SALMONID DISEASE STUDIES

Federal Aid Project F-394R-9

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Job Progress Report

Colorado Division of Wildlife

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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.

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State: **Colorado**

Project No. **F-394-R9**

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

Period Covered: **July 1, 2009 - June 30, 2010**

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) and/or Visible Implant (VI) 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 2009, FRH personnel checked all of the Hofer[1] (GR), Harrison Lake (HL), Hofer X Harrison Lake (GRXHL) and Hofer X Colorado River Rainbow (GRXCRR) brood fish (2, 3 and 4 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 18, 2009, the fish from the first group of GRXCRR females were ripe and ready to spawn. Before each fish was spawned, it was examined for the proper identification (fin-clip, PIT or VI 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, HL, GRXHL and GRXCRR 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 Galen 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, HL, GRXHL and GRXCRR rainbow trout.

The FRH 2009/20010 on-site rainbow trout production spawn started on November 18, 2009 with ripe GRXCRR females. The last group of HL females were spawned on January 18, 2009. With a goal in the fall to produce @ 400,000 eyed eggs, the egg take far exceeded the production needs with 629,100 eyed eggs produced (Table 1.1) and another 393,000 transferred out as green eggs. With the availability of both ripe males and females of several year classes and combinations of previous years crosses (F1 and B2) of GