735 fish were swum over the course of the six month swimming experiment.

Three days prior to swimming, five fish were chosen randomly from each of the tanks to be swum in the swimming experiment. Each fish was marked with a Visual Implant Elastomer (VIE) tag for individual identification at each of the swimming times. The five unique identification colors used were green, red, pink, orange, and green/orange. Fish were marked in both the adipose fin and in the adipose tissue behind the right eye. Green/orange fish were marked with orange in the adipose fin, green along the base of the dorsal fin, green in the adipose tissue behind the right eye, and orange in the adipose tissue behind the left eye. Identification of the colors was visually possible without aid for the first two swimming periods. As fish grew, the marks became harder to see, and identification of the colors was made using a UV light and UV reflection filtering glasses. Orange reflected orange/yellow, green reflected yellow, red reflected burnt orange, and pink reflected bright red, and identification of the reflecting colors was made easier by identifying the reflections in the dark. Approximately 10% of the tags were no longer visible at 134 days post-exposure. If a tag was lost, a fish was randomly chosen from the same tank to be swum in place of the missing color in order to keep sample sizes consistent out of each tank at each of the four time periods.

Two Loligo® swimming flumes were used to conduct the swimming experiments, one for infected fish and one for control fish. The following protocol was used for each individual fish, in either of the two flumes, on any given swimming day: First, a fish was identified and removed from a tank and placed in the swimming flume chamber. The time at which the fish was placed in the chamber and the temperature of the flume was recorded. The flume was then started on the lowest speed setting of 2 cm/sec and run for one hour in order to allow the fish to acclimate to the flowing conditions of the flume and recover from handling. At the conclusion of the one hour acclimation period, flume speed was increased to 5 cm/sec, the starting speed for the swimming trials; this was also the starting time for the swimming trial. After ten minutes, the flume speed was increased by 5 cm/sec. This procedure continued until the end of the swimming trial. The swimming trial was considered completed when the fish was no longer able to swim against the current and became impinged on the screen at the back of the swimming chamber. At this time the flume was stopped and the fish was removed. The flume speed and length of time at that speed were also recorded. Weights and lengths were taken on the fish before it was placed into a well aerated bucket of water where it was allowed to recover before being returned to the tank.

A rating scale was also created to rank an individual in terms of the number of deformities that it had. Rating was determined after the swimming trial while the fish was being handled for measuring weights and lengths. A ranking of "1" meant that the individual had no visual deformities, nor displayed any whirling behavior in the tank or the swimming chamber. A ranking of "2" meant that the individual had one visual deformity, most commonly, cranial, opercular, or lower jaw deformities, blacktail, or displayed whirling behavior either in the tank or in the swimming chamber. A rating of "3" meant that the individual had two visual deformities, or had a spinal deformity

between 0 and 15 degrees. A rating of "4" meant that an individual had three visual deformities, or had a spinal deformity between 15 and 45 degrees. A rating of "5" meant that an individual had four or more visual deformities, had a spinal deformity that was greater than 45 degrees, or had multiple spinal deformities of varying degrees of severity. The rating scale was used to determine if individuals with fewer deformities swam better than individuals with more deformities.

The critical swimming velocity (U_{crit}), or fatigue speed, was calculated for each individual using the equation,

$$U_{crit} = V_p + (t_f/t_i)*V_i$$

where V_p is the penultimate velocity reached at fatigue (cm/s), t_f is the time elapsed from the velocity increase to fatigue, t_i the time between velocity increments (in this case, 10 minutes), and V_i is the velocity step (in this case, 5 cm/sec). The U_{crit} was then used to calculate body lengths per second for each individual, which was calculated by dividing the U_{crit} by the total length of the individual. Body lengths per second was used as the standard measure because it removes the variation in body length between individuals. Analysis of the swimming results was done using an ANOVA test and a Duncan's Multiple Range Test in SAS Proc GLM.

Pond Predation Experiment

The pond predation experiment was begun on March 12, 2008 and conducted in ponds located at the Foothills Fisheries Laboratory on the Colorado State University Foothills Campus in Fort Collins, Colorado. The ultimate goal of this experiment was to determine which of the strains used in the experiments described above could recognize and avoid predation.

The rainbow trout, both infected and control, came from the previous exposure experiments conducted in 2007. After the conclusion of the exposure experiment, fish within a strain were divided into multiple tanks so that each tank contained fish that were roughly the same size. Each tank was then fed a different amount of feed, depending on their size difference from the average. The goal was to get all of the crosses to roughly the same size. Because the GR strain individuals had grown much faster during the exposure experiment, these fish were kept in cooler water (average of 4˚C) and fed much less per week than were the other strains. The F1, F2 and B2 crosses were smaller than the GR strain individuals, but larger than the CRR strain individuals. These three crosses were held in cool water (average of 7˚C) and fed different feed amounts depending on whether the tanks contained small, medium or large individuals within that cross. The CRR strain individuals were much smaller than the GR strain individuals. This strain was kept in larger round tanks in warmer water (average 10.5˚C) and fed a larger amount of feed to promote growth. The CRR tanks did not respond to the larger amount of dry feed, and therefore, their diets were supplemented by live feed, including eggs, fry and fingerlings supplied by several hatcheries around Colorado.

The growth phase prior to the start of the pond experiments lasted roughly three months. Two weeks prior to the start of the pond experiment, individuals from all five strains were weighed and measured to determine which fish were to be used in the pond experiment. Because only 40 fish per strain were left in the control tanks at the end of the exposure experiment, the control fish were limiting in terms of the number of fish that could be used per strain. The minimum number of fish was 36 individuals, seen in the CRR and B2 strains. All 36 individuals were used from these two control strains, and the same number was chosen from the GR, F1 and F2 strains so that the averages and ranges of sizes were as close as possible. The same process was used to sort through the infected fish, choosing 36 individuals from each strain that were within the average and ranges set for the control individuals.

Four ponds were used for the predation experiment, two control and two infected. The locations of the control and infected ponds, within the four, were chosen using a random number generator. The ponds are numbered in order from east to west, with Pond 1 containing the large control rainbows, Pond 2 containing the small whirling disease infected rainbows, Pond 3 containing the large whirling disease infected rainbows, and Pond 4 containing the small control rainbows. Each pond contained 18 fish of each strain. Pond 1 included CRR individuals with an average length of 20.1 cm, GR individuals with an average length of 27.8 cm, F1 individuals with an average length of 26.5 cm, F2 individuals with an average length of 27.3 cm, and B2 individuals with an average 25.8 cm. Pond 2 included CRR individuals with an average length of 15.3 cm, GR individuals with an average length of 25 cm, F1 individuals with an average length of 22.8 cm, F2 individuals with an average length of 21.7 cm, and B2 individuals with an average 21.1 cm. Pond 3 included CRR individuals with an average length of 20 cm, GR individuals with an average length of 27.4 cm, F1 individuals with an average length of 26.3 cm, F2 individuals with an average length of 26.9 cm, and B2 individuals with an average 25.7 cm. Pond 4 included CRR individuals with an average length of 15.1 cm, GR individuals with an average length of 25.1 cm, F1 individuals with an average length of 22.9 cm, F2 individuals with an average length of 21.9 cm, and B2 individuals with an average 21.1 cm. All CRR individuals were marked with a pink VIE tag in the right eye, GR individuals with a red VIE tag in the left eye, F1 individuals with a green VIE tag in the right eye, F2 individuals with an orange VIE tag in the left eye, and B2 individuals with a green VIE tag in the left eye and an orange VIE tag in the right eye. The rainbows were placed in their respective ponds on March 7, 2008.

The pike for the experiment were caught out of Lake Ladora on the Rocky Mountain Arsenal National Wildlife Refuge in Denver, Colorado on March 10, 2008. A group of 16 people, consisting of Colorado Division of Wildlife personnel, U.S. Fish and Wildlife personnel, and volunteer fisherman, were used to catch the pike. A total of 22 pike over the 26 inch minimum (in order to have a 3:1 predator to prey ratio) were caught, and 12 pike ranging between 28 and 32 inches was brought back to Fort Collins for use in the experiment. Three pike were placed in net pens in each of the four ponds before introduction to the ponds to give them time to acclimate to the pond environment,

and to allow them to digest whatever food may have been in their stomachs before they were caught (Table 2.1).

Pond 1	Pond 2	Pond 3	Pond 4
31.75	28.75	29	27.75
30.5	28.5	29	28.5
31.25	28.25	29.25	28.5

Table 2.1. Length (in inches) for each of three pike placed in the net pens in four separate ponds used in the predation experiment on March 10, 2008.

The two larger pike of the three were introduced into the ponds two days later, on March 12, 2008, which marked the beginning of the pond experiment. A 31.75 inch and 31.25 inch pike were introduced into Pond 1, a 28.75 inch and 28.5 inch pike were introduced into Pond 2, a 29.25 inch and 29 inch pike were introduced into Pond 3, and two 28.5 inch pike were introduced into Pond 4. Pike size for each of the ponds was chosen based on whether the pond contained large or small rainbows which had been previously introduced to the pond.

Over the course of the experiment, the ponds were seined several times to determine how many of the rainbows had been lost to predation. The goal was to have 50% predation of the rainbows in each of the ponds. If there was no differential predation, all of the strains would have approximately equal numbers at the end of the experiment, whereas if there was differential predation, at least one, if not two, strains would be completely missing, while the other strains would be relatively untouched. In addition, secchi disk depth, temperature, and dissolved oxygen where measured in each pond every day. This is an ongoing experiment, which has been changed to track the trout population as it declines to zero to determine if the patterns seen in the first 50% of rainbows predated continues in the second 50%.

Protein and Lipid Analysis

Protein and lipid analyses were run on 100 fish, ten of each cross, infected and control, to determine if there were differences in the way the fish process their food. To start, a range of fish sizes were selected out of each cross. The fins were removed in order to ease the grinding process. The standard lengths of the fish (minus the heads) were taken on each fish after fin removal. The fish were ground, frozen, in a food processor, and alcohol (95% ETOH) was added during the grinding process to help break up the chunks and clean the processor. The samples were then placed into a large oven set at 60˚F and dried for approximately five days. Once the samples no longer lost weight during the drying process, the samples were removed from the oven. The ground material dried into a hard, round disk that was broken up and ground down to a fine

powder using a food chopper and mortar and pestle. The powder was then placed into individually labeled bags, and ready for analysis.

Lipid analyses were conducted in the Animal Science Laboratory run by Terry Engle at Colorado State University. Two lipid bags per individual were labeled and filled with approximately one gram of sample. First, the bags were weighed and the scale tared. The sample was then added to the bag. Once the goal weight of the bag was reached, the bag was removed from the scale and sealed using a heat sealer. The second bag, which was used as a replicate, was treated in the same manner and the weight was measured to the same tenth of a gram. The bag weight and the sample weight were added together to obtain a total weight. Twelve bags were run through the lipid analysis machine at a time. The lipid analysis machine used 350 ml of petroleum ether to remove the lipids from the sample in the bag and was run for 30 minutes. The run time was ten minutes longer than a usual run for beef and other mammals because the fish were suspected to have more lipids, requiring a longer run time. Upon conclusion of a run, the bags were removed from the machine, placed under a flume hood to cool and dry for two hours, and then placed in an oven set at 100˚F to dry completely. After the four hour drying period, the samples were placed in a decanter that kept the samples from absorbing moisture from the air, and cooled to room temperature. The bags were then weighed and total weight recorded. Total lipid content for a bag was calculated using the equation,

$$TL = ((W_{sample} - (W_{final} - W_{bag}))/W_{sample})*100$$

where W_{sample} was the weight of the sample put into a bag, W_{final} was the final weight of the bag containing the sample after a run, and W_{bag} was the initial weight of the bag not containing the sample. This equation gave percent lipid content for each bag. If the two bag replicates for an individual were off by more than 15 percent, than the samples were rerun. The two replicates for each individual were then averaged together to get one estimate of total lipid content for each individual.

Protein analyses were conducted in the Animal Science Laboratory run by Terry Engle at Colorado State University. As with the lipid analysis, two replicates were run per individual. The same 100 samples were run with the exception of a few samples where there was not enough sample after the lipid run. For these few samples, fish were reground from those crosses missing individuals. Aluminum tins were filled with approximately 0.1 grams of sample and placed in wells in the protein analysis machine. The samples were then incinerated, and the various components of the protein, nitrogen and carbohydrates were caught in gas filled tubes and analyzed for their content. The results given were percent protein, percent nitrogen, and percent carbohydrate of the sample. The two replicates for each individual were then averaged together to get one estimate of the aforementioned percentages for each individual.

Results

Exposure Experiment

Fish in the exposure experiment were held for an average of 2,240 degree-days post-exposure before sacrificing for disease evaluation. The CRR strain had significantly higher mean myxospores per fish than did any of the other strains. The B2 strain had significantly higher mean myxospores per fish than did the F2, F1 or GR strains, but were significantly lower than the CRR strain in mean myxospore count. The F2, F1 and GR strains did not differ significantly from each other in mean myxospore count, but all had significantly lower mean myxospore counts than the CRR or B2 strains (Table 2.2). In all of the strains, the control families did not show any spores.

Cross	Spore Count	Confidence Interval
CRR (N=10)	187,209	(171,222, 203,196)
B2 (N=20)	97,588	(83,402, 111,774)
F2 (N=20)	46,227	(40,621, 51,883)
F1 (N=20)	9,566	(7,603, 11,529)
GR (N=10)	275	(211, 339)

Table 2.2. Mean myxospore counts and confidence intervals by strain, for the 2007 *Myxobolus cerebralis* exposure experiment. N-value represents number of replicate tanks per strain.

Variation in mean myxospores per family also differed among the strains. The GR strain showed the lowest range of variability in their mean myxospore counts, ranging from 0 to 1,177 mean myxospores per family. The F1 strain showed slightly higher variation, ranging from 0 to 51,418 mean myxospores per family. Variation doubled between the F1 and F2 strains, with the F2 strain ranging from 0 to 135,064 mean myxospores per family. The largest variation in mean myxospore count was seen in the B2 and CRR strains, with the B2 strain ranging from 0 to 338,128 mean myxospores per family, and the CRR strain ranging from 15,090 to 350,423 mean myxospores per family.

The strains also showed variation in percent mortality and number and kinds of deformities seen in the infected and control fish (Table 2.3). The control families showed significantly higher mortality than did the infected families in the GR strain. In the CRR, F2 and B2 strains, mortality was significantly higher in the infected families than in the control families. There was no significant difference in mortality between the infected and control families within the F1 strain.

There were no significant differences in percent deformities between the infected and control families of the GR strain. In the CRR, F1, F2 and B2 strains, there was a

significantly higher number of deformities seen in the infected families than in the control families (Table 2.3). The most common deformity experienced by all the strains was a cranial deformity. In the CRR and F2 strains, infected families exhibited significantly higher cranial deformities than did the control families. The GR, F1 and B2 strains did not differ significantly in the number of cranial deformities between infected and control families. The two most common deformities, other than cranial deformities, in order of number of fish exhibiting the deformity, were spinal deformities and opercular deformities. The F2, B2 and CRR strains exhibited a significantly higher number of spinal deformities in infected families than in the control families; there was no significant difference in the number of spinal deformities in the infected and control families in the F1 and GR strains. Infected and control families in the GR strain did not differ significantly in the number of opercular deformities, whereas in the other four strains, infected families exhibited a significantly higher number of opercular deformities than the control families. Other deformities seen in a much smaller proportion of fish included exopthalmia, lower jaw deformities, cleft peduncles and missing eyes. Blacktail, experienced in only the CRR, F2 and B2 strains, was exhibited by a significantly higher number of fish in the infected families than in the control families, and the CRR strain experienced a significantly higher occurrence of blacktail than did the F2 or B2 strains (Table 2.4).

Strain	N	% Mortality	N	% Deformity
Infected GR	250	3.6	241	96.5
Control GR	100	10.0	90	98.1
Infected CRR	250	12.8	218	100.0
Control CRR	100	2.0	98	20.7
Infected F1	500	1.8	491	85.6
Control F1	100	2.0	98	55.2
Infected F2	500	8.8	433	88.4
Control F2	100	2.0	98	25.9
Infected B2	500	6.2	472	85.8
Control B2	100	3.0	97	29.3

Table 2.3. Percent mortality and percent of individuals with deformities by strain, in both the infected and control fish, in the 2007 *Myxobolus cerebralis* exposure experiment.

Final weights and lengths were also recorded for the infected and control families within each of the strains. These results are presented separately from the growth experiment results because the growth experiment was not carried out to the conclusion of the exposure experiment.

	N	Cranial	Spinal	Exo.	Lower Jaw	Opercular	Peduncle	No Eye	Black Tail
Infected GR	241	97.8	9.6	8.1	5.9	17.6	0.7	0.0	0.0
Control GR	90	98.0	3.9	2.0	0.0	9.8	0.0	3.9	0.0
Infected CRR	218	91.4	85.2	8.6	8.6	82.0	0.0	0.0	35.2
Control CRR	98	33.3	50.0	0.0	8.3	8.3	0.0	0.0	0.0
Infected F1	491	94.4	19.7	4.4	3.6	17.3	0.8	0.0	0.0
Control F1	93	93.8	6.3	0.0	0.0	3.1	0.0	3.1	0.0
Infected F2	433	95.0	37.1	5.9	7.2	39.8	0.0	0.0	4.5
Control F2	98	46.7	0.0	0.0	53.3	0.0	0.0	0.0	0.0
Infected B2	472	85.5	55.7	5.5	5.5	43.0	0.9	0.9	7.7
Control B2	97	100.0	5.9	0.0	0.0	0.0	0.0	5.9	0.0

Table 2.4. Percentage of fish exhibiting each of the deformities, both control and infected, within each of the strains in the 2007 *Myxobolus cerebralis* exposure experiment. Percentages represent the percentage of fish exhibiting a given deformity out of the total number of fish that exhibited a deformity, not the percentage of all the fish examined upon conclusion of the exposure experiment.

Within the F1, F2, B2 and CRR strains, there were no significant differences in weight between the infected and control families in terms of grams per fish. In the GR strain, the control families weighed significantly more than did the infected families. In addition, the GR strain, both infected and control individuals, weighed significantly more than all of the other strains. The F1 strain, infected and control individuals, did not differ significantly in weight from the control individuals in the F2 strain. F1 strain infected individuals did not differ significantly in weight from either the infected or control individuals in the F2 strain. F2 strain individuals, infected and control, did not differ significantly in weight from the B2 strain control individuals. Finally, the B2 strain infected individuals did not differ significantly in weight from either the infected or control individuals in the CRR strain.

Within the F1, F2 and CRR strains, there were no significant differences in total length per individual between the infected and control fish. In the GR and B2 strains, the control families were significantly longer in terms of total length per individual than were the control families. In addition, the GR strain, both infected and control individuals, were significantly longer than any of the other strains. The F1 strain, infected and control individuals, did not differ significantly in total length from the control individuals in the F2 strain. F1 strain infected individuals did not differ significantly in total length from either the infected or control individuals in the F2 strain. F2 strain infected individuals did not differ significantly in weight from the B2 strain control individuals. Finally, the B2 strain infected individuals were significantly shorter than the GR, F1 and F2 strains, and significantly longer than the CRR strain infected and control individuals.

Growth Experiment

Growth in the growth experiment was measured and analyzed in two ways, average batch weight per strain and average grams per individual per strain. In terms of average batch weight per strain, the F1, F2, B2 and CRR strains did not differ significantly between infected and control families. The control families in the GR strain weighed significantly more, in terms of their batch weight per tank, than did the infected individuals. In addition, both infected and control individuals weighed significantly more than all of the other strains. The F1 individuals, both infected and control, also weighed significantly more than the F2, B2 and CRR strains. The B2 control and infected individuals did not differ significantly in batch weight per tank from the F2 strain infected or control individuals, or the CRR strain control individuals. Finally, the F2 strain control individuals did not differ significantly in batch weight per tank from the B2 strain infected individuals or the CRR strain infected and control individuals. The same general pattern was seen in the grams per individual per strain with the exception that the F2 strain infected and control individuals, along with the B2 strain infected and control individuals, weighed significantly more than the CRR strain control individuals. The reciprocal families in the F1 cross, as well as those in the B2 cross, did not show any significant differences in growth.

A large amount of variation is seen within a family of all of the strains. However, more variation is seen in some of the strains than others. In terms of length, the GR and CRR show only a small amount of variation, whereas the F1, F2 and B2 groups show an increasing amount of variation in length, respectively. In terms of weight, more variation is seen in the GR strain. The CRR strain shows similar variation in weight as is seen with length. The F1, F2 and B2 strains also generally show the same pattern in weight as is seen with length, with variation increasing from the F1 to F2, and F2 to B2 strains.

The feed conversion ratio was the lowest in the GR strain individuals, both infected and control. Conversely, feed efficiency was highest in infected and control individuals within the GR strain. The feed conversion ratio in the control individuals of the CRR, F1 and B2 strains were similar, as were the feed efficiencies for these same individuals. The feed conversion ratio for the control individuals within the F2 strain was slightly higher than the CRR, F1 and B2 strains. The feed efficiency for these same individuals was slightly lower than the CRR, F1 and B2 strains. The feed conversion ratio for the infected CRR strain individuals was much higher than the infected individuals in the F1, F2 and B2 strains, with increasing feed conversion ratios in the F1, F2 and B2 strains respectively. Conversely, the feed efficiency for the infected CRR strain individuals was much lower than the infected individuals in the F1, F2 and B2 strains, with decreasing feed efficiencies in the F1, F2 and B2 strains respectively (Table 2.5).

Cross	N		F.C.R		F.E	
	Control	Infected	Control	Infected	Control	Infected
GR	90	241	1.06	1.08	0.94	0.93
CRR	98	218	1.39	1.96	0.72	0.51
F1	98	491	1.31	1.19	0.76	0.84
F2	98	433	1.53	1.26	0.65	0.79
B2	97	472	1.42	1.44	0.70	0.69

Table 2.5. Feed conversion ratios (F.C.R.) and feed efficiency (F.E.) for infected and control fish within each of the strains in the growth experiment.

Swimming Experiment

Critical swimming speed reached, in terms of body lengths per second, decreased within all five strains as fish length increased. Previous studies on swimming with rainbow trout have shown that this result is not uncommon. There was no significant difference in critical swimming speed between infected and control fish for any of the strains, at any of the four time periods. Therefore, analyses of swimming data combined infected and control fish from a strain into an overall representation of the stain, which was used for a comparison across the strains.

At all time periods, the CRR strain reached a significantly faster speed, in terms of body lengths per second, than did the GR strain. In the first time period, fourteen days post-exposure to whirling disease, the F1, F2 and B2 crosses did not differ significantly from each other, or the CRR strain. The F2 cross reached significantly higher speeds than the GR strain. In the second time period, thirty days post-exposure to whirling disease, the CRR strain reached significantly higher speeds than did the F1, F2, B2 or GR strains. The F1, F2, B2 and GR strains did not differ significantly from each other in this time period. Between the second and third time period, signs of disease began to become more prominent in all of the crosses. In the third and fourth time periods, after signs of disease became more prominent, the CRR strain reached the highest speeds, and the GR strain reached significantly lower speeds, and the F1, F2 and B2 crosses fell in between these two speeds, not differing significantly from the CRR, the GR, or each other.

The deformity rating at the final swimming time, when infection severity was highest of the four time periods, only had a small effect in three of the strains. In two of these, the effect was seen only in infected fish, and in one, in both infected and control fish. The F2 strain infected fish ranged in deformity rating from "1" to "4", with seven individuals having a rating of"1", ten individuals having a rating of "2", two individuals having a rating of"3", and one individual having a rating of"4". Those individuals having a rating of three did not differ significantly in critical swimming speed from those individuals having a rating of "1", "2", or "4". However, the individual with a rating of "4" reached a significantly lower critical swimming speed than those individuals with a

rating of "1' or "2". The B2 strain infected fish ranged in deformity rating from "1" to "5" with nine individuals having a rating of "1", three individuals having a rating of "2", five individuals having a rating of "3", two individuals having a rating of "4", and one individual having a rating of"5". Those individuals having a rating of "2", "three" or "4", did not differ significantly in critical swimming speed from each other, or individuals having a rating of "1" or "5". However, the individual with a rating of "5" reached a significantly lower critical swimming speed than those individuals having a rating of "1". The CRR infected fish ranged in deformity rating from "1" to "5", with one individual having a rating of "1", three individuals having a rating of "2", ten individuals having a rating of "3", four individuals having a rating of "4", and two individuals having a rating of "5". Those individuals having a rating of "3", "4" or "5" did not differ in critical swimming speed from each other or those individuals having a rating of "1" or "2". The individual with a rating of "1" reached a significantly lower critical swimming speed than the individuals having a rating of "2". The CRR control fish ranged in deformity rating from "1" to "3", with individuals having a rating of "1", zero individuals having a rating of "2", and one individual having a rating of "3". The individual with a rating of "3" reached a significantly lower critical swimming speed than did the individuals with a rating of "1". The GR strain, both infected and control, the F1 strain, both infected and control, the F2 strain control, and the B2 strain control fish did not show any significant differences in swimming speed due to the number or severity of deformities.

Pond Experiment

The condition of all four ponds has been kept as constant as possible throughout the course of the experiment. Pond 1 had an average secchi depth of 103 cm, ranging from 42.5 cm to 178 cm, an average dissolved oxygen level of 7.90 ppm (parts per million), ranging from 4.2 ppm to 10.74 ppm, and an average temperature of 8.86˚C, ranging from 4˚C to 14.2˚C. Pond 2 had an average secchi depth of 136.75 cm, ranging from 60 cm to 178.5 cm, an average dissolved oxygen level of 7.24 ppm, ranging from 3.75 ppm to 10.34 ppm, and an average temperature of 8.98˚C, ranging from 4.2˚C to 14.3˚C. Pond 3 had an average secchi depth of 118.82 cm, ranging from 47.5 cm to 178 cm, an average dissolved oxygen level of 7.51 ppm, ranging from 4.13 ppm to 9.84 ppm, and an average temperature of 9.26˚C, ranging from 4.2˚C to 14.7˚C. Pond 4 had an average secchi depth of 128.31 cm, ranging from 45.25 cm to 178 cm, an average dissolved oxygen level of 7.63 ppm, ranging from 4.67 ppm to 10.54 ppm, and an average temperature of 9.47, ranging from 3.6 to 15.5. In addition, calibration temperatures, which generally reflect the environmental temperature, comments on weather conditions, processes, such as seining and running water, and comments on the biotic environment around the ponds was recorded every day

The first seining event in the ponds took place on March 19, 2008, approximately one week after the experiment started. This was used as the baseline data to determine how quickly the rainbows may be consumed by the pike. After seining Pond 3 the first time, the fish were counted and returned to the pond. The second pass through pond 3 took place after seining pond 4 which allowed the fish in pond 3 to redistribute

throughout the pond. Pond 3 was seined twice to determine if seining would give an accurate, repeatable measure of the number of fish left in the pond. The results of the two seining events in pond 3 were very similar (Table 2.6), indicating that seining was a good method of capture for accurately measuring the populations in the ponds.

Species	Pond 1	Pond 2	Pond 3	Pond 3 (2)	Pond 4
Rainbow Live	69	67	75	73	61
Pike	2	0	1	2	2
Rainbow Dead	0	0	1	0	0

Table 2.6. Results showing the number of each species caught in each of the ponds in the first seining event that took place on March 19, 2008.

The second seining event took place on March 26, 2008, approximately two weeks after the experiment started. In this seining event, the number of which cross was recorded for each of the ponds. In addition, two passes were made through each pond in order to get a more accurate removal estimate of the trout population left in the ponds (Table 2.7). The proportion of each cross left in the ponds was also estimated. In the control ponds, Ponds 1 and 4, of the 36 individuals that were stocked per strain, 94% of the GR individuals, 94% of the F1 individuals, 100% of the F2 individuals, and 89% of the B2 individuals were still left in the ponds, compared to the CRR individuals, which only had 50% of the stocked population left in the ponds. In the infected ponds, Ponds 2 and 3, 94% of the GR individuals, 94% of the F1 individuals, 94% of the F2 individuals, and 89% of the B2 individuals stocked were still left in the ponds, compared to the CRR individuals, which only had 56% of the stocked population left in the ponds. Total, 94% of the GR individuals, 94% of the F1 individuals, 97% of the F2 individuals, 89% of the B2 individuals and 53% of the CRR individuals, of the 72 individuals stocked per strain, were left at this time. Numbers of trout in each pond were similar to or slightly higher than the first seining event, likely due to the addition of the second pass through each pond which helped to estimate the population more accurately.

The third seining event took place on April 9, 2008, approximately four weeks after the experiment started. Again, the number of which cross was recorded for each of the ponds after a two pass removal (Table 2.8). The proportion of each cross remaining in the ponds was also estimated. In the control ponds, 83% of the GR individuals, 83% of the F1 individuals, 80% of the F2 individuals, and 72% of the B2 individuals stocked were still left in the ponds, compared to the CRR individuals, which only had 39% of the stocked population left in the ponds. In the infected ponds, 92% of the GR individuals, 83% of the F1 individuals, 86% of the F2 individuals, and 80% of the B2 individuals stocked were still left in the ponds, compared to the CRR individuals, which only had 44% of the stocked population left in the ponds. In total, 88% of the GR individuals, 83% of the F1 individuals, 83% of the F2 individuals, 76% of the B2 individuals and 42% of the CRR individuals were left at this time. An average of 10 fish per pond, five fish per pike, was consumed between the second and third seining events.

Species	Pond 1		Pond 2		Pond 3		Pond 4	
	Pass 1	Pass 2	Pass 1	Pass 2	Pass 1	Pass 2	Pass 1	Pass 2
GR	17	1	17	0	17	0	16	0
CRR	8	2	8	2	10	0	8	0
F1	16	0	17	0	17	0	18	0
F2	18	0	17	0	17	0	18	0
B2	16	0	14	0	18	0	16	0
Pike	1	1	0	2	2	0	2	0
	75	3	73	2	79	0	76	0

Rainbows	78	75	79	76
Pike	2	2	2	2

Table 2.7. Results showing the number of each strain/species caught in the two passes through each pond in the second seining event that took place on March 26, 2008. The results of the two passes through each pond are combined and summarized at the bottom.

Species	Pond 1		Pond 2		Pond 3		Pond 4	
	Pass 1	Pass 2	Pass 1	Pass 2	Pass 1	Pass 2	Pass 1	Pass 2
Hofer	16	0	16	0	17	0	14	0
CRR	7	0	9	2	4	1	7	0
F1	15	0	14	0	16	0	15	0
F2	14	1	13	1	17	0	13	1
B2	14	0	11	0	18	0	12	0
Pike	1	1	2	0	2	0	2	0
	66	1	63	3	72	1	61	1

Rainbows	67	66	73	62
Pike	2	2	2	2
	Eggs in pike	Milt in pike		Milt in pike

Table 2.8. Results showing the number of each strain/species caught in the two passes through each pond in the third seining event that took place on April 9, 2008. The results of the two passes through each pond are combined and summarized at the bottom along with comments on the spawning condition of the pike in each pond.

The fourth seining event took place on April 23, 2008, approximately six weeks after the experiment began. Again, the number of fish for each cross was recorded for each of the ponds after a two pass removal. In addition, weights and standard, fork and total lengths were recorded for each individual. The proportion of each cross left in the ponds was also estimated. In the control ponds, 61% of the GR individuals, 64% of the F1 individuals, 64% of the F2 individuals, and 42% of the B2 individuals stocked were

still left in the ponds, compared to the CRR individuals, which only had 6% of the stocked population left in the ponds. In the infected ponds, 72% of the GR individuals, 81% of the F1 individuals, 56% of the F2 individuals, and 69% of the B2 individuals stocked were still left in the ponds, compared to the CRR individuals, of which only 11% of the stocked population was left in the ponds. In total, 67% of the GR individuals, 72% of the F1 individuals, 60% of the F2 individuals, 56% of the B2 individuals and 8% of the CRR individuals were left at this time. An average of 20 fish per pond, ten fish per pike, was consumed between the third and forth seining events.

These predation trials are an ongoing experiment. The goal is to track which of the strains are disappearing over time, and how the proportions relate to one another over time. This experiment will be concluded once the rainbow trout are no longer present in the pond, or are present in low enough numbers that consumption by the pike has ceased.

Protein and Lipid Analysis

The protein and lipid analyses used calculated the percent total lipids, and the percent protein and nitrogen content, in a given amount of a dry sample. For lipids, there was no significant difference in percent lipid content in the CRR infected, B2 control, CRR control, and F1 control individuals. The GR control and infected individuals had a significantly lower percent lipid content than did the F2 infected and control individuals. In addition, the GR infected individuals had a significantly lower percent lipid content than did the F1 infected and B2 infected individuals. For protein, the GR control and infected, and F2 control individuals had a significantly higher percent protein content than did the F1 infected and control, B2 infected and control, CRR infected and control, and F2 infected individuals. For nitrogen, the GR control and infected, and F2 control individuals had a significantly higher percent nitrogen content than did the F1 infected and control, B2 infected and control, CRR infected and control, and F2 infected individuals.

Discussion

The ultimate goal of this research project was to determine which of the strains perform better, in terms of certain physiological characteristics that are important for survival, when exposed to and not exposed to whirling disease. The myxospore count results revealed that the CRR rainbow trout are very susceptible to whirling disease, having higher spore counts, higher mortality, and a greater number of deformities as a result of the disease. In addition, the more CRR genetics a cross has, the less resistance it shows when exposed to whirling disease, as is the case with the B2s. Conversely, the more GR genetics a strain has, as in the case of the F1s, the more resistance to whirling disease the cross exhibits. The pattern that is likely to develop from this trend is that heritability of resistance also decreases when a cross has more of the CRR and less of the GR genetics.

Growth characteristics tend to follow the same trend. The GR individuals were the largest of the strains in both length and weight at the end of the growth experiment. In conjunction with this, the GR individuals had the highest percent protein and lowest percent lipid content of the strains, as well as the lowest feed conversion ratio and highest feed efficiency of all the strains. This is likely a result of a century of selection in the German Hofer fish hatchery where the GR was grown as a food fish. Hatchery practices have likely resulted in selection of the largest and fastest growing fish for spawning. The F1 individuals were the second largest of the strains in both length and weight. They also had the second lowest feed conversion ratio and second highest feed efficiency of all the strains. This is likely a result of their genetic makeup, which consists of approximately 50% of the GR genetic alleles. The fewer GR alleles the cross has, the slower the growth, the higher the food conversion ratio and the lower the feed efficiency. The CRR individuals were the slowest growing individuals, having a fairly high feed conversion ratio and lower feed efficiency, especially in the infected individuals. This is probably caused by a combination of the historical growth characteristics of the strain, and a trade-off between growth and the body's ability to cope with whirling disease, diverting energy needed to convert food into body mass to combating the disease.

A different pattern was revealed in the swimming experiments. In this case, the CRR strain was able to reach higher critical swimming speeds than was the GR strain. This is likely a result of the selection pressure that requires wild-strain fish to adapt to changing water conditions in natural river systems where high flows and seasonal fluctuations are common. These conditions are a strong contrast to the constant, slower running, water conditions of hatchery raceways to which the GR strain has been confined for over a century. The fact that there are no differences in critical swimming speeds between infected and control individuals within a strain indicates that whirling disease is not likely to affect the ability of a fish to reach typical critical swimming speeds. However, whirling disease probably still has an effect on swimming, especially when individuals that are heavily infected display whirling behavior. Whirling behavior in a river situation may cause the fish to be swept downstream if they are unable to correct themselves fast enough. Given that the F1, F2 and B2 crosses reached swimming speeds that did not differ from either the GR or CRR, these crosses are likely able to survive the same flow conditions as the wild CRR.

The pond experiment has also yielded some unexpected results. The original theory was that since the CRR strain is a wild strain, they were more likely to be able to identify and avoid predators. Conversely, the GR strain, having not been exposed to piscine predators for over a century, may not recognize a predator nor avoid it if it approached. However, based on the results, the CRR is the most susceptible to predation of all of the strains. One explanation for this is the large difference in size between the CRR individuals, and the individuals of the other strains stocked into the ponds. Because the CRR is a very slow growing fish, it was not possible to grow them to the same size as the other strains, especially the GR, before the start of the pond experiment. Because of their smaller size, the pike may have been more likely to consume these fish based on their gape size. The CRR strain fish in both the infected and control ponds were less numerous than the other strains at this stage of the experiment. If this were simply a

function of disease effects, the proportion of CRR individuals should be much higher in the control ponds than in the infected ponds. The individuals in the infected ponds are heavily infected, more likely to whirl, thereby attracting attention and making it hard for them to escape from an approaching predator. Also, the other strains being in equal proportions, and fairly close in size, indicates that one strain is not more susceptible to predation than another. The final results of this experiment will provide more insight as to which of these strains, if any, are more susceptible to predation, especially now that most of the smaller fish in the ponds have been selectively eaten.

The results of the pond experiment do suggest that there is a minimum stocking size for susceptibility to predation. The majority of the individuals that have been consumed thus far have been on the smaller range of those stocked, whereas the larger individuals have been disappearing at a much slower rate. Larger size at stocking is an important concept to recognize, not only in the case of waters that contain pike as the top predator, but also in waters that have predators, such as brown trout, that can be just as voracious. As further introduction to wild situations occurs, this will be a major component in the survival of these fish, in addition to their ability to survive exposure to whirling disease.

Based on the results of this experiment, we conclude that the F1 cross is the best candidate for repopulating Colorado's rivers. This cross has the lowest spore counts of the tested strains. The F1 strain has better growth than the pure CRR strain, and its swimming ability does not differ from the CRRs. In addition, it is still well represented in the pond experiment suggesting that these individuals may be able to identify and avoid predation. Their rate of growth allows them to grow fast enough to possibly exceed many of the wild predator's gape limitations. In addition, lower spore counts allow them to survive better when infected with whirling disease. The production of fewer mature myxospores will also result in fewer spores contributed back to natural systems where they are stocked. Finally, the F2 cross performed similarly in the infection and swimming trials, and still had a faster growth rate than the B2 or CRR strains. This indicates that some of the GR resistance and growth characteristics can be passed through the F1 generation onto subsequent generations, possibly leading to a self-sustaining wild trout population in areas where one has not existed for over a decade. Further research with these fish in the field will lead to a better understanding of their survival under natural conditions, both physiologically and in the face of whirling disease.

Experiment 3: Heritability of Myxospore Count, Genetic Correlations, and Effective Number of Genes Involved in Resistance in Whirling Disease Resistant and Susceptible Strains of Rainbow Trout

Quantitative genetics is a form of genetics that operates under the basic idea that phenotypic variation and expression of a trait is dependent on two factors, the underlying genetics of the trait, and the environment in which an individual strain or population exists. Quantitative genetics, as a whole, operates under the idea that trait expression and transmission can be measured without the necessity of DNA, in other words, by examining the phenotypic expression of the trait. It allows the researcher to both understand how the trait works, and how it is passed from generation to generation, without knowing the exact gene or set of genes that control for the trait. The quantitative genetics method is invaluable in situations such as this, where the genes involved in such processes as resistance to whirling disease in the Hofer (GR) strain are still unknown. By examining the phenotypic variability in myxospore count, heritability of myxospore count, genetic correlations between myxospore count and other physical and physiological processes, the effective number of genes involved in resistance can be estimated.

In this experiment, variation in myxospore count was examined in five strains of rainbow trout, the Hofer (GR) trout strain, the Colorado River rainbow (CRR) trout strain, and three intermediate strains, the F1, F2 and B2 strains. The GR strain is a domesticated hatchery strain from Germany that is grown as a food fish for human consumption. For over a century, the GR strain has been exposed to the whirling disease parasite, *Myxobolus cerebralis,* in the Hofer Rainbow Trout farm in Bavaria. Through hatchery selection processes, this strain has developed a resistance to whirling disease, as those individuals that survived exposure to the disease were selected to spawn subsequent generations. However, as a result, domestication selection has also occurred, as individuals that survived well under hatchery conditions were also selected to spawn subsequent generations. Due to this type of selection, the GR strain is considered domesticated, and it is suspected that it no longer possess the characteristics necessary for survival in natural systems. In addition, the GR strain is known to be inbred, and may not possess the genetic variability needed to adapt to changing conditions in the wild. The CRR strain is a wild rainbow trout strain that has historically been used to stock many of Colorado's streams and rivers because of its ability to survive and reproduce in the wild. However, the CRR strain is one of the most susceptible strains of rainbow trout to whirling disease, and has experienced large population declines as a result of exposure to whirling disease. In addition, little to no natural recruitment has occurred in the wild in areas where a high *M. cerebralis* infection exists.

A selective breeding program was initiated to create several generational strains by crossing the GR and CRR strains, with the ultimate goal of creating a strain of rainbow trout that would have the correct combination of resistant and wild rainbow trout characteristics that would allow it to survive and reproduce in areas where a high *M. cerebralis* infection exists. Three intermediate strains have been created. The F1 strain is the first filial generational cross between the GR and CRR strains, and is created by

spawning a GR individual (male or female) with a CRR individual (male or female). Based purely on Mendelian segregation, this strain is 50 percent GR and 50 percent CRR, expressing characteristics of both strains. The F2 strain is the second filial generational cross between the GR and CRR strains, and is created by spawning an F1 male from one family with an F1 female from a different family. This strain is also effectively 50 percent GR and 50 percent CRR. However, any given genotype in this strain has a 25 percent chance of being homozygous GR, expressing only GR-like characteristics, a 50 percent chance of heterozygous GR-CRR, expressing characteristics of both, or a 25 percent chance of being homozygous CRR, expressing only CRR-like characteristics. The B2 strain is the first generational backcross between the F1 and CRR strains, and is created by spawning an F1 individual (male or female) with a CRR individual (male or female). This strain is effectively 25 percent GR and 75 percent CRR, with any given genotype having a 50 percent chance of being heterozygous GR-CRR, expressing characteristics of both, or a 50 percent chance of being homozygous CRR, expressing only CRR-like characteristics. The genetic variation possible due to recombination and linkage characteristics of the genes in these strains leads to a lot of phenotypic variation in myxospore count, which in turn can be used to calculate heritability of myxospore count, and to understand how resistance characteristics are passed on to subsequent generations of these intermediate strains.

Heritability of a character determines the degree of resemblance between relatives, and is calculated using either a full- or half-sibling analysis, or a parent-offspring regression. Heritability estimates are used as a guide to predict which individuals to spawn and how the selected trait will change in subsequent generations. This change can occur either through natural selection in the wild, or through a selective breeding program under hatchery conditions. It is important to understand that heritability calculations are based on the variability seen within a given trait across related individuals within a strain, and therefore, it is the variability seen within the strains that lends an estimate of heritability of myxospore count. Heritability of myxospore count as a result of exposure to *M. cerebralis* was evaluated using a single pair mating design. The development of all the strains from pairs of individuals resulted in unique families containing full sibling offspring for each strain. The full sibling analysis includes both an additive and dominance variance component, and is therefore an estimation of heritability in the broad sense, which measures the extent to which phenotypic variation is determined by genotypic variation. Variance components used in the calculations were estimated using ANOVA. Myxospore count was log transformed prior to analysis.

In addition to heritability, genetic variation within individuals allows estimation of the correlation between characteristics. Deformity development as a result of exposure to whirling disease, growth, and swimming ability of both exposed and unexposed individuals, were previously examined for each of the five strains described above, and correlations were estimated between these characteristics and myxospore count. Three correlations can be estimated from the data: genetic, environmental and phenotypic. Genetic correlations estimate the degree to which two traits are affected by the same genes or pairs of genes, or in other words, the amount to which the two traits covary

genetically. Environmental correlations estimate the degree to which two traits respond to variation in the same environmental factors. Phenotypic correlations estimate the degree to which the expressions of two traits covary. Each of these correlations gives information on how different characteristics of interest will respond together in subsequent generations. Variance components from the heritability calculations described above, as well as covariance components between traits estimated from ANCOVA, were used to calculate all three correlations.

A line-cross analysis was used to calculate the effective number of factors (n_e) by which the resistance characteristics in the GR and CRR strains differed, estimated by the Castle-Wright estimator. The quantity n_e is equivalent to the number of freely segregating loci with equal effects that would yield the observed pattern in the two genetic lines, and assumes independent assortment. It explains whether phenotypic variation is caused by a large number of genes with relatively small effects or a few major genes with large effects. It is also an important determinant in artificial selection programs of whether a search for informative markers is likely to be successful. Low values of n_e would suggest that genes responsible for resistance are contained on relatively few chromosomes and higher values suggest that resistance is spread over several or all chromosomes. In addition, the line-cross analysis was used to determine if an additive or additive-dominance model best fit the data. The additive model assumes that all genetic effects are additive within and between loci, where the F1 and F2 lines exhibit median phenotypic expressions between the two parental lines, and the backcrosses exhibit median phenotypic expressions between the F1 and parental line. The additive-dominance model assumes that some genetic effects are the result of dominance in one parent. Dominance results in phenotypes that are more similar to the dominant parent. It was also used to determine if dominance (from the additive-dominance model) accounted for a significant proportion of variance in the strain means.

Variation in myxospore count, both within and between families of the strains, indicated that heritability was estimable for all of the strains. Expectations, based on the variance in myxospore count and response to disease in terms of average myxospore count, for each of the strains were developed based on the predictions of the additive genetic model. The GR strain was expected to have a low variation in myxospore count, and a low response to the disease, because the genes involved in resistance to whirling disease should be approaching fixation in this strain. The CRR strain was expected to have a low variation in myxospore count, and a high response to the disease, because the development of resistance genes should not have occurred yet for this strain; each individual in this strain was expected to be equally susceptible to the disease. The F1 strain was expected to have a low variation in myxospore count, and an intermediate response to the disease between the GR and CRR strains, because the individuals in this strain should have obtained half of their genes from the GR strain, and the other half from the CRR strain. The F2 strain was expected to have a similar response to the disease as the F1 strain, but the highest variation in myxospore count of all of the strains due to the differences in segregation and recombination of the parental genes in the individuals of this strain. Finally, the B2 strain was expected to have an intermediate variation in myxospore count to the F2 and CRR strains, and an intermediate response to the disease

between the F1 and CRR strains, due to the differences in segregation and recombination of genes in the individuals of this strain as a result of the backcrossing between the F1 and CRR strains. The F1 and F2 strains deviated from these expectations, with the F1 strain having a slightly higher variation in myxsopore count and lower response to the disease than expected, and the F2 strain exhibiting a lower variation in myxospore count than expected and differing from the F1 strain in their response to the disease (Figure 2.3).

The F2 strain had a broad sense heritability estimate for myxospore count as a result of exposure to whirling disease of 0.34 ± 0.21; the F1 and GR strains were similarly low in their heritability estimates for myxospore count with estimates of 0.42 ± 0.23 and 0.34 ± 0.21, respectively. The B2 strain had a higher broad sense heritability estimate than the F2 strain, with an estimate of 0.93 ± 0.28. Interestingly, the CRR strain had a higher broad sense heritability estimate than expected at 0.89 ± 0.28 (Table 2.9). A heritability estimate of 0.3 or larger is considered a high heritability estimate.

The heritability estimates for all of the strains are considered high (greater than 0.3), indicating that there is a high selection differential in all of the strains. This means that through selection, whether it occurs through the selective breeding program or by natural selection in the wild, the allele frequencies of the population can be changed in subsequent generations, increasing resistance in future generations. The lower heritability estimate and lack of variability in myxospore count, in the GR strain indicates that selection for resistance has already occurred under hatchery conditions, and that the genes controlling for lowered myxospore count in the GR strain are approaching fixation. The fact that heritability estimates remain low in the F1 and F2 strains indicates that heritability remains similar in the first few generations, meaning that resistance to whirling disease will not be lost as quickly in the first few generational crosses of the GR and CRR strains. Finally, the higher than expected heritability estimate in the CRR strains indicates that either the CRR strain has some innate resistance to the disease, or that over the last two decades of exposure in Colorado, this strain has started to develop a resistance to the disease.

Genetic correlations between myxospore count and deformities were rarely significantly different from zero. Genetic correlations between myxospore count and physiological characteristics were also rarely significantly different from zero. The only significant genetic correlation with a physiological trait was between myxospore count and swimming performance in CRR, and the correlation was negative. Environmental correlations between myxospore count and deformity were higher than the genetic correlations, and often significantly different from zero, in the F2 and B2 strains. The environmental correlation between myxospore count and weight was also higher than the genetic correlation, and significantly different from zero in the F2 and B2 strains; however, the environmental correlations between myxospore count and length, and myxospore count and swimming ability were low and not significantly different from zero. Phenotypic correlations between myxospore count and deformity were similarly higher than the genetic correlations, and often significantly different from zero, in the F2 and B2 strains. The phenotypic correlation between myxospore count and weight was

also higher than the genetic correlation, and significantly different from zero in the F2 and B2 strains; however, the phenotypic correlations between myxospore count and length, and myxospore count and swimming ability were low and not significantly different from zero (Table 2.10).

The low genetic correlations between myxospore count and physiological characteristics indicate that it is possible to select for lowered myxospore count without selecting for/against or changing the other physiological traits. The higher environmental correlations between myxospore count and deformity formation indicate that there is not likely a genetic basis for deformity formation, but that the environmental conditions that the fish is experiencing are more likely responsible for whether or not a certain deformity will be expressed in that individual. The higher phenotypic correlations between myxospore count and deformity formation indicate that a deformity is more likely to occur with increasing myxospore count.

The effective number of factors (n_e) by which the GR and CRR strains differ in relation to myxospore development is 9 ± 5. The test statistic for the likelihood-ratio test between the additive and additive-dominance model was not significant ($P = 0.0836$), indicating that the model of best fit for the data was the additive model. However, there is still some evidence that dominance may play a role in how the resistance characteristics of the GR strain are passed on to the F1 and F2 strains. Dominance appears to break down in the B2 strain, leading to the large amount of variation in myxospore count seen in the families of this strain.

This is the first estimate of the number of genes involved in resistance in the GR strain. Though researchers have been able to make a connection between the interferon system and resistance in the GR strain, the specific genes involved in resistance are till unknown. Since the estimated number of loci involved was low, it is reasonable to believe that a search for informative molecular markers should provide information on the exact location of the loci involved in resistance to whirling disease.

Further work with genetics is planned for the future. Because we have built up a large amount of genetic material from both this and previous experiments conducted throughout the course of the selective breeding program, it may be possible to use AFLPs (Amplified Fragment Length Polymorphisms), SNPs (Single Nucleotide Polymorphisms), or other sequencing techniques to identify differences in the GR and CRR strains nuclear or mitochondrial genomes, and identify the exact locations of the genes involved in resistance. In addition, it may be possible to track the changes in allele frequencies over time for the CRR strain, both through previous experiments and in the future, to determine if genetic resistance characteristics appear as exposure of this strain to whirling disease in the state of Colorado continues. Finally, the heritability estimates can be used to aid in selecting individuals from the current broodstock of the GR and F1 strains for use as parents to spawn future generations, utilizing the selection potential in these strains to increase resistance in future generations.

References

Becker, W. A. 1992. Manual of Quantitative Genetics. Academic Enterprises: Pullman, WA.

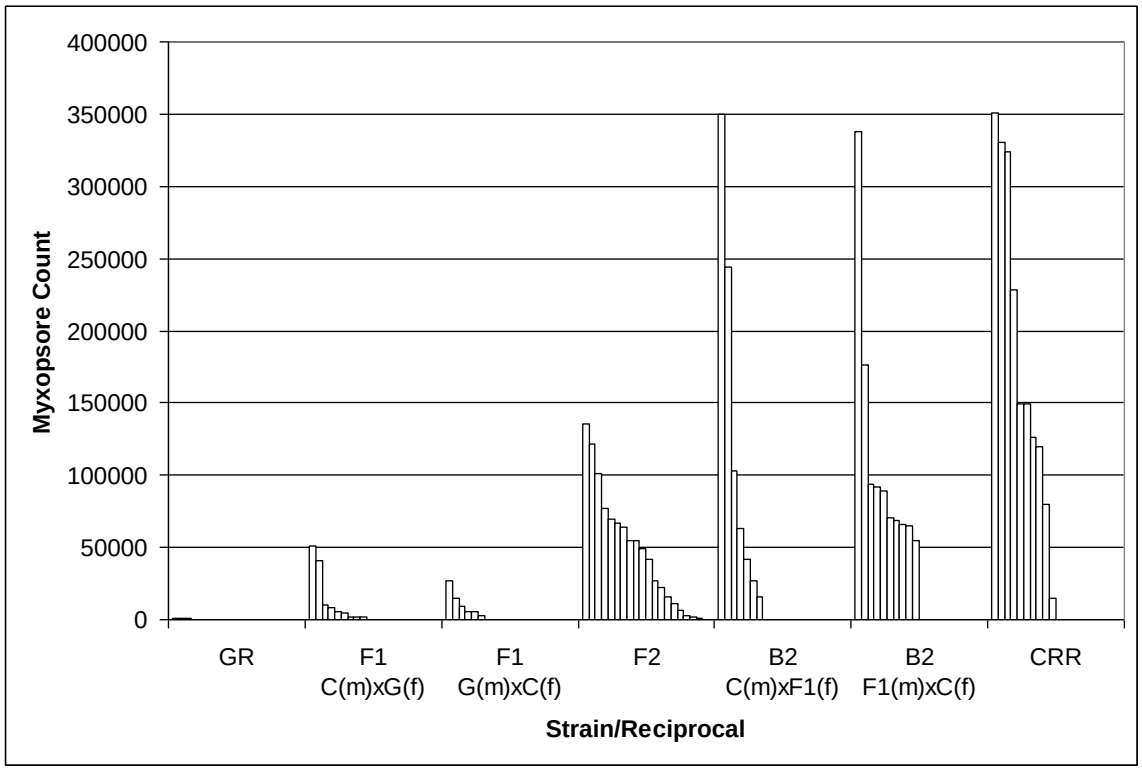


Figure 2.3. Comparison of myxospore count per family for each of five strains (with reciprocal families split out for the F1 and B2 strains) exposed to *M. cerebralis*. Ten families are represented in the GR and CRR strains, as well as in the reciprocals of the F1 and B2 strains, and 20 families are represented in the F2 strain. Notice that despite expectations, variance is low in the F1 and F2 families compared to the B2 families. In addition, variance is higher than expected in the CRR strain.

Table 2.9. Broad sense heritability estimates of myxospore count as a result of exposure to *M. cerebralis*, standard errors (as calculated using the formula from Becker (1992), representing 2 SE), and 95% confidence intervals (for ± 2 SE), for the five strains of rainbow used in the *M. cerebralis* exposure experiment.

Strain	H^2 Myxospore Count	Standard Error	95% Confidence Interval
GR	0.34	0.21	(0.13, 0.55)
F1	0.42	0.23	(0.19, 0.64)
F2	0.34	0.21	(0.13, 0.55)
B2	0.93	0.28	(0.66, 1.21)
CRR	0.89	0.28	(0.61, 1.17)

Table 2.10. Genetic, environmental and phenotypic correlations between myxospore count and deformity or physiological characteristic, and standard errors (in parentheses), for the five strains of rainbow trout used in the *M. cerebralis* exposure experiment. A "------" indicates that the correlation for that deformity or physiological characteristic was inestimable for that strain. A "=====" indicates that there was no heritability for the trait within a given strain, and therefore, genetic correlations could not be estimated. Significance is indicated by an "*".

Deformity/ Characteristic	GR	F1	F2	B2	CRR
Overall					
Genetic	=====	0.01 (0.02)	-0.001 (0.01)	-0.0001 (0.007)	=====
Environ.	=====	0.23 (0.10)*	0.19 (0.11)*	0.68 (0.38)*	=====
Phenotypic	=====	0.14 (0.07)*	0.14 (0.08)*	0.15 (0.06)*	=====
Cranial					
Genetic	=====	0.02 (0.02)	0.002 (0.01)	0.003 (0.006)	=====
Environ.	=====	0.26 (0.11)*	0.20 (0.11)*	0.78 (0.42)*	=====
Phenotypic	=====	0.15 (0.06)*	0.15 (0.08)*	0.15 (0.06)*	=====
Spinal					
Genetic	-0.04 (0.06)	0.02 (0.03)	0.01 (0.01)	0.005 (0.006)	-0.007 (0.02)
Environ.	0.34 (0.12)*	0.23 (0.10)*	0.20 (0.11)*	0.72 (0.41)*	------
Phenotypic	0.26 (0.10)*	0.16 (0.07)*	0.14 (0.08)*	0.13 (0.06)*	0.45 (0.13)*
Exopthalmia					
Genetic	0.19 (0.13)*	0.01 (0.02)	0.01 (0.02)	0.002 (0.006)	=====
Environ.	0.32 (0.15)*	0.12 (0.10)*	0.08 (0.11)	0.30 (0.37)	=====
Phenotypic	0.27 (0.10)*	0.08 (0.07)*	0.07 (0.09)	0.05 (0.06)	=====
Lower Jaw					
Genetic	0.14 (0.18)	=====	0.006 (0.005)*	0.001 (0.007)	-0.001 (0.01)
Environ.	0.19 (0.14)*	=====	0.12 (0.13)	0.37 (0.33)*	------
Phenotypic	0.18 (0.10)*	=====	0.07 (0.07)	0.08 (0.07)	0.34 (0.13)*
Opercular					
Genetic	0.18 (0.13)*	0.03 (0.02)	0.01 (0.009)	0.01 (0.009)	0.01 (0.01)
Environ.	0.47 (0.13)*	0.20 (0.11)*	0.19 (0.12)*	0.53 (0.33)*	0.98 (0.50)*
Phenotypic	0.38 (0.09)*	0.13 (0.06)*	0.13 (0.08)*	0.13 (0.07)*	0.29 (0.13)*
Blacktail					
Genetic	=====	=====	0.01 (0.01)	0.007 (0.007)	0.02 (0.01)
Environ.	=====	=====	0.04 (0.11)	0.20 (0.31)	------
Phenotypic	=====	=====	0.03 (0.09)	0.05 (0.07)	0.27 (0.11)*
Weight					
Genetic	0.07 (0.13)	0.006 (0.02)	0.005 (0.009)	0.004 (0.01)	0.006 (0.01)
Environ.	0.16 (0.17)	0.15 (0.10)*	0.15 (0.13)*	0.37 (0.31)*	0.58 (0.58)
Phenotypic	0.13 (0.10)*	0.10 (0.07)*	0.09 (0.07)*	0.09 (0.07)*	0.16 (0.15)*
Length					
Genetic	0.05 (0.16)	0.002 (0.02)	0.002 (0.008)	0.001 (0.009)	0.002 (0.01)
Environ.	0.05 (0.15)	0.05 (0.10)	0.05 (0.14)	0.12 (0.29)	0.16 (0.56)
Phenotypic	0.05 (0.11)	0.03 (0.07)	0.03 (0.07)	0.03 (0.07)	0.05 (0.16)
Swimming					
Genetic	=====	0.03 (0.51)	=====	0.01 (0.07)	-0.35 (0.17)*
Environ.	=====	0.06 (0.63)	=====	------	------
Phenotypic	=====	0.03 (0.24)	=====	0.01 (0.21)	------

HOFER-HARRISON LAKE CROSSES

Past evaluations of pure Hofer (GR) and Harrison strains of rainbow trout

Much of the laboratory work from 2006 through 2008 was focused on the GR-Colorado River rainbow trout cross varieties. That strain has been primarily designated to be used for re-establishing wild rainbow trout populations in rivers. The GR and GR-Harrison Lake varieties were tested in 2005 and 2006 as varieties for use as catchable products in put-and-take fisheries. With the increased use of the variety in the CDOW hatchery system, GR-Harrison strain fish are being used to fill requests for plants in put-grow-and-take waters as fingerlings.

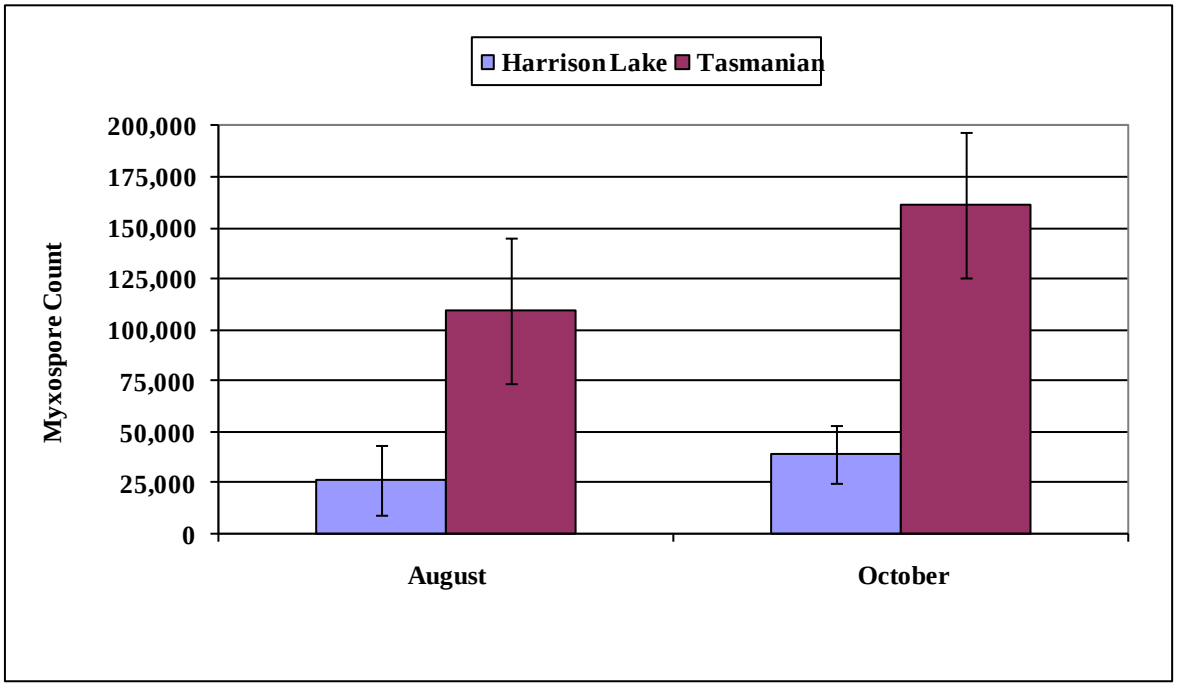
Evaluations of the pure Harrison and GR-Harrison varieties for resistance to *M. cerebralis* had been previously limited to experiments conducted in 2003 and 2004. In 2003, the Harrison Lake strain was compared with Big Thompson rainbow trout, Colorado River cutthroat trout, and Colorado River rainbow trout in a laboratory setting. Fingerlings from each strain were exposed to 2,358 TAMs per fish, divided into three replicate groups of 30 fish each, and placed into 76 L glass tanks fed with 1 L/min well water at 13 ˚C and reared for five months. On a scale of 0-4 Colorado River rainbows had the highest infection severity with an average of 4.00, followed by Big Thompson rainbows with a score of 3.93, Colorado River cutthroats with a score of 3.87, and Harrison Lake rainbows with a score of 3.60. PTD testing resulted in significantly different ($F_{[3,8]} = 17.04$, $P = 0.0008$) myxospore counts among the four strains (Table 2.11). Duncan's multiple range test with an alpha level set at 0.05 identified Big Thompson rainbows as developing significantly more myxospores than the other strains. Colorado River cutthroats and Colorado River rainbows were not significantly different from each other. Harrison Lake rainbow trout developed significantly fewer spores than Big Thompson and Colorado River rainbows, but not significantly less than the Colorado River cutthroats.

Table 2.11. PTD and PCR results of Colorado River cutthroat, Colorado River rainbow, Harrison Lake rainbow, and Big Thompson River rainbow exposed to *M. cerebralis* at a dose of 2,358 TAMS per fish as two month-old fry after five months.

	PTD Results		PCR Results	
Strain	**Myxospore counts**	**Percent positive**	**Infection Score**	**Percent Positive**
Colorado River Cutthroat	278,725	100.0	3.6	100.0
	249,319	100.0	4.0	100.0
	85,672	100.0	4.0	100.0
Average	**204,572**	**100.0**	**3.9**	**100.0**
Colorado River Rainbow	273,671	93.3	4.0	100.0
	496,380	100.0	4.0	100.0
	235,931	93.3	4.0	100.0
Average	**335,327**	**95.5**	**4.0**	**100.0**
Harrison Lake Rainbow	188,487	100.0	3.0	80.0
	132,519	73.3	4.0	100.0
	91,563	60.0	3.8	100.0
Average	**137,523**	**77.7**	**3.6**	**93.3**
Big Thompson Rainbow	758,254	100.0	3.8	100.0
	586,701	100.0	4.0	100.0
	681,945	100.0	4.0	100.0
Average	**675,633**	**100.0**	**3.9**	**100.0**

The reported resistance of the Harrison Lake rainbow trout and the encouraging preliminary results of the lab experiment in 2003 led to a second experiment in which the Harrison Lake rainbow trout would be exposed to chronic low levels of infection at an infected trout rearing facility. A total of 750 Harrison Lake and 750 Tasmanian rainbow trout of the same size and age were transported to the Poudre Rearing Unit. These fish were placed together in the lower raceways at the facility where exposure to *M. cerebralis* was expected to occur. Fish were reared for four months before the first collection of 60 fish was made for PTD analysis. Samples were collected again at six, eight, 10 and 12 months to test for *M. cerebralis* infection severity. This sampling protocol allowed comparison between the Tasmanian and Harrison Lake strains, and identification of changes in myxospore counts over time in both strains in a chronic low-level exposure environment. Because of the cold water at the facility, *M. cerebralis* could not be identified in any of the fish until the 10 month sample. In both the 10 and 12 month samples, the Harrison Lake variety had significantly lower infection than the Tasmanian strain as tested with PTD (Figure 2.4).

Figure 2.4. Myxospore counts for Harrison Lake and Tasmanian rainbow trout reared at the Poudre Rearing Unit for 10 and 12 months.

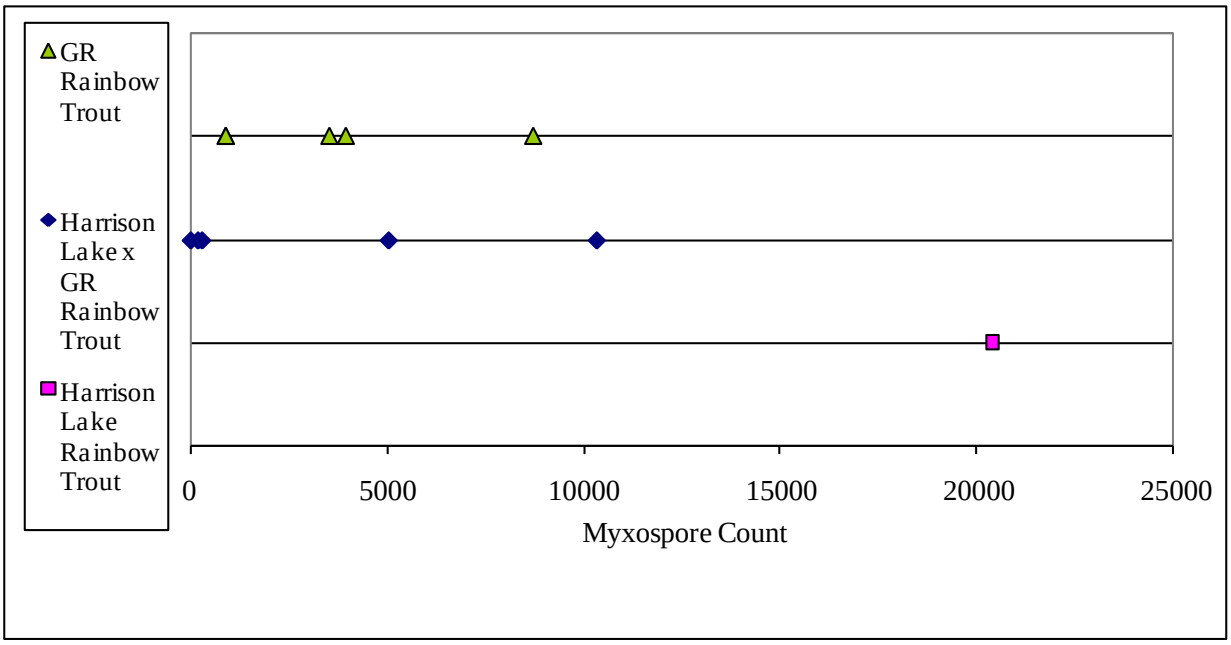


In 2004, a laboratory experiment was conducted to test not only the pure Harrison Lake strain, but also the GR and Harrison Lake (50:50) cross, and pure Hofer strain rainbow trout. In this experiment, individual families (single male/female matings) were used as replicates, with 30 fish per family. Fish from each group were exposed to an average of 2,000 triactinomyxons per fish as two-month old fry. The fish were then reared for five months. Ten fish from each family were randomly selected for myxospore counts. The GR rainbow trout and Harrison Lake x Hofer rainbow trout developed the lowest spore counts of the groups tested (Table 2.12). The Harrison Lake rainbow trout also performed well in this experiment, but the mean spore count of 20,398 myxospores per fish was higher than the Hofer-cross variety. The Hofer x Harrison Lake crosses were very resistant to the parasite, with an average myxospore count of only 3,168 per fish in the five families tested. The families created from this cross were relatively uniform in their resistance to *M. cerebralis* (Figure 2.5).

Table 2.12. Overall myxospore counts, prevalence of infection, and mortality in GR, Colorado River Rainbow, Harrison Lake rainbow, and crosses of those strains exposed to 2,000 TAMs per fish.

Strain	Families	N	Spore Count	PTD	Mortality
			Mean	Infected (%)	(%)
GR Rainbow	5	50	3,593	30.0	0.8
GR (f) x Harrison Lake Rainbow (m)	5	50	3,168	30.0	5.0
Harrison Lake Rainbow	1	10	20,398	40.0	16.7

Figure 2.5. Myxospore counts for individual families of Hofer, Harrison Lake, and 50:50 crosses of the strains.



In 2009, experiments were designed to further gauge the susceptibility of the GR, Harrison, and crosses of the strains to *M. cerebralis*. Three separate experiments were set up to run at the same time, with eight varieties of fish. These included pure GR, pure Harrison Lake (HL), pure Tasmaninan rainbow (TAS), GR-HL (50:50) cross, GR-HL (75:25) cross, GR-HL (87.5:12.5) cross, a GR-Snake River cross (HHN), and Bellaire rainbow-Snake River cutthroat (50:50) cross (RXN). All of the lots were coded wire tagged prior to the experiments, so positive identification of each fish to strain would be possible without physical separation. These eight varieties were reared to the same size and as close to the same age as possible prior to the experiments. These experiments were started in the summer of 2009, so all of the experiments are not entirely complete.

Resistance Experiment 1: Aquarium Experiment

This laboratory experiment was set up to evaluate the parasite load of each of the eight varieties of fish exposed to known doses of the parasite in a controlled setting. In this experiment, the fish were separated by strain to preclude strain interactions on growth and parasite loads that may occur due to elevated stress in the less aggressive strains.

Methods

Twenty fish of each of the eight strains were placed in two replicates of eight 76 L aquariums. One set of eight aquariums was used as the treatment group, and one set of eight aquariums was used as the control group. Weights and lengths of each fish were recorded at the beginning of the experiment. In both this experiment and in Resistance Experiment 2, the exposure levels were between 1,500 and 3,000 TAMs per fish depending on the actual number available in the filtrate. This varied depending on the TAM production from the infected *T. tubifex* cultures used. These levels are known from previous experiments to cause relatively high infection in susceptible fish. In this experiment, the treatment group was exposed to 2,956 TAMs per fish on July 15, 2009.

The fish were reared in the aquariums with 2 liters per minute flow-through of ambient-temperature lake water. The fish in this experiment were sacrificed on January 15, 2010 for infection evaluation at six months post-exposure. Weights and lengths of all fish surviving to the end of the experiment were recorded, and parasite load for each fish was measured by pepsin-trypsin digest (PTD).

Results

Mortality was low for most of the test lots. In the aquarium holding the GR-Harrison (75:25) fish, the standpipe was dislodged, which resulted in the death of 13 of the 20 fish. However, the remaining seven survived until the end of the experiment and were evaluated. The single lot that experienced other mortality was the pure Harrison

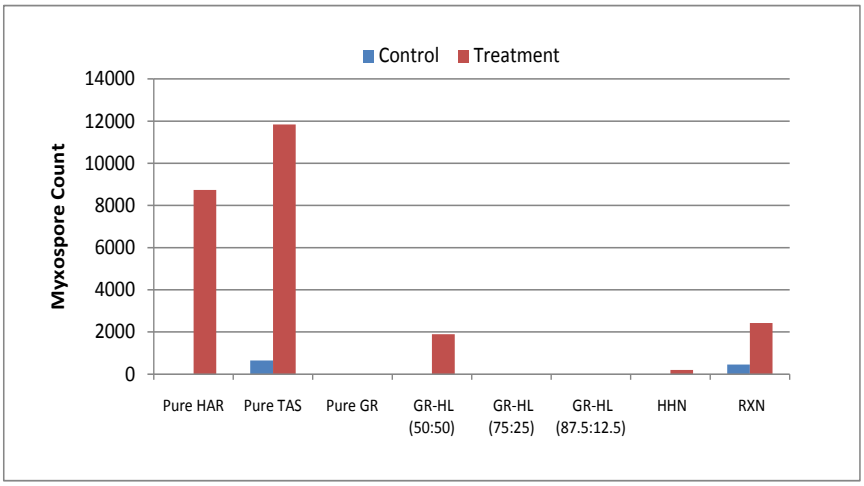
Lake lot, in which three individual fish died over the course of the six month rearing period. Growth of the eight varieties (both control and treatment) was variable (Table 2.13). As with many of the previous experiments in which GR strain rainbow trout have been reared, this particular strain, and crosses with high GR background outgrew the other varieties.

Table 2.13. Weights and lengths of eight varieties of rainbow and rainbow-cutthroat crosses at the beginning and end of Experiment 1.

		Pure HAR	Pure TAS	Pure GR	GR:HL 50:50	GR:HL 75:25	GR:HL 87.5:12.5	HHN	RXN
Control July 2009	**Length (mm)**	92	114	120	116	117	111	105	98
	Weight (g)	8.2	17.6	21.0	17.8	18.5	16.5	12.0	10.1
Treatment July 2009	**Length (mm)**	91	113	114	115	112	114	104	96
	Weight (g)	7.6	17.6	17.5	17.6	15.4	17.6	12.2	9.9
Average	**Length (mm)**	91.5	113.5	117.0	115.5	114.5	112.5	104.5	97.0
	Weight (g)	7.9	17.6	19.3	17.7	17.0	17.0	12.1	10.0
Control April 2010	**Length (mm)**	163	191	222	201	205	199	198	187
	Weight (g)	46.4	85.1	134.9	89.3	98.3	93.4	85.4	72.5
Treatment April 2010	**Length (mm)**	162	197	237	213	206	207	200	178
	Weight (g)	47.6	92.4	150.5	113.8	94.7	99.4	94.3	65.9
Average	**Length (mm)**	162.5	194.0	229.5	207.0	205.5	203.0	199.0	182.5
	Weight (g)	47.0	88.8	142.7	101.6	96.5	96.4	89.9	69.2
Net Growth	**Length (mm)**	71.0	80.5	112.5	91.5	91.0	90.5	94.5	85.5
	Weight (g)	39.1	71.2	123.4	83.9	79.5	79.4	77.8	59.2

Myxospore counts among the treatment groups once again demonstrated the resistance of the GR strain and crosses of the GR strain to be highly resistant to the parasite (Figure 2.6). The Tasmanian strain was once again shown to be very vulnerable to the parasite, and the HL strain also had relatively high infection levels compared to the GR strains. The HHN strain performed quite well, with only one fish of 20 identified as infected, with an average myxospore count of 193 for the strain. An unexpected result among the control fish in the Tasmanian and the RXN strain was observed in this experiment. Two fish of the 20 in the Tasmanian control group were identified as infected, with an average of 647 myxospores for the group. One of the 20 fish in the RXN group was also identified as infected, with an average myxospore count of 457 for the group. It is possible that the fish became infected during the rearing period from exposure to TAMs that were drawn in through the laboratory intake from the lake and not killed by the UV system. Another, less likely possibility is that these strains were exposed in the facilities where they originated. If exposure did occur due to contamination in the laboratory intake, it is surprising that none of the fish in the Harrison Lake variety control group were identified as infected. In either case, the control group should be considered to be lightly exposed rather than not exposed in this experiment.

Figure 2.6. Myxospore counts at six months post-exposure for eight varieties of rainbow and rainbow-cutthroat crosses. Treatment group exposed to 2,956 TAMs per fish.



Resistance Experiment 2: Mixed Lot Experiment

This laboratory experiment had the same goals as the first experiment, but was conducted with all eight strains reared together to avoid any tank effect that might occur as a result of rearing single strains in each tank.

Methods

Twenty-five fish of each variety were placed into each of four 200 gallon circular tanks for a total of 200 fish per tank. Starting weights and lengths were recorded for each group. Two tanks were designated as treatment tanks, and two were designated as control tanks. The first treatment tank was exposed to an average of 1,603 TAMs per fish on July 22, 2009. The second treatment tank was exposed to 1,775 TAMs per fish on Aug 8, 2009. The fish were reared in the circular tanks for the duration of the experiment. The first replicate was reared for eight months, and sacrificed on March 22, 2010. The second replicate was reared for ten months, and sacrificed on June 5, 2010. Both the treatment and control tanks for the second replicate were evenly divided into four tanks after the first replicate was sacrificed to avoid crowding in the second replicate during the additional two months of rearing.

Results

Lengths and weights of each of the different strains of fish are shown in Figure 2.7and 2.8. Large differences in growth between the replicate groups grown out to ten months were not much different than groups reared eight months. Results were very similar to Experiment 1, in which varieties with more Hofer background grew much better than those with without Hofer background. Pure Harrison and RXN varieties exhibited the poorest growth as measured by both length and weight.

Myxospore results for both the eight and ten month samples resulted in Harrison Lake and Tasmanian strain fish harboring much higher levels of infection than the Hofer crosses, or the RXN variety(Figure 2.9). In the case of myxospore counts, the additional two months of rearing dramatically increased parasite load in both of these strains. The Hofer, Hofer crosses, and RXN varieties also exhibited increase in parasite load, but not to the extent as was observed in the Harrison Lake and Tasmanian strains.

Figure 2.7. Lengths for eight varieties of rainbow and rainbow-cutthroat crosses during Experiment 2.

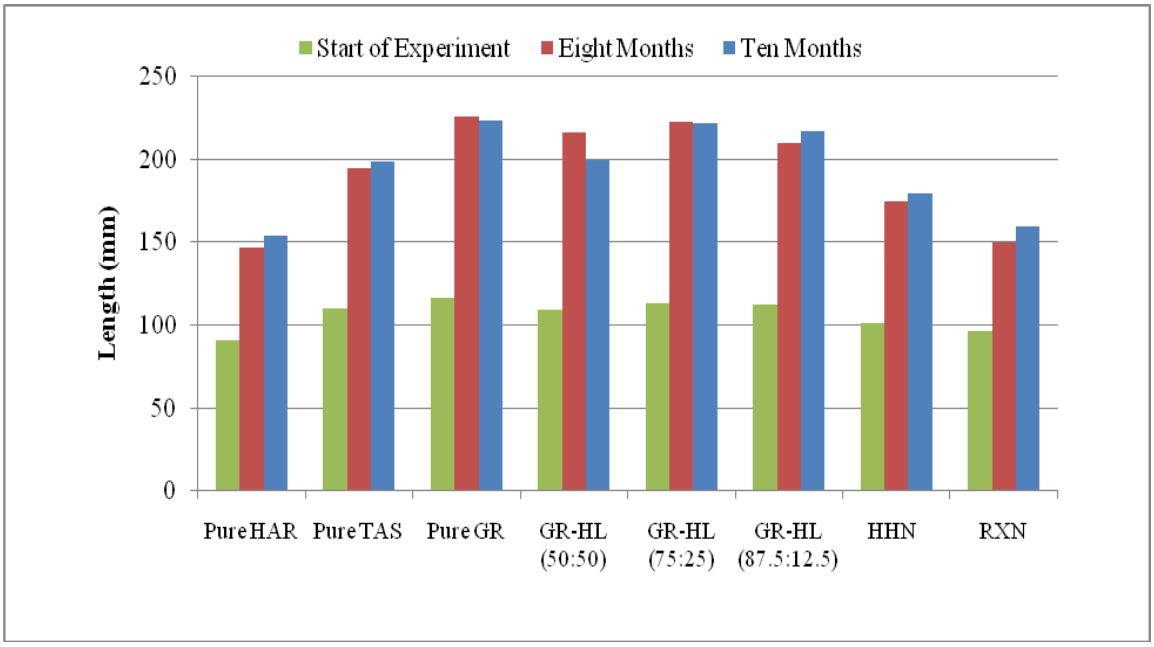


Figure 2.8. Weights for eight varieties of rainbow and rainbow-cutthroat crosses during Experiment 2.

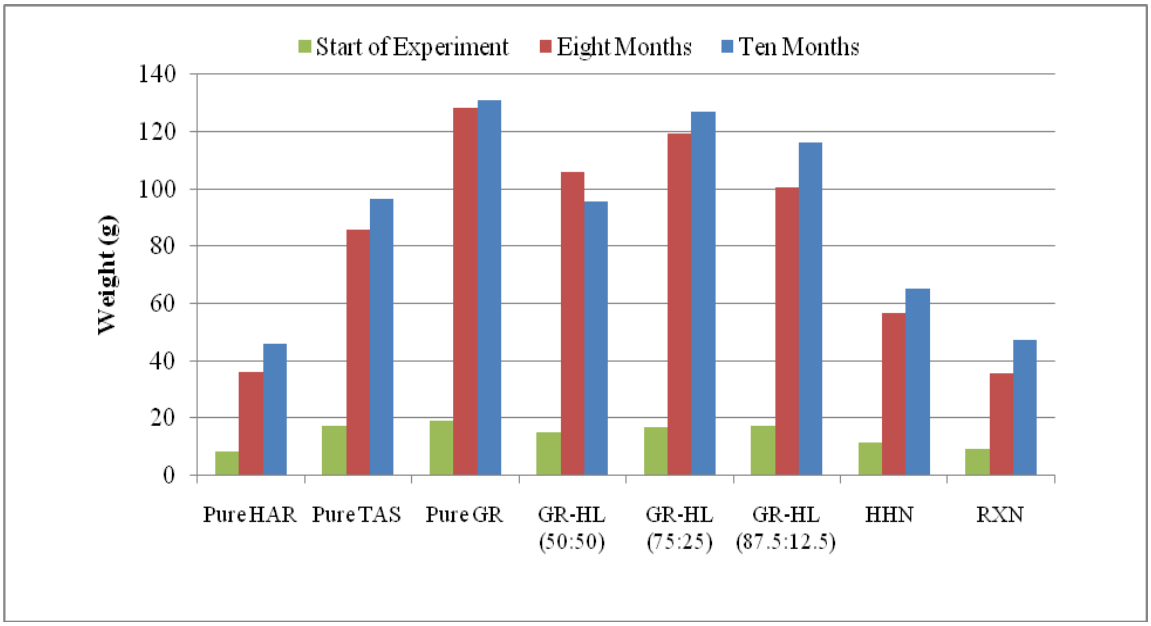
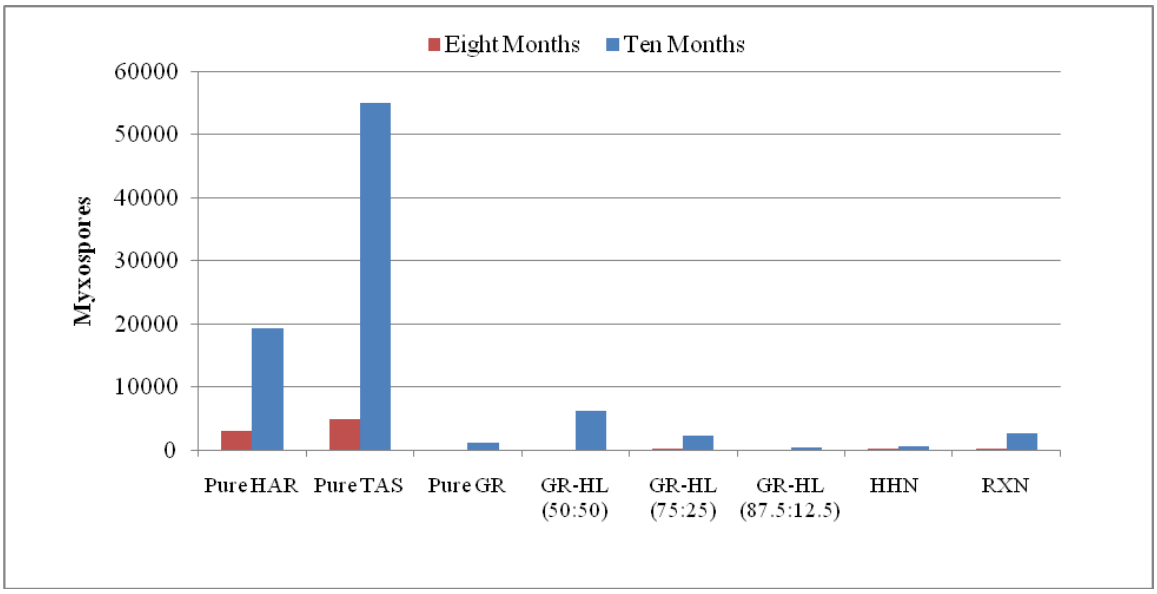


Figure 2.9. Myxospore counts for eight varieties of rainbow and rainbow-cutthroat crosses during Experiment 2 at eight and ten months post-exposure.



Resistance Experiment 3: Poudre Pond Experiment

This experiment was conducted to determine what level of infection and growth would occur with each of the eight varieties reared together in a more natural setting that is known to have high ambient levels of *M. cerebralis*.

Methods

This experiment was an extension of the two laboratory experiments in which all eight varieties were reared in two earthen ponds at the Poudre Rearing Unit. One thousand fish of each variety were stocked into each pond, for a total of 8,000 fish per pond. Samples were collected at eight months and 12 months post-release. In addition, mortalities were collected throughout the study period. At the eight-month collection time, the ponds were still covered with ice, making random sampling a challenge. Ice was broken at the upstream end of each pond and a gill net was set. Thirty and 32 fish were collected in this manner from Pond 1 and Pond 2, respectively. Hook-and-line sampling was used to capture an additional 32 and 31 fish from Pond 1 and Pond 2, respectively. During the 12-month collection each pond was seined, with 62 and 55 fish collected from Pond 1 and Pond 2, respectively. All samples collected from the ponds were weighed and measured, and then coded wire tags were extracted from the fish to identify the strain. The individual fish were then numbered, individually bagged. A sub-set was submitted for PTD testing.

Results

Catch results for the eight-month sample by gear type are summarized in Table 2.14. No Harrison Lake rainbow trout were found during the eight-month post-release sample among the 125 fish collected. Only five pure Tasmanian strain fish were found, and six GR-Harrison (50:50) crosses. The other strains were relatively uniform in catch, ranging from 18 (14.4%) to 26 (20.8%).

The eight-month length results suggest that the GR strain and high proportion GR crosses such as the GR-Harrison (75:25) and GR-Harrison (87.5:12.5) had slightly better growth as measured in length compared to the other strains (Figure 2.10). Each of these strains averaged over 210 mm in length at eight months. Weight measurements demonstrated even greater advantage to the GR strain and high proportion crosses, with all three averaging over 100 grams (Figure 2.11).

The 12-month length results were very similar, with the GR and high proportion GR crosses having the best growth as measured by both weight and length. The high proportion GR varieties were also present in the sample at higher rates than the other strains (Table 2.15). The exception was the HHN variety, in which growth as measured by both weight and length was more comparable to the RXN variety.

Table 2.14. Total catch for the eight-month post-release sample at Poudre Ponds.

	Pure HAR	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%)

Table 2.15. Total catch for the 12-month post-release sample at Poudre Ponds.

	Pure HAR	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%)

Figure 2.10. Lengths of eight rainbow and rainbow-cutthroat trout cross varieties upon release, eight and 12 months post-release at the Poudre Rearing Ponds.

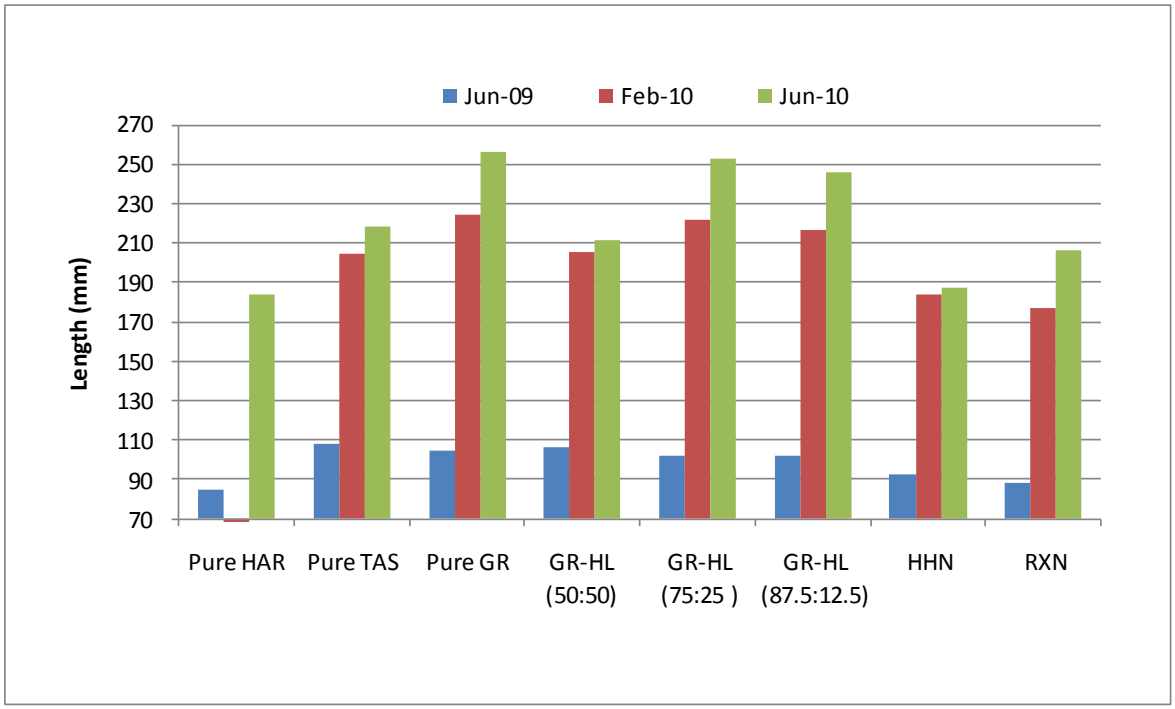


Figure 2.11. Weights of eight rainbow and rainbow-cutthroat trout cross varieties upon release, eight and 12 months post-release at the Poudre Rearing Ponds.

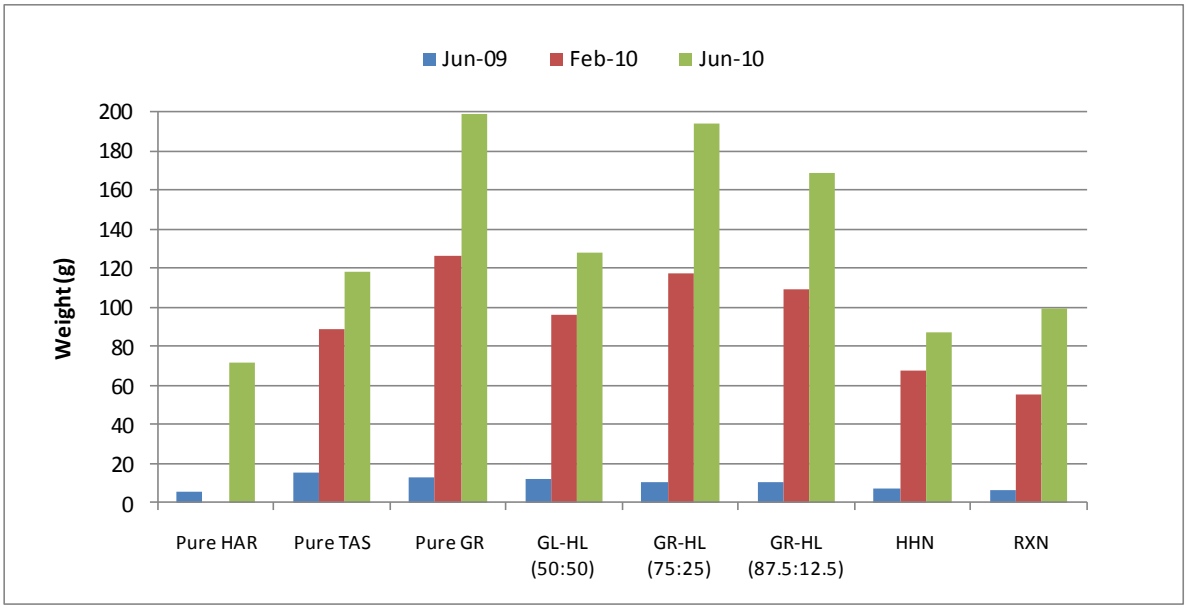
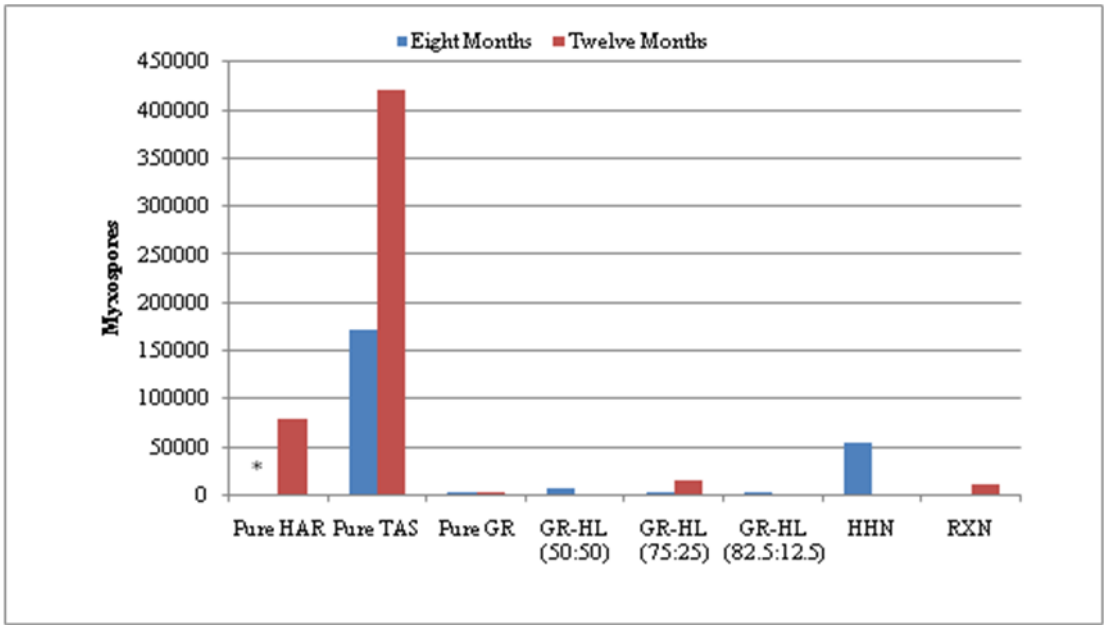


Figure 2.12. Myxospore count by strain at the Poudre Rearing Unit at eight months and 12 months post-release.



Myxospore count results were very similar to the other experiments in which these strains were evaluated (Figure 2.12). 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 parasites per fish. This high spore level, as observed in a highly infected natural environment, would unquestionably lead to amplification of *M. cerebralis* in waters stocked with this suceptible strain.

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

Job Objective: These experiments are focused on the performance of the Hofer (GR) strain and GR-Harrison strain as domestic production fish compared with other commonly used production fish.

Hatchery Performance Evaluations: Performance of a whirling disease resistant rainbow trout strain at two *Myxobolus cerebralis*-positive trout rearing facilities

Abstract

A recently identified strain of rainbow trout with resistance to whirling disease (GR) was compared with Tasmanian and Bellaire rainbow trout strains in two separate trout rearing facilities to evaluate its performance and susceptibility to *M. cerebralis* infection under standard rearing conditions. Fish were brought to the facilities as either advanced fingerlings or as eyed eggs. Growth in the GR strain was significantly faster than in these other two domestic strains. Infection severity and prevalence in the GR strain was significantly lower than in the other two strains. These results demonstrate that the GR strain may be a useful replacement for more susceptible strains in facilities with a history of *M. cerebralis* infection.

Introduction

Whirling disease, caused by *Myxobolus cerebralis* is known to cause severe declines in wild rainbow trout populations, particularly in the Intermountain West (Nehring and Walker 1996, Vincent 1996). The parasite has become established in many fish culture facilities as well. For example, fish in 10 of Colorado's 14 state-operated trout rearing facilities were identified as infected with the parasite as recently as 1997 (Rich Kolecki, Colorado Division of Wildlife Chief of Hatcheries, personal communication). While infections from the parasite in hatchery situations do not typically result in heavy mortality, other detrimental effects such as compromised growth, performance, and conformation of the infected fish can occur. This can result in reduced marketability of the fish in commercial operations. Possible spread of the parasite from infected facilities can also be a damaging consequence. Spread of *M. cerebralis* through human transfer of infected fish is well documented, and considered to be one of the primary routes of dispersal (Hoffmann 1990, Modin 1998, Bartholomew and Reno 2002). Stocking of infected fish has been shown to increase the likelihood of *M. cerebralis* establishment (Schisler 2002), and greatly increase the ambient parasite load and infection severity in fish in the near vicinity and downstream of the stocked locations (Nehring 2006). In some states, regulations require that facilities harboring the parasite be depopulated and the parasite eliminated from the water supply or the facilities be closed.

Fish culture problems related to whirling disease infection can be alleviated in many cases through improved management practices to reduce or eliminate the parasite (Hoffman 1990). Solutions include using well water or water treatment to ensure parasite-free water supplies. Hatchery renovation, such as installation of concrete raceways or lining earthen ponds can also help eliminate habitat for the intermediate host, *Tubifex tubifex*. These practices can greatly reduce the incidence and prevalence of infection in some locations. In Colorado, hatchery improvements have eliminated the parasite from seven of the facilities previously identified as positive for the parasite. The parasite cannot be eliminated in some facilities because of reliance on infected water sources. In these situations, hatchery managers may be somewhat limited in their ability to reduce infection prevalence and severity.

Many previous studies have demonstrated that rainbow trout are quite vulnerable to whirling disease (Thompson et. al 1999, Hedrick et al. 1999, Vincent 2002), and until recently, all rainbow trout strains were considered to be very susceptible to the parasite. The discovery of whirling disease-resistant rainbow trout strains (Hedrick et al. 2003, Schisler et al. 2006, Wagner et al. 2006) has provided hope that effects of the parasite could be further alleviated through the use of these resistant strains in trout rearing facilities where *M. cerebralis* cannot be completely eradicated. The potential use of these strains as a method to reduce impacts due to *M. cerebralis* has generated considerable interest. Performance of the GR strain in typical fish culture situations in the United States has not yet been evaluated, and verification of the resistance of these strains to whirling disease under normal culture conditions is needed to determine if their use is a viable option. This study was designed to evaluate the growth and survival of the GR strain when compared with other standard domestic strains in representative hatchery situations.

Methods

GR strain rainbow trout were evaluated at two separate state-operated *M. cerebralis*-positive trout rearing facilities. Both of these facilities rely on surface water, and have a history of infection in fish reared at these locations. Bellaire strain and Tasmanian strain rainbow trout are commonly used in Colorado as a catchable rainbow trout product for put-and-take and put-grow-and-take fisheries. In both of the trials described herein, the Bellaire and Tasmanian strain lots were reared through their normal production cycle, and matched with equal numbers of the GR strain to compare the growth and infectivity between the strains.

Chalk Cliffs Rearing Unit. – The Chalk Cliffs Rearing Unit is located in the upper Arkansas River drainage near Nathrop, Colorado, at an elevation of 2,438 meters. The facility was first identified as positive for *M. cerebralis* in March, 1988. The facility relies on surface water from Chalk Creek, and fish are reared in a series of raceways and earthen ponds. Warm springs in Chalk Creek result in an increased ambient temperature through the winter months (Figure 3.1). Myxospore counts in fish collected from the ponds on the facility during annual disease inspections have at times averaged over one million per fish. Improved management practices such as regular removal of moralities and rotation of active ponds, with periodic drying and excavating, have helped reduce myxospore counts in recent years. However, because of its reliance on surface water, the Chalk Cliffs facility cannot be completely rid of the parasite.

Eyed eggs of the GR and Tasmanian strain rainbow trout were transported to the facility in December, 2005. The eggs hatched within a day of each other, and fry were reared together in 0.2 x 3.5 m troughs in a hatchery building, fed with 38-53 liters per minute surface water. At six months post-hatch, the fish were moved to 1 x 50 m raceways with a flow of 4,920 liters per minute for further growth, then to a 0.47 hectare pond for final grow-out at 11 months post-hatch. Growth was measured periodically throughout the rearing period, starting at four months post-hatch, by using wet weights. Direct length measurements for statistical comparisons were made at nine and a half months and one year post-hatch.

Samples were collected to test for *M. cerebralis* infection and prevalence at three months post-hatch (1,002 degree-days ºC). Ten fish from each lot were collected and euthanized with tricaine methanesulfonate for histological analysis. Because of the size of the fish, they were fixed whole in Davidson's solution for 48 hours and then transferred to 70% ethanol. The bodies were embedded in paraffin, sectioned and stained with hematoxylin and eosin by standard procedures (Humason 1979). Two sections, one 30 microns deeper than the other, for each fish were evaluated for the presence of microscopic lesions due to *M. cerebralis.* The severity of microscopic lesions present in stained tissue sections were evaluated by the MacConnell-Baldwin scale using a scale from 0 – 5 with 5 representing the most severe lesions and 0 indicating no abnormalities seen (Hedrick et al. 1999b, Baldwin et al. 2000).

At five months post-hatch (1,934 degree-days ºC), 10 fish of each strain were again collected for histological analysis and 10 fish of each strain were collected for PTD analysis. Heads were removed from the sample fish. Whole heads designated for histological sectioning were preserved in Davidson's solution. Histological procedures were conducted as described above. If used for PTD analysis, heads were placed in individually labeled plastic bags, and then held at – 20^{o}C until processing. The samples were then soaked in water at 45°C to soften the tissues, and then skeletal elements were separated from soft tissue by agitation in a wrist-action electric shaker using glass marbles as hammers. The samples were then decanted through disposable 190 µm calculi filters and rinse water was added back to the skeletal elements for purification and concentration by PTD (Markiw and Wolf 1974) and myxospore quantification (O'Grodnick 1975).

A third sample was collected during the facilities annual disease inspection, at nine months post-hatch (3,468 degree-days ºC). This collection occurred after the fish had been in the raceways for three and a half months (1,402 degree-days ºC). Thirty fish of each strain were collected for testing with PTD. These fish were processed for whole-head analysis as described previously.

Proc GLM in SAS system software was used to conduct tests in a general linear model framework for differences in growth and infection severity (dependent continuous variables) between strains (independent classification variable) for data collected during each sampling event. Wet weights over the course of the grow-out period were also compared using Proc GLM, in a simple regression analysis. This analysis used strain as an independent classification variable, days post-hatch as an independent continuous variable, and weight as a dependent continuous variable. Alpha was set at 0.05 for all tests of differences in growth and infection severity.

Poudre Rearing Unit - The Poudre Rearing Unit is located at an elevation of 2,347 meters above sea level in the Cache la Poudre Canyon, Northwest of Fort Collins, Colorado. The facility relies on surface water drawn from the Cache la Poudre River, which results in very slow growth at the facility during the winter months, when temperatures drop to near freezing from September through April (Figure 3.2). The facility has been positive for *M. cerebralis* since June of 1988. Fish produced at the Poudre Rearing Unit are typically brought to the facility in the late summer or fall of the year from the Bellvue Hatchery, near Laporte, Colorado, as 8 to 16-cm fingerlings. Fish reared at the facility are used as a catchable product or as replacement brood fish. GR and Bellaire strain rainbow trout were brought to the Poudre Rearing Unit in late July, 2005, as 15-cm (43-45 g) *M. cerebralis*-negative fingerlings. Each lot consisted of 1,550 fish. The GR rainbow trout were seven months post-hatch, and the Bellaire rainbow trout were nine and a half months post-hatch. The age difference was necessary to match the sizes of the fish, due to the rapid early growth of the GR trout. Increase in size and age at exposure has been demonstrated to reduce infection severity in rainbow trout (Markiw 1992, Ryce et al. 2005). The GR rainbow trout were younger and therefore presumably more susceptible to infection as a function of age than the Bellaire rainbow trout when brought to the facility. The adipose fin was removed from GR

rainbow trout two weeks prior to transport to ensure the fish could be easily identified when samples were collected.

The fish were held together in a single (1.8 m x 30.4 m) raceway, with a flow of 3,218 to 3,407 liters per minute. Fish were fed ad libitum with demand feeders during the summer months, and a daily maintenance ration 0.5%-2% body weight during the winter months when temperatures were below 2º C. Growth was monitored for one year at the facility.

Samples were collected for histological examinations and myxospore counts four months (970 degree-days ºC) after the fish were transported to the facility. Thirty fish of the GR and Bellaire rainbow trout were collected for the evaluations. The fish were euthanized with tricaine methanesulfonate, then weighed and measured. Heads were removed from the fish and split in two equal halves along the dorsal midline for histological analysis and pepsin-trypsin digest (PTD).

Subsequent samples of 30 fish of each strain were collected at eight months (1,039 degree-days ºC) and one year (1,617 degree-days ºC) after the fish were brought to the facility. The entire head of each fish was collected and processed with PTD as described above in these samples. Average myxospore counts were compared between the two strains for each of the three sampling events. As with the data collected from the Chalk Cliffs evaluation, Proc GLM in SAS System software was used to test for differences in growth and infection severity for data collected during each sampling event, and alpha was set at 0.05 for all tests.

Results

Chalk Cliffs Rearing Unit

Growth – At the Chalk Cliffs Rearing Unit, growth as measured by average weight in the GR strain was much faster than the Tasmanian strain (Figure 3.3). A simple linear regression model with days post-hatch as an independent continuous variable and strain as an independent classification variable resulted in a very good fit (R^2 = 0.8914). Both strain ($F_{[1, 19]}$ =23.70, $P < 0.0001$) and days post-hatch ($F_{[1, 19]}$ =132.23, $P < 0.0001$) were found to be significant parameters in this model. More complicated models were explored, with similar results. Growth differences were also quite different when direct length measurements were compared. At nine and a half months post-hatch, average length of GR strain was 23.6 cm (n = 60, SD = 1.5), and 18.5 cm (n = 60, SD = 2.4) for the Tasmanian strain. At one year post-hatch, the GR strain averaged 28.4 cm (n = 50, SD = 2.8), while the Tasmanian strain averaged 22.3 cm (n = 50, SD = 3.3). These differences were significant between the two strains during both the first ($F_{[1, 118]}$ =199.26, $P < 0.0001$) and second ($F_{[1, 98]}$ = 100.85, $P < 0.0001$) sampling events.

M. cerebralis Infection – Statistical test results for comparison of the infection severity and prevalence in the two strains for all of the sampling events at the Chalk

Cliffs Rearing Unit are provided in Table 3.1. Samples collected at three months post-hatch were identified as negative with histology in both the GR and Tasmanian rainbow trout. Samples collected at five months post-hatch also resulted in negative results for both histology and PTD in both strains. At nine and a half months post-hatch, infection prevalence in the GR strain was 73.3%, and prevalence in the Tasmanian strain was 96.7% Average whole-head myxospore count in the GR strain was 5,175 (n = 30, SD = 7,643), compared with 48,883 (n = 30, SD = 50,825) in the Tasmanian strain. The differences in myxospore counts were highly significant ($F_{[1, 58]} = 21.70$ $P < 0.0001$).

Poudre Rearing Unit

Growth - At the Poudre Rearing Unit, size in the GR rainbow trout was closely matched to the Bellaire rainbow trout for the first four months at the facility, with the GR rainbow trout averaging 24.3 cm (n = 30, SD = 2.1) versus 23.6 cm (n = 30, SD = 2.2) for the Bellaire rainbow trout. These differences were not significant ($F_{[1, 58]} = 1.86$, $P = 0.1779$). At eight months, growth in the GR strain was slightly better (26.1 cm, n = 30, SD = 17.2) than the Bellaire (24.9 cm, n = 30, SD = 4.0), but the difference was not significant ($F_{[1, 58]} = 2.31$, $P = 0.1340$). By one year on the facility, the GR rainbow trout (35.0 cm, n = 30, SD = 4.3) were significantly ($F_{[1, 58]} = 19.07$, $P < 0.0001$) larger than the Bellaire strain (30.4 cm, n = 30, SD = 3.7).

Average weights of the two strains followed the same pattern as the lengths. After four months on the facility, the GR rainbow trout averaged 173.0 g (n = 30, SD = 48.0), and the Bellaire rainbow trout averaged 171.0 g (n = 30, SD = 44.5). These differences were not significant ($F_{[1, 58]} = 0.04$, $P = 0.8373$). After eight months on the on the facility, the GR strain averaged 190.5 g (n = 30, SD = 40.1), while the Bellaire strain averaged 180.1 g (n = 30, SD = 60.5). Again, the weights were not significantly different ($F_{[1, 58]} = 0.61$, $P = 0.4370$). When sampled at one year on the facility, the GR strain averaged 493.1 g (n = 30, SD = 132.9 g), and the Bellaire strain averaged 375.4 g (n = 30, SD = 122.1). Despite being an equivalent size, but younger than the Bellaire rainbow trout at the beginning of the grow-out period, the GR strain were significantly heavier ($F_{[1, 58]} = 12.74$, $P = 0.0007$) than the Bellaire strain.

M. cerebralis Infection - Testing for *M. cerebralis* resulted in identification of significantly higher prevalence and infection severity in the Bellaire rainbow trout than in the GR strain at the Poudre Rearing Unit (Figure 3.4). After four months, no infection was found in the head cartilage of any of the GR rainbow trout with histology, while lesions were found in 43.3% of the Bellaire rainbow trout. Histological scores in the Bellaire rainbow trout were low, with an average of 0.57 (SD = 0.73) on the McConnell-Baldwin scale of 0 – 5. No myxospores were found in any of the GR rainbow trout (n = 30) after four months. Prevalence of infection in the Bellaire strain as measured by PTD was 46.7% (n = 30), with an average (half-head) myxospores count of 3,657 (SD = 7,044).

Samples collected for PTD analysis after eight months resulted in only three of the GR rainbow trout identified as infected, with an average whole-head myxospore count of 3,440 (n =30, SD = 20,445). All of the Bellaire rainbow trout were found to be infected, with an average whole-head myxospore count of 84,993 (n = 30, SD = 86,791). Samples collected after the two strains had been reared for one year on the facility identified none of the GR rainbow trout (n = 30) as infected, while 90% (n = 30) of the Bellaire rainbow trout were identified as infected, with an average myxospore count of 361,099 (SD = 376,794) per fish.

Statistical test results for comparison of the infection severity and prevalence in the two strains for all of the sampling events at the Poudre Rearing Unit are provided in Table 3.2. The GR strain produced significantly ($P < 0.05$) lower infection severity and prevalence for each of the sampling events and testing methods.

Discussion

Growth of the GR strain rainbow trout was significantly better than both the Tasmanian strain rainbow trout at the Chalk Cliffs Rearing Unit and the Bellaire rainbow trout at the Poudre Rearing Unit. Growing conditions and temperature regimes were quite different at the two facilities, but the growth advantage of the GR strain was apparent at both locations. At the Chalk Cliffs Rearing Unit, where the two strains were reared under identical conditions from hatch, the GR strain reached stocking size of 23 cm, four months sooner than the Tasmanian strain. At the Poudre Rearing Unit, the GR and Tasmanian strain maintained relatively equal growth rates until the spring following transport to the facility, when accelerated growth occurred in the GR strain. This resulted in the GR strain fish outgrowing the Bellaire rainbow trout by the end of the evaluation, even though the Bellaire strain fish were two and a half months older than the GR strain fish at the beginning of the rearing period.

Infection prevalence and severity were significantly lower in the GR strain than both the Tasmanian and Bellaire strains by histological evaluation and myxospore counts in each of the sampling events in which the parasite was detected. Infection differences between the Tasmanian and the GR fish reared at the Chalk Cliffs facility were quite large, albeit not as pronounced by the end of the experiment as at the Poudre Rearing Unit. Initial exposure to *M. cerebralis* at Chalk Cliffs likely occurred after the fish were moved from the hatchery building to the raceways, at about six months post-hatch. The large differences in infection severity between the Bellaire and GR strains at the Poudre Rearing Unit are underscored by the increasing myxospore count in the Bellaire rainbow trout throughout the evaluation period. High prevalence of infection in the Bellaire rainbow trout also demonstrates the high susceptibility of the strain to infection. Only three of the GR strain fish were identified as *M. cerebralis*-positive in the Poudre Rearing Unit evaluations. These were found during the sample collected at eight months after the beginning of the rearing period. These results indicate that the infection was quite low, but present at marginally detectable levels, in the GR strain fish. With much

larger sample sizes, more infected GR fish would likely have been found at each of the sampling events.

Potential consequences of rearing and stocking a highly susceptible strain such as the Bellaire or Tasmanian rainbow trout are obvious. The parasite burden from these infected fish has the potential to greatly amplify the infection in both waters downstream from the facility if escapement occurs and in waters where the fish are stocked.
The results of these evaluations demonstrate that the GR strain has reduced infection prevalence and severity, as well as greater growth potential compared with other typical hatchery strains. The GR strain is a prospective replacement for these strains used for purely production purposes in areas where *M. cerebralis* is endemic, specifically where the parasite cannot be completely eradicated from water supplies.

Additional benefit could be realized from use of this or other resistant strains in facilities that do not harbor the parasite if fish are eventually stocked into waters where the parasite exists. Fish that are negative for the parasite, which are stocked into waters where *M. cerebralis* is endemic, can and do become infected after release (Nehring 2006). If these fish are not captured and removed before mature myxospores are developed, the end result can be an increase in parasite burden in the system. This reduces, and could completely negate, the benefits of stocking *M. cerebrali*s-negative fish where the parasite already exists. A strain such as the GR, which does not develop high levels of infection after release into waters where *M. cerebralis* is endemic, would help reduce the overall parasite burden in the receiving water.

Figure 3.1. Daily mean temperature, cumulative temperature units, and sample collections at the Chalk Cliffs Rearing Unit from January 2005 to January 2006.

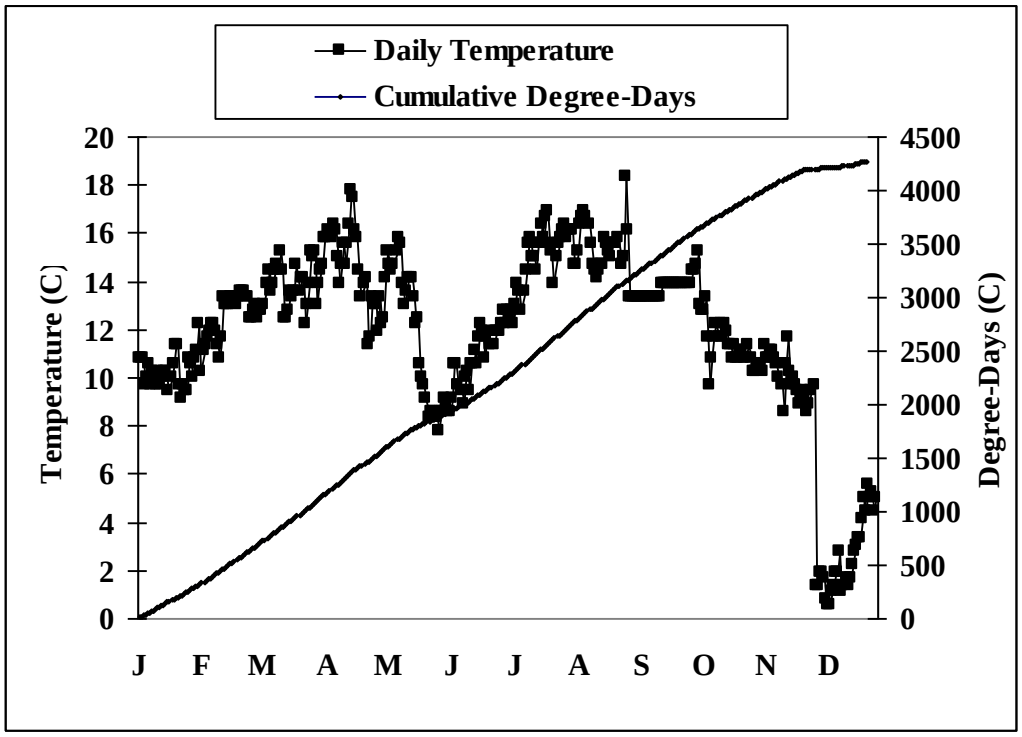


Figure 3.2. Daily mean temperature, cumulative temperature units, and sample collections at the Poudre Rearing Unit from July 2005 to July 2006.

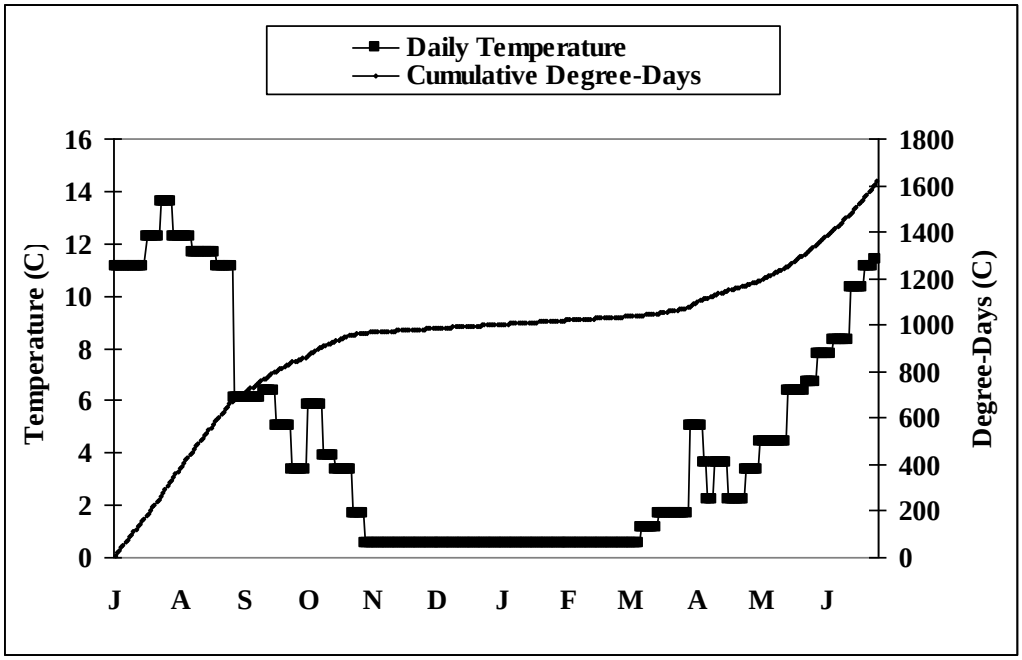


Figure 3.3. Growth rates for the GR and Tasmanian strain rainbow trout at the Chalk Cliffs Rearing Unit.

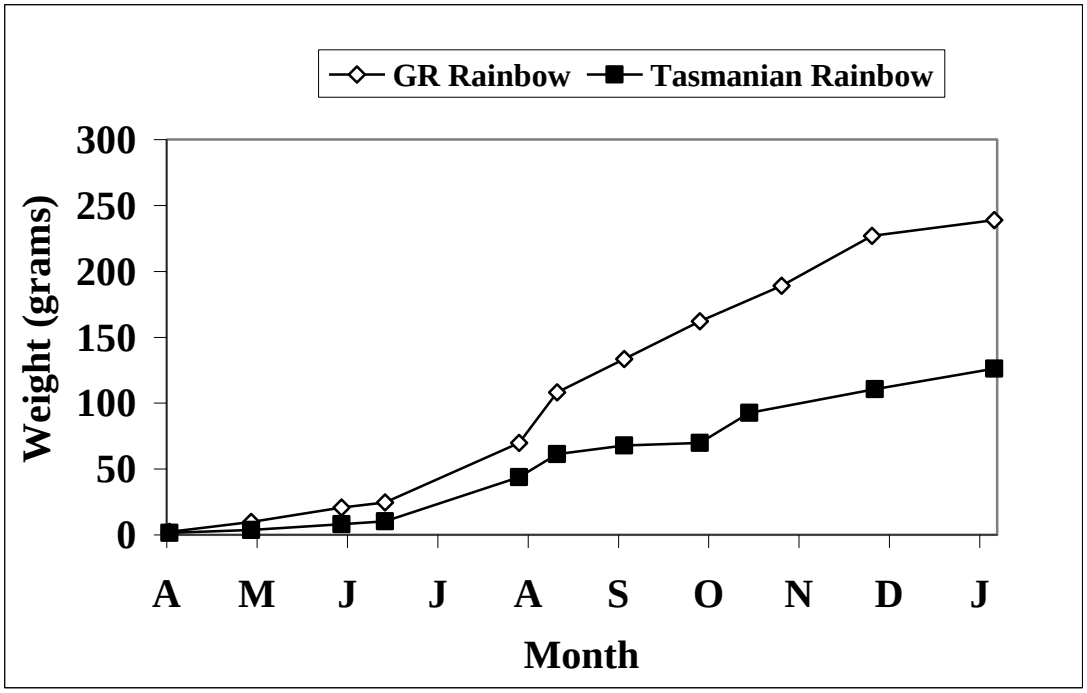


Figure 3.4. Myxospore counts for GR and Bellaire rainbow trout at four months, eight months, and one year at the Poudre Rearing Unit.

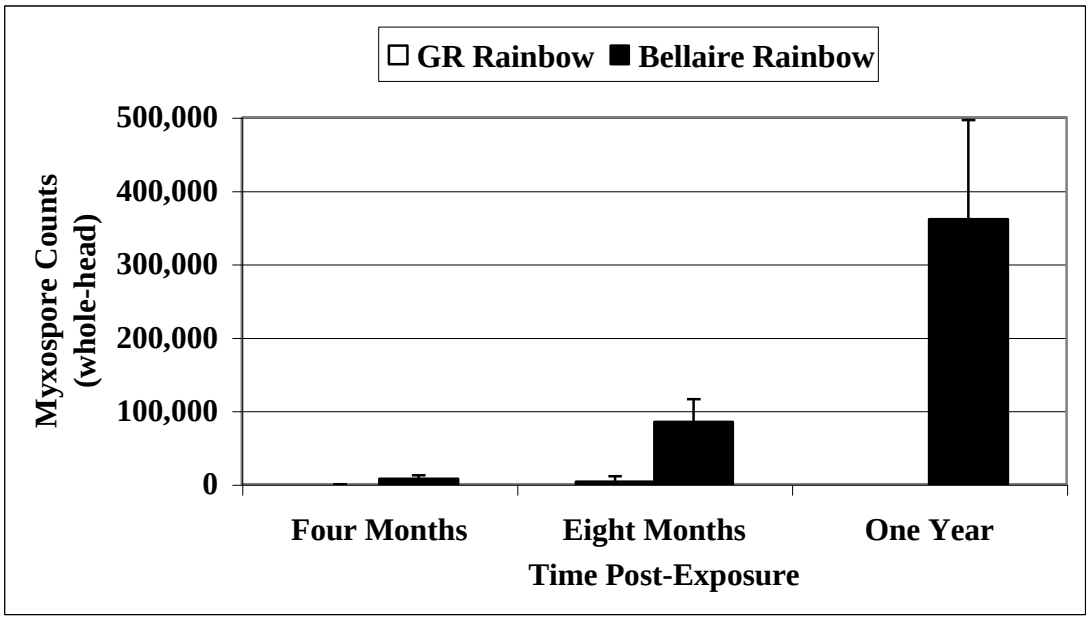


Table 3.1. Results of *M. cerebralis* infection evaluations at the Chalk Cliffs Rearing Unit. F-tests for comparisons of infection severity between the Tasmanian and GR strain rainbow trout are provided for each sample event.

	Tasmanian Rainbow Trout			**GR Rainbow Trout**			**F-Test**
	N	Infected (%)	Severity	N	Infected (%)	Severity	
Sample 1 (1,002 degree-days ºC)							
Histology Score (whole- body)	10	0	0	10	0	0	$F_{[1, 18]} = 0.0$, $P = 1.0$
Sample 2 (1,934 degree-days ºC)							
Histology Score (whole-head)	10	0	0	10	0	0	$F_{[1, 18]} = 0.0$, $P = 1.0$
Myxospore Count (whole-head)	10	0	0	10	0	0	$F_{[1, 18]} = 0.0$, $P = 1.0$
Sample 3 (3,468 degree-days ºC)							
Myxospore Count (whole-head)	30	96.7	48,883	30	73.3	5,175	$F_{[1, 58]} = 21.70$, $P < 0.0001$

Table 3.2. Results of *M. cerebralis* infection evaluations at the Poudre Rearing Unit. F-tests for comparisons of infection severity between the Bellaire and GR strain rainbow trout are provided for each sample event.

	Bellaire Rainbow Trout			**GR Rainbow Trout**			**F-Test**
	N	Infected (%)	Severity	N	Infected (%)	Severity	
Sample 1 (970 degree-days ºC)							
Histology Score (half-head)	30	43.3	0.57	30	0	0	$F_{[1, 58]} = 16.11$, $P = 0.0002$
Myxospore Count (half-head)	30	46.7	3,657	30	0	0	$F_{[1, 58]} = 8.09$, $P = 0.0061$
Sample 2 (1,039 degree-days ºC)							
Myxospore Count (whole-head)	30	100.0	84,993	30	10	3,440	$F_{[1, 58]} = 24.66$, $P < 0.0001$
Sample 3 (1,617 degree-days ºC)							
Myxospore Count (whole-head)	30	90.0	361,099	30	0	0	$F_{[1, 58]} = 27.55$, $P < 0.0001$

References

Bartholomew, J. L., and P. W. Reno. 2002. The history and dissemination of whirling disease. Pages 3-24 *in* J. L. Bartholomew 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. Diseases of Aquatic Organisms 55: 37-44.

Hedrick, R. P., T. S. McDowell, K. Mukkataira, M. P. Georgiadis, and E. MacConnell. 1999. Susceptibility of selected inland salmonids to experimentally induced infections with *Myxobolus cerebralis*, the causative agent of whirling disease. Journal of Aquatic Animal Health 11: 330-339.

Hoffmann, G. L. 1990. *Myxobolus cerebralis*, a worldwide cause of salmonid whirling disease. Journal of Aquatic Animal Health 2: 30-37.

Markiw, M. E. 1992. Experimentally induced whirling disease I. Dose response of fry and adults of rainbow trout exposed to the triactinomyxon stage of *Myxobolus cerebralis.* Journal of Aquatic Animal Health 4: 40-43.

Modin, J. 1998. Whirling disease in California: A review of its history, distribution, and impacts, 1965-1997. Journal of Aquatic Animal Health 10:132-142.

Nehring, R. B. 2006. Colorado's cold water fisheries: whirling disease case histories and insights for risk management. Special Report Number 79. February 2006. Colorado Division of Wildlife, Denver, Colorado.

Nehring, R. B., and P. G. Walker. 1996. Whirling disease in the wild: the new reality in the intermountain west. Fisheries (Bethesda) 21: 28-32.

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.

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., and E. P. Bergersen. 2002. Evaluation of risk of high elevation Colorado waters to the establishment of *Myxobolus cerebralis*. Pages 33-41 *in* J. L. Bartholomew and J. C. Wilson, editors. Whirling disease: reviews and current topics. American Fisheries Society, Symposium 29, Bethesda, Maryland.

Thompson, K. G., R. B. Nehring, D. C. Bowden, and T. Wygant. 1999. Field exposures of seven species or subspecies of salmonids to *Myxobolus cerebralis* in the Colorado River, Middle Park, Colorado. Journal of Aquatic Animal Health 11: 312-329.

Vincent, E. R. 1996. Whirling disease and wild trout: the Montana experience. Fisheries (Bethesda) 21: 32-34

Vincent, E. R. 2002. Relative susceptibility of various salmonids to whirling disease with emphasis on rainbow and cutthroat trout. Pages 109-115 *in* J. L. Bartholomew and J. C. Wilson, editors. Whirling disease: reviews and current topics. American Fisheries Society, Symposium 29, Bethesda, Maryland.

Wagner, E. J., C. Wilson, R. Arndt, P. Goddard, M. Miller, A. Hodgson, R. Vincent, and K. Mock. 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.

Hatchery Performance Evaluations: Summaries of growth data for GR and GR-cross varieties at participating State Fish Hatcheries

Summary of Results

Multiple lots of GR, GR-Harrison, and GR-Colorado River rainbow trout were produced at the Research Hatchery during 2006 and 2007. Many of these lots were distributed to the Fish Production Section for rearing as brood fish replacements or as part of the typical fish production schedule. Field trials with catchable plants of the pure GR strain strongly suggest that the GR strain or slightly outbred varieties of this strain would be good replacements for existing domestic strains currently used for catchable production. Performance of these strains in the State of Colorado hatchery system is important. The large numbers of fish produced each year by the Colorado Division of Wildlife represents a substantial investment. Efficient hatchery production is necessary to minimize costs and produce the greatest benefit to anglers. Growth and anecdotal information is reported here for those locations where data was compiled and reported by the respective hatchery managers. All data collected thus far has been generally positive with respect to the use of these strains as replacements for other strains that are more susceptible to *Myxobolus cerebralis* infection.

Bellvue-Watson

Two lots of pure GR strain rainbow trout have been reared at the Bellvue-Watson Rearing Unit, in 2006 and 2007. Growth results of the GR strain compared with other production rainbow strains are depicted in Figure 3.5. The lots reared at this facility are reported to be exceptionally fast-growing, with only five to six months required to reach the subcatchable size (13 cm; 5 inches). The pure GR strain tends to swim near the surface, making them more vulnerable to bird predation. Some questions have been raised about the susceptibility of the GR strain to formalin due to higher mortality is some lots treated for gill parasites with the chemical. Outbreeding of the pure GR strain with other strains such as the Harrison Lake strain seems to result in reduction of the unconventional swimming behavior.

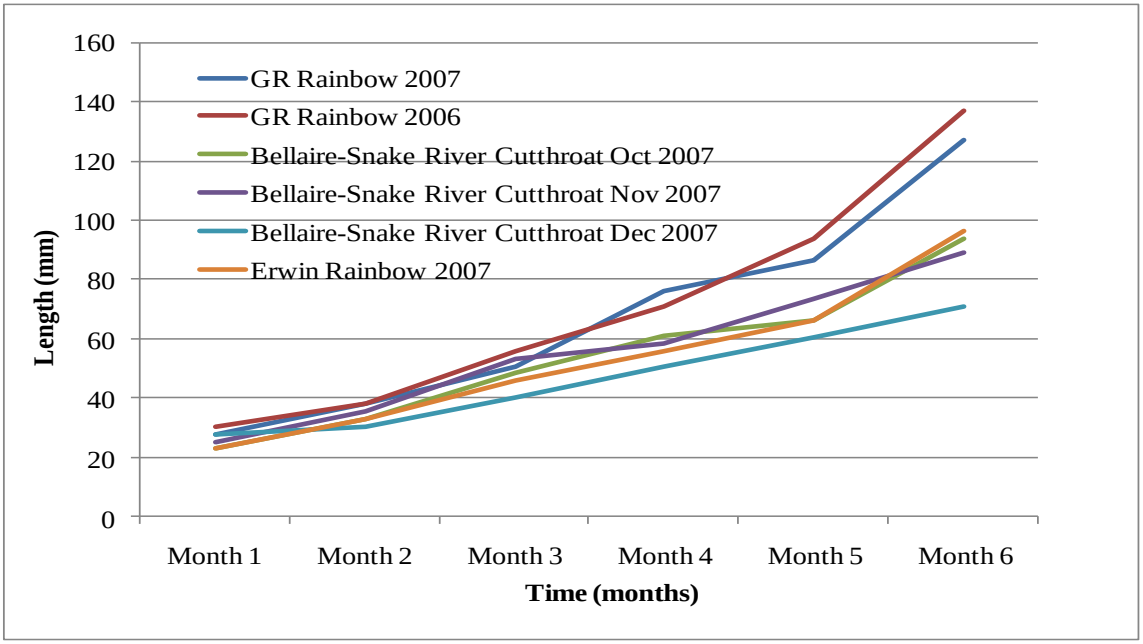


Figure 3.5. Growth of GR rainbow, Bellaire-Snake River cutbows, and Erwin rainbow trout at Bellvue-Watson in 2006 and 2007.

Crystal River Fish Hatchery

The ongoing field and laboratory trials have suggested that the GR-Harrison (75:25) strain rainbow trout would be a potentially good production fish and could be used to replace some existing production strains that normally carry much higher myxospore burdens when exposed to *M. cerebralis*. Eggs of this variety were sent to the Crystal River Hatchery in both 2006 and 2007 to be used as a future brood source. Growth results of the GR strain compared with other strains used as brood lots are depicted in Figure 3.6.

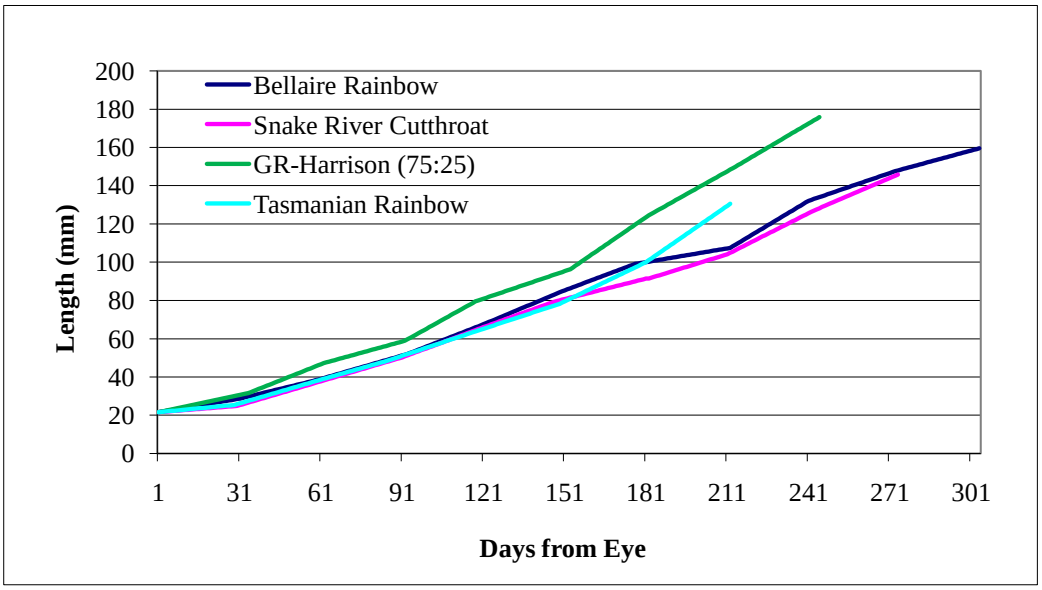


Figure 3.6. Growth of Bellaire rainbow, Snake River cutthroat, GR-Harrison (75:25) cross rainbow and Tasmanian rainbow trout at Crystal River Hatchery in fall, 2007, through spring, 2008.

Mt. Shavano Fish Hatchery

GR-Harrison (50:50) strain rainbow trout were sent to the Mount Shavano rearing Facility to be reared as part of a normal production run for stocking purposes. Growth results of the GR-Harrison strain compared with other strains used as brood lots are depicted in Figure 3.7. The GR-Harrison strain did grow quite well at the facility, but not as fast as the Puget Sound strain (Trout Lodge).

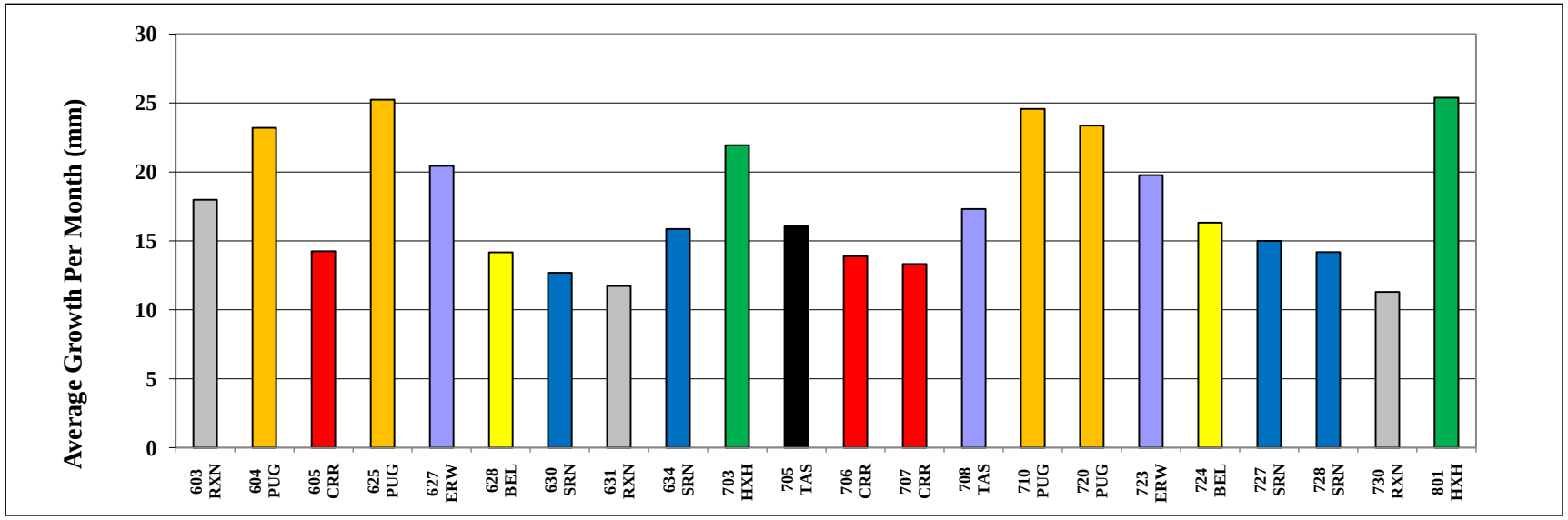


Figure 3.7 . Growth of Bellaire-Snake River cutbow (RXN), Puget Sound rainbow (PUG), Colorado River rainbow (CRR), Erwin rainbow (ERW), Bellaire rainbow (BEL), Snake River cutthroat (SRN), GR-Harrison (50:50) cross rainbow (HXH), and Tasmanian rainbow trout at Mount Shavano Rearing Facility in 2007.

Durango Fish Rearing Unit

A lot of GR-Colorado River rainbow (B2) eyed eggs were sent to Durango Fish Hatchery on December 24, 2008. Growth through the end of June, 2008 was slightly better than Bellaire strain rainbow trout (Figure 3.8).

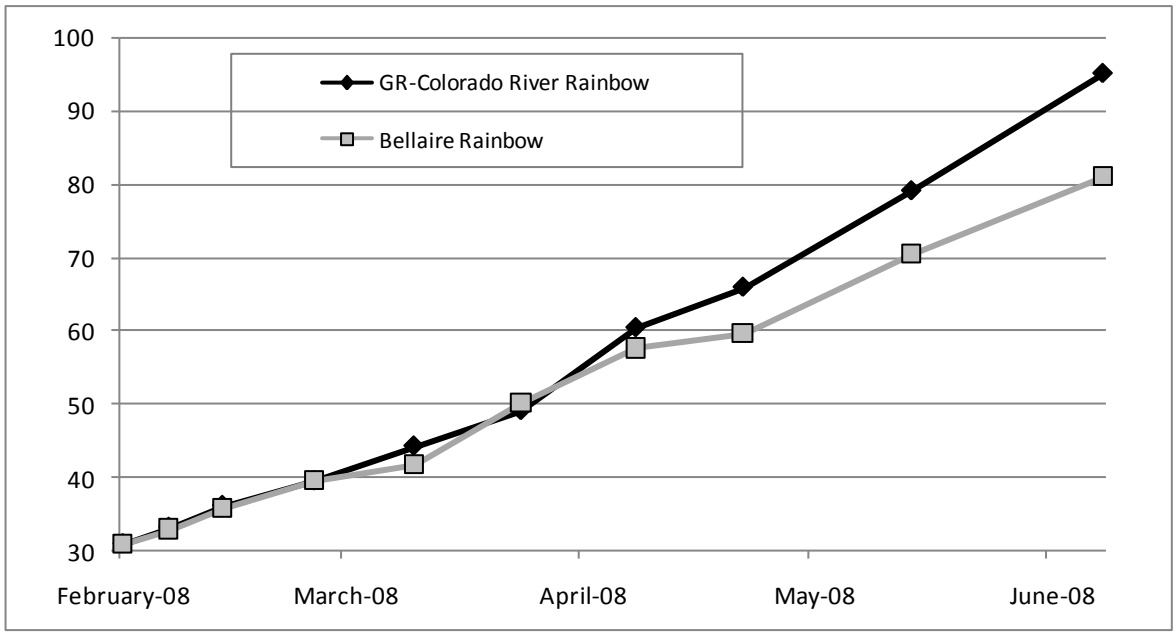


Figure 3.8. Growth of GR-Colorado River rainbow (F1 strain) compared with Bellaire rainbow at Durango Fish Rearing Unit, from February through June, 2008.

Field Performance Evaluations: Comparison of GR and Tasmanian strain rainbow trout as catchable plants in two put-and-take waters in Colorado.

Introduction

The GR strain rainbow trout has been identified as a strain that is highly resistant to *M. cerebralis* (Hedrick et al. 2003, Schisler et al. 2006). Other characteristics observed in laboratory experiments such as aggressive feeding behavior and rapid growth suggests that the strain may be useful as a catchable rainbow trout product. The GR rainbow trout appears to be a suitable replacement for other domestic strains used in Colorado from the standpoint of hatchery performance. However, performance of the GR strain compared to other standard domestic strains after it has been released to receiving waters has not yet been evaluated. In Colorado, approximately 3.2 million catchable-sized rainbow trout are produced per year for recreational angling. Catchable production fish raised for put-and-take use in Colorado are usually Tasmanian or Bellaire strain rainbow trout. In 2006, a study was designed to compare the GR rainbow trout with another standard domestic strain, the Tasmanian rainbow trout, as a catchable product in typical put-and-take waters. In 2007, the GR-Harrison strain was evaluated in the same manner as the pure GR strain in 2006.

Methods

2006

Hatchery Rearing - GR and Tasmanian strain rainbow trout were reared in parallel from egg to catchable size at Chalk Cliffs Rearing Unit, a facility that is positive for *M. cerebralis*. Eggs from both strains were hatched during the same week, and the conditions were identical for both strains throughout the rearing period of 16 months.

Stocking and Creel Surveys - Two front-range reservoirs, Flatiron and Pinewood reservoirs, were used as study locations for the catch and return to creel portion of the experiment. Both reservoirs are typical of coolwater reservoirs on the front range of Colorado in which fish are stocked for immediate recreational angling and harvest. These reservoirs, located northwest of Berthoud, Colorado, are typical high-use locations managed as put-and-take fisheries. Historical stocking rates have been from 15,000-30,000 catchable rainbow trout per year in each of the reservoirs. Pinewood Reservoir has also been stocked with 200-800 tiger musky (*Esox lucius x Esox masquinongy*) fingerlings per year.

A reduced number of fish were stocked into the reservoirs for the purposes of this experiment, rather than the full allocation of catchable and subcatchable fish normally stocked into these reservoirs. Fish of each strain were stocked in the reservoirs every two to four weeks from the beginning of April until the end of June. Equal numbers of each strain were stocked into each reservoir during each stocking event, with the exception of the last plant (Table 3.3).

	Flatiron Reservoir		Pinewood Reservoir	
	GR	Tasmanian	GR	Tasmanian
April 5, 2006	1000	1000	1000	1000
May 4, 2006	874	874	874	874
May 17, 2006	700	700	700	700
June 7, 2006	700	700	700	700
June 28, 2006	861	861	612	362
Totals	4135	4135	3886	3636

Table 3.3. GR and Tasmanian strain rainbow trout stocked from April through June, 2006, at Flatiron and Pinewood reservoirs.

One half of the fish stocked on each occasion were of the GR strain, and the other half were of the Tasmanian strain. The fish had been marked prior to stocking with fin clips to identify the fish by strain, GR fish with adipose clips, and Tasmanian stain with pelvic fin clips.

A creel schedule was created in which anglers were surveyed on both weekend days of every week, and two randomly chosen weekdays per week for the months of April through August. Two weeks at the end of March, 2006, were also included in the survey, prior to the official start of the study, to familiarize the creel clerks with the process. Angler counts were conducted five times daily throughout the daylight hours. Angler interviews were conducted between count times. Because the strains were differentially marked with fin clips, the creel clerk could easily distinguish between the two strains and catch estimates were made for both strains. During the angler interviews, additional questions were asked to determine if there was an angler preference between the strains. If there was a preference, the anglers were asked to describe which characteristics were most important in making that determination.

Supplemental Questionnaire Information - Supplemental questions were also asked to provide information on other unrelated topics. These were questions requested by management or hatchery section personnel. One question was an inquiry as to the number of days the angler ice-fished in the previous year. This was asked because relatively little statewide data exists on the proportion of anglers in Colorado that participate in ice fishing.

A second question was asked to determine if there was a preference for fish flesh color in catchable rainbow trout. This was asked because some preliminary work conducted by the Colorado Division of Wildlife Hatchery Section with Roxanthin-

enhanced feed demonstrated that flesh color in catchable-sized trout could be changed from white to pink for nominal cost per pound (Matt Schehrer, Mt. Shavano hatchery manager, personal communication).

Holdover Evaluation - Boat-mounted electroshocking was conducted at the end of the summer fishing season to evaluate fish remaining of each strain. Proportions of fish remaining were compared between strains. Samples were collected from surviving fish for analysis with pepsin-trypsin digest to determine myxospore counts in holdover fish.

Creel Survey Analysis - As part of the Technical Assistance portion of this Federal Aid project (Job 5), a new creel survey computer program was developed, based on the Colorado Division of Wildlife's DOS-based version of the original program. This program was used to generate creel survey analysis results for this study.

2007

Hatchery Rearing - Fry of both the Tasmanian and GR-Harrison strains were hatched under identical conditions at the Chalk Cliffs Rearing Unit in the same manner as the Tasmanian and pure GR fish in 2006. A pelvic fin clip was used to mark the Tasmanian strain fish, and an adipose fin clip was used to mark the GR-Harrison strain fish. At eight months of age, and at 16 months of age, 30 fish of each strain were collected from the facility for growth and infection severity evaluations. Weight and length of each fish was recorded, and the pepsin-trypsin digest method was used to quantify the myxospore load of each fish.

Stocking and Creel Surveys - Flatiron and Pinewood reservoirs were again used as the study areas for the comparisons. A creel survey schedule was generated to sample all weekend days, and two weekdays per week from April through September. The experimental fish were stocked three times in each reservoir from April through June, 2008 (Table 3.4). Fish were sorted to size match as closely as possible, although the average size of the GR-Harrison strain was larger due to their more rapid growth than the Tasmanian strain. Snake River finespot cutthroat trout x Bellaire rainbow trout (RXN) were stocked for management purposes after the experimental stocking was complete.

The creel survey schedule for 2007 was similar to that in 2006, with two weekdays and both weekend days being surveyed during each week, although during 2007 the survey was conducted from April through September. Angler counts were conducted as in 2006, except the frequency was reduced to four per day rather than five. Interviews were conducted between counts as in 2006. The fin clips used for 2007 were the same as in 2006, so the clerk was able to distinguish between strain of fish based on the fin clips, as in 2006.

Supplemental Questionnaire Information - Supplemental questions were asked during the interviews exactly as in 2006. More emphasis was placed on obtaining responses to each question during the interviews to produce larger samples all of the questions asked.

Flatiron Reservoir

	GR		Tasmanian		RXN	
	Number	Length	Number	Length	Number	Length
April 11, 2007	1000	11.4	1000	9.3	0	.
April 30, 2007	1001	11.8	1001	10.1	0	.
June 18, 2007	1281	12.6	1281	11.8	0	.
July 15, 2007	0	.	0	.	1254	10.3
July 22, 2007	0	.	0	.	1863	10.3
August 14, 2007	0	.	0	.	1834	10.3
September 5, 2007	0	.	0	.	1486	11.9
Totals	3282		3282		6437	

Pinewood Reservoir

	GR		Tasmanian		RXN	
	Number	Length	Number	Length	Number	Length
April 11, 2007	1000	11.4	1000	9.7	0	.
April 30, 2007	1001	11.8	1001	10.1	0	.
June 18, 2007	1281	12.6	1281	10.5	0	.
July 18, 2007	0	.	0	.	1430	9.9
July 24, 2007	0	.	0	.	1930	10.4
August 13, 2007	0	.	0	.	707	11.6
August 14, 2007	0	.	0	.	1834	10.3
September 5, 2007	0	.	0	.	1364	10.9
September 18, 2007	0	.	0	.	809	10.1
Totals	3282		3282		8074	

Table 3.4. GR and Tasmanian rainbow trout and RXN cutbow trout stocked from April through September, 2007, at Flatiron and Pinewood reservoirs.

Results

2006

Hatchery Rearing - The GR strain rainbow trout developed an average myxospore count of 5,175 (SD = 7,644) and the Tasmanian rainbow trout developing an average myxospore count of 48,883 (SD = 50,825) after 10 months of growth at the Chalk Cliffs rearing facility. Growth in the GR strain rainbow trout was significantly faster than in the Tasmanian rainbow trout with the GR strain reaching an average length of 282 mm (11.1 inches) and the Tasmanians reaching an average length of 234 mm (9.2 inches) at the time the first fish were stocked from the facility.

Catch by Strain - Raw data indicated that a much higher percent of the GR rainbow trout were captured than the Tasmanian rainbow trout (Figures 3.9 and 3.10). This was especially true during the months that stocking occurred. After stocking was halted, numbers of fish captured of each strain were more closely matched. Total catch reported was 34.6% higher for the GR strain than the Tasmanian strain in Pinewood Reservoir (549 GR versus 359 Tasmanian strain reported catch). Total reported catch was 19.2% higher for the GR strain than the Tasmanian strain in Flatiron Reservoir (1,011 GR versus 817 Tasmanian strain reported catch).

Creel Survey Analysis - Reports were produced by using the Creel Survey Analysis Program (C-SAP) in 2006 for the Flatiron and Pinewood data and reported in the 2007 Federal Aid Report. Those reports were re-run with the newest version of the program in 2007 for this report. Fish returns by strain were compared with numbers of fish stocked to determine the rate of return for each of the two strains (Figure 3.13).

Holdover Evaluation - Low numbers of both strains were found during the end-of-season electrofishing samples. In Flatiron Reservoir, only two GR and three Tasmanian rainbow trout were collected. In Pinewood Reservoir, only six GR and 26 Tasmanian rainbow trout were collected. These front-range reservoirs are subject to intense fishing pressure that typically results in seasonal depletions of stocked fish. It is notable, however, that more Tasmanian rainbow trout remained in both reservoirs at the end of the experiment. These results support the creel survey data, which indicated that the GR rainbow trout were caught more readily than the Tasmanian strain. Myxospores found in the Tasmanian rainbow trout averaged 122,074 (SD = 70,628) per fish, while those found in the GR rainbow trout averaged 210 (SD = 595) per fish at the conclusion of the experiment. In reservoirs where large numbers of holdover fish or mortality occurs, contribution of myxospores to the system could be quite different for the two strains. This could occur due to both the higher holdover rate and higher average myxospore count in the Tasmanian rainbow trout. Holdovers were not evaluated in 2007.

Angler Preference - Responses for each question were summarized separately. Not all questions were answered by all contacts, so number of respondents is not the same for each question. When asked about strain preference based on the fin clip marks,

22.6% of the 1,831 respondents chose the GR rainbow, compared with 3.2% that chose the Tasmanian rainbow. The remaining 74.2% had no preference. When asked about which characteristics they preferred with regard to the two strains, fighting ability was reported as the most important by 25.0% of 1,843 respondents. Only 1.8% reported that fish size was the most important characteristic. Catch rate was regarded as most important by 1.2% of the respondents, and appearance was most important to 0.3% of the respondents.

Other Questionnaire Responses - Angler participation in ice–fishing among the respondents was low. Of the 1,880 respondents, only 113 (6.0%) had ice-fished in the previous year. Average number of days fished per person that participated in ice-fishing was 5.06. When asked which color flesh was preferred, the anglers overwhelmingly chose pink flesh as the color of choice. Of the 1,918 respondents, 1,221 preferred pink flesh, 407 preferred white, and 141 preferred red. Only 149 anglers had no preference.

2007

Hatchery Rearing - Growth of the GR-Harrison strain in the 2007 lot was substantially greater than in the Tasmanian strain. Average length was 145 mm (SD = 19.1) in the Tasmanian strain compared with 182 mm (SD = 28.9) in the GR-Harrison strain after eight months (Table 3.5). At 16 months, average length of the Tasmanian strain fish was 221 mm (SD = 37.0), and average length of the GR-Harrison strain was 315 mm (SD = 28.6) (Table 3.6). Weight differences were even more dramatic, with average weight at eight months for Tasmanians at 35.8 g (SD = 13.5 compared with 75.7 g (SD = 27.1) for the GR-Harrison strain. At 16 months, average weight was 123.6 g (SD = 51.7), compared with 332.4 g (SD = 94.20) for the GR-Harrison strain.

Tasmanian rainbow trout developed an average myxospore count of 5,106 (SD = 8,999) after eight months on the facility (Table 3.7). No myxospores were found in any of the GR-Harrison strain trout tested. The Tasmanian rainbow trout developed average spore counts of 158,437 (SD = 239,901) after 16 months of growth at the Chalk Cliffs rearing facility. No myxospores could be found in any of the GR strain rainbow trout reared in 2007 (Table 3.8).

Catch by Strain - In 2007, raw data followed the same pattern for the GR-Harrison strain as was observed with the pure GR strain in the previous year. Higher catch was observed for the GR-Harrison strain than the Tasmanian strain at both reservoirs. At Flatiron Reservoir, 27.7% higher catch was reported for the GR-Harrison strain than for the Tasmanian strain (784 reported catch for the GR-Harrison strain versus 567 reported catch for the Tasmanian strain). At Pinewood Reservoir, a 24.7% higher catch was reported for the GR-Harrison strain than for the Tasmanian strain (548 reported catch for the GR-Harrison strain versus 440 reported catch for the Tasmanian strain).

Creel Survey Analysis - As with data collected in 2006, creel analysis reports for 2007 data were created using the newest version of the Creel Survey Analysis Program. Fish returns by strain were compared with numbers of fish stocked to determine the rate of return for each of the two strains (Figure 3.13).

Angler Preference - As in 2006, responses for each question were summarized separately. In 2007, the creel clerk was instructed to ensure that a response was provided for each question, which improved the reporting for the survey questions. When asked about strain preference based on the fin clip marks, 9.5% of the 2,441 respondents chose the GR-Harrison rainbow, compared with 1.1% that chose the Tasmanian rainbow. The remaining 89.3% had no preference. These responses were very similar to those received in 2006 (Figure 3.14). When asked about which characteristics they preferred with regard to the two strains, fighting ability was reported as the most important by 9.2% of 2,441 respondents. Only 1.1% reported that fish size was the most important characteristic. Catch rate was regarded as most important by 0.2% of the respondents, and appearance was most important to 0.5% of the respondents. These results were also strikingly similar to those received in 2006 (Figure 3.15).

Other Questionnaire Responses - Angler participation in ice–fishing among the respondents was low. Of the 2,441 respondents, only 160 (6.6%) had ice-fished in the previous year. When asked which color flesh was preferred, the anglers overwhelmingly chose pink flesh as the color of choice. Of the 2,441 respondents, 1,733 preferred pink flesh, 341 preferred white, and 149 preferred red. Only 218 anglers had no preference. Again, these results are nearly identical to the questionnaire responses received in 2006 (Figure 3.16).

Tasmanian			GR-Harrison		
Fish #	Length (mm)	Weight (g)	Fish #	Length (mm)	Weight (g)
1	153	38	1	184	72.5
2	160	44	2	152	37.5
3	149	34	3	200	84.8
4	122	22	4	223	133.1
5	154	41	5	205	96.2
6	135	28	6	190	70.3
7	158	42	7	198	85.2
8	145	30	8	165	47.8
9	173	50	9	194	89
10	161	46	10	179	70
11	115	18	11	163	51
12	129	23	12	210	105
13	168	53	13	204	100
14	169	51	14	172	62
15	144	34	15	204	87
16	150	36	16	205	94
17	169	63	17	185	74
18	165	51	18	210	116
19	128	23	19	137	30
20	145	37	20	163	48
21	149	38	21	154	42
22	132	25	22	196	82
23	143	33	23	187	74
24	123	23	24	197	91
25	180	66	25	220	122
26	130	21	26	177	58
27	144	32	27	175	68
28	137	25	28	142	33
29	97	10	29	93	72
30	127	23	30	137	30
Average	145.7586207	35.758621	Average	182.2068966	75.703448
St. Dev.	5.738528374	0.0787635	St. Dev.	7.173499864	0.1667477

Table 3.5. Length and weight of Tasmanian and GR-Harrison (50:50) strain rainbow trout at the Chalk Cliffs Fish Rearing Unit, August 15, 2006.

Tasmanian			GR-Harrison		
Fish #	Length (mm)	Weight (g)	Fish #	Length (mm)	Weight (g)
1	212	106	1	365	500
2	235	130	2	355	520
3	216	102	3	345	460
4	255	165	4	275	220
5	207	100	5	345	420
6	255	180	6	330	360
7	230	120	7	310	320
8	255	175	8	285	220
9	205	102	9	350	460
10	236	140	10	350	440
11	225	120	11	335	420
12	210	100	12	315	300
13	265	200	13	305	300
14	215	100	14	315	340
15	225	120	15	300	260
16	145	30	16	350	440
17	245	150	17	320	360
18	220	130	18	335	400
19	220	120	19	305	280
20	215	90	20	270	220
21	115	35	21	305	300
22	200	90	22	305	300
23	185	65	23	300	280
24	165	60	24	310	300
25	220	130	25	305	300
26	205	75	26	250	180
27	290	265	27	325	320
28	270	215	28	275	200
29	270	170	29	295	220
30	250	155	30	280	240
Average	221.0689655	123.62069	Average	314.8275862	332.41379
St. Dev.	8.703502579	0.2722923	St. Dev.	12.39478686	0.732189

Table 3.6. Length and weight of Tasmanian and GR-Harrison (50:50) strain rainbow trout at the Chalk Cliffs Fish Rearing Unit, March 27, 2007.

Case History Number			06-179		Location			Chalk Cliffs Hatchery	
Date Collected			08/15/06		Water Code				
Lot	Species	Age (Months)	Sample #	No. of Spores	No. of Grids	Measured Volume of Suspension (ml)	Final Volume	Spores per Head	Comments
1	TAS		1	0	18	0.00	0.00	0	Bag marked J
			2	1	18	3.00	3.03	1,683	
			3	8	18	3.00	3.03	13,467	
			4	0	18	0.00	0.00	0	
			5	2	18	3.00	3.03	3,367	
			6	2	18	3.00	3.03	3,367	
			7	0	18	0.00	0.00	0	
			8	1	18	3.00	3.03	1,683	
			9	0	18	0.00	0.00	0	
			10	5	18	3.00	3.03	8,417	
			11	0	18	0.00	0.00	0	
			12	0	18	0.00	0.00	0	
			13	2	18	3.00	3.03	3,367	
			14	18	18	3.00	3.03	30,300	
			15	0	18	0.00	0.00	0	
			16	0	18	0.00	0.00	0	
			17	0	18	0.00	0.00	0	
			18	18	18	3.00	3.03	30,300	
			19	12	18	3.00	3.03	20,200	
			20	0	18	0.00	0.00	0	
			21	1	18	3.00	3.03	1,683	
			22	0	18	0.00	0.00	0	
			23	0	18	0.00	0.00	0	
			24	0	18	0.00	0.00	0	
			25	0	18	0.00	0.00	0	
			26	13	18	3.00	3.03	21,883	
			27	6	18	3.00	3.03	10,100	
			28	2	18	3.00	3.03	3,367	
			29	0	18	0.00	0.00	0	
			30	0	18	0.00	0.00	0	
						Average Spores per Head		5,106	
						St. Dev.		8,999.9	
2	GR-HAR		1	0	18	0.00	0.00	0	Bag marked K
			2	0	18	0.00	0.00	0	
			3	0	18	0.00	0.00	0	
			4	0	18	0.00	0.00	0	
			5	0	18	0.00	0.00	0	
			6	0	18	0.00	0.00	0	
			7	0	18	0.00	0.00	0	
			8	0	18	0.00	0.00	0	
			9	0	18	0.00	0.00	0	
			10	0	18	0.00	0.00	0	
			11	0	18	0.00	0.00	0	
			12	0	18	0.00	0.00	0	
			13	0	18	0.00	0.00	0	
			14	0	18	0.00	0.00	0	
			15	0	18	0.00	0.00	0	
			16	0	18	0.00	0.00	0	
			17	0	18	0.00	0.00	0	
			18	0	18	0.00	0.00	0	
			19	0	18	0.00	0.00	0	
			20	0	18	0.00	0.00	0	
			21	0	18	0.00	0.00	0	
			22	0	18	0.00	0.00	0	
			23	0	18	0.00	0.00	0	
			24	0	18	0.00	0.00	0	
			25	0	18	0.00	0.00	0	
			26	0	18	0.00	0.00	0	
			27	0	18	0.00	0.00	0	
			28	0	18	0.00	0.00	0	
			29	0	18	0.00	0.00	0	
			30	0	18	0.00	0.00	0	
						Average Spores per Head		0	
						St. Dev.		0.0	

Table 3.7. Myxospore count results for Tasmanian and GR-Harrison rainbow trout at the Chalk Cliffs Fish Rearing Unit, August 15, 2006.

Case History Number			07-094		Location			Chalk Cliffs Rearing Unit	
Date Collected			03/27/07		Water Code			29341	
Lot	Species	Age (Months)	Sample #	No. of Spores	No. of Grids	Measured Volume of Suspension (ml)	Final Volume	Spores per Head	Comments
1	GR-HAR	16	1	0	18	0.00	0.00	0	
			2	0	18	0.00	0.00	0	
			3	0	18	0.00	0.00	0	
			4	0	18	0.00	0.00	0	
			5	0	18	0.00	0.00	0	
			6	0	18	0.00	0.00	0	
			7	0	18	0.00	0.00	0	
			8	0	18	0.00	0.00	0	
			9	0	18	0.00	0.00	0	
			10	0	18	0.00	0.00	0	
			11	0	18	0.00	0.00	0	
			12	0	18	0.00	0.00	0	
			13	0	18	0.00	0.00	0	
			14	0	18	0.00	0.00	0	
			15	0	18	0.00	0.00	0	
			16	0	18	0.00	0.00	0	
			17	0	18	0.00	0.00	0	
			18	0	18	0.00	0.00	0	
			19	0	18	0.00	0.00	0	
			20	0	18	0.00	0.00	0	
			21	0	18	0.00	0.00	0	
			22	0	18	0.00	0.00	0	
			23	0	18	0.00	0.00	0	
			24	0	18	0.00	0.00	0	
			25	0	18	0.00	0.00	0	
			26	0	18	0.00	0.00	0	
			27	0	18	0.00	0.00	0	
			28	0	18	0.00	0.00	0	
			29	0	18	0.00	0.00	0	
			30	0	18	0.00	0.00	0	
						Average Spores per Head		0	
						St. Dev.		0	
2	TAS	16	1	0	18	10.00	10.03	0	
			2	27	18	10.00	10.03	150,450	
			3	0	18	10.00	10.03	0	
			4	73	18	10.00	10.03	406,772	
			5	3	18	10.00	10.03	16,717	
			6	2	18	10.00	10.03	11,144	
			7	45	18	10.00	10.03	250,750	
			8	10	18	10.00	10.03	55,722	
			9	5	18	10.00	10.03	27,861	
			10	177	18	10.00	10.03	986,283	
			11	1	18	10.00	10.03	5,572	
			12	4	18	10.00	10.03	22,289	
			13	20	18	10.00	10.03	111,444	
			14	52	18	10.00	10.03	289,756	
			15	12	18	10.00	10.03	66,867	
			16	6	18	10.00	10.03	33,433	
			17	149	18	10.00	10.03	830,261	
			18	3	18	10.00	10.03	16,717	
			19	9	18	10.00	10.03	50,150	
			20	0	18	10.00	10.03	0	
			21	3	18	10.00	10.03	16,717	
			22	30	18	10.00	10.03	167,167	
			23	91	18	10.00	10.03	507,072	
			24	4	18	10.00	10.03	22,289	
			25	42	18	10.00	10.03	234,033	
			26	1	18	10.00	10.03	5,572	
			27	14	18	10.00	10.03	78,011	
			28	17	18	10.00	10.03	94,728	
			29	28	18	10.00	10.03	156,022	
			30	25	18	10.00	10.03	139,306	
						Average Spores per Head		158,437	
						St. Dev.		239,901.1	

Table 3.8. Myxospore count results for Tasmanian and GR-Harrison rainbow trout at the Chalk Cliffs Fish Rearing Unit, March 27, 2007.

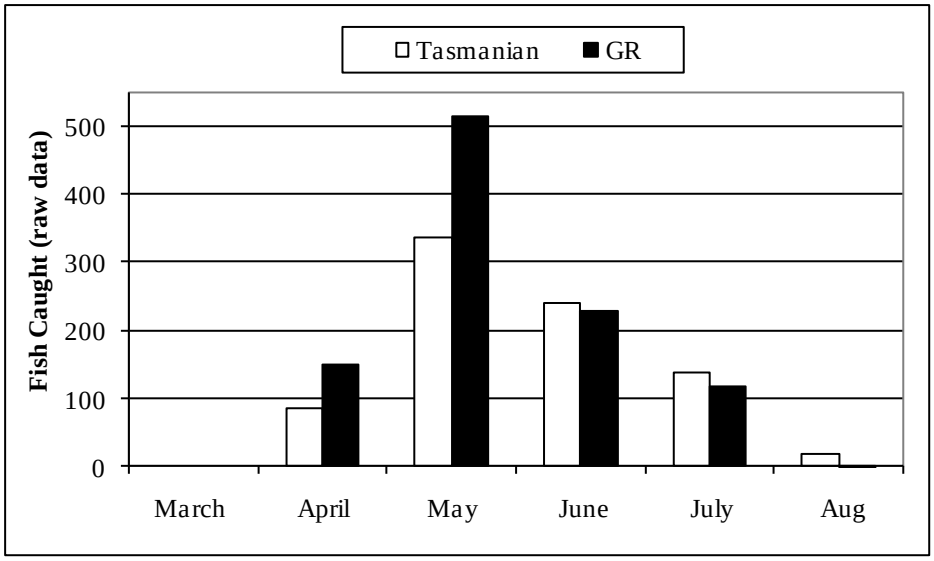


Figure 3.9. Catch data (raw data) for number of rainbow trout caught by strain at Flatiron Reservoir in 2006.

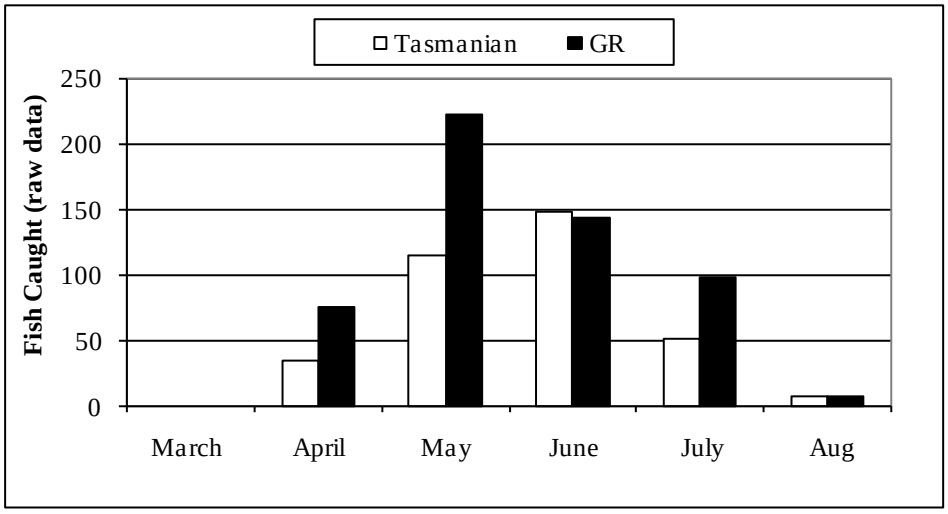


Figure 3.10. Catch data (raw data) for number of rainbow trout caught by strain at Pinewood Reservoir in 2006.

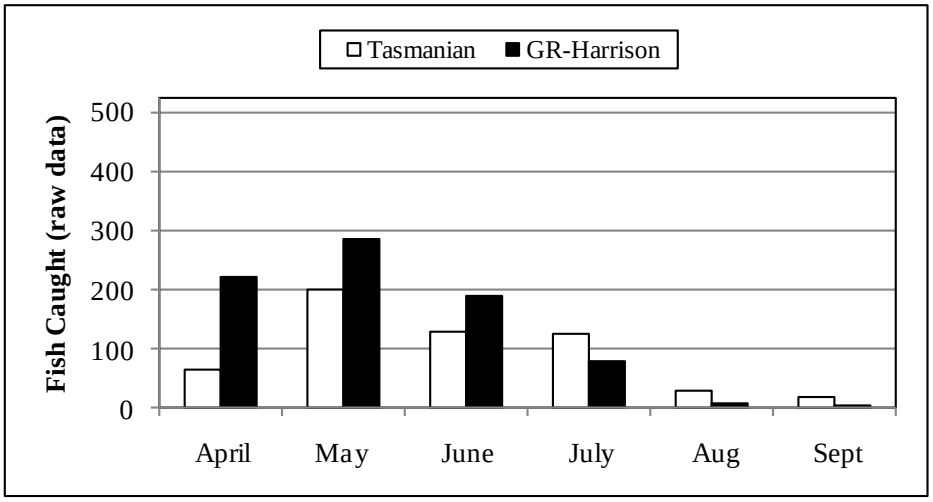


Figure 3.11. Catch data (raw data) for number of rainbow trout caught by strain at Flatiron Reservoir in 2007.

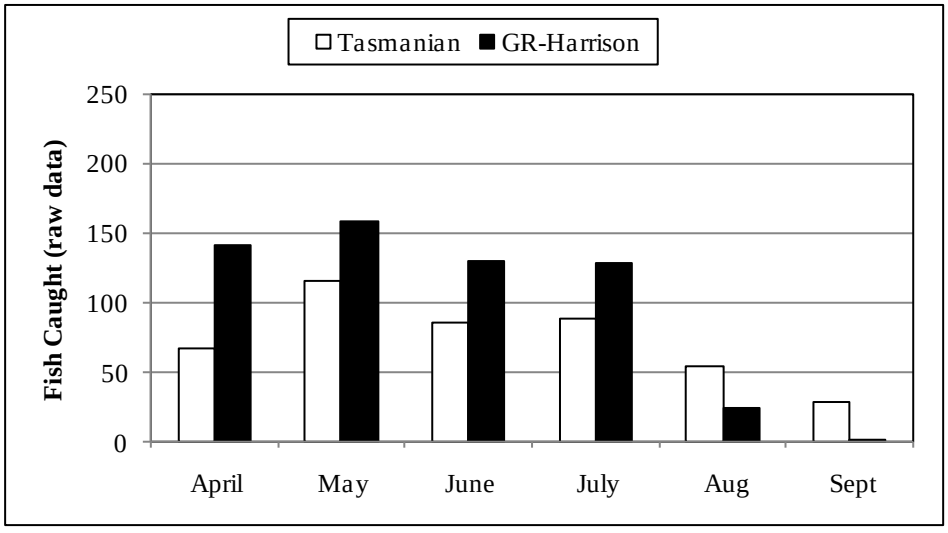


Figure 3.12. Catch data (raw data) for number of rainbow trout caught by strain at Pinewood Reservoir in 2007.

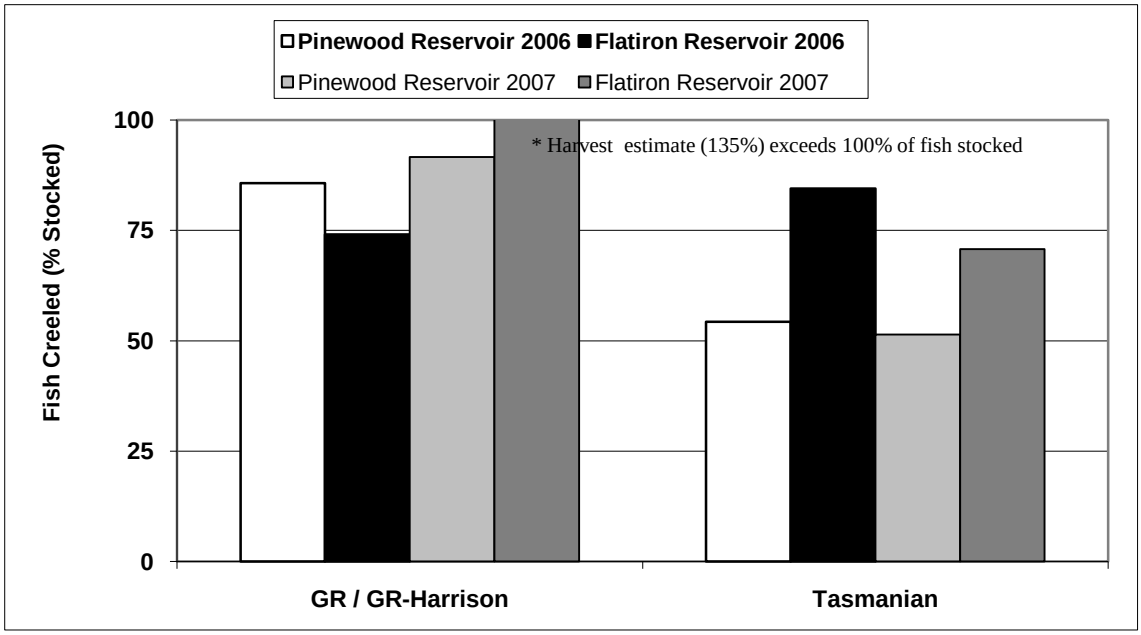


Figure 3.13. Proportion of fish returned to creel by strain for Flatiron and Pinewood reservoirs in 2006 and 2007 as estimated by the C-SAP Creel Survey Program.

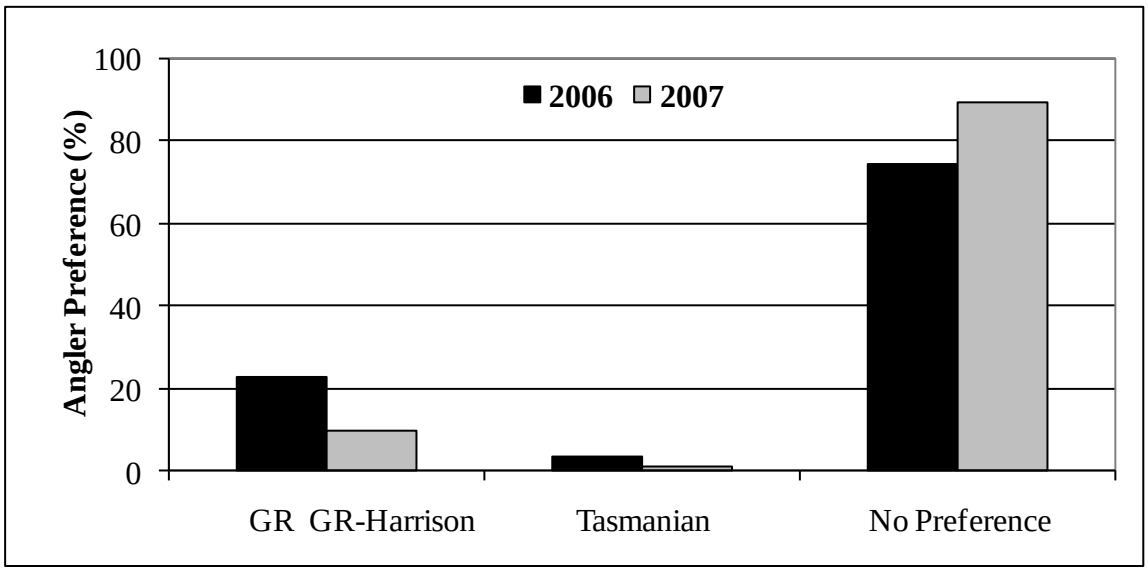


Figure 3.14. Angler preference by strain, as defined by fin clip, for Flatiron and Pinewood reservoirs in 2006 and 2007.

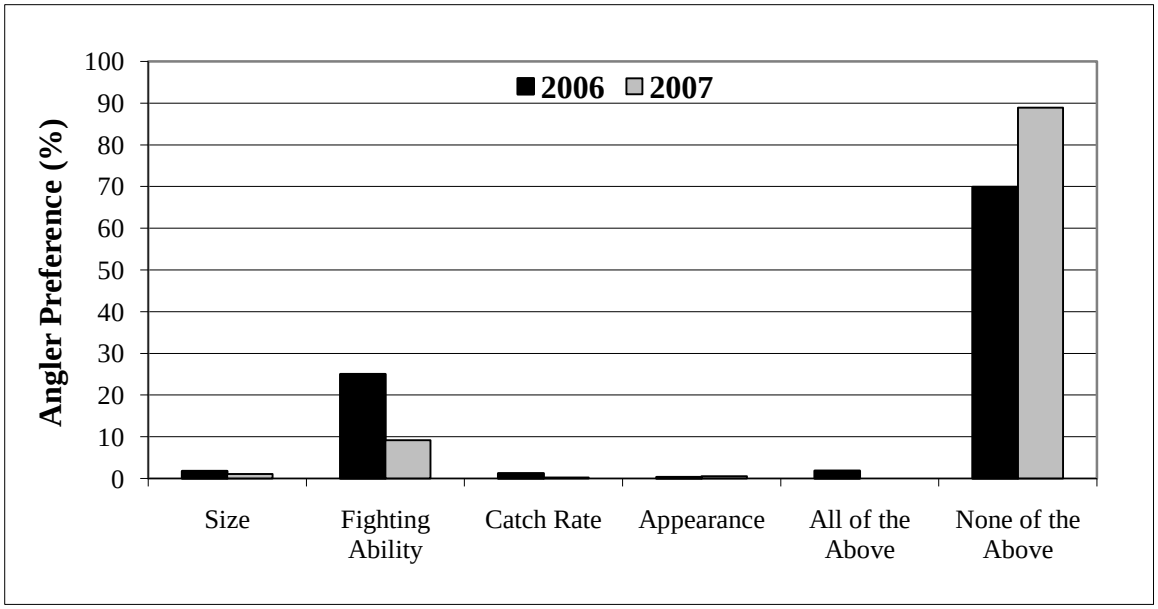


Figure 3.15. Characteristics of fish contributing to angler preference at Flatiron and Pinewood reservoirs in 2006 and 2007.

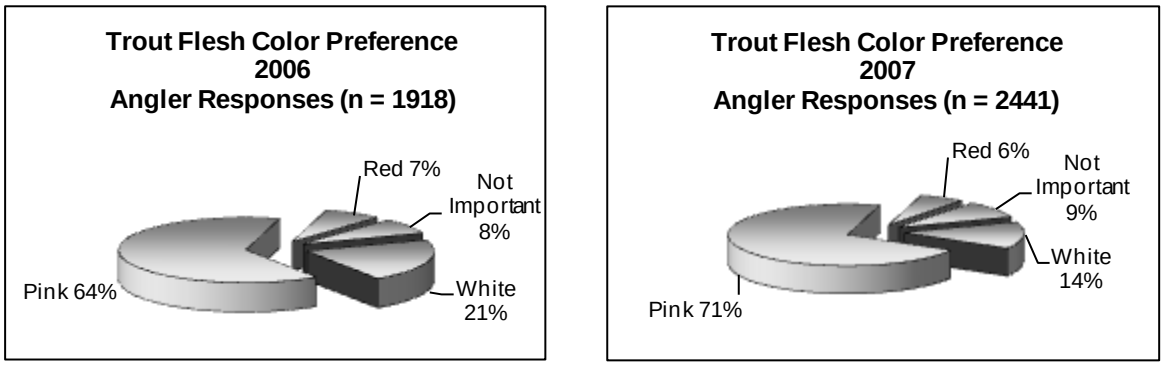


Figure 3.16. Angler preference for trout flesh color, Flatiron and Pinewood reservoir questionnaire results, 2006 and 2007.

Discussion

Rapid growth, high return to creel and angler satisfaction, and low myxospore production all support the conclusion that the GR or GR-Harrison strain could be useful replacements for other domestic strains used in Colorado for catchable rainbow trout production. The unofficial evaluations reported by the State Fish Production facilities support the conclusion that these strains of fish will be acceptable replacements as well.

The myxospore counts obtained from samples taken at the Chalk Cliffs Rearing Unit are very encouraging. The identification of no myxospores at all in the GR-Harrison lot, compared with 90% infection prevalence and an average spore count of 158,437 in the Tasmanian lot after 16 months on the facility, is very encouraging. These sorts of differences in infection severity further support the argument that resistant strains are a useful tool in reducing parasite burden in stocked fish.

Total catch, as defined by the raw data and by the creel survey estimates, was higher for the GR and GR-Harrison strain during both years and at both reservoirs. The only exception was for the Creel Survey estimate at Flatiron Reservoir in 2006, where the overall catch estimate for the Tasmanian strain was slightly higher than that of the GR strain. The average across both years and both reservoirs resulted in a total return to creel of 96.5% of the stocked GR and GR-Harrison fish, and a total return to creel of 65.25% of the stocked Tasmanian strain fish. This difference represents a higher recreational value provided by the GR strains in addition to the lower potential spore burden added to the system.

Lower returns were observed with both strains in Pinewood Reservoir during both years of the evaluations. Pinewood Reservoir is a little farther for fishermen to travel and the camping facilities are not as extensive, which could influence angler use. The outlet of Pinewood reservoir is also not conducive to retaining fish in the reservoir, with a vortex-like outlet structure having the potential to draw out fish. The principle difference between the two reservoirs, however, is the presence of large numbers of tiger muskies in Pinewood Reservoir. The impact of these fish on the catchable and fingerling plants in the reservoir is unknown, although predation on hatchery-produced trout would presumably be quite high.

Reported angler preference by strain favored the GR and GR-Harrison groups over the Tasmanian strain in both years, although the vast majority of anglers did not have a preference. GR strain and GR-Harrison strain were larger on average than the Tasmanian strain fish when stocked. This was unavoidable because of the rapid growth of the GR strain in the Chalk Cliffs Rearing Unit prior to stocking. While the anglers did not perceive fish size to be a major factor in preference between the two strains in either year, it is possible that the larger size of the fish affected their perception of the strain. Surprisingly, fighting ability was the only reported factor that influenced the angler's preference to any measurable degree during both years of the survey. Another interesting anecdote from the survey is that anglers preferred fish with pink flesh over white flesh by

a very large margin during both years of the survey, suggesting that feed additives may indeed be worthwhile to increase angler satisfaction.

References

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. Diseases of Aquatic Organisms 55: 37-44.

Schisler, G. J., Myklebust, K. A., and R. P. Hedrick. 2006. Inheritance of resistance to *Myxobolus cerebralis* among F1-generation crosses of whirling disease resistant and susceptible strains of rainbow trout. Journal of Aquatic Animal Health 18:109-115.

Field Performance Evaluations: Parvin Lake Fingerling Stocking Experiments

Introduction

Earlier experiments demonstrated that the GR and GRxHL crosses have excellent growth and return-to-creel when stocked as catchable-sized fish. The Colorado Division of Wildlife is aggressively transitioning its brood facilities to produce larger numbers of GR or GRxHL 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.

Parvin Lake, (Figure 3.17) 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-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 3.18). 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.

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 3.19). 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. This compares well with the figures from 2006, when over 60% of the total catch was white suckers.

Figure 3.17. Parvin Lake, Colorado.

Figure 3.18. Number of catostomids and salmonids caught at Parvin Lake the inlet trap (May-July) for years where data are available.

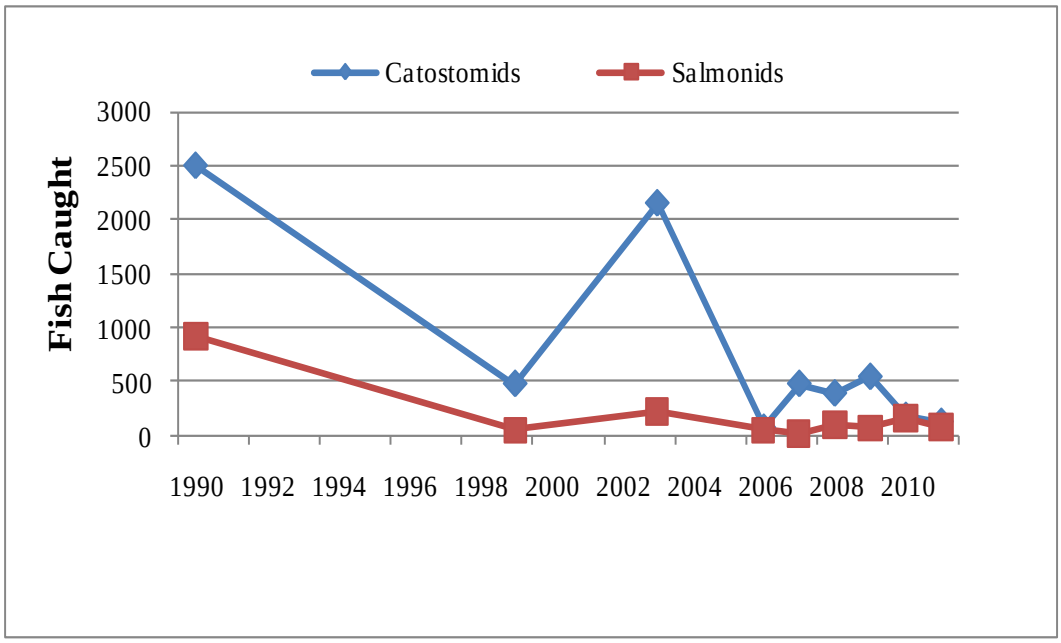
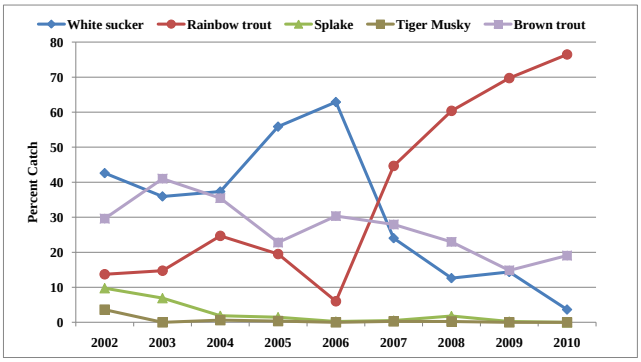


Figure 3.19. Percent of catch by species during fall electroshocking surveys for the years 2002 - 2010.



Methods

In order to evaluate survival and growth of multiple different varieties of fingerling trout, an initial live-release experiment was conducted in 2007. Preliminary returns of the different varieties, as well as fingerling strain availability were used to determine which varieties would be used for subsequent plants. In 2007, 2,800 fish each of the GR, HL, GRxHL (50:50), GRxHL (75:25), and Bellaire rainbow trout x Snake River cutthroat trout cross RXN (50:50) varieties were batch-marked with coded wire tags to identify returned fish by strain. These fish were reared as closely as possible to the same size before stocking. However, because of the rapid growth of the GR strain, and the very slow growth of the Harrison strain, sizes were not exactly matched (Table 3.9). The fish were all stocked at the same time into Parvin Lake on August 14, 2007.

In 2008, 2,050 fish of each GR, HL, GRxHL (50:50), GRxHL (75:25), and Bellaire rainbow trout x Snake River cutthroat trout cross RxN (50:50) were again batch-marked with coded wire tags. Similar difficulties with matching sizes of the Harrison Lake strain with the other varieties were encountered during the rearing period. These fish were stocked into Parvin Lake on July 31, 2008.

Fish stocked in 2009 included all of the eight varieties described in 2007 and 2008, along with the addition of the pure Tasmanian rainbow trout, the GRxHL (87.5:12.5) cross and the HHN cross as described in Job 2 (Table 3.10). The fish were stocked on August 12, 2009, and as in previous years, released in the lake inlet.

Collections of these 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 and 2010, 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. An attempt was made to collect 30 fish per event for each age class of marked fish, which was typically accomplished by shocking the entire perimeter of the lake over a three-hour time period.

Table 3.9. 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
GRxHL (50:50)	75.5	2800	104	GRxHL (50:50)	78.2	2050	117
GRxHL (75:25)	76.6	2800	104	GRxHL (75:25)	81.7	2050	117
RXN (50:50)	125	2800	122	RXN (50:50)	103	2050	127

Table 3.10. Coded-wire tagged fish stocked in Parvin Lake during 2009.

2009 Plants								
Strain	HL	TAS	GR	GRxHL (50:50)	GRxHL (75:25)	GRxHL (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

Results

Collections of fish from the 2007 plant (Figure 3.21) resulted in the RXN strain being most consistently more abundant in the samples than the other strains, contributing to 46.6% (198 fish) of the overall catch of 425 fish. The Harrison Lake strain contributed to 20.9% (89 fish) of the overall catch. The GRxHL (50:50 cross) contributed to 17.9% (76 fish) of the overall catch. The GRxHL (72:25 cross) contributed to 8.2% (35 fish) of the overall catch, and the pure GR strain contributed to 6.4% (27 fish) of the overall catch.

Collections of fish from the 2008 plant resulted in the RXN and GRXHL (50:50) cross being more abundant in the samples than the other strains (Figure 3.22). The RXN strain contributed to 38.8% (94 fish) of the overall catch of 242 fish. The Harrison Lake strain contributed to 17.4% (42 fish) of the overall catch. The GRxHL (50:50 cross) contributed to 29.3% (71 fish) of the overall catch. The GRxHL (72:25 cross) contributed to 9.9% (24 fish) of the overall catch, and the pure GR strain contributed to 4.5% (11 fish) of the overall catch.

Collections of fish from the 2009 plant were still relatively equal through June of 2011. A total of 318 fish were collected, with Harrison Lake being the most abundant at 19.5% of the catch (62 individuals). RXN and HHN were also present in high numbers, with 48 (15.1%) and 45 (14.2%), respectively. Catch for the three GRxHL crosses (50:50, 75:25, and 87.5:12.5) was 37 (11.6%), 35 (11.0%), and 28 (8.8%). Catch for the Tasmanian strain was 37 (11.6%), and catch for pure GR strain fish was only 26 individuals (8.2%).

Growth of the five strains was relatively equal for all strains for the 2007, 2008, and 2009 plants (Figures 3.24-3.26). The exception was the Harrison Lake strain, which grew slower than the other varieties in all year-classes. The pure GR strain were such a small proportion of the catch in both year-classes that it was difficult to evaluate growth. In fact, no GR strain fish from the 2008 plant were found after October of 2008.

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 collections 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 3.11). Figure 3.25 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 3.11. Myxospore results for five strains stocked in 2007 and 2008 for each collection period in 2009. 'NC' means no samples were collected for that strain and sample time.

	RXN		HL		GR-HL (50:50)		GR-HL (75:25)		GR	
	2007 Plant	**2008 Plant**	**2007 Plant**	**2008 Plant**	**2007 Plant**	**2008 Plant**	**2007 Plant**	**2008 Plant**	**2007 Plant**	**2008 Plant**
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**

Figure 3.20. Overall averages of myxospore counts for the 2007 and 2008 plants of five strains of trout during 2009 and 2010.

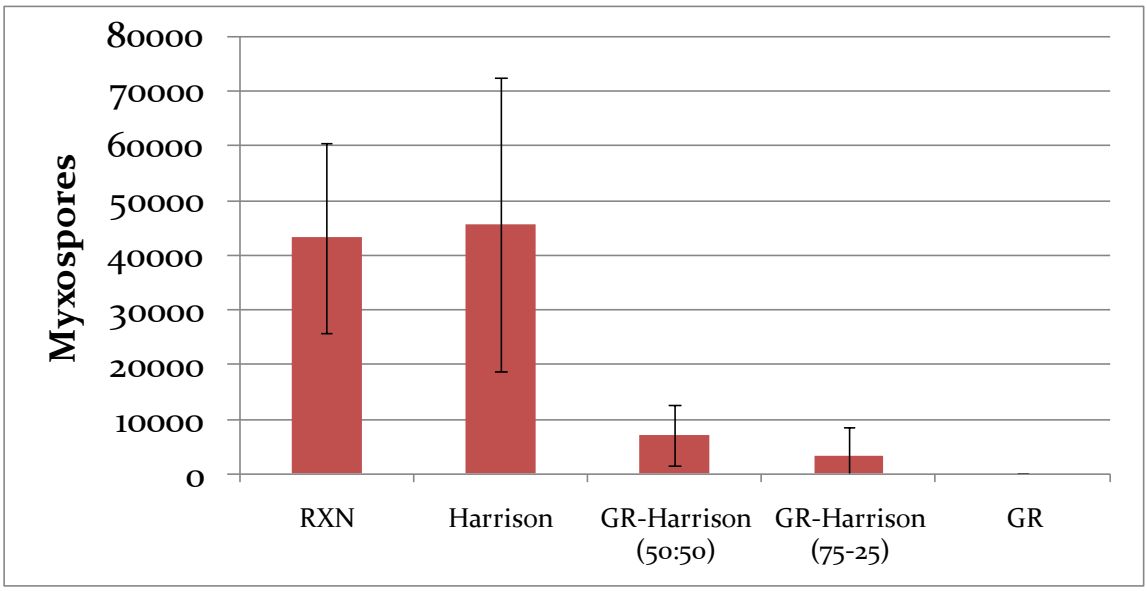


Figure 3.21. Cumulative catch for each of the five varieties of fingerling rainbow trout stocked in Parvin Lake in August, 2007.

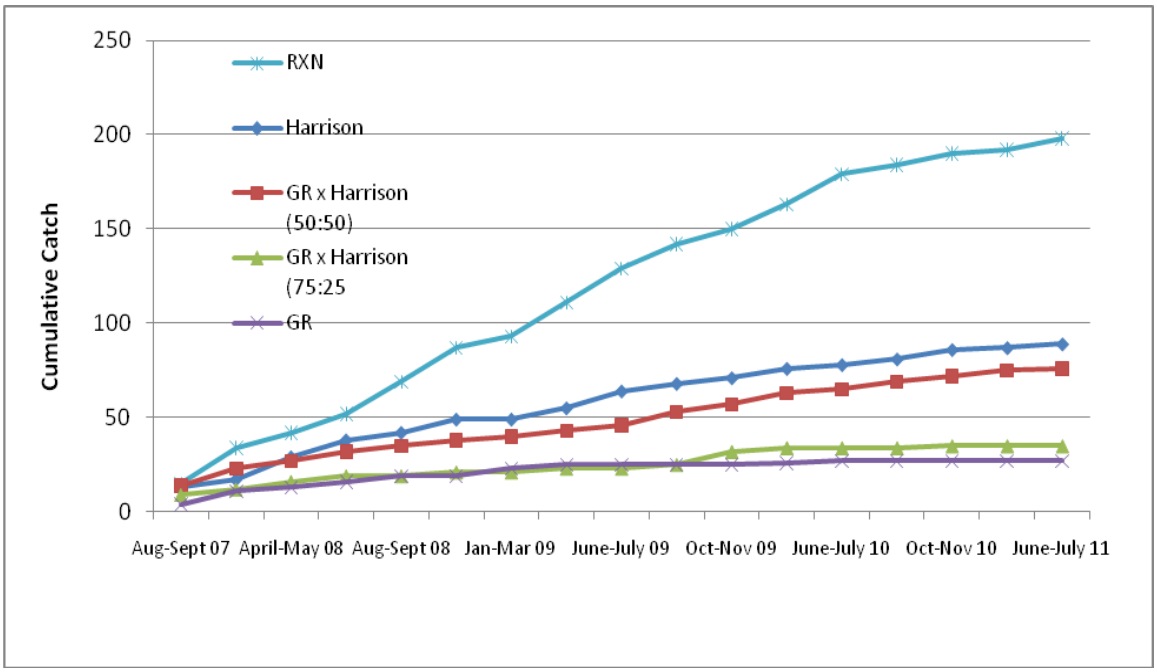


Figure 3.22. Cumulative catch for each of the five varieties of fingerling rainbow trout stocked in Parvin Lake in July, 2008.

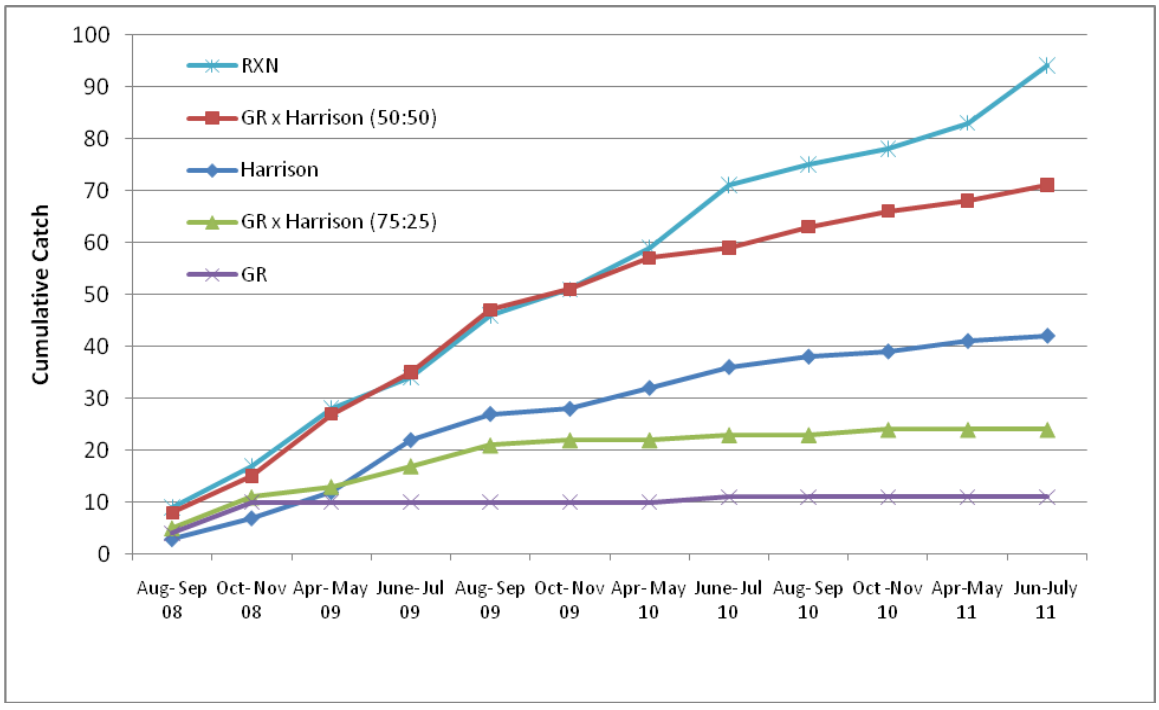


Figure 3.23. Cumulative catch for each of the eight varieties of fingerling rainbow trout stocked in Parvin Lake in July, 2009.

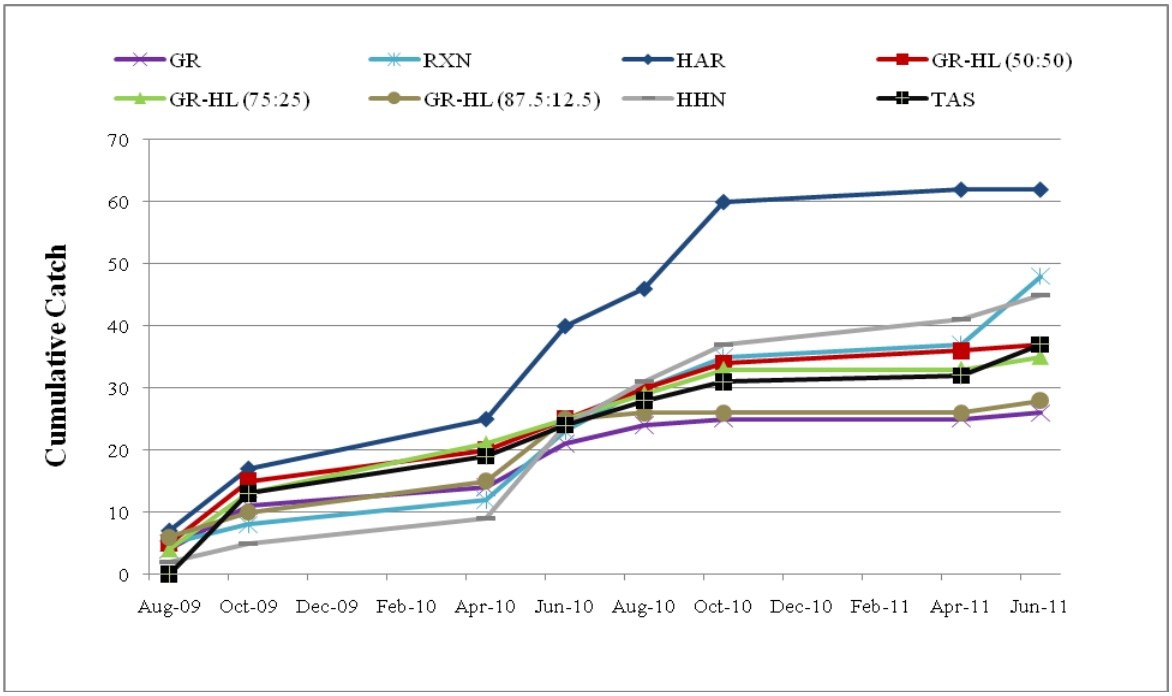


Figure 3.24. Fish length from 2007 through 2011 for each of the five varieties stocked in Parvin Lake in 2007.

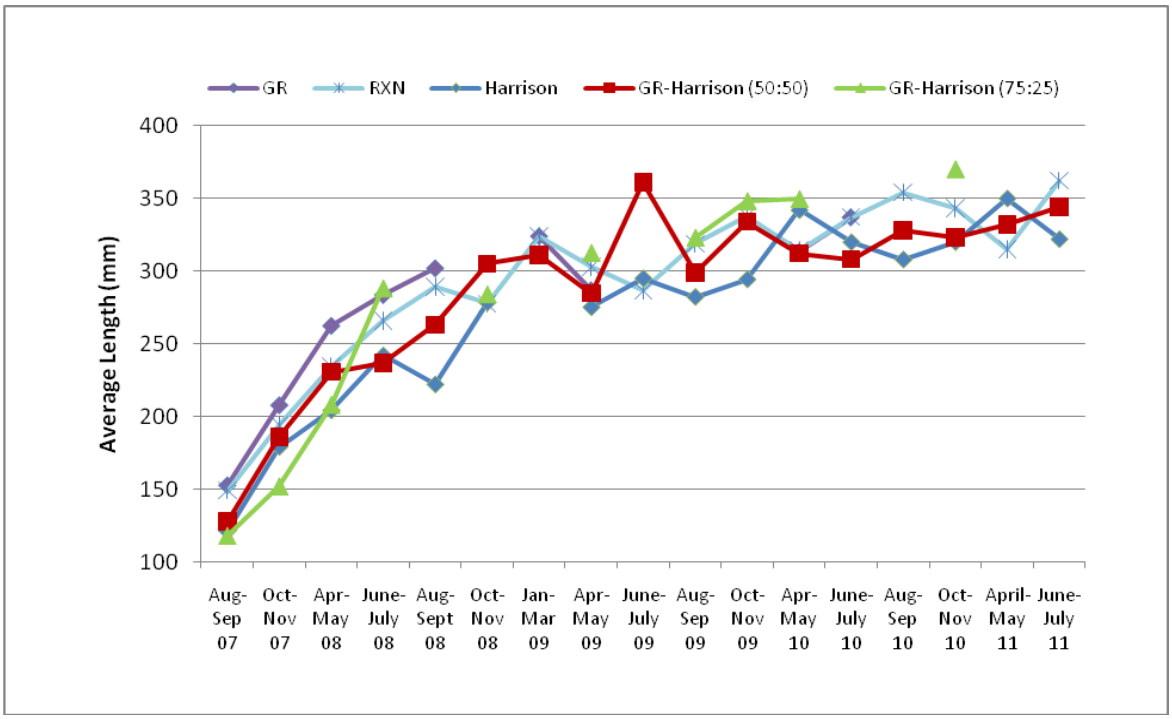


Figure 3.25. Fish length from 2008 through 2011 for each of the five varieties stocked in Parvin Lake in 2008.

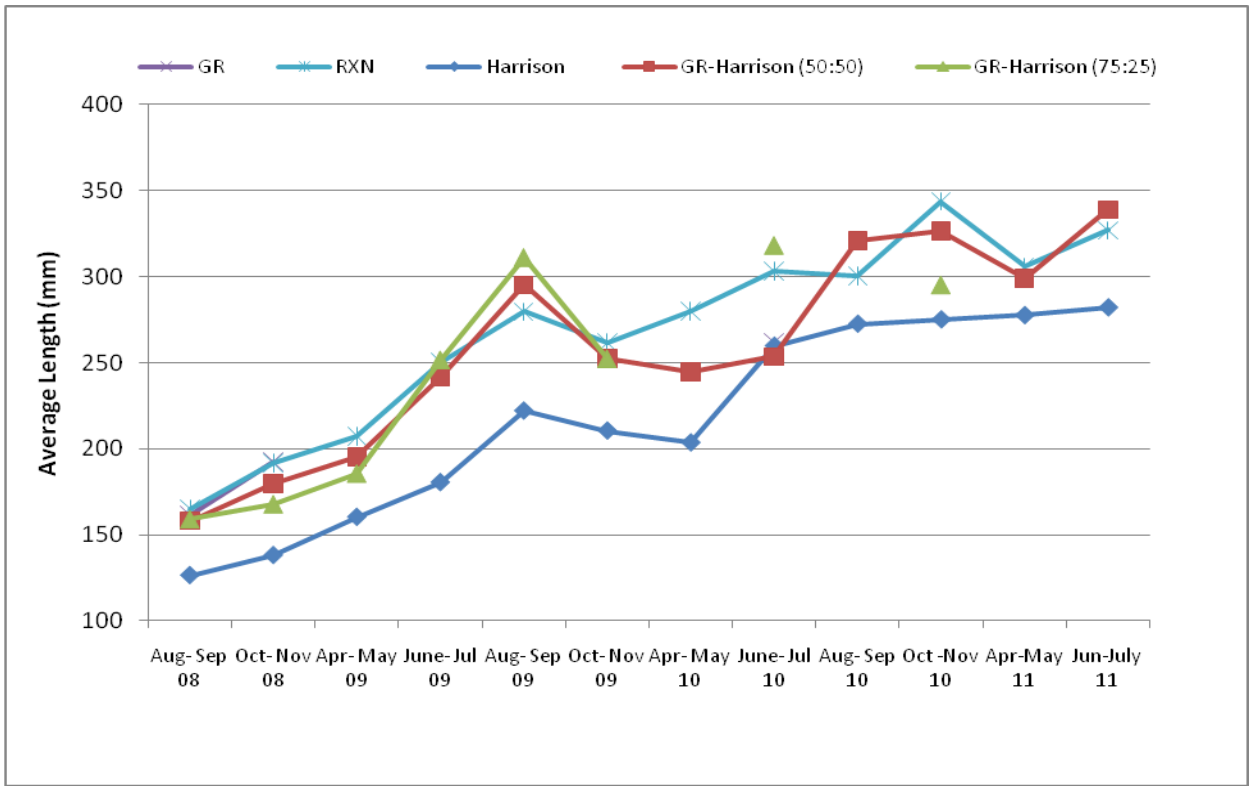
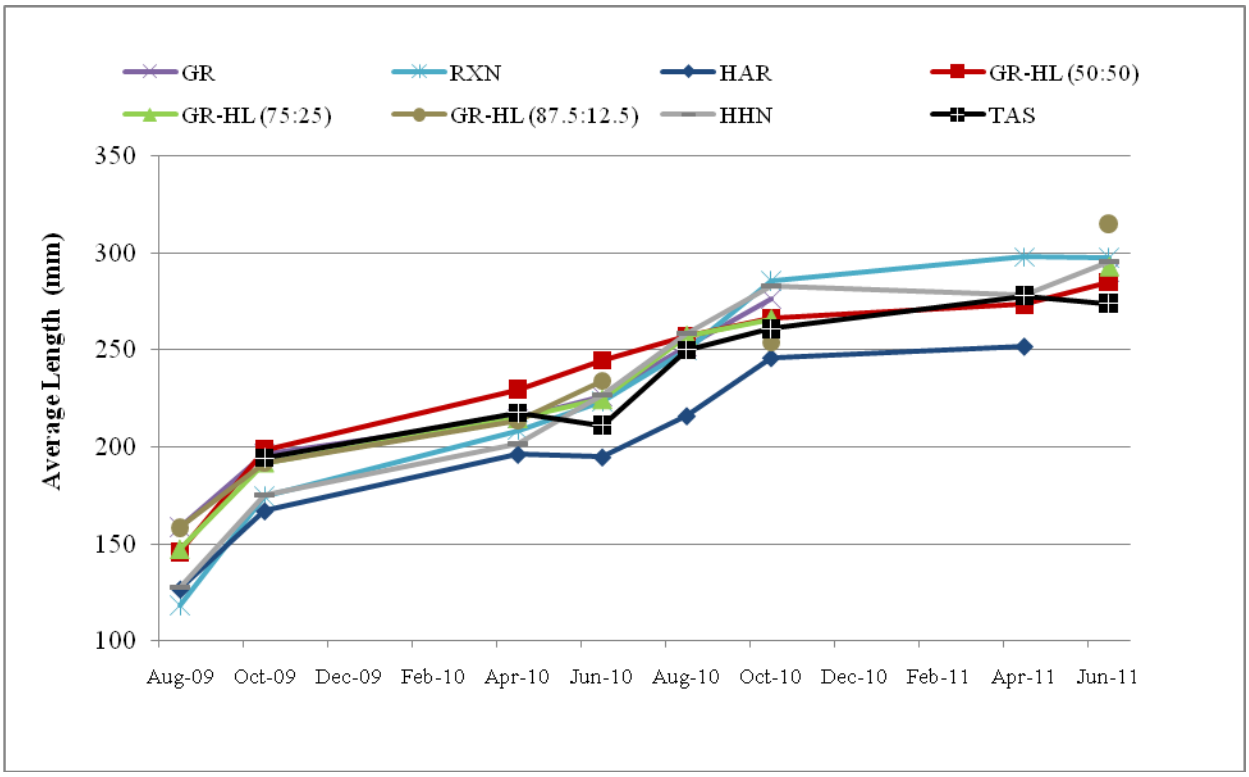


Figure 3.26. Fish length from 2008 through 2011 for each of the five varieties stocked in Parvin Lake in 2009.



Discussion

Given the relatively large size of the pure GR strain fish in both the 2007 and 2008 stocking events, their low return suggests that they may be more vulnerable to predation pressure than the other strains. This strain did poorly in the 2007 plant, and extremely poorly in the 2008 plant. The Harrison Lake variety was at a distinct disadvantage during both stocking events due to their smaller size, particularly in the 2007 stocking event, but managed to appear more often in the catch than all the other strains with the exception of the RXN fish in the 2007 plant. In general, it appears that a higher ratio of HL to GR in the crosses is advantageous to post-stocking survival with fingerling plants. The RXN group was much more abundant in the catch from the 2007 plan than the other strains. 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.

The myxospore counts found in the 2007 and 2008 plant collections are quite different among the strains. The GR and GR-HL crosses had a clear advantage with respect to infection severity. The Harrison Lake and the RXN strains both had much higher average myxospore counts.

Given the relatively high survival of the GR-HL (50:50) cross in both the 2007 and 2008 plants, and the low myxospore counts compared to the pure Harrison and the RXN varieties, the GR-HL (50:50) appears to be the best fit for fingerling reservoir plants in areas where *M. cerebralis* exists to optimize survival and minimize *M. cerebralis*.

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. The HHN strain has similar growth and lower parasite load than the RXN variety, and may have a similar survival rate. The strain is thus far performing well as a fingerling plant. Future study is warranted on this variety.

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

Job Objective: These experiments are designed to establish, develop and evaluate "wild" strain whirling disease resistant rainbow trout for reintroduction into areas where self-sustaining populations have been lost due to whirling disease.

Past Evaluations

A substantial effort has been exerted in the last several years to incorporate the Hofer (GR) resistant strains into both domestic and wild rainbow trout programs. Specific work conducted during the 2008-2011 field seasons is presented below.

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.

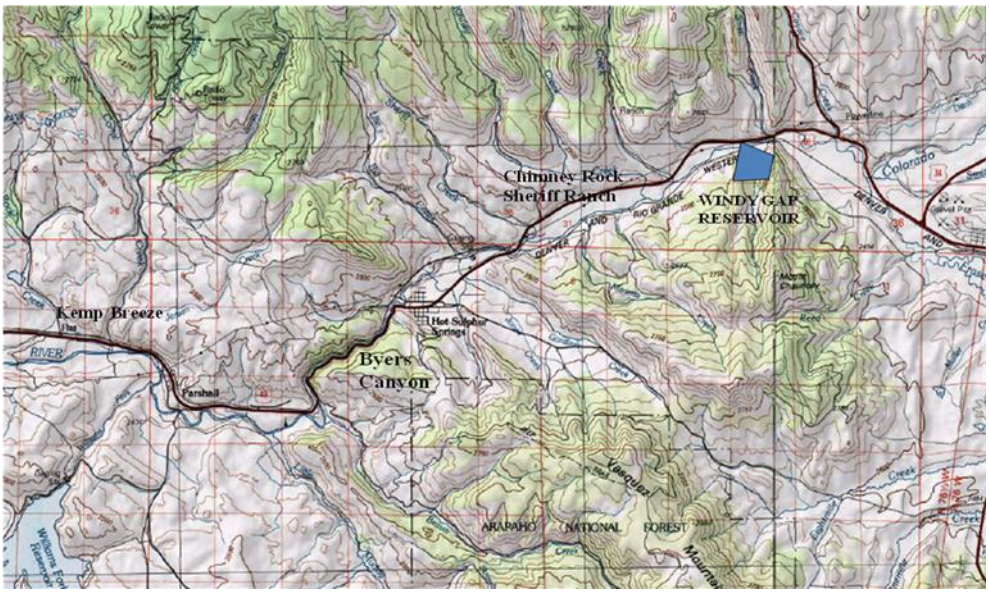
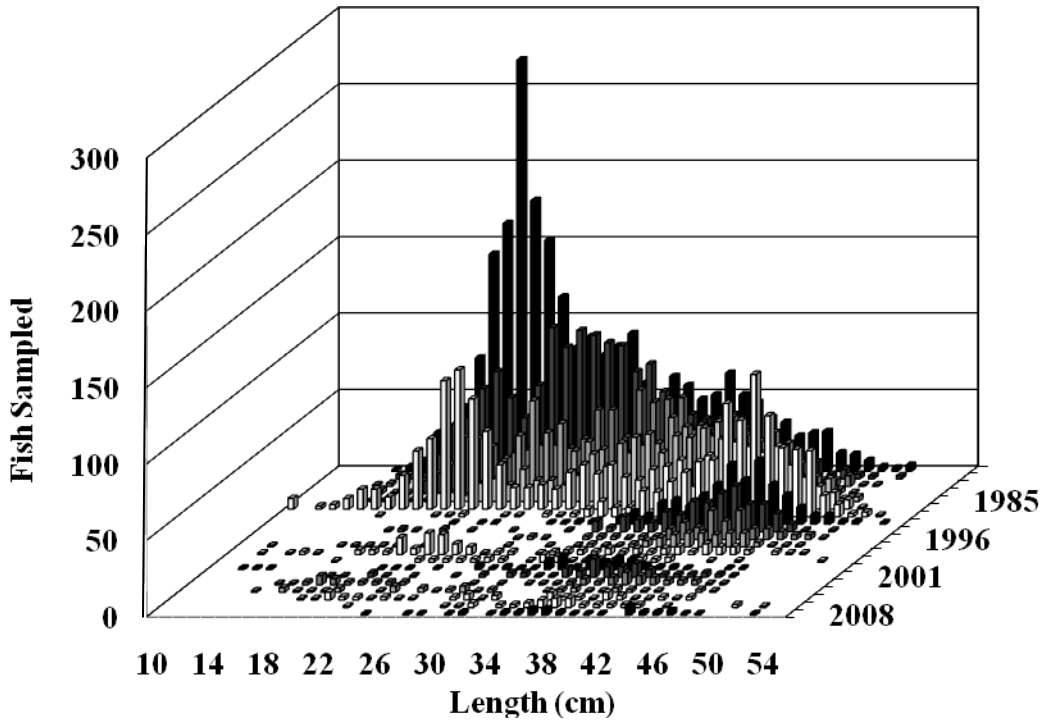


Figure 4.2. Upper Colorado River historic rainbow trout length-frequencies at Kemp-Breeze State Wildlife Area.

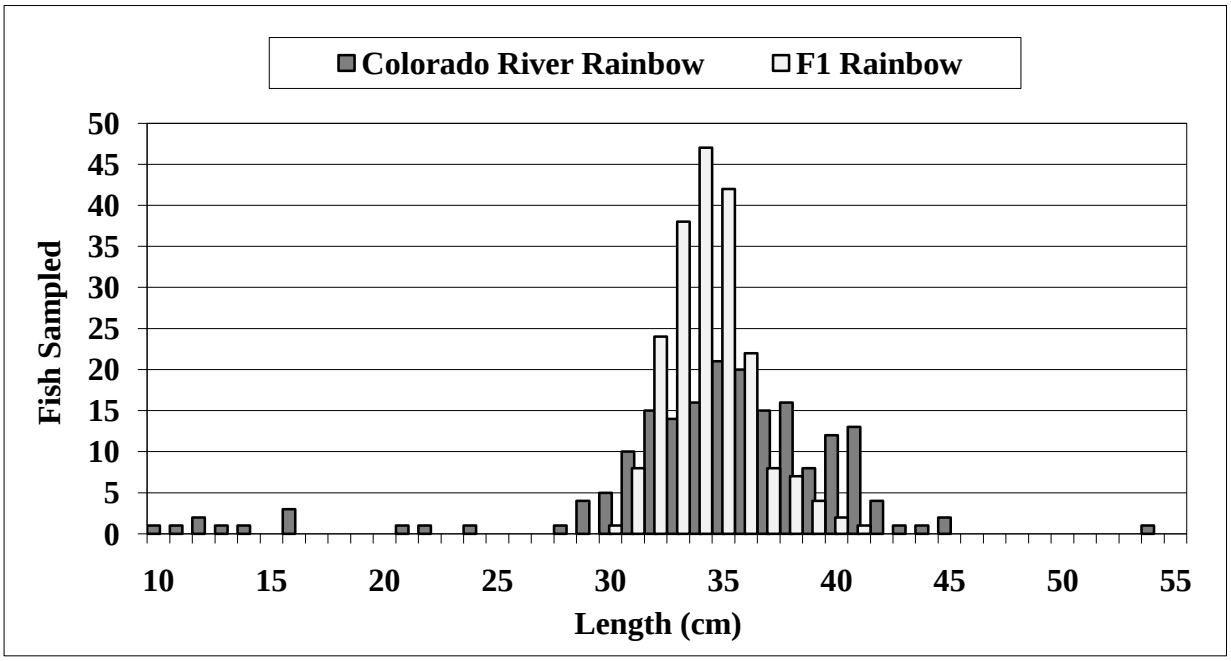


Initial Introductions and Sampling

In 2006, a single lot of GR x CRR 50:50 (F1) rainbow trout was stocked in the upper Colorado River, at 23.5 cm (9.4 in) total length (TL), to evaluate the survival of these larger fish (relative to previous plants) in an area dominated by brown trout, and with an extremely high prevalence of *Myxobolus cerebralis*. All rainbow trout were tagged with an individually numbered fine-filament Floy tags, and secondarily adipose clipped for identification in the event of tag loss, used to track individual growth and survival of the introduced fish. This introduction of rainbow trout has been monitored using annual population estimates. An extensive population estimate was conducted in the Chimney Rock/Sheriff Ranch section of the river in spring 2008. This sampling event was designed to evaluate the growth and survival of the F1 fish stocked in 2006, and also to determine what proportions of the fish were sexually mature. The population estimate consisted of a mark-recapture procedure conducted over 6.28 river km (3.9 river mi). Brown trout, which have increased dramatically in the river with the decline in rainbow trout numbers, were present in the reach at a density of 1,308 fish per km (2,092 fish per mi). CRRs (residual wild fish and fish present due to repeated stocking of CRR fingerlings) were estimated to exist at a density of 109 fish per km (175 fish per mi). The F1 rainbow trout from the 2006 plant were present at a density of 93 fish per km (148 fish per mi). They averaged 34.3 cm (13.5 in) TL, ranging from 30.0 to 40.9 cm (11.8 to 16.1 in) TL. In 2008, the fish from this single plant of 3,000 F1 fish comprised nearly half of the entire rainbow trout population in this stretch of river (Figure 4.3).

Of the 257 F1 fish examined, 32 (12.5 %) were found to be sexually mature. Of these, nine were females and 23 were males. The relatively high proportion of surviving F1 fish, and the onset of sexual maturity of many of these fish, was very encouraging. Typically, rainbow trout become sexually mature at age two or three under hatchery conditions, and later in natural environments. The identification of sexually mature rainbow trout from the 2006 stocking event appeared favorable with respect to re-establishing a wild rainbow trout population in this location. Fingerling fish were also collected in 2007 and 2008 and tested for the presence of GR rainbow trout genes using a quantitative trait loci (QTL) mapping and assignment technique. Details of the QTL mapping technique, and current results are presented in the "Genetic Techniques" section of Job No. 4.

Figure 4.3. Number of F1 and Colorado River rainbow trout encountered, by length, during the spring 2008 mark-recapture population estimate in the Chimney Rock/Sheriff Ranch section of the upper Colorado River.



2009 Field Season

In January 2009, a second introduction of 5,000 F1 rainbow trout, averaging 20.9 cm (8.2 in) TL and 107 g (0.2 lbs), occurred in the upper Colorado River. Prior to being introduced, all rainbow trout were tagged with an individually numbered fine-filament Floy tag, used to track individual growth and survival of the introduced fish. Fish were secondarily marked with an adipose clip for identification in the event of tag loss. Approximately two-thirds of the rainbow trout were introduced to the river via the Windy Gap Reservoir bypass flume, in which water was open and flowing, while the other third were introduced through a hole in the ice below Hitchin' Post Bridge, approximately one mile downstream of Windy Gap Reservoir. The objective of this second introduction was to increase the adult whirling disease resistant rainbow trout population in the upper Colorado River, making spawning between resistant rainbow trout more likely in this section of river.

On April 28 and 30, 2009, a population estimate was again conducted on the Chimney Rock/Sheriff Ranch stretch of the upper Colorado River. Two raft-mounted electrofishing units, one fixed-boom electrode unit and one throw electrode unit, were used for both the mark and recapture runs. All trout captured during the mark run were given a caudal fin punch for identification on the recapture run. All of the brown trout captured on the mark run were measured to the nearest millimeter. In addition, ten brown trout from each 10 millimeter size class, 150 mm and larger, were weighed to the nearest

gram. All rainbow trout captured on the mark run were measured to the nearest millimeter and weighed to the nearest gram. If an individual had a Floy tag, the number on the tag and tag color were recorded. If the individual could be identified as one from a previous plant, as evidenced by a missing adipose fin, but did not have a Floy tag, the fish was retagged with a new Floy tag and the number was recorded. In addition, the sex and reproductive status of each rainbow trout, if easily identifiable, were recorded. On the recapture run, all of the brown trout captured were measured to the nearest millimeter. Weights were recorded to the nearest gram for fish in any of the size classes that had not been completed on the mark run. All rainbows were measured to the nearest millimeter, weighed to the nearest gram, and checked for Floy tag number and color, sex, and reproductive status.

Population estimate were calculated using the Petersen estimator (with the Bailey (1951) modification). Brown trout were present in the reach at a density of 1,209 fish per km (1,934 fish per mi). CRRs, including residual wild fish and fish present due to repeated stocking of CRR fingerlings, were estimated to exist at a density of 30 fish per km (48 fish per mi). F1 rainbow trout from the 2006 plant were present at a density of 41 fish per km (66 fish per mi). No F1 rainbow trout from the January 2009 plant were encountered during the population estimate. Other fish species encountered during the estimate included speckled dace (*Rhinichthys osculus*), white sucker (*Catostomus commersoni*), longnose sucker (*Catostomus catostomus*), bluehead sucker (*Catostomus discobolus*), and brook trout *(Salvelinus fontinalis*).

Average length of the 2,229 brown trout encountered during the estimate was 32.7 cm (12.8 in) TL, ranging from 7 to 53.7 cm (2.8 to 21.1 in) TL. The 92 F1 rainbow trout encountered averaged 36.8 cm (14.5 in) TL, ranging from 32.7 to 44 cm (12.8 to 17.3 in) TL. The 84 CRR encountered averaged 36.5 cm (14.4 in) TL, ranging from 14 to 49.5 cm (5.5 to 19.5 in) TL (Figure 4.4). F1 rainbows averaged 532 g in weight, ranging from 290 to 1,030 g, and CRR averaged 520 g, ranging from 124 to 1,254 g. As with the population estimate in 2008, the F1 fish stocked in 2006 comprised a large proportion of the total rainbow trout population in the study area (Figure 4.5).

Of the 92 F1 fish that were handled during the population estimate, 32 (14 females and 22 males) were found to be sexually mature and ripe. An additional 20 females were sexually mature, but in pre-spawn status (green). Twenty-nine fish were green and of unknown sexual status, but appeared that they could be potentially ripe later in the spring. Only seven were clearly immature and did not appear to be potentially sexually mature in 2009. Eighty-three CRR individuals were handled during the population estimate, and of those, 22 were found to be sexually mature and ripe (14 females, eight of which were already spent, and eight males). An additional 16 green females and 39 green fish of unknown sexual status were present. Six sexually immature CRR individuals were also encountered.

Figure 4.4. Length-frequency distribution of brown trout, Colorado River rainbow trout, and F1 (2006 plant) rainbow trout encountered during the spring 2009 mark-recapture population estimate in the Chimney Rock/Sheriff Ranch section of the upper Colorado River.

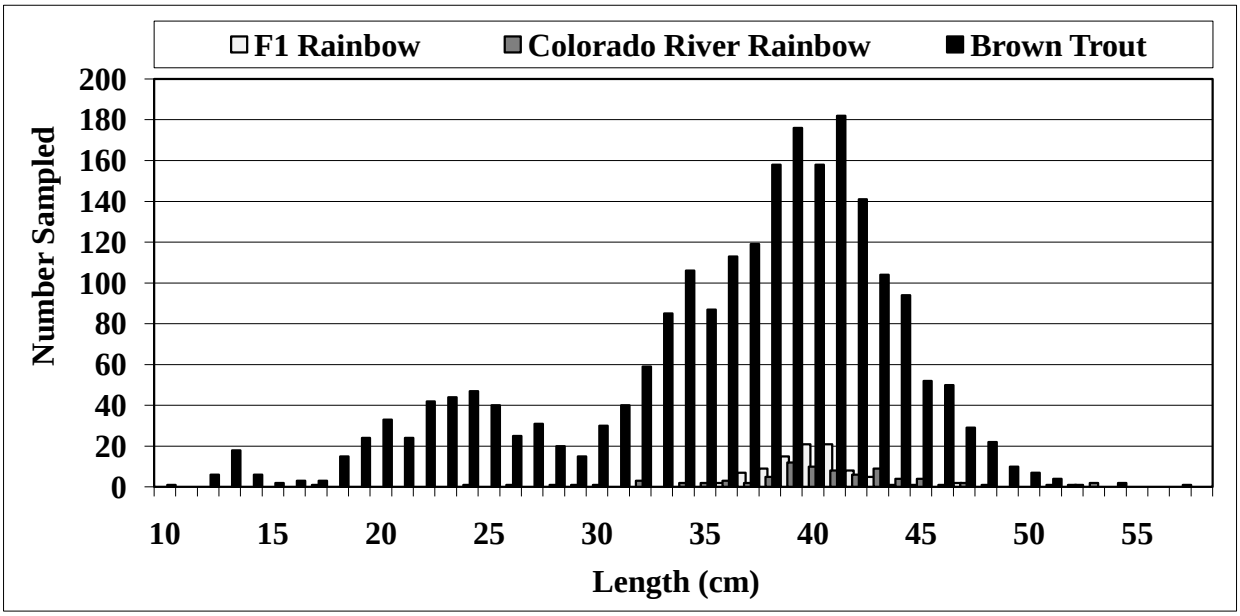
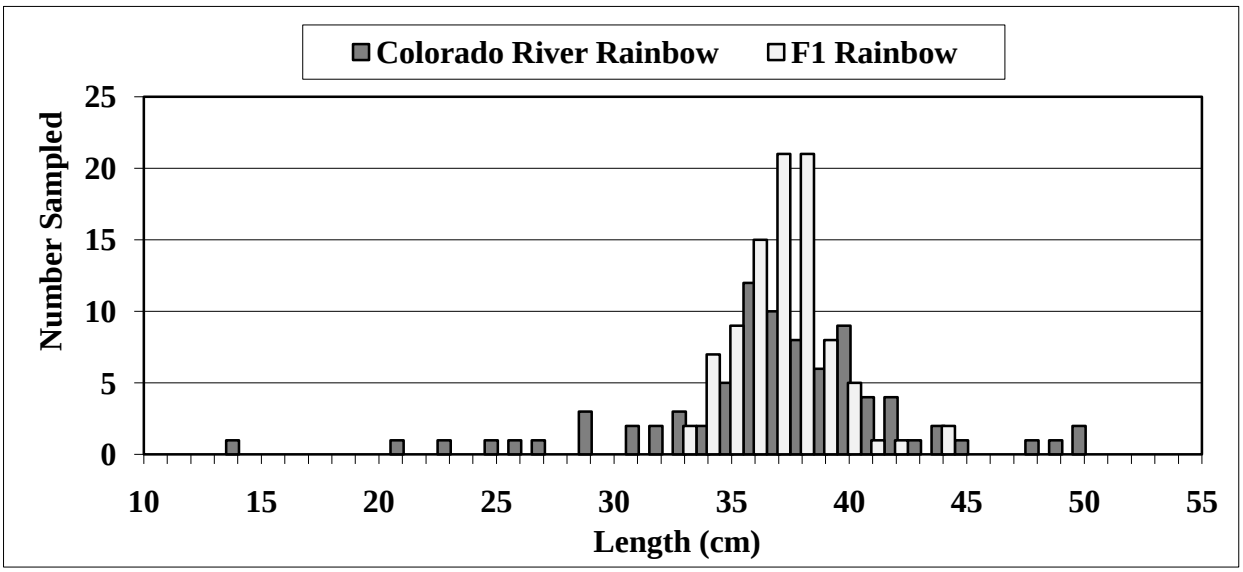


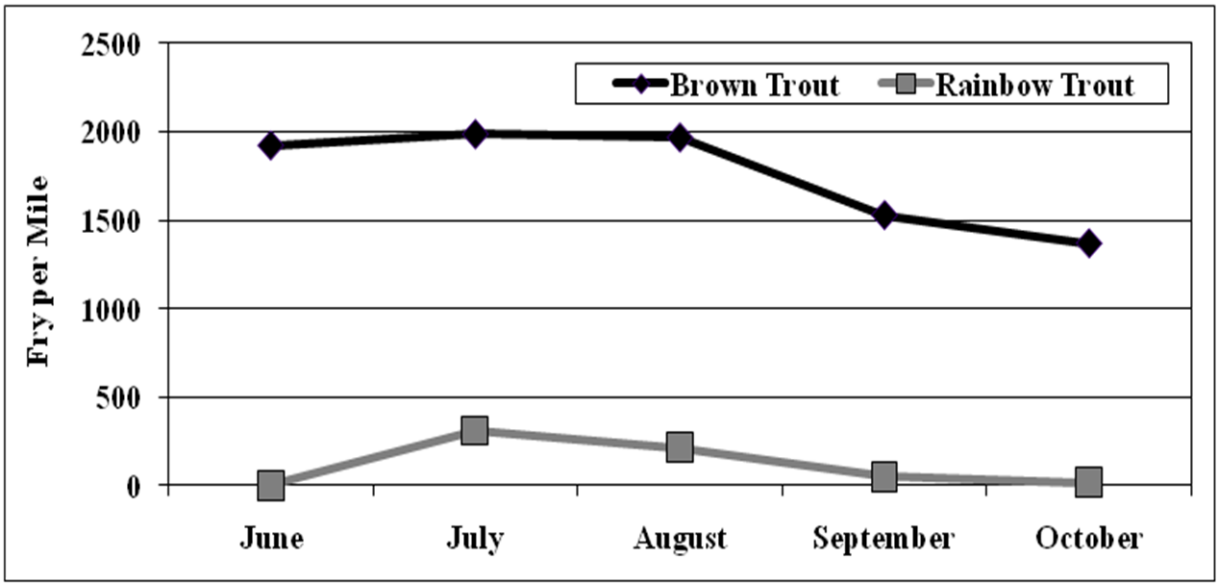
Figure 4.5. Length-frequency distribution for Colorado River and F1 (2006 plant) rainbow trout encountered during the spring 2009 mark-recapture population estimate in the Chimney Rock/Sheriff Ranch section of the upper Colorado River.



Fry estimates were conducted once a month, June through October 2009. Standard, three-pass, 50 ft removal estimates were conducted at seven stations throughout the upper Colorado River, with three sites downriver of Byers Canyon (Kemp-Breeze, Lone Buck, and Paul Gilbert State Wildlife Areas), and four sites within the 6.28 km (3.9 mi) study reach on the Chimney Rock and Sheriff Ranches (Sheriff Ranch, Lower and Upper Red Barn, and Hitchin' Post Bridge). Two LR-24 Smith-Root backpack electrofishing units were used to complete the fry estimates. All fry caught within the 50 ft sections were identified as brown trout or rainbow trout, measured, and examined for signs of whirling disease. In addition, spot shocking was conducted during the estimates for additional disease status information. Fin clips were taken from all rainbow trout fry for genetic analysis. During the October fry estimates, 30 brown trout and 10 rainbow trout were collected for myxospore enumeration.

Seventy-seven rainbow trout fry were encountered over the five-month fry evaluations, in comparison to 22 rainbow trout fry encountered in 2008, and 14 rainbow trout fry encountered in 2007. Of those rainbow trout fry encountered, 36 were found in the 50 foot study sites, and 41 were found in areas outside of the study sites during spot shocking. Fry density estimates were calculated using the three-pass removal equations of Seber and Whale (1970). Brown trout fry densities peaked in July, with an estimate of 1,234 fry per km (1,986 fry per mi), dropping to 849 fry per km (1,366 fry per mi) in October. Rainbow trout fry densities also peaked in July, with an estimate of 193 fry per km (310 fry per mi), dropping to 9 fry per km (15 fry per mi) in October (Figure 4.6). Seven percent of the brown trout fry encountered during the fry estimates showed signs of whirling disease, whereas 19.4 percent of the rainbow trout fry encountered showed signs of disease. The average myxospore count of the brown trout fry collected in October was 9,105 myxospores per fish, compared with 47,708 myxospores per fish for the rainbow trout fry.

Figure 4.6. Upper Colorado River brown trout and rainbow trout fry density estimates for the months of June to October 2009.



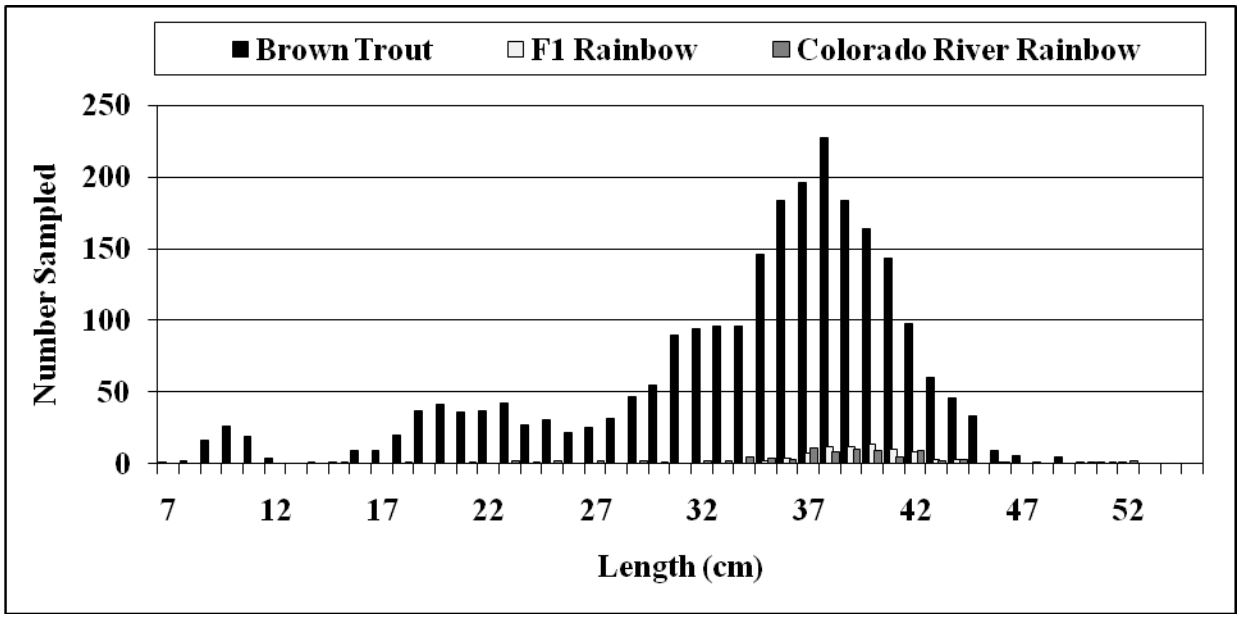
2010 Field Season

On May 14 and 18, 2010, a population estimate was conducted on the same 6.28 km (3.9 mi) stretch of the upper Colorado River through the Chimney Rock/Sheriff Ranches as in 2008 and 2009. Two raft-mounted fixed-boom electrofishing units were used for both the mark and recapture runs. Marking, identification and data collection procedures were conducted in the same manner as in 2009.

The population estimate was calculated using the Petersen estimator (with the Bailey (1951) modification). Brown trout were present in the reach at a density of 672 fish per km (1,081 fish per mi). CRRs, including residual wild fish and fish present due to repeated stocking of CRR fingerlings, were estimated to exist at a density of 20 fish per km (33 fish per mi). F1 rainbow trout from the 2006 plant were present at a density of 11 fish per km (17 fish per mi). No F1 rainbow trout from the January 2009 plant were encountered during the population estimate. White suckers were present in the reach at a density of 65 fish per km (105 fish per mi). Other fish species encountered during the population estimate included speckled dace and longnose suckers.

Average length of the 2,421 brown trout encountered during the estimate was 33.8 cm (13.3 in) TL, ranging from 4.4 to 51.8 cm (1.7 to 20.4 in) TL. The 78 F1 rainbow trout encountered averaged 39.3 cm (15.5 in) TL, ranging from 34.5 to 45.6 cm (13.6 to 18 in) TL. The 91 CRR trout encountered averaged 37 cm (14.6 in) TL, ranging from 14.3 to 59 mm (5.6 to 23.3 in) TL (Figure 4.7). F1 rainbows averaged 582 g in weight, ranging from 351 to 880 g, and CRR averaged 484 g, ranging from 29 to 930 g. The F1 fish stocked in 2006 comprised a much smaller proportion of the total rainbow trout population in the study area than they had in either 2008 or 2009 (Figure 4.8).

Figure 4.7. Length-frequency distribution for brown trout, Colorado River Rainbow trout, and F1 (2006 plant) rainbow trout encountered during the spring 2010 mark-recapture population estimate in the Chimney Rock/Sheriff Ranch section of the upper Colorado River.



Of the 78 F1 fish that were handled during the population estimate, 46 (22 females and 24 males) were found to be sexually mature and ripe, while 22 (20 females and two males) had already spawned. An additional three females and three males were sexually mature, but in pre-spawn status (green). Only four were clearly immature and did not appear to be potentially sexually mature in 2010. Ninety-one CRR individuals were handled during the population estimate, and of those, 23 were found to be sexually mature and ripe (13 females and ten males), while 24 (22 females and two males) had already spawned. An additional two green females and eight green males were present. Thirty-four sexually immature CRR individuals were also encountered (Figure 4.9).

Figure 4.8. Length-frequency distribution for Colorado River and F1 (2006 plant) rainbow trout encountered during the spring 2010 mark-recapture population estimate in the Chimney Rock/Sheriff Ranch section of the upper Colorado River.

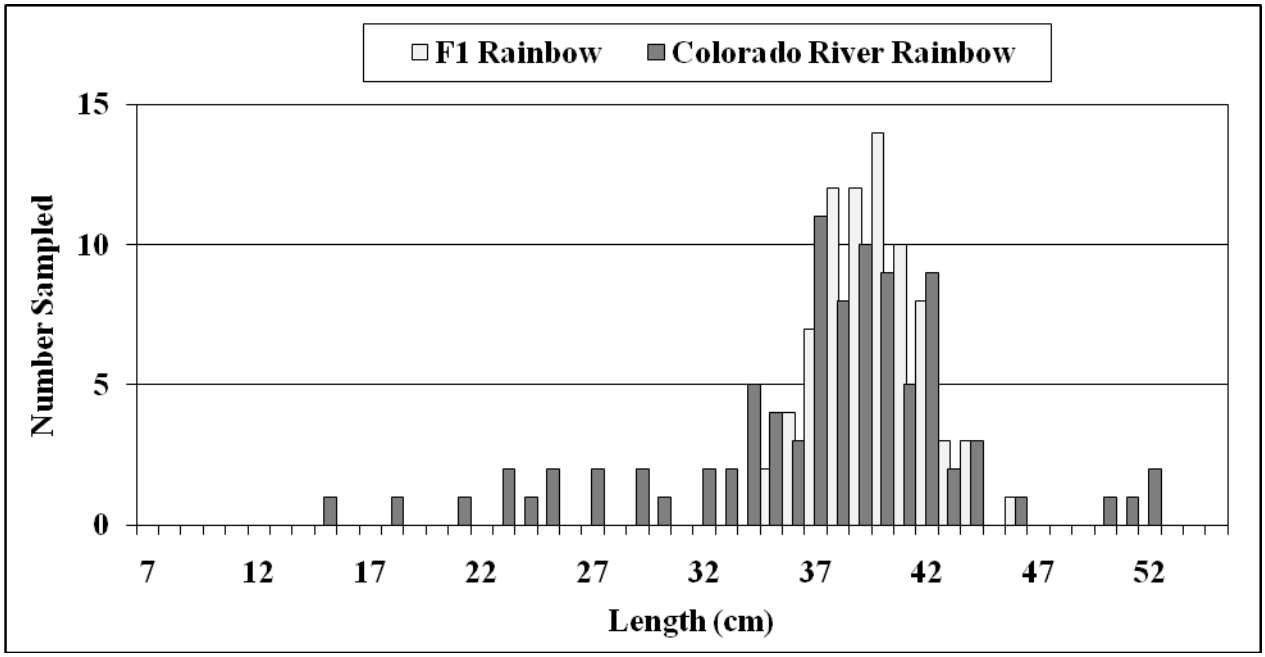
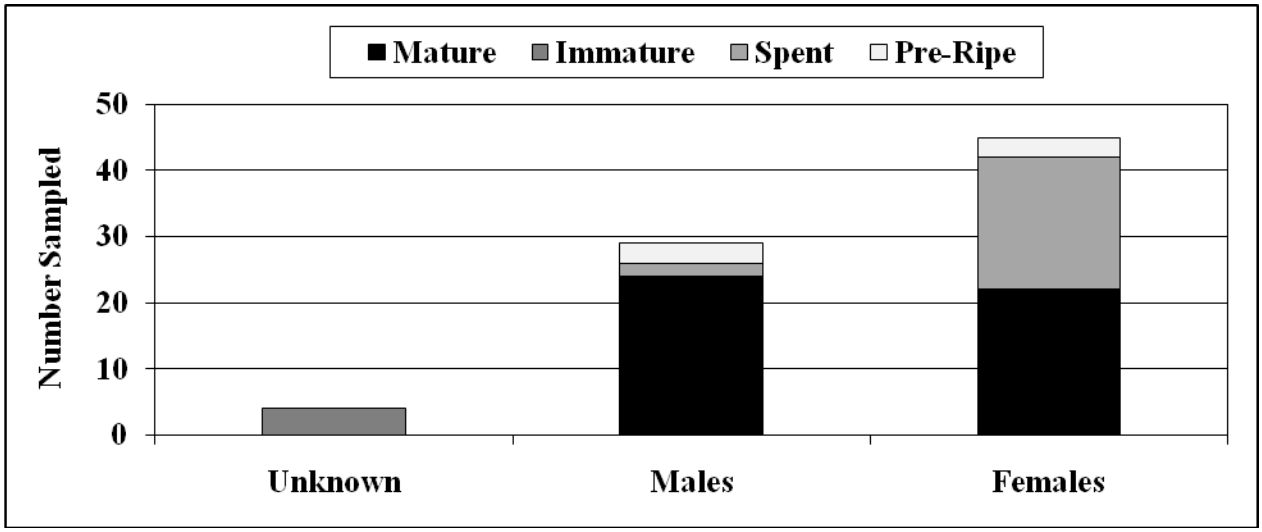


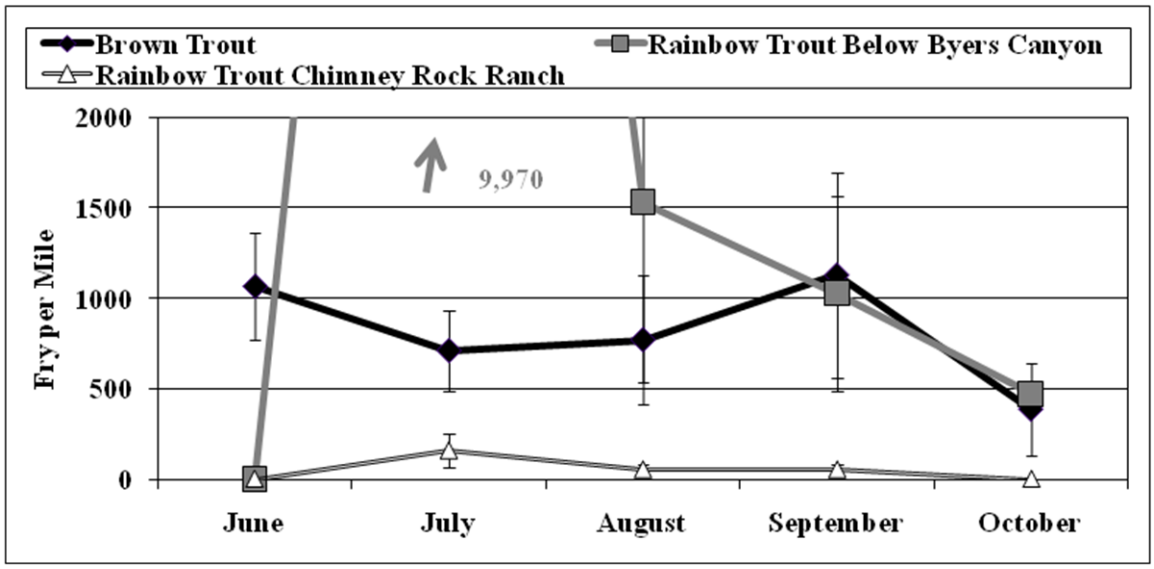
Figure 4.9. Number of F1 adult rainbow trout encountered during the spring 2010 mark-recapture population estimate in the Chimney Rock/Sheriff Ranch section of the upper Colorado River that were immature, mature (ripe), spent, and pre-ripe (green).



In June 2010, 1,947 F1 rainbow trout, averaging 17.2 cm (6.8 in) TL and 58.1 g (0.1 lbs), were introduced to the upper Colorado River. Prior to being introduced, all rainbow trout were tagged with an individually numbered fine-filament Floy tag, and secondarily adipose clipped for identification in the event of tag loss, used to track individual growth and survival of the introduced fish. Approximately one-third of the fish were introduced at each of three locations: the Sheriff Ranch, located at the lower end of the Chimney Rock/Sheriff Ranch section, Red Barn, located in the middle of the Chimney Rock/Sheriff Ranch section, and Hitchin' Post Bridge, located toward the upper end of the Chimney Rock/Sheriff Ranch section. This plant was used to boost adult whirling disease resistant rainbow trout numbers throughout this section of river, following the unsuccessful introduction of fish in the winter of 2009.

Fry estimates were conducted once a month, June through October 2010. Standard, three-pass, 50 ft removal estimates were conducted at seven standard stations throughout the upper Colorado River, with three sites downriver of Byers Canyon, and four sites within the 6.28 km (3.9 mi) study reach on the Chimney Rock and Sheriff Ranches. Two LR-24 Smith-Root backpack electrofishing units were used to complete the fry estimates. All fry caught within the 50 ft sections were identified as brown trout or rainbow trout, measured, and examined for signs of whirling disease. In addition, spot shocking was conducted during the estimates for additional disease status information. Fin clips were taken from all rainbow trout fry for genetic analysis. During the October fry estimates, 28 brown trout, two rainbow trout, and five brook trout (from Corral Creek on the Chimney Rock Ranch) were collected for myxospore enumeration.

Figure 4.10. Upper Colorado River brown trout and rainbow trout fry density estimates, above and below Byers Canyon, for the months of June to October 2009.



Three hundred and seventy-five rainbow trout fry were encountered over the five-month fry evaluations, 329 in the three sites below Byers Canyon and 46 in the four sites above Byers Canyon, in comparison to 77 rainbow trout fry encountered in 2009, 22 rainbow trout fry encountered in 2008, and 14 rainbow trout fry encountered in 2007. Below Byers Canyon numbers were significantly higher than those above Byers Canyon because of an introduction of approximately 200,000 rainbow trout fry along the margins of the river below Byers Canyon in July. Of those rainbow trout fry encountered, 339 were found in the 50 foot study sites, and 36 were found in areas outside of the study sites during spot shocking. Spot shocking only occurred around the four sites located above Byers canyon in the Chimney Rock/Sheriff Ranch study area.

Fry density estimates were calculated using the three-pass removal equations of Seber and Whale (1970). Brown trout fry densities peaked in September, with an estimate of 701 fry per km (1,127 fry per mi), dropping to 239 fry per km (384 fry per mi) in October. Rainbow trout fry densities peaked in July, both above and below Byers Canyon, with an estimate of 6,195 fry per km (9,970 fry per mi) below Byers Canyon, and an estimate of 99 fry per km (160 fry per mi) above Byers Canyon. These estimates dropped to 294 fry per km (473 fry per mi) below Byers Canyon, and 0 fry per km (0 fry per mile) above Byers Canyon, by the end of October (Figure 4.10). Nine percent of the brown trout fry encountered during the fry estimates showed signs of whirling disease, whereas 6.6 percent of the rainbow trout fry encountered showed signs of disease. The average myxospore count of the brown trout fry collected in October was 29,187 myxospores per fish, compared with 90,839 myxospores per fish for the rainbow trout fry, and 29,696 myxospores per fish for the brook trout fry.

2011 Field Season

On May 2 and 4, 2011, a population estimate was conducted on the same 6.28 km (3.9 mi) stretch of the upper Colorado River through the Chimney Rock/Sheriff Ranches as in 2008, 2009, and 2010. Two raft-mounted fixed-boom electrofishing units were used for both the mark and recapture runs. Marking, identification and data collection procedures were conducted in the same manner as in 2010. Due to high water conditions (averaging 856 cfs, compared to 253 cfs in 2008, 426.8 cfs in 2009, and 270.6 cfs in 2010), approximately 900 less fish were encountered on both the mark and recapture runs than had been encountered in previous years.

The population estimate was calculated using the Petersen estimator (with the Bailey (1951) modification). Brown trout were present in the reach at a density of 525 fish per km (845 fish per mi). CRRs, including residual wild fish and fish present due to repeated stocking of CRR fingerlings, were estimated to exist at a density of five fish per km (nine fish per mi). F1 rainbow trout from the 2006 and 2010 plants were present at a density of four fish per km (six fish per mi). No F1 rainbow trout from the January 2009 plant were encountered during the population estimate. In addition, a small number of cutbows (cutthroat trout x rainbow trout; RxN) were encountered during the estimate, one of which was 61.4 mm (24.2 in) TL, and weighed 2,670 g. It is suspected that these fish

may be escapees from Granby Reservoir, located about six miles upstream of Windy Gap Reservoir. An estimate of the number of RxNs in the section could not be obtained because no marked RxNs were encountered on the recapture run. Other fish species encountered during the population estimate included white sucker, speckled dace, and longnose sucker.

Average length of the 1,155 brown trout encountered during the estimate was 34.4 cm (13.5 in) TL, ranging from 9.7 to 51 mm (3.8 to 20.1 in) TL. The 23 F1 rainbow trout encountered averaged 36.9 mm (14.5 in) TL, ranging from 24.3 to 61.4 mm (9.6 to 24.2 in) TL. The 38 CRR trout encountered averaged 37 mm (14.6 in) TL, ranging from 25.3 to 47.9 mm (10 to 18.9 in) TL (Figure 4.11). F1 rainbows averaged 539 g in weight, ranging from 122 to 882 g, and CRR averaged 511 g, ranging from 172 to 1,148 g. The F1 fish stocked in 2006 and 2010 comprised about half of the total rainbow trout population in the study area, with numbers of both F1 and CRR trout in the study area being extremely low (Figure 4.12).

Figure 4.11. Length-frequency distribution for brown trout, Colorado River Rainbow trout, and F1 (2006 and 2010 plant) rainbow trout encountered during the spring 2011 mark-recapture population estimate in the Chimney Rock/Sheriff Ranch section of the upper Colorado River.

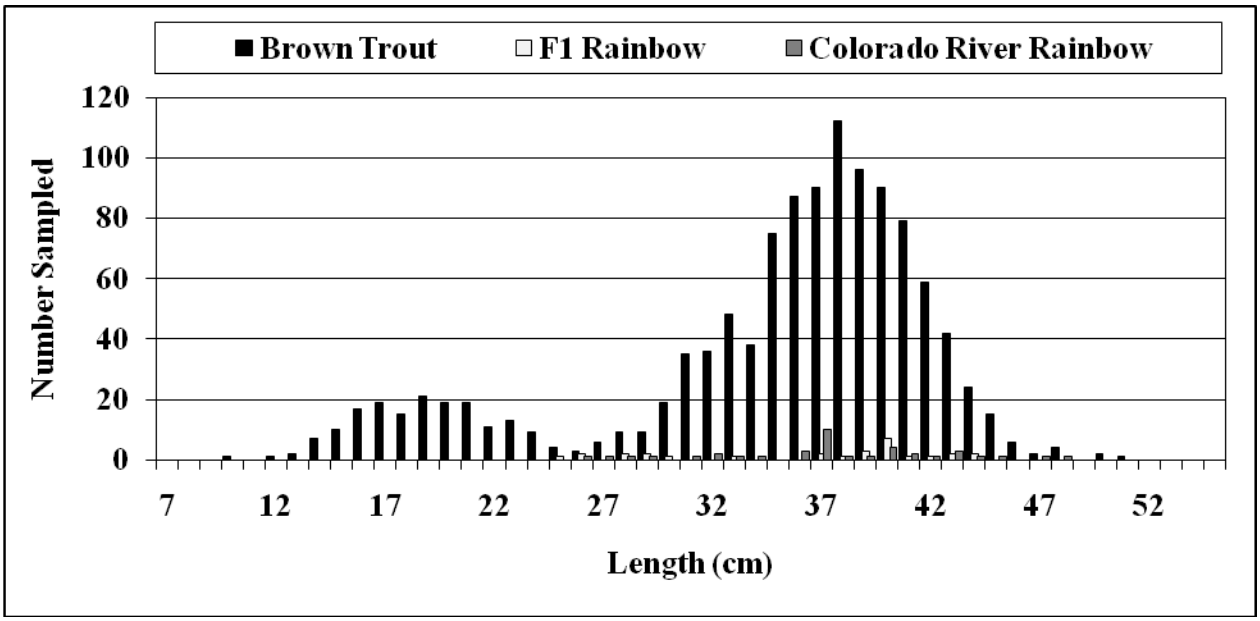
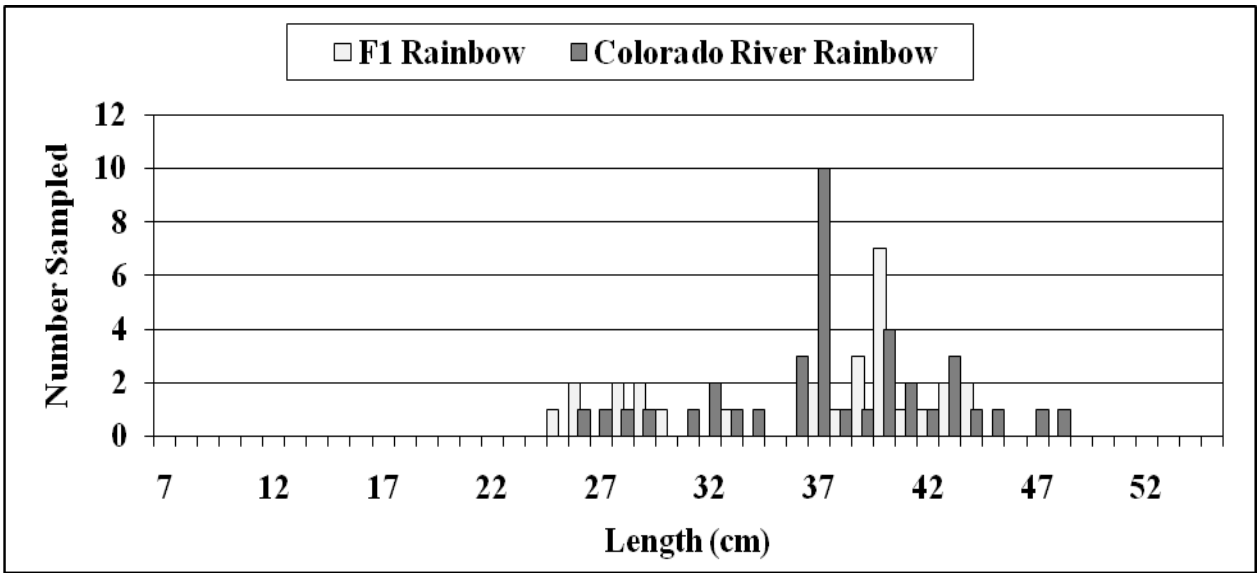
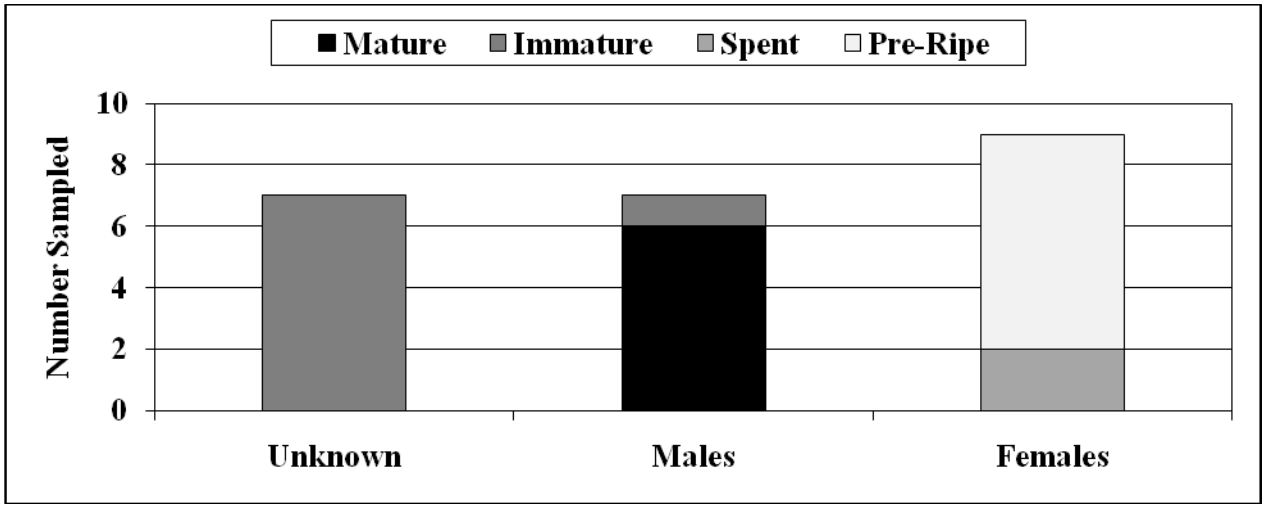


Figure 4.12. Length-frequency distribution for Colorado River and F1 (2006 and 2010 plant) rainbow trout encountered during the spring 2011 mark-recapture population estimate in the Chimney Rock/Sheriff Ranch section of the upper Colorado River.



Of the 23 F1 fish that were handled during the population estimate, six (males) were found to be sexually mature and ripe, while wo (females) had already spawned. An additional seven females were sexually mature, but in pre-spawn status (green). Seven were clearly immature and did not appear to be potentially sexually mature in 2011. Thirty-eight CRR individuals were handled during the population estimate, and of those, ten were found to be sexually mature and ripe (six females and four males), while one (female) had already spawned. An additional ten green females and seven green males were present. Ten sexually immature CRR individuals were also encountered (Figure 4.13).

Figure 4.13. Number of F1 adult rainbow trout encountered during the spring 2011 mark-recapture population estimate in the Chimney Rock/Sheriff Ranch section of the upper Colorado River that were immature, mature (ripe), spent, and pre-ripe (green).



Survival and Growth of Introduced Rainbow Trout

Encounter histories for the introduced rainbow trout were constructed using the data from population estimates conducted between 2006 and 2011. Survival (φ) and detection probability (p) estimates for the introduced rainbow trout population were obtained using a Cormack-Jolly-Seber (CJS) open capture-recapture model in program MARK (White and Burnham 1999). Detection probability was assumed to vary by year, discharge (entered as a covariate), and sampling season (fall or spring). Discharge was obtained from the Northern Colorado Water Conservancy District historical water records (Northern Colorado Water Conservancy District 2011): 38 cfs in 2006, 60 cfs in 2007, 253 cfs in 2008, 427 cfs in 2009, 271 cfs in 2010, and 856 cfs in 2011. Sampling in different seasons was thought to affect detection probability; the river was sampled in the fall in 2006 and 2007, and in the spring from 2008 on. Models in which detection probability was kept constant were also run. Survival was assumed to vary by year, introduction size (the combination of length and weight – entered as covariates), Floy tag color (three colors – pink, grey, and green – entered as covariates), season of introduction (two seasons – winter and summer – entered as covariates), and all additive combinations of time, size, color, and season. Models were ranked using Akaike's Information Criterion corrected for small sample sizes (AICc). Estimates of survival and detection probability were obtained through model averaging of the models with an AIC weight greater than zero.

The top model contained size at introduction and Floy tag color as the variables that most affected survival rates of the introduced rainbow trout in the upper Colorado River (AICc = 1794.691, AICc weight = 0.988). The second best model, the likelihood of which was 84 times less than the top model, also included both size at introduction and Floy tag color, as well as season of introduction, as the variables affecting survival (AICc = 1803.546, AICc weight = 0.012). Survival rate did not vary between years. Estimated yearly survival rate in the upper Colorado River of the introduced rainbow trout was 0.017 (± 0.12). The top model showed that detection probability was most likely to vary by year (within which any number of factors could vary detection probability including discharge, water clarity, conductivity, electrofishing equipment type and power output, sampling crew variations, etc.), with the second best model showing detection probability varying with discharge (cfs). Detection probability was lowest in 2006 and 2007, when sampling occurred in the fall using backpack electrofishing units, with detection probabilities of 0.067 and 0.023, respectively. Detection increased in 2008 when sampling started occurring in the spring, using electrofishing rafts, with a detection probability of 0.228, and remained high in subsequent years, with a detection probability of 0.336 in 2009, 0.391 in 2010, and 0.246 in 2011.

The same encounter histories used for the survival estimation described above were reversed to estimate seniority (γ), the probability that a fish encountered in any given year was present in the year previous. Seniority and detection probability estimates for the introduced rainbow trout population were obtained using a CJS open capture-recapture model in program MARK (White and Burnham 1999). Sampling in different seasons was thought to affect detection probability; the river was sampled in the fall in

2006 and 2007, and in the spring from 2008 on. A model in which detection probability was kept constant was also run. Seniority was assumed to remain constant from year to year. Models were ranked using AICc. Estimates of seniority and detection probability were obtained through model averaging of the models with an AIC weight greater than zero.

The top model had season as the variable that most affected detection rates of the introduced rainbow trout in the upper Colorado River (AICc = 2108.892, AICc weight = 0.922). The model in which detection probability was constant was 11 times less likely than the first model (AICc = 2113.834, AICc weight = 0.078). As a result of seniority being set as a constant, seniority rate did not vary between years. Estimated yearly seniority of the introduced rainbow trout in the upper Colorado River was 0.631 (± 0.04).

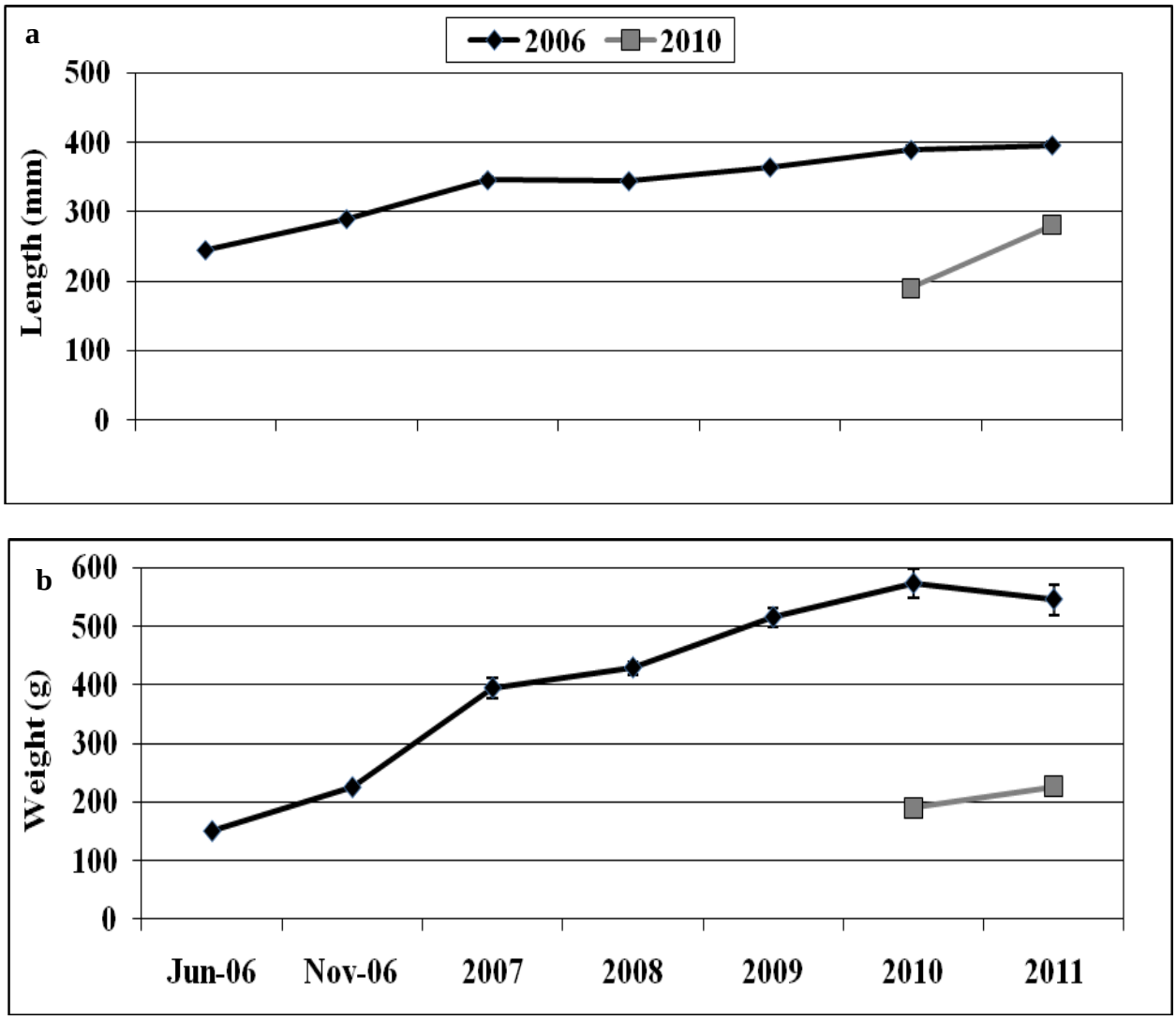
The finite rate of population increase (λ) can be calculated in any given year using survival and seniority estimates, using the equation

$$\lambda_i = \frac{\varphi_i}{\gamma_{i+1}},$$

where λ_i is the finite rate of population increase in year i, φ_i is the survival estimate in year i, and γ_{i+1} is the seniority estimate in year $i + 1$. The finite rate of increase describes trends in population growth, with λ values greater than one indicating that the population is increasing, and λ values less than one indicating that the population is decreasing. Because both survival and seniority estimates were continuous, a single estimate of λ is produced. The finite rate of population increase of the rainbow trout population in the upper Colorado River is 0.027. This number is very low, and supports data collected from the field showing that survival is very low and that the introduced rainbow trout population is declining.

Growth of the introduced rainbow trout was calculated using the length and weight information collected on a per individual basis during the population estimates conducted between 2006 and 2011. Each cohort (introduction year) was treated separately, and only the individuals that were recaptured in subsequent population estimates were used to obtain estimates of individual specific growth. Both the fish stocked in 2006 and in 2010 showed an increase of about 10 cm (3.9 in) TL in their first year in the river. However, the 2006 fish showed a much larger increase in weight in their first year in the river than did the 2010 fish. In general, 2007 seemed to be a fairly unproductive year for the fish planted in 2006, with growth rates increasing between 2008 and 2010. The fish stocked in 2006 appear to be reaching the maximum size that can be sustained in the upper Colorado River, with the rate of increase in length decreasing between 2010 and 2011, and no weight gain occurring between 2010 and 2011 (Figure 4.14).

Figure 4.14. Individual specific increases in length (mm; a) and weight (g; b), obtained from Floy tagged rainbow trout encountered during mark-recapture population estimates conducted in the Chimney Rock/Sheriff Ranch section of the upper Colorado River between 2006 and 2010.



Conclusions

The F1s stocked as catchable-sized fish in 2006 continue to be encountered in the upper Colorado River. In addition, F1s stocked in 2010 appear to have survived introduction and remained in the reach in 2011. However, overall numbers of adult F1 rainbow trout per mile are at an all time low since beginning these experiments in 2006. There are several reasons for this, including the lack of survival of the winter 2009 introduction of F1 rainbow trout, the low yearly survival of introduced rainbow trout (1.7%), and the lack of recruitment to the adult rainbow trout population despite the

occurrence of natural reproduction in the upper Colorado River. Fry estimates in 2011 indicate that natural reproduction of rainbow trout is occurring in the upper Colorado River; however, there is still a nearly complete loss of rainbow trout fry in the Chimney Rock/Sheriff Ranch section by late October. Recruitment to the adult population still appears to be non-existent, a result of a lack of survival of naturally produced offspring beyond the fall. However, stocking resistant rainbow trout fry does appear to increase the number of rainbow trout fry present in the river in October, as seen in the fry populations examined below Byers Canyon. Sampling in 2011 will determine if this also translates to an increase in recruitment to the age-1 rainbow trout population below Byers Canyon.

The adult resistant rainbow trout population in the Chimney Rock/Sheriff Ranch section needs to be increased if a self-sustaining rainbow trout population is going to be established in this section of the upper Colorado River. Rainbow trout numbers are low enough that we are likely to see an Allee effect in this section of river in 2011; that is, numbers are so low that the adult rainbow trout may be unable to find each other to spawn, and therefore, little to no natural reproduction may occur this year. Larger introductions, occurring in the summer when survival is higher, will likely be needed in the near future to boost the adult spawning rainbow trout population in the Chimney Rock/Sheriff Ranch section of the river. In addition, depending on the results of the fry introductions below Byers Canyon, large fry introductions may also be used to increase recruitment to the adult spawning population in this section of the river.

Gunnison River

Introduction

The rainbow trout population in the Gunnison River has dramatically declined since the introduction of whirling disease in the early 1990's (Figure 4.15). Like the upper Colorado River, multiple years of stocking pure Colorado River rainbow trout fingerlings has not resulted in any measurable increase in rainbow trout density or biomass. In fact, rainbow trout numbers have continued to decline, and brown trout numbers have increased to historical highs. A series of stocking events in the Gunnison River have occurred since 2004 in which equal numbers of pure Colorado River rainbow trout and GR-CRR cross fish have been differentially marked and stocked together to evaluate relative survival rates of the strains, and as an attempt to re-establish a wild self-sustaining population in this location.

Introductions and Evaluations (2004-2008)

In 2004, GR-CRR 50:50 (F1) fish were marked with red Visible Implant Elastomer (VIE) tags, and pure CRRs were similarly marked with green VIE tags. During this initial introduction, 10,104 CRR (13.6 cm TL) and 10,115 F1 (11.9 cm TL) rainbow trout were stocked as fingerlings into the Ute Park section of the Gunnison Gorge (Figure 4.16). The fish were mixed together prior to stocking to prevent bias due

to handling, and then spread throughout the stream section using a helicopter. In 2005, GR-CRR 25:75 (B2) fish were stocked, rather than F1s, along with pure CRRs. The B2s were marked with an adipose clip, and pure CRRs were marked with a right pelvic clip. Five thousand of each variety (15.2 cm TL) were stocked as fingerlings. In 2006, B2s (17.3 cm TL) were stocked as larger fingerlings to determine if slightly larger B2s would perform better than those from the original (2005) plant of B2s. Pure CRRs were not marked in this plant; B2s were given an adipose clip and a red VIE tag. In 2007, the number of fish stocked was increased to 20,000 each of the pure CRR and F1 rainbow trout, stocked as 14.7 cm fingerlings. Coded wire tags were used to batch-mark both F1s and CRRs. Additionally, F1s were secondarily adipose clipped for identification in the event that the coded wire tag was lost.

Figure 4.15. Historic rainbow trout and brown trout population estimates (fish per mile) for the Ute Park section of the Gunnison Gorge.

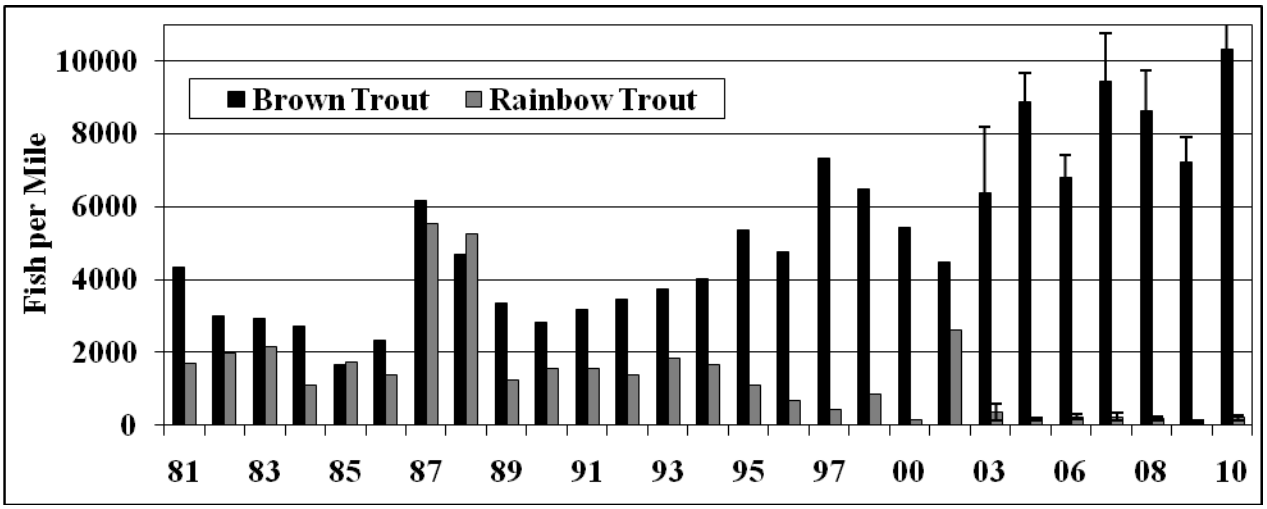
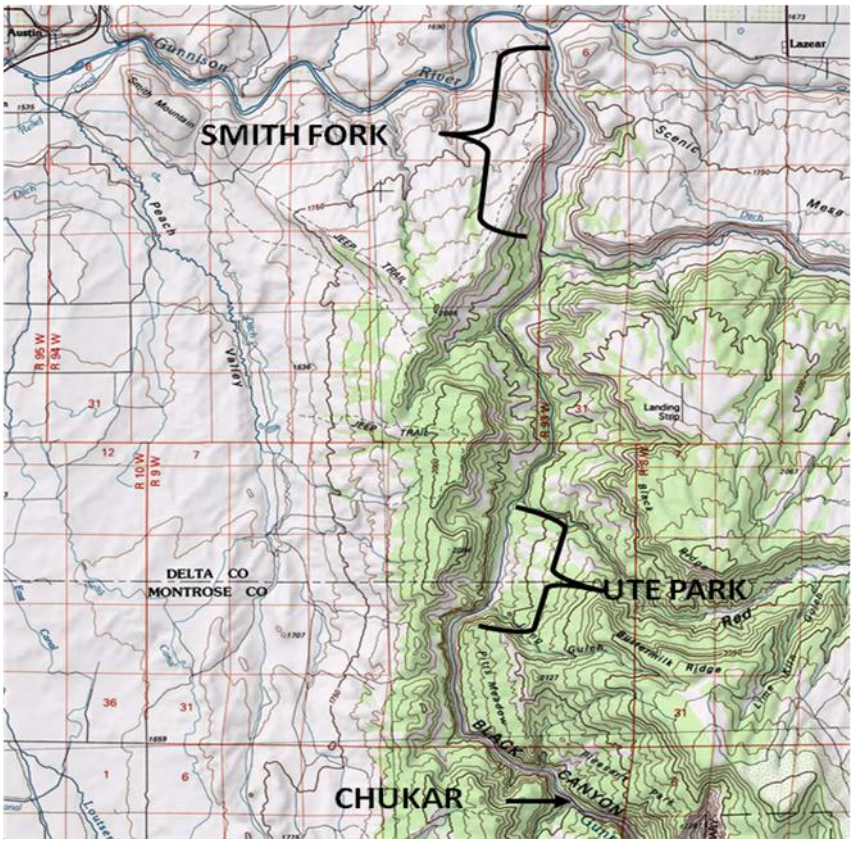


Figure 4.16. Gunnison River study area.



Growth, survival, and infection severity of the introduced strains were evaluated from samples collected during the annual population estimate conducted the year following the introduction. Estimates were obtained using mark-recapture sampling with boat-mounted electrofishing gear. All rainbow trout were carefully examined for evidence of VIE marks, fin clips, and coded wire tags. Subsamples of fish were collected for myxospore evaluation using the pepsin-trypsin digest (PTD) method in 2005 and 2006.

The 2005 population estimate indicated that survival of both varieties of fish stocked in 2004 was relatively low, with only 12 of the pure CRR, and 24 of the F1 fish encountered in the 2,375 m sampling area. The sampling resulted in an estimate of ten CRR per km (16 CRR per mi). The estimate for the F1 strain was 14 fish per km (22 fish per mi). The CRRs averaged 24.8 cm (9.8 in) TL, and the F1s averaged 28.3 cm (11.1 in) TL. All of the pure CRR individuals collected were found to be infected, with an average myxospore count of 124,603 (± 129,406) myxospores per fish. Only six of the ten F1 individuals collected were found to be infected, with an average myxospore count of 4,055 (± 8,336) myxospores per fish.

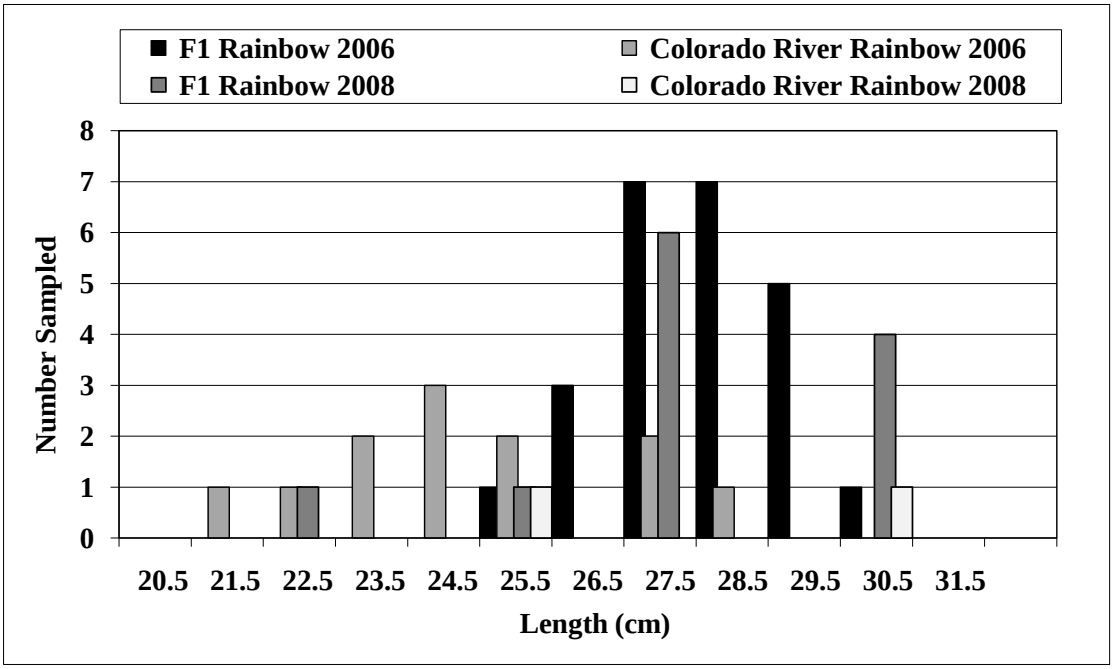
Survival and population estimates were difficult to assess directly in 2006, for fish stocked in 2005, because of mark loss (fin regeneration or poor marks) in both the CRR

and B2 strains. Amplified Fragment Length Polymorphism (AFLP) testing, a molecular technique that can help distinguish between individuals of the same species with different genetic lineages, was used to identify a subsample of unmarked fish as either B2 or CRR. Applying the ratio of fish identified as each strain in the subset to the overall population estimate of fish resulted in an estimate of 33 fish per km (53 fish per mi) for the CRRs, and 22 fish per km (35 fish per mi) for the B2s. PTD testing identified an average of 83,929 myxospores (± 149,719) in the pure CRRs planted in 2005. The average myxospore count among the B2s was 40,480 (± 48,121) myxospores per fish.

In 2007, poor mark retention once again made estimating numbers of pure CRR and GR-cross fish difficult. The overall population estimate of rainbow trout (over 15 cm TL) was 135 fish per km (217 fish per mi). Of the 144 fish sampled, 16 (11.1%) were identified as either F1 or B2, indicated by either the presence of red VIE tags or adipose clips, while only three (2.1%) were identified as pure CRR, indicated by the presence of green VIE tags. In 2008, the population estimate for rainbow trout (over 15 cm TL) was 111 fish per km (178 fish per mi). Fish stocked in 2007 were clearly identifiable due to the coded wire tags and fin clips. Of the 157 rainbow trout that were sampled, 12 F1s and two CRRs from the 2007 plant were positively identified, producing an estimate of seven F1s and two CRRs per km (12 F1s and three CRRs per mile). Average length of the F1s (27.7 cm TL) was similar to the CRRs (27.5 cm TL) in 2008, after one year in the river.

Overall, poor survival estimates were quite evident for both the pure CRR and the GR-cross fish in each year of stocking. Predation by brown trout, loss of marks, and emigration from the study area were likely contributing factors. However, in both years (2006 and 2008) where F1s and CRRs were positively identified, and could be compared directly from the stocking event in the previous year, the F1s were much more abundant than the pure CRRs (Figure 4.17).

Figure 4.17. Length-frequency distribution of the rainbow trout strains encountered in the Gunnison River in 2006 and 2008 where direct comparisons of pure Colorado River rainbow trout and F1 strain rainbow trout that could be made as a result of positive identification as fish stocked in the previous year.



The results of this field evaluation demonstrated that the F1s can survive at least as well as the CRRs when planted as fingerlings. The results also demonstrated that myxospore counts, developed after stocking, are much lower in the F1s than in the CRRs. The myxospore counts in B2s released into the wild were similar to those found in laboratory experiments, and while lower than the spore counts from the pure CRRs, were also higher than observed in the F1s. This reinforces the notion that allowing natural selection, acting on F1 offspring, to occur in the wild may be a more effective method to producing sufficient resistance and wild behaviors than creating subsequent crosses (such as the B2s) artificially.

2009 Field Season

Brown trout numbers remained high, and rainbow trout numbers low, in the Ute Park section of the Gunnison Gorge in 2009. Brown trout were estimated to be present in the section at a density of 4,699 fish per km (7,562 fish per mi). Nine fish were positively identified as either F1s or B2s stocked in past years, and were estimated to be present in the reach at a density of 3 fish per km (5 fish per mi). Wild rainbow trout, those that could not be positively identified as CRRs, F1s, or B2s, were estimated to be present in the study reach at a density of 44 fish per km (70 fish per mi; Figure 4.18). Despite low numbers of rainbow trout, three age classes were seen in the rainbow trout

population for the first time since the introduction of whirling disease in the early 1990s (Figure 4.19).

Figure 4.18. Length-frequency distribution of brown trout, GR-cross (HxC) rainbow trout, and wild rainbow trout encountered during the fall 2009 mark-recapture population estimate in the Ute Park section of the Gunnison Gorge.

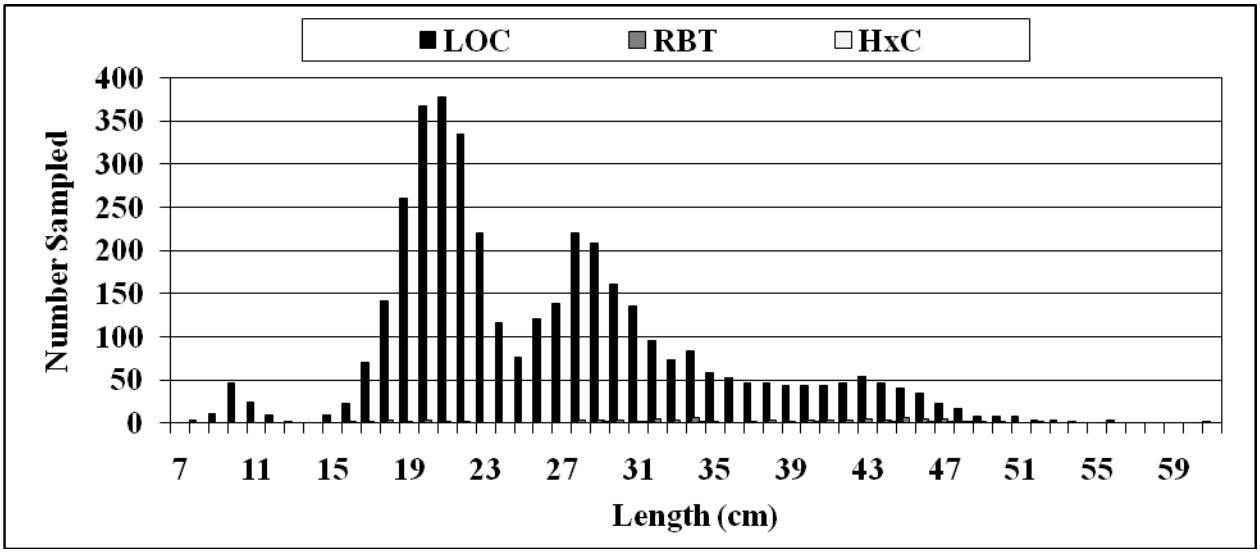
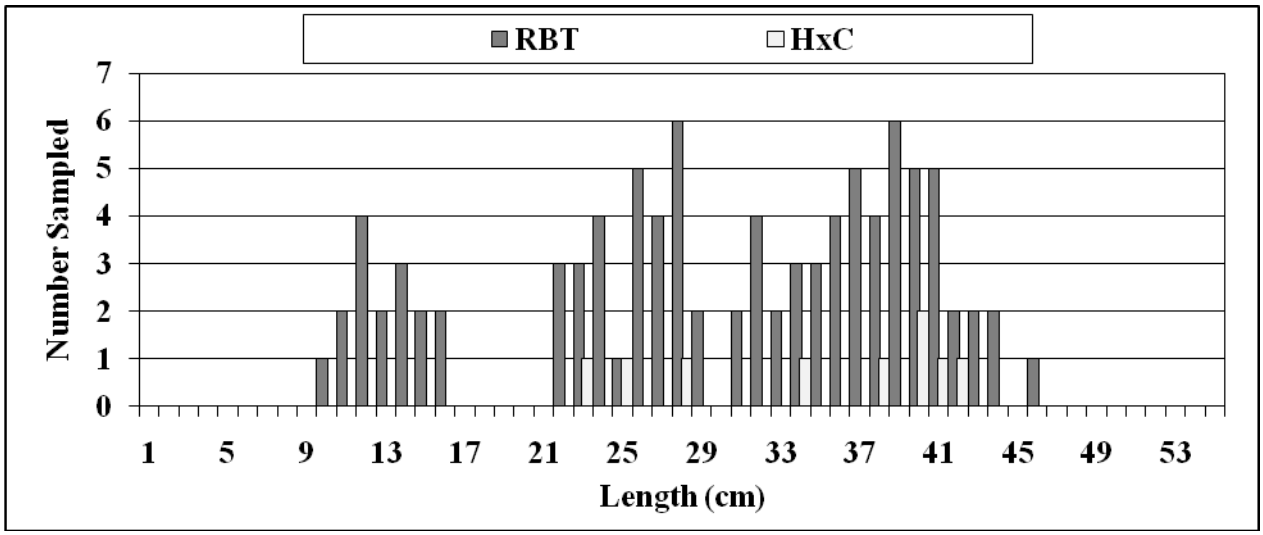


Figure 4.19. Length-frequency distribution of GR-cross (HxC) and wild rainbow trout encountered during the fall 2009 mark-recapture population estimate in the Ute Park section of the Gunnison Gorge.

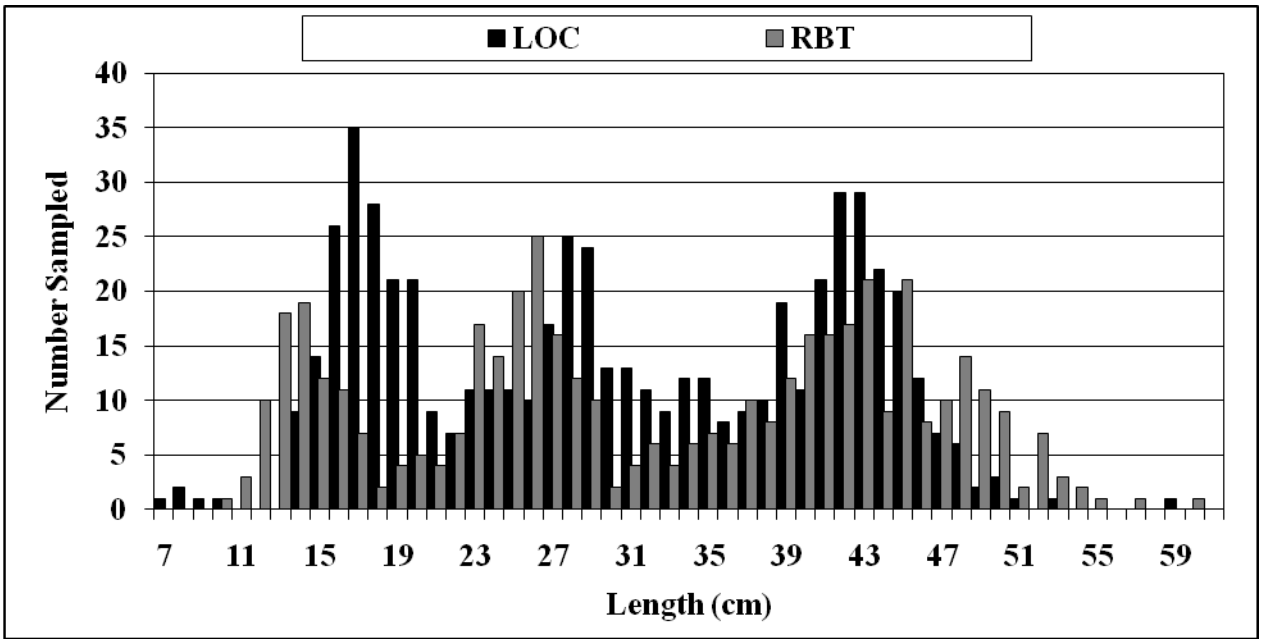


During fry evaluations conducted in July 2009, 90 rainbow trout fry were found at several sites throughout the Gunnison Gorge. Fin clips were taken from all rainbow trout fry for genetic analysis. Fry population estimates conducted in August estimated that brown trout were present in the Gunnison Gorge at a density of 803.4 fish per km (1,293 fry per mi), decreasing to 345.4 fish per km (556 fish per mi) in October. Rainbow trout fry were present in the Gunnison Gorge at a density of 523 fish per km (816 fish per mi) in August, decreasing to 347 fish per km (556 fish per mi) in October. Brown trout fry were removed from one 50 foot section in the Ute Park section of the Gunnison Gorge during the August fry evaluations. At the time of the removal, two rainbow trout fry were found in this section. This same section contained 18 rainbow trout fry when fry evaluations were repeated in October. These results suggested that brown trout fry removal may increase rainbow trout fry survival and retention in the Gunnison Gorge; therefore, a larger scale replicate of the removal was conducted in the Ute Park section of the Gunnison Gorge in 2010 (see below).

The East Portal of the Gunnison River is located downstream of the Crystal Dam, at the upstream end of the Black Canyon of the Gunnison National Park, and is currently being managed as a potential GR-cross wild brood stock location. In 2007, 4,100 F1 rainbow trout, averaging six inches in length, were stocked into the a two mile section of the East Portal, with introductions of F1 rainbow trout continuing in 2008 (42,000 rainbow trout averaging 4.7 in) and 2009 (5,000 rainbow trout averaging 4.7 in). The introduced rainbow trout have exhibited high survival in the East Portal, comprising half of the overall fish population (Figure 4.20). In September 2009, brown trout were estimated to be present in the East Portal at a density of 1,616 fish per km (2,601 fish per mi), with rainbow trout estimated to be present at a density of 1,548 fish per km (2,492 fish per mi). During the recapture run of the population estimate, a small-scale brown trout removal was conducted, moving captured brown trout below a diversion structure located downstream of the study section. During the recapture run, 225 brown trout, or about 5.4% of the population, were removed from the two mile section of the East Portal.

The high survival of the rainbow trout in the East Portal of the Gunnison River can be partially attributed to the lower whirling disease infectivity in this part of the river. In addition to high adult survival, the adults appear to reproducing, and the offspring recruiting to the adult population. Brown trout removal may prove to be effective in increasing rainbow trout numbers in the East Portal. These results are promising, and could lead to the establishment of a wild, self-sustaining GR-cross brood stock in the East Portal of the Gunnison River.

Figure 4.20. Length-frequency distribution of brown trout and rainbow trout encountered during the fall 2009 mark-recapture estimate in the East Portal of the Gunnison River.



2010 Field Season

A large-scale brown trout fry removal project was initiated in the Ute Park section of the Gunnison Gorge in 2010. A one mile section of the Gunnison River was selected for the experimental manipulation. The upstream end of the section was located just below Buttermilk rapid, with the downstream end extending just downriver of the BLM tepee (Figure 4.21). Removal of brown trout fry occurred over the full length of the section on the west side of the river; no removal occurred on the east side of the river. It was assumed that the river was wide and swift enough to prevent recolonization of brown trout fry from the east side of the river. In addition, the section was split into two half-mile sections; rainbow trout fry were stocked in the lower half-mile section, and were not stocked in the upper half-mile section. This provided four treatment areas: (1) no brown trout fry removal and no rainbow trout fry stocking (NR, NS), (2) no brown trout fry removal and rainbow trout fry stocking (NR, S), (3) brown trout fry removal and no rainbow trout fry stocking (R, NS), and (4) brown trout fry removal and rainbow trout fry stocking (Figure 4.21).

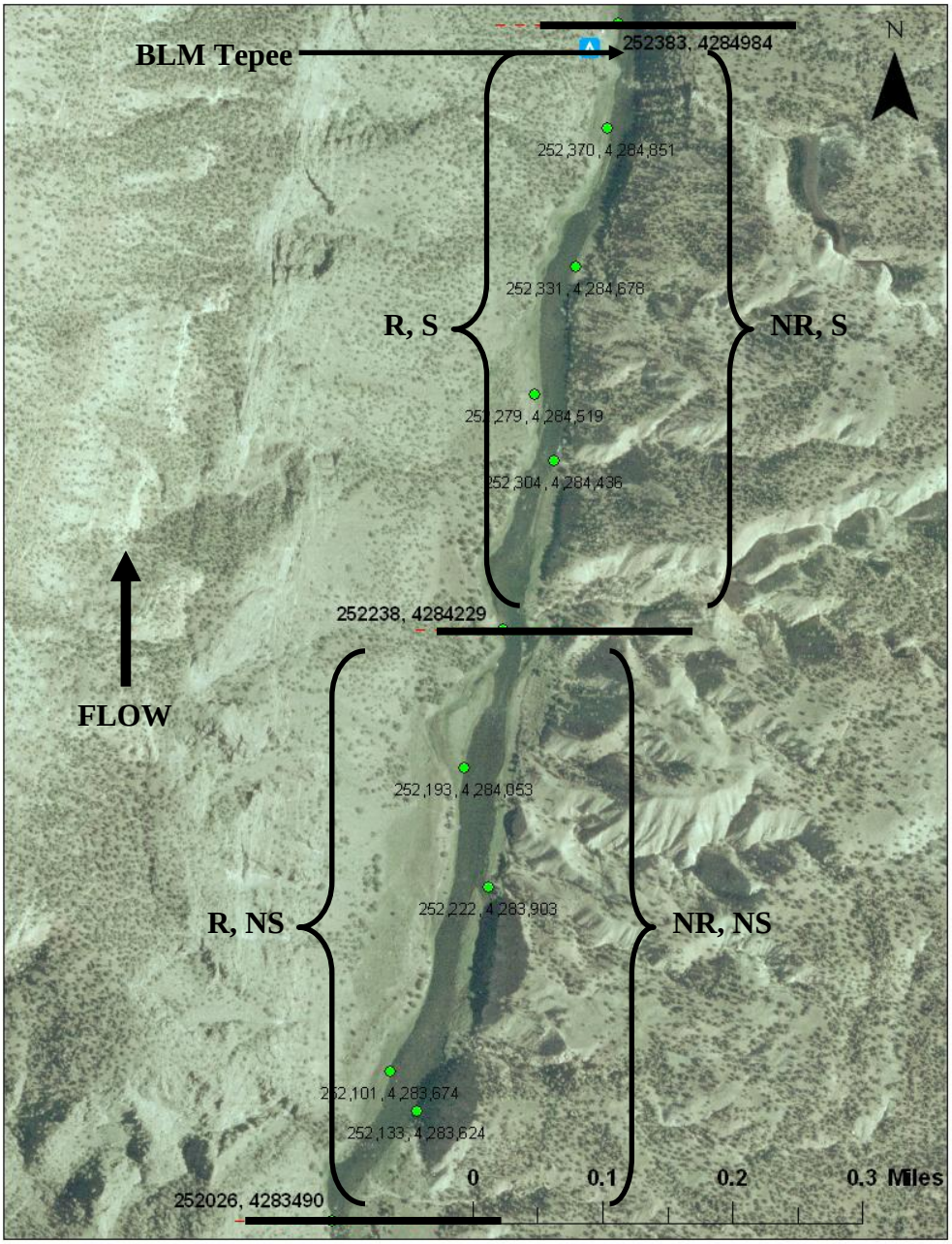
Brown trout fry removal occurred during the last week of June 2010. Prior to the removal, two fry population estimation sites, 50 ft in length, were established in each of the four treatment areas; the first represented "good" fry habitat (lots of fry expected prior to estimates) and the second represented "moderate" fry habitat (less fry expected prior to estimates), to determine the range of fry distribution throughout the study section.

Population estimates were conducted using two Smith-Root LR-24 backpack electrofishing units running side-by-side to complete a two-pass removal estimate. Total lengths were obtained from all fish encountered during the population estimates. The removal was accomplished using two Smith-Root LR-24 backpack electrofishing units, and occurred in four quarter-mile sections in which one backpack electrofishing unit and two netters scoured the shallow bank habitat for fry. Therefore, the fry habitat throughout the entire section was shocked only once during the removal. All rainbow trout fry encountered during the removal were immediately returned to the river; brown trout fry were not. A total of 4,267 brown trout fry were removed over the course of the three day removal, representing 32% of the estimated brown trout fry population on the west side of the river.

Rainbow trout fry were packed into the gorge on horseback on June 25, 2010. A total of 21,000 rainbow trout fry were brought in 12 bags. Three bags were taken to each of the four 50 ft population estimation sections in the lower half mile of the river for stocking. The 50 ft sections were used as focal points for the stocking to ensure that rainbow trout were introduced to the sections were the fry population estimates would be repeated in October. The fish were distributed both up and downriver from the 50 ft sections, with the rainbow trout being introduced in groups of 10 to 50 every couple of feet. After stocking, rainbow trout were observed swimming in the margins of the river, feeding, and reacting to shadows normally.

Fry population estimates were conducted in October to evaluate the success of the brown trout fry removal and rainbow trout fry stocking. A raft-mounted bank electrofishing unit with three electrodes was used to complete the estimates, and three removal passes were made through each of the eight previously established sites (two in each of the treatment areas). In August 2010, a major flood occurred in the Gunnison Gorge, changing the habitat of several of the established fry sites. To account for effects of the flood, and to gain a better understanding of how the fry redistributed after the flood, a third randomly chosen site was sampled in each of the treatment areas to increase sample size. The amount of silt was qualified (lots, some, little or none) for each of the sites to be used as a covariate in the analysis. Lengths were taken from all fish encountered during the sampling. In addition, fin clips were taken for genetic analysis from the sites in which rainbow trout fry were not stocked in June (sites within the NR, NS and R, NS treatment areas).

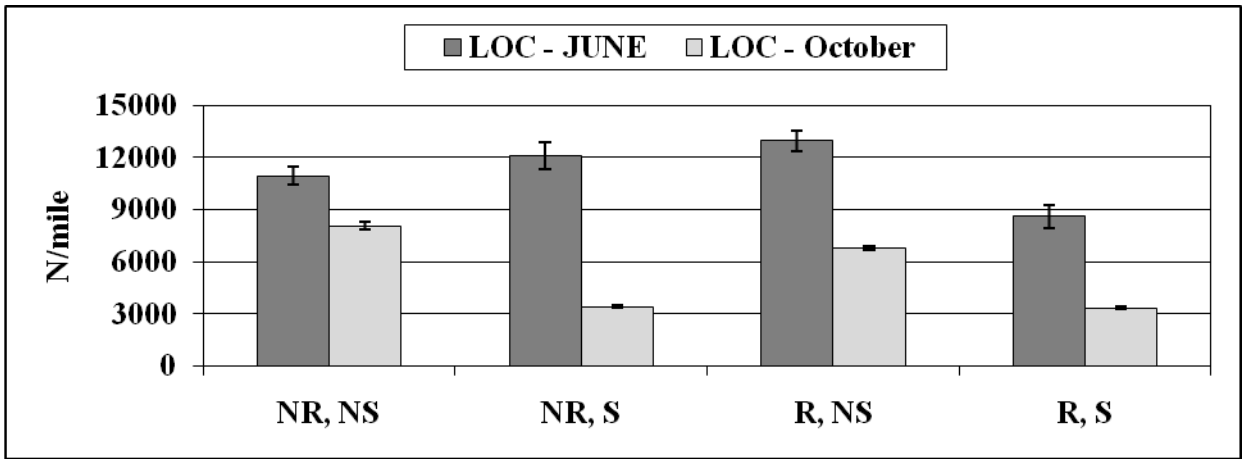
Figure 4.21. Map of the experimental set-up for the brown trout fry removal experiment conducted in the Ute Park section of the Gunnison Gorge. The two dots and GPS locations within each of the treatment areas represent the fry population estimation sites established in June 2010, and resampled in October 2010.



A Huggin's closed capture mark-recapture model in program MARK (White and Burnham 1999) was used to directly estimate detection probabilities (*p*); in addition abundance (*N*)was obtained as a derived parameter. Models with continuous detection probability, as well as with detection probability varying by length (entered as a covariate

in the input file), site, or species (rainbow trout or brown trout), and all additive combinations therein, were used to obtain estimates of detection probability and abundance in June. Models in which detection probability was continuous, or varied by site, species, the qualitative variable silt (silt), whether or not the site had been stocked with rainbow trout fry (stocking), whether or not brown trout removal had occurred in the site (removal), treatment (combination of stocking and removal), or length (entered as a covariate), and all additive combinations therein, were used to obtain estimates of detection probability and abundance in October. Models were ranked using AICc. For the data collected in June, the continuous detection probability model was the top model (AICc = 269.493, AICc weight = 0.291). For the data collected in October, the top model had detection probability varying by site (AICc = 1108.523, AICc weight = 0.183). In addition, the importance of each model parameter was calculated using cumulative AICc weights. For the June data, length was the parameter that most affected detection probability (cumulative AICc weight = 0.438), followed by site (cumulative AICc weight = 0.301), with species having less of an effect on detection probability than either length or site (cumulative AICc weight = 0.271). Site was the variable that most affected detection probability in October (cumulative AICc weight = 0.894). Silt (cumulative AICc weight = 0.311), species (cumulative AICc weight = 0.287), length (cumulative AICc weight = 0.269), stocking (cumulative AICc weight = 0.202), and removal (cumulative AICc weight = 0.194) all had less of an effect on capture probability than did site, and treatment had a very small effect on detection probability (cumulative AICc weight = 0.074). Estimates of detection probability and abundance were obtained through model averaging of the models with an AIC weight greater than zero.

Figure 4.22. Estimated brown trout (LOC) fry abundance (per mile) in the four treatment sections, for the months of June and October, in the Ute Park section of the Gunnison Gorge.



Brown trout fry abundance decreased in all four of the treatment sections between June and October (Figure 4.22). In June, the number of brown trout fry per mile was similar on both sides of the river (removal versus non-removal sections). Both sections showed a similar decrease in brown trout fry between June and October, and did not differ in the number of fry per mile in October, indicating that the removal was not

necessarily responsible for the decline. Similarly, brown trout fry experienced a similar decrease in number of fry per mile in sections that were and were not stocked with rainbow trout fry, indicating that the addition of rainbow trout fry was not necessarily responsible for the decline (Figure 4.23). Overall, there did not appear to be a relationship between treatment and decline in brown trout fry per mile between June and October.

Figure 4.23. Estimated brown trout (LOC) fry abundance (per mile) in the (a) removal (R) and non-removal (NR) sections, and (b) the sections stocked (S) and not stocked (NS) with rainbow trout fry, for the months of June and October, in the Ute Park section of the Gunnison Gorge.

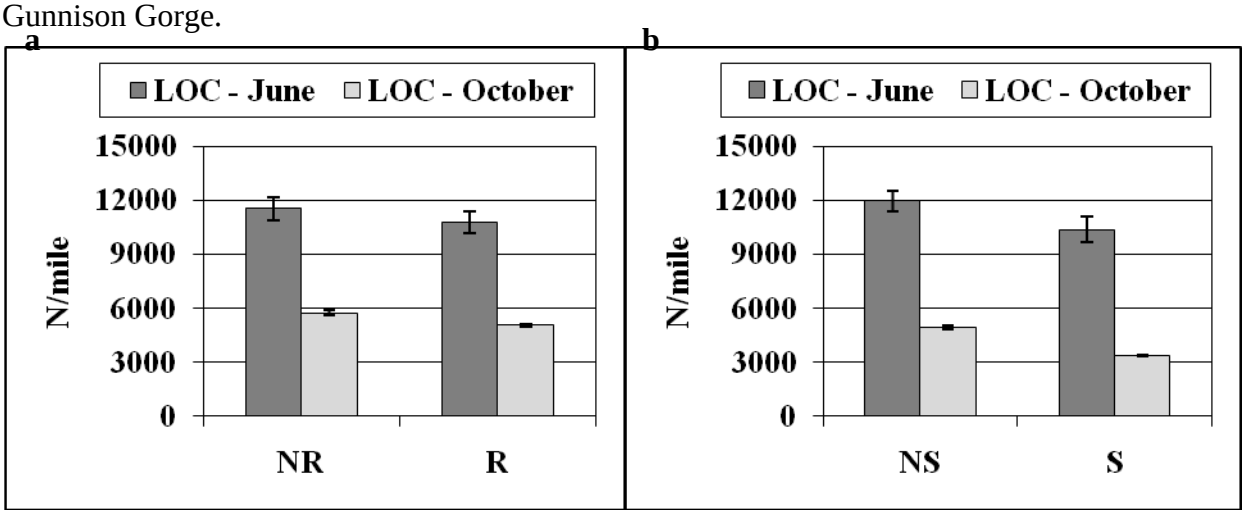
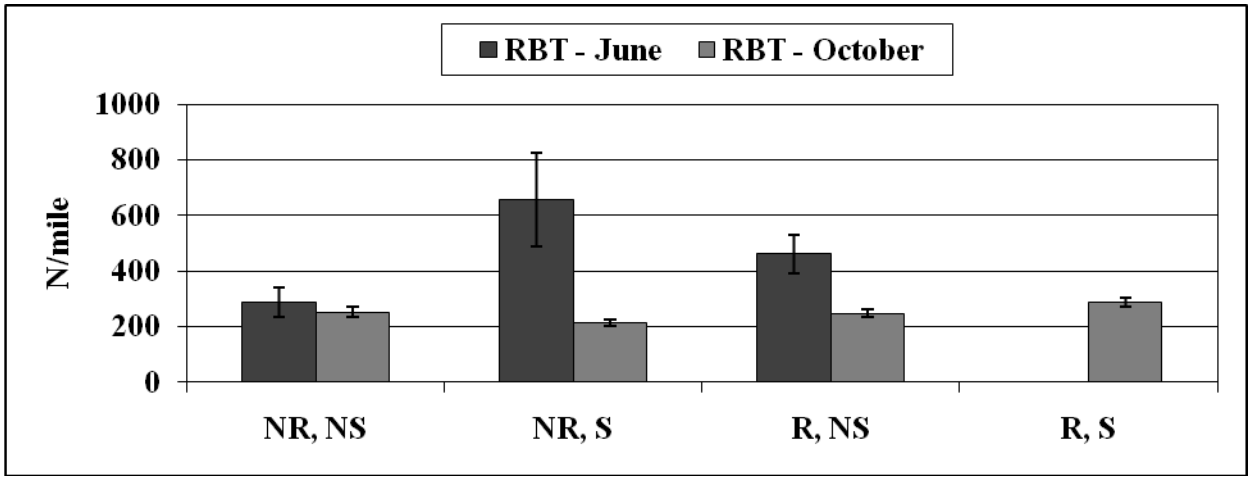


Figure 4.24. Estimated rainbow trout (RBT) fry abundance (per mile) in the four treatment sections, for the months of June and October, in the Ute Park section of the Gunnison Gorge.



There was a significant decline in the number of rainbow trout fry per mile in the non-removal/stocked and removal/not stocked treatments, no difference in fry per mile in the non-removal/not stocked treatment, and a significant increase in fry per mile in the removal/stocked treatment between June and October (Figure 4.24). The increase in the treatment in which brown trout were removed *and* rainbow trout fry were stocked indicates that the combination of the two management actions may have a positive effect on rainbow trout fry survival in the Gunnison Gorge. In the sections where brown trout fry removal did not occur, and sections in which rainbow trout fry were not stocked, there was a significant decline in the number of rainbow trout fry per mile between June and October. However, in sections where rainbow trout were stocked, and sections where brown trout were removed, there was not a significant change in the number of rainbow trout fry per mile between June and October (Figure 4.25). These results indicate that either management action may be effective in increasing rainbow trout fry survival in the Gunnison Gorge. The results of this study were significantly influenced by the flood that occurred in the Gunnison Gorge in August 2010, specifically the change in the quality of fry habitat due to siltation (Figure 4.26). Therefore, a similar experiment is scheduled to occur in the Smith Fork section of the Gunnison Gorge in 2011.

Figure 4.25. Estimated rainbow trout (RBT) fry abundance (per mile) in the (a) removal (R) and non-removal (NR) sections, and (b) the sections stocked (S) and not stocked (NS) with rainbow trout fry, for the months of June and October, in the Ute Park section of the Gunnison Gorge.

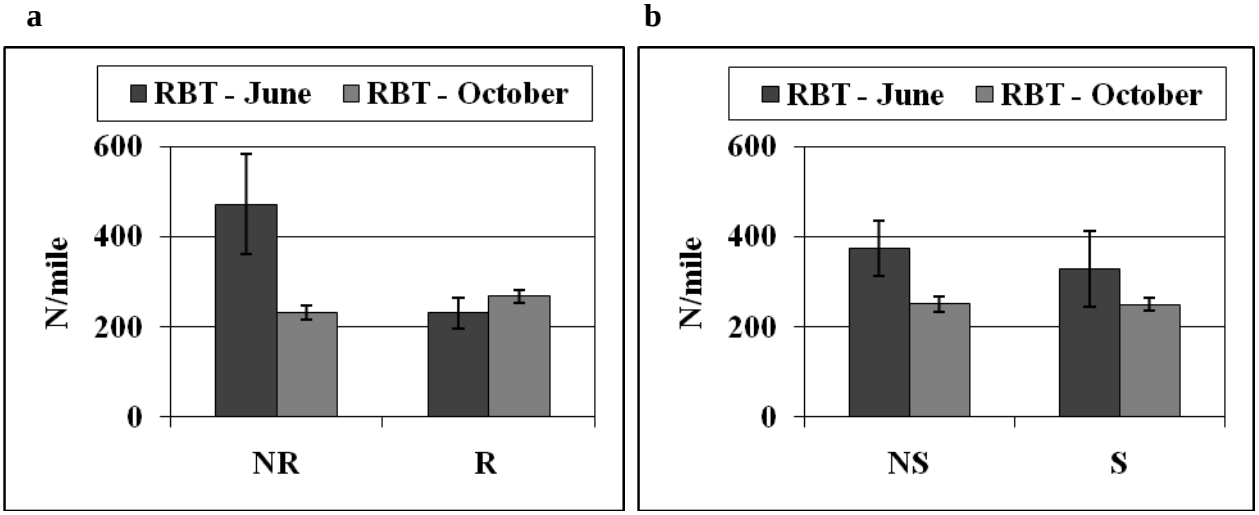
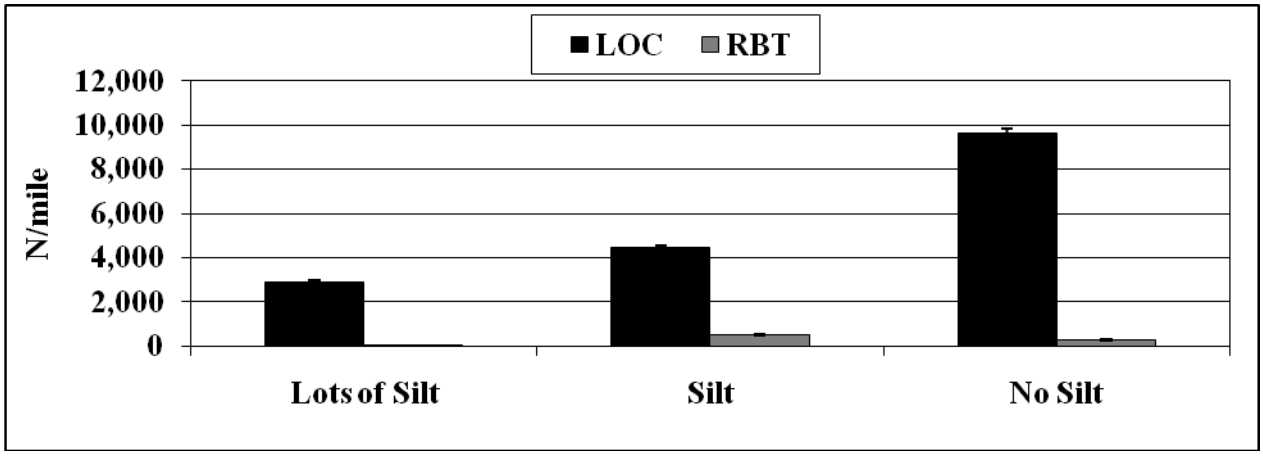


Figure 4.26. Effects of silt in fry habitat on the October 2010 estimated fry abundance (per mile) in the Ute Park section of the Gunnison Gorge.



Brown trout numbers remained high, and rainbow trout numbers low, in the Ute Park section of the Gunnison Gorge in 2010. Brown trout biomass was up 9.2%, and density was up 43.4%, from 2009. Brown trout were estimated to be present in the section at a density of 6,438 fish per km (10,342 fish per mi). Despite low numbers of rainbow trout, rainbow trout biomass was up 45.5%, and density was up 95.2%, from 2009. No rainbow trout were positively identified as either F1s or B2s stocked in past years. Therefore, wild rainbow trout, those that could not be positively identified as CRRs, F1s, or B2s, were estimated to be present in the study reach at a density of 127 fish per km (205 fish per mi; Figure 4.27). Five age classes were observed in the rainbow trout population in 2010, an increase from the three age classes represented in the rainbow trout population in 2009 (Figure 4.28).

Figure 4.27. Length-frequency distribution of brown trout and wild rainbow trout encountered during the fall 2010 mark-recapture population estimate in the Ute Park section of the Gunnison Gorge.

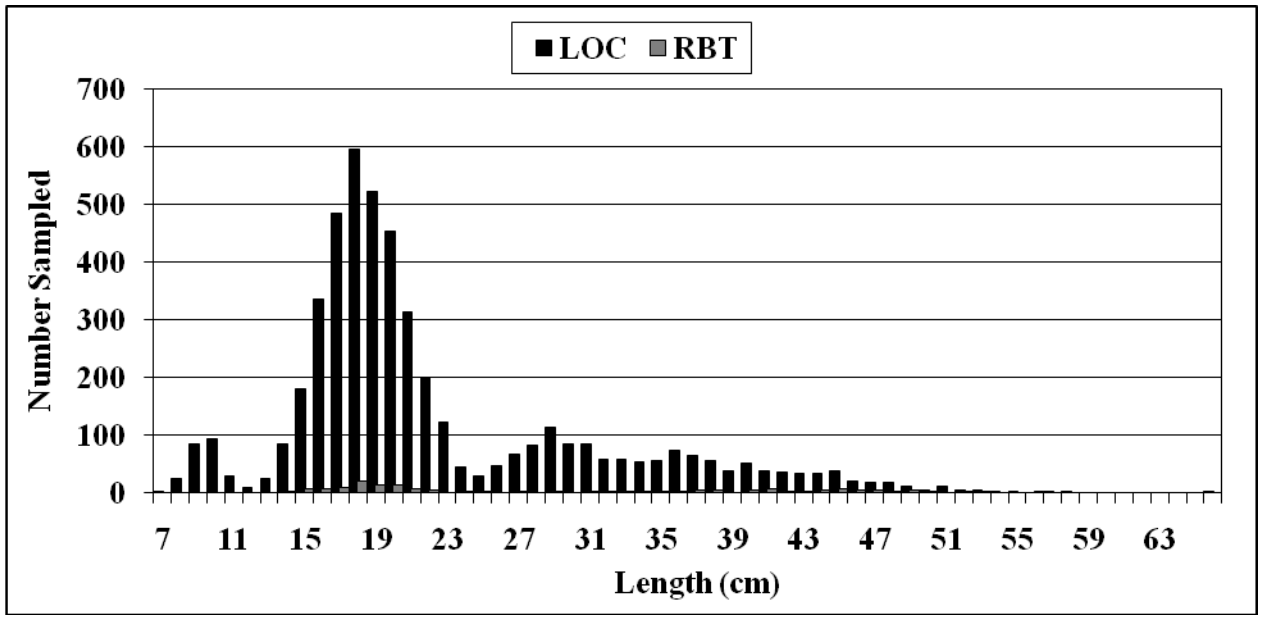
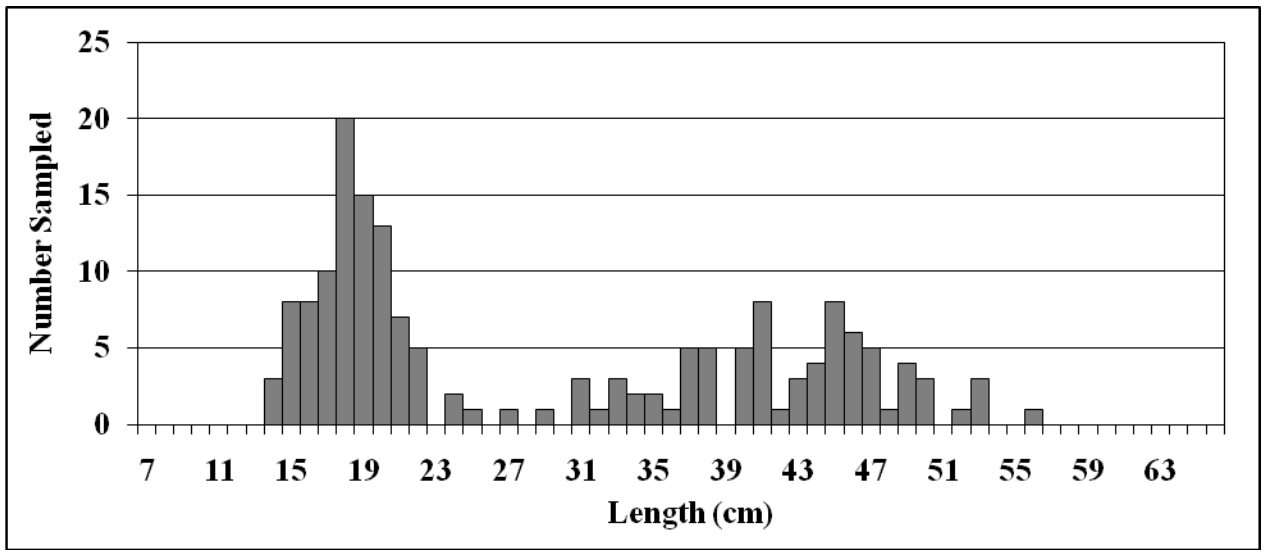


Figure 4.28. Length-frequency distribution of wild rainbow trout encountered during the fall 2010 mark-recapture population estimate in the Ute Park section of the Gunnison Gorge.



Conclusions

Brown trout numbers continue to remain high in the Gunnison River, a factor likely contributing to the low survival and persistence of the introduced rainbow trout. Tag loss has made identification of previously introduced rainbow trout nearly impossible. However, the increase in "wild" rainbow trout witnessed in the Ute Park section of the Gunnison Gorge in 2010 is encouraging, considering no adult rainbow trout have been introduced to this section of river since January 2009. In addition, the presence of five age classes of rainbow trout indicates that not only is reproduction occurring in the Ute Park section of the Gunnison Gorge, but recruitment to subsequent age classes must also be occurring. Genetic tests will be used to confirm both reproduction and recruitment of GR-cross fish in the Gunnison Gorge.

The brown trout fry removal experiment conducted in the Ute Park section of the Gunnison Gorge had some encouraging results. Despite a flood changing fry habitat conditions prior to resampling, rainbow trout fry appeared to be more abundant in the treatment section in which both brown trout fry were removed and rainbow trout fry were stocked. The results of this experiment, and potential implications for future whirling disease resistant rainbow trout management, has prompted this experiment to be repeated in the Smith Fork section of the Gunnison River, as well as a section of the upper Colorado River near Hot Sulpher Springs and a section of the Laramie River, in 2011.

Genetic Techniques

Introduction

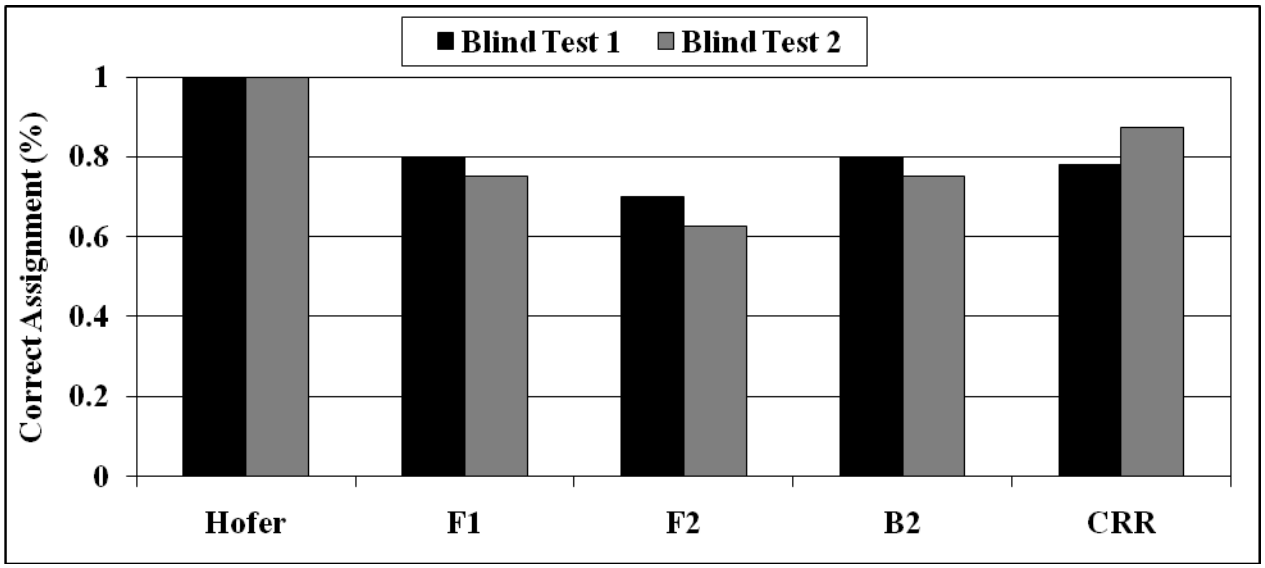
A suite of microsatellite markers capable of distinguishing fish of the GR lineage, including pure GR, F1, F2, and backcross generations (B2 – F1 x CRR – and BC1 – F1 x GR), from other rainbow trout strains, specifically the CRR, have recently been developed and tested. These markers were developed to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

Tests for Accuracy

Initial tests for accuracy were run using known samples through the NewHybrids program to determine how often an individual was correctly assigned to the known GR-cross, with a probability of 80% or greater. One hundred percent of the GR strain individuals were correctly assigned as pure GR, whereas 93.5 % of the pure CRR individuals were correctly assigned as pure CRR; most commonly, pure CRR individuals were misidentified as B2 individuals. For the pure strains, 87.5% of the F1 individuals were correctly assigned as F1s, and were most commonly misidentified as F2s. Similarly, 87.2% of the B2 individuals were correctly assigned as B2s, and were most commonly misidentified as F2s. Finally, 80% of the F2 individuals were correctly assigned as F2s, and both individuals incorrectly assigned were misidentified as B2s. These results indicated that the microsatellite markers and associated NewHybrids probability tests were capable of distinguishing between pure and hybrid individuals (99% of the time), and that the majority of F1, F2 and B2 individuals could be correctly assigned.

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

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



Colorado River

Genetic samples were collected from rainbow trout fry encountered during electrofishing efforts in 2007-2010. In 2007 (n = 15), all positively identified fry were identified as CRR; the genetic background of one individual was unknown. The proportion of fry in the sample that were positively identified as CRR dropped below 80% in 2008 (n = 21), remaining below 80% in 2009 (n = 74), and dropped to just below 50% in 2010 (n = 57). GR-cross fish began to appear in the sample in 2008; B2 fish were the first to appear, indicating that spawning between F1 adults, stocked in 2006, and residual CRR adults occurred in 2008. Unclassifiable hybrids, F2s, and F1s appeared in the sample in 2009, comprising 5% of the sample, indicating that adult F1 rainbow trout were spawning with each other in the upper Colorado River. Over 50% of the fry sample in 2010 consisted of GR-cross fish, with over 20% consisting of hybrids, F2s, and F1s (Figure 4.30).

Figure 4.30. Proportion of fry categorized as unknown, pure CRR, or GR-cross fish, collected from the upper Colorado River, 2007-2010.

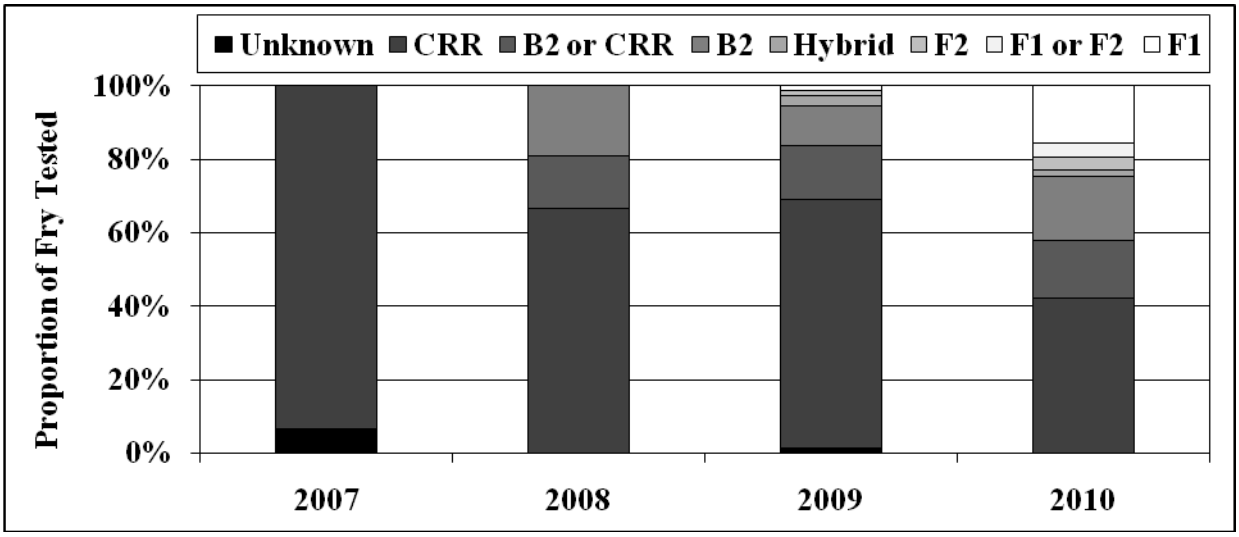
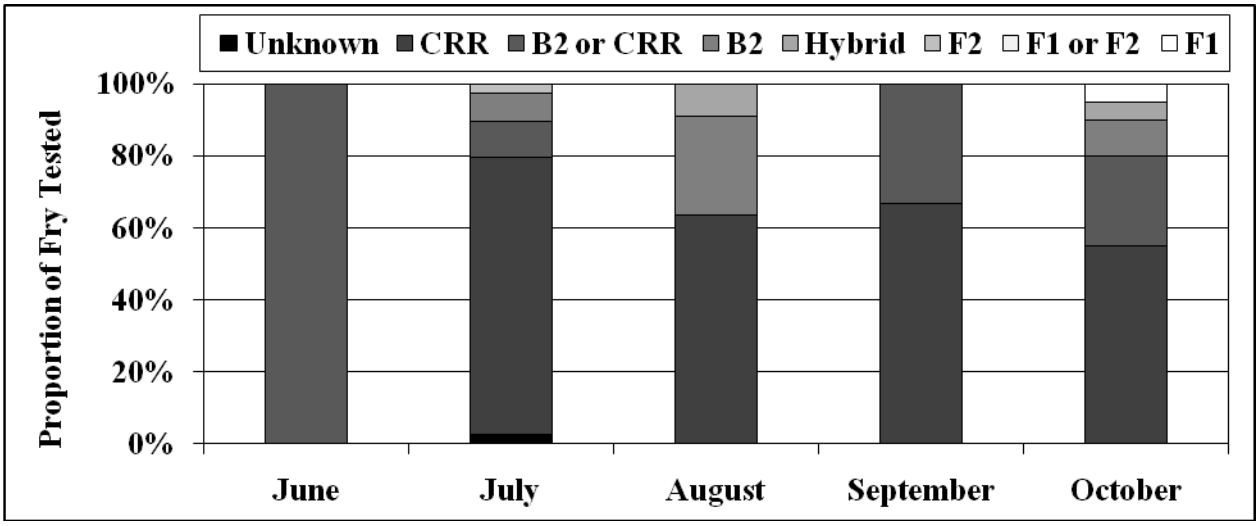


Figure 4.31. Proportion of fry categorized as unknown, pure CRR, or GR-cross fish, collected from the upper Colorado River, July-August, 2009.

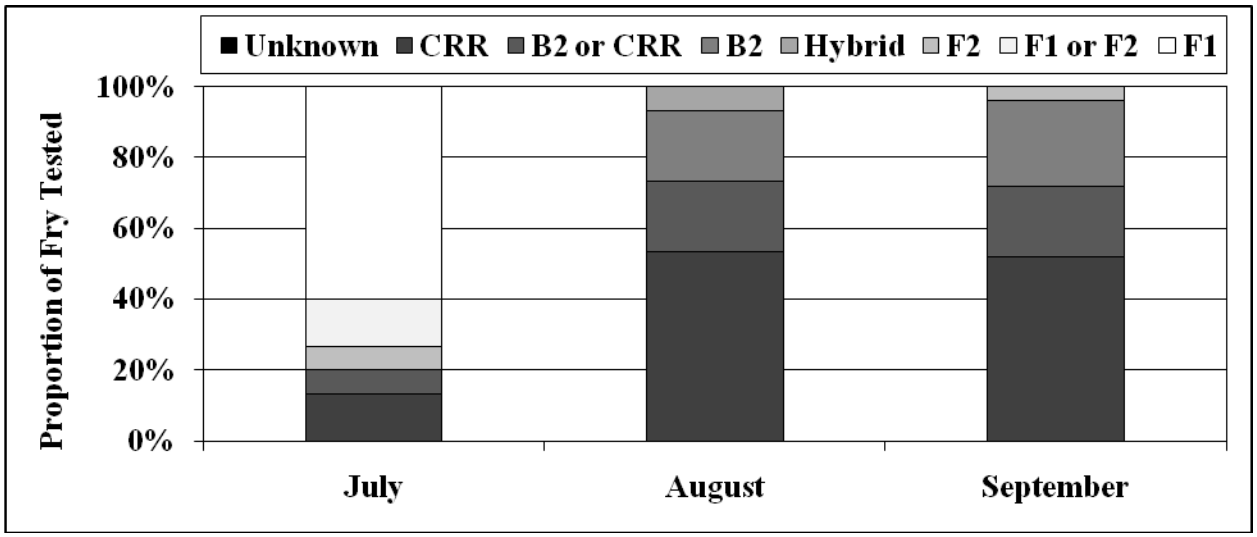


The proportion of CRR and GR-cross individuals in the sample not only changed across years, but also across months within a year. In July 2009, positively identified CRRs comprised over 75% of the sample; this proportion was reduced to just over 60% in August and September 2009, and was lowest in October 2009 at just over 50%. GR-cross fish were positively identified in the samples in July, August and October, with samples in September possibly being either CRR or B2 fish. The proportion of positively identified GR-cross fish was highest in August at just under 40%; however, 20% of the sample in October still consisted of positively identified GR-cross fish (Figure 4.31). The reduction in the proportion of CRR in the sample from July to October was expected

as these fish are most susceptible to whirling disease infection, and are subject to increasing mortality over time as a result.

In 2010, no rainbow trout fry were collected in June or October. The proportion of positively identified CRR individuals in the sample was lowest in July at less than 20%; this proportion increased to around 50% in August, and remained at around 50% in September. GR-cross fish represented the largest proportion of the sample in July, comprising 80% of the sample; this proportion decreased to about 25% positively identified GR-cross fish in August, remaining the same in September (Figure 4.32). The decrease in the proportion of GR-cross fish in August and September was unexpected, as these fish are more resistant to whirling disease infection, and less susceptible to mortality due to infection over time. The decrease in GR-cross fish could have resulted from a number of factors, including mortality from causes other than whirling disease, or low detection of rainbow trout in general. Despite the decrease in proportion of GR-cross fish, these fish were present in a higher proportion of the sample in September 2010 then they were in 2009.

Figure 4.32. Proportion of fry categorized as unknown, pure CRR, or GR-cross fish, collected from the upper Colorado River, July-August, 2009.



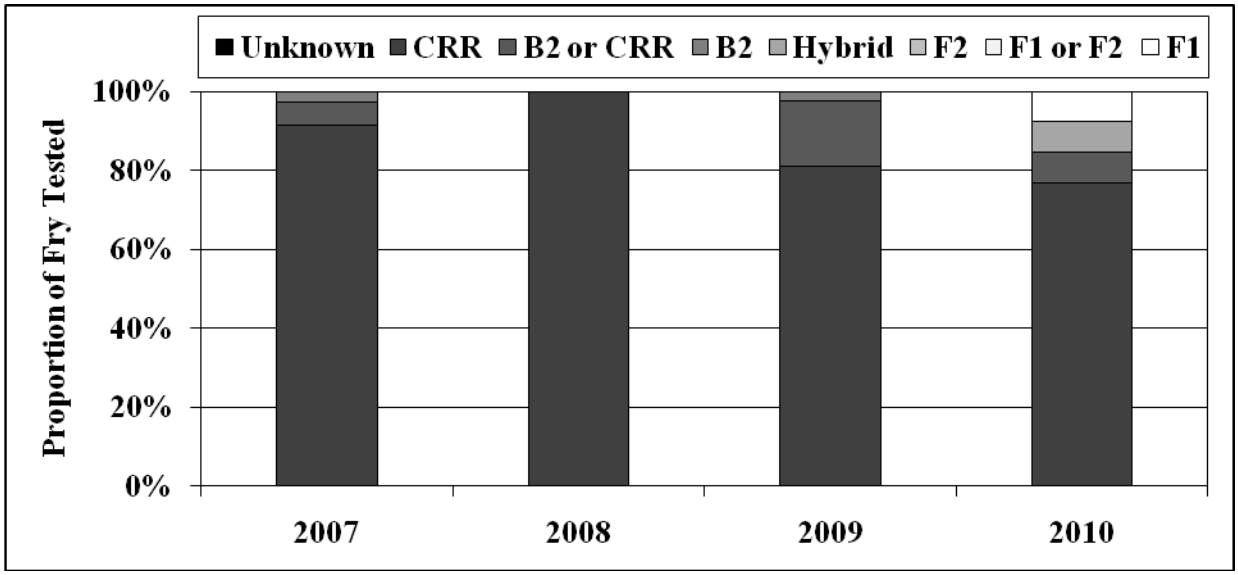
In general, fluctuating numbers of GR-cross fry in the first few years of reproduction is expected in the upper Colorado River. As GR-cross fish become more established, proportions are expected to change from a more CRR to a more GR-cross dominated rainbow trout fry community.

Gunnison River

Genetic samples were collected from rainbow trout fry encountered during electrofishing efforts in 2007-2010. In 2007 (n = 35), over 90% of the sample was positively identified as CRR; the genetic background of two individuals was undeterminable between B2 or CRR, and only one individual was positively identified as a B2 individual. The proportion of fry in the sample that were positively identified as

CRR was 100% in 2008 (*n* = 21), dropped to around 80% in 2009 (*n* = 42), and dropped to just below 80% in 2010 (*n* = 13). GR-cross fish were present in the sample in small proportions in 2007, 2009, and 2010 (Figure 4.33).

Figure 4.33. Proportion of fry categorized as unknown, pure CRR, or GR-cross fish, collected from the Gunnison River, 2007-2010.



Despite a large number of introductions of F1 and B2 fish to the Ute Park section of the Gunnison Gorge, GR-cross offspring are still poorly represented in the rainbow trout fry population. CRR offspring appear to dominate the rainbow trout fry community, indicating that the residual and stocked CRR are spawning, and their offspring are surviving. Genetic analysis of age-1 rainbow trout from the Ute Park section of the Gunnison Gorge is currently being completed, the results of which will show whether CRR or GR-cross fish are recruiting to the age-1 rainbow trout population.

References

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

Northern Colorado Water Conservancy District. 2011. Retrieved May 31, 2011 from http://www.ncwcd.org/datareports/westflow.asp.

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

White, G. C., and K. P. Burnham. 1999. Program MARK: Survival estimation from populations of marked animals. Bird Study 46 Supplement, 120-138.

Job No. 5: Technical Assistance

Job Objective: Provide information on impacts of fish disease on wild trout populations to fisheries managers and hatchery personnel of the Colorado Division of Wildlife and other resource agencies. Provide specialized information or assistance to the Hatchery Section. Contribute editorial assistance to various professional journals and other organizations upon request.

Technical Assistance Projects

The work described in this Federal Aid Project is closely associated with work conducted by Ron Hedrick, Bernie May, and Melinda Baerwald at the University of California-Davis to identify markers for WD resistance in select families of fish. The Colorado Division of Wildlife continues to work with these individuals, as well as with other agencies, such as the Utah Department of Natural Resources, the California Department of Fish and Game and the Montana Department of Fish, Wildlife, and Parks, to enhance and accelerate research on rainbow trout strains.

Development and testing of the C-SAP creel survey analysis computer program was a major part of this technical assistance during this project cycle. The original C-SAP program was last updated in February of 1990 and the software had become increasingly difficult to run on newer computers. The data entry portion of the program was problematic and interpretation of the reports was complicated. Accurate creel information and efficient data entry were necessary for this particular project and the Colorado Division of Wildlife as a whole would benefit from an updated format of the program. As a result, efforts were initiated to create a Windows-based version of the original C-SAP program. The new version, written in the Microsoft .NET platform, was released in several different early versions, and is currently being run statewide on the December 16, 2009 release. Training biologists in operating and conducting analysis with the program was large part of the technical assistance provided.

Another technical assistance project that has generated interest among other State and Federal agencies is a small-scale experiment designed to evaluate the efficacy of quaternary ammonia compounds for the disinfection of equipment to prevent the distribution of New Zealand mud snails (*Potamopyrgus antipodarum*). A summary of this work was published in the North American Journal of Fisheries Management: "Schisler, G. J., N. K. M. Vieira, and P. G. Walker. 2008. Application of Household Disinfectants to Control New Zealand Mudsnails. North American Journal of Fisheries Management 28:1172–1176." The recommendations for disinfection found in this publication were adopted by several agencies, including the United States Forest Service.

Additional work associated with the effects of whirling disease on mountain whitefish (*Prosopium williamsoni)* was conducted to help fisheries managers better understand the relationship between the parasite and this salmonid species. A summary this work was written as an internal CDOW document "Schisler, G. J. 2010. Effects of

whirling disease (*Myxobolus cerebralis*) exposure on juvenile mountain whitefish (*Prosopium williamsoni*). Research Report. Colorado Division of Wildlife Fish Research Section. Fort Collins, CO."

2006-2007 Technical Assistance Milestones

1) National American Fisheries Society meeting on September 12, 2006 in Lake Placid, New York.
2) Colorado Wildlife Commission Meeting in Steamboat Springs, Colorado, on August 10, 2006.
3) Continuing Education Biology Teachers group at Parvin Lake Research Station on July 10, 2006.
4) United States Geological Survey meeting in Fort Collins, Colorado on November 2, 2006.
5) 13th Annual Whirling Disease Symposium: Resistance on Two Fronts! Denver, Colorado, February 12-13, 2006.
6) Colorado-Wyoming annual American Fisheries Society meeting, February 26-March 1, 2007, in Fort Collins, Colorado.
7) Interviews and materials for popular articles were provided for several periodicals including Colorado Hunting and Fishing News, The Denver Post, The Scientist, Fly Rod and Reel, High Country Angler, and Southwest Fly Fishing.
8) A professional journal article was published in 2006; "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."

2007-2008 Technical Assistance Milestones

1) Schisler, G. J. 2007. Resistant rainbow trout brood stock development for fisheries management in Colorado. Trout Unlimited-Cherry Creek Anglers, July 21, 2007. Parvin Lake Research Station. Red Feather Lakes, CO.
2) Schisler, G. J. 2007. Resistant rainbow trout brood stock development for fisheries management in Colorado. Colorado State University Student Chapter of the American Fisheries Society. October 17, 2007. Fort Collins, CO.
3) Schisler, G. J. 2008. Resistant rainbow trout brood stock development for fisheries management in Colorado. American Fly Fishing Trade Association meeting. January 4, 2008. Denver, CO.
4) Schisler, G. J. 2007. Resistant rainbow trout brood stock development for fisheries management in Colorado. Colorado Aquaculture Association Meeting. January 18, 2008. Mt. Princeton, CO.
5) Schisler, G. J., K. B. Rogers, and R. P. Hedrick. 2008. Early development of mountain whitefish (*Prosopium williamsoni*) and effects of *Myxobolus cerebralis* exposure. 14th Annual Whirling Disease Symposium: Solving the Puzzle, Denver, Colorado, February 4-5, 2008.

6) Kowalski, D. A, R. B. Nehring, and G. J. Schisler. 2008. Preliminary results on the introduction of *Myxobolus cerebralis* resistant rainbow trout in the Gunnison River, Colorado. 14th Annual Whirling Disease Symposium: Solving the Puzzle. February 4-5, 2008**,** Denver, CO.
7) Fetherman, E. F., D. L. Winkelman, and G. J. Schisler. 2008. The physiological effects of whirling disease in resistant and susceptible crosses of rainbow trout. 14th Annual Whirling Disease Symposium: Solving the Puzzle. February 4-5, 2008**,** Denver, CO.
8) Fetherman, E. F., D. L. Winkelman, and G. J. Schisler. 2008. The physiological effects of whirling disease in resistant and susceptible crosses of rainbow trout. Colorado-Wyoming Annual American Fisheries Society meeting, March 3-7, 2008 Cheyenne, WY.
9) Fetherman, E. F., D. L. Winkelman, and G. J. Schisler. 2008. The physiological effects of whirling disease in resistant and susceptible crosses of rainbow trout. Western Division Annual American Fisheries Society meeting, May 4-9, 2008 Portland, OR.
10) Bartholomew, J., G. Schisler, R. B. Nehring, R. Hedrick, and M. El-Matbouli. 2008. Fisheries management approaches for control of *Myxobolus cerebralis:* resistant rainbow trout and worms. Western Division Annual American Fisheries Society meeting, May 4-9, 2008 Portland, OR.
11) Interviews and materials for popular articles were provided for several periodicals including Sports Afield, North American Fisherman Magazine, and American Angler Magazine.
12) Additional media interviews and popular articles have been published in the Denver Post, Rocky Mountain News, Fort Collins Coloradoan, Vail Daily News, Summit Daily News, Glenwood Springs Post-Independent, Pueblo Chieftain, and many other newspapers.
13) A full–feature article appeared in Headwaters Magazine. The project was also mentioned on CBS News 4 television and Denver 9 News.

2008-2009 Technical Assistance Milestones

1) Schisler, G. J. 2008. Resistant rainbow trout brood stock development for fisheries management in Colorado. Red Feather Lakes Historical Society, July 16, 2007. Parvin Lake Research Station. Red Feather Lakes, CO.
2) Schisler, G. J. 2008. Resistant rainbow trout brood stock development for fisheries management in Colorado. Colorado State University Student Chapter of the American Fisheries Society. December 3, 2008. Fort Collins, CO.
3) Schisler, G. J. 2008. Resistant rainbow trout brood stock development for fisheries management in Colorado. Chimney Rock Ranch Club, July 24, 2008. Denver, CO.
4) Schisler, G. J. 2009. Resistant rainbow trout brood stock development for fisheries management in Colorado. Colorado Aquaculture Association Meeting. January 24, 2009. Mt. Princeton, CO.

5) Schisler, G. J., J. Ewert, B. Atkinson, K. Rogers, K. Thompson, R. B. Nehring, and E. Fetherman. 2009. Whirling disease resistant rainbow trout Colorado River project update. 15th Annual Whirling Disease Symposium: Conserving coldwater fisheries, Denver, CO, February 5-6, 2009**.**
6) Schisler, G. J., K. B. Rogers, and R. P. Hedrick. 2009. Early development of mountain whitefish *(Prosopium williamsoni*) and effects of *Myxobolus cerebralis* exposure. 15th Annual Whirling Disease Symposium: Conserving coldwater fisheries, Denver, CO, February 5-6, 2009.
7) Schisler, G. J., K. B. Rogers, and R. P. Hedrick. 2009. Early development of mountain whitefish (*Prosopium williamsoni*) and effects of *Myxobolus cerebralis* exposure. Whitefish summit, Silverthorne, Colorado, January 6, 2009**.**
8) Kowalski, D. A, R. B. Nehring, and G. J. Schisler. 2008. Preliminary results on the introduction of *Myxobolus cerebralis* resistant rainbow trout in the Gunnison River, Colorado. 15th Annual Whirling Disease Symposium: Conserving coldwater fisheries. February 5-6, 2009**,** Denver, CO.
9) Fetherman, E. F., D. L. Winkelman, and G. J. Schisler. 2008. The physiological effects of whirling disease in resistant and susceptible crosses of rainbow trout. 15th Annual Whirling Disease Symposium: Conserving coldwater fisheries 5-6, 2008**,** Denver, CO.
10) Fetherman, E. F., D. L. Winkelman, and G. J. Schisler. 2008. The physiological effects of whirling disease in resistant and susceptible crosses of rainbow trout. Colorado-Wyoming Annual American Fisheries Society meeting, February 23-26, 2009, Loveland, CO.
11) Fetherman, E. F., D. L. Winkelman, and G. J. Schisler. 2008. The physiological effects of whirling disease in resistant and susceptible crosses of rainbow trout. Western Division Annual American Fisheries Society meeting, May 3-7, 2009 Albuquerque, NM .
12) Several popular articles have appeared as a result of interviews this year on this project such as North Forty News (May 2008), TROUT Magazine (Spring 2008), North American Fisherman Magazine (April 2008).
13) Schisler, G. J., N. K. M. Vieira, and P. G. Walker. 2008. Application of Household Disinfectants to Control New Zealand Mudsnails. North American Journal of Fisheries Management 28:1172–1176.

2009-2010 Technical Assistance Milestones

1) Fetherman, E. R., D. L. Winkelman, and G. J. Schisler. 2009. Physiological Effects of Whirling Disease and Heritability of Myxospore Count in Susceptible and Resistant Strains of Rainbow Trout. Annual Meeting of the Colorado Aquaculture Association. Mt. Princeton Hot Springs, CO. January 26, 2009.
2) Fetherman, E. R., D. L. Winkelman, and G. J. Schisler. 2009. Physiological Effects of Whirling Disease and Heritability of Myxospore Count in Susceptible and Resistant Strains of Rainbow Trout. 15th Annual Whirling Disease Symposium: Conserving Coldwater Fisheries. Denver, CO. February 4-5, 2009.

3) Fetherman, E. R., D. L. Winkelman, and G. J. Schisler. 2009. Physiological Effects of Whirling Disease and Heritability of Myxospore Count in Susceptible and Resistant Strains of Rainbow Trout. 2009 Annual Meeting of the Colorado-Wyoming Chapter of the American Fisheries Society. Loveland, CO. February 23-26, 2009.
4) Fetherman, E. R., D. L. Winkelman, and G. J. Schisler. 2009. Physiological Effects of Whirling Disease and Heritability of Myxospore Count in Susceptible and Resistant Strains of Rainbow Trout. 2009 Annual Meeting of the Western Division of the American Fisheries Society. Albuquerque, NM. May 3-7, 2009.
5) Fetherman, E. R., and G. J. Schisler. 2010. Whirling Disease Resistant Rainbow Trout 2009 Project Update. Annual Meeting of the Colorado Aquaculture Association. Mt. Princeton Hot Springs, CO. January 22, 2010.
6) Fetherman, E. R., D. L. Winkelman, and G. J. Schisler. 2010. Whirling Disease Resistant Rainbow Trout 2009 Project Update. 2010 Annual Meeting of the Colorado-Wyoming Chapter of the American Fisheries Society. Laramie, WY. March 1-3, 2010.
7) Fetherman, E. R., D. L. Winkelman, and G. J. Schisler. 2010. Whirling Disease Resistant Rainbow Trout 2009 Project Update. Whirling Disease Symposium. 2010 Annual Meeting of the Western Division of the American Fisheries Society. Salt Lake City, UT. April 19-23, 2010.
8) Schisler, G.J., J. Ewert, B. Atkinson, K. Rogers, K. Thompson, R. B. Nehring, and E. Fetherman. 2009. Whirling disease resistant rainbow trout Colorado River project update. 15th Annual Whirling Disease Symposium: Conserving coldwater fisheries, Denver, CO, February 5-6, 2009**.**
9) Schisler, G.J., J. Ewert, B. Atkinson, K. Rogers, K. Thompson, R. B. Nehring, and E. Fetherman. 2009. Whirling disease resistant rainbow trout Colorado River project update. Annual Meeting of the Colorado Aquaculture Association. Mt. Princeton Hot Springs, CO. January 26, 2009.
10) Schisler, G. J. and E. R. Fetherman. Post-release evaluation of resistant rainbow trout. Whirling Disease Symposium. 2010 Annual Meeting of the Western Division of the American Fisheries Society. Salt Lake City, UT. April 19-23, 2010.
11) Schisler, G. J. and E. R. Fetherman. Post-release evaluation of resistant rainbow trout. Annual Meeting of the Colorado Aquaculture Association. Mt. Princeton Hot Springs, CO. January 22, 2010.
12) Several popular articles have appeared as a result of interviews this year on this project such as North Forty News (July 2010).

2010-2011 Technical Assistance Milestones

1) Schisler, G. J. 2010. Effects of whirling disease (*Myxobolus cerebralis*) exposure on juvenile mountain whitefish (*Prosopium williamsoni*). Research Report. Colorado Division of Wildlife Fish Research Section. Fort Collins, CO.

2) Baerwald, M. R., Petersen, J. L., Hedrick, R. P., Schisler, G. J., and B. May. 2010. A major effect quantitative trait locus for whirling disease resistance identified in rainbow trout *Oncorhynchus mykiss* Heredity (2010) 1-7.
3) Fetherman, E. R., D. L. Winkelman, G. J. Schisler, K. Davies, and K. Kehmeier. 2011. Brown trout removal in the Cache la Poudre River: The next step in whirling disease resistant rainbow trout management. 2011 Annual Meeting of the Colorado-Wyoming Chapter of the American Fisheries Society and the Colorado Chapter of the Wildlife Society. Fort Collins, Colorado. February 22-25, 2011.
4) Fetherman, E. R., D. L. Winkelman, and G. J. Schisler. 2011. Whirling disease resistant rainbow trout in Colorado: Introductions, monitoring, and brown trout removal. Annual Meeting of the Colorado Aquaculture Association. Mt. Princeton Hot Springs, Colorado. January 21, 2011.
5) Fetherman, E. R. 2010. Brown trout removal in the Cache la Poudre River: The next step in whirling diseases resistant rainbow trout management? Bi-weekly meeting of the Colorado State University student chapter of the American Fisheries Society. Fort Collins, Colorado. October 20, 2010.
6) Assistance was provided to the CDOW Hatchery Section to develop hatchery brood stock SOP's on a statewide basis and for individual facilities or wild spawn takes.
7) Work was initiated on an internal CDOW document describing Aquatic Section history and processes for determining fish production and stocking rates in Colorado.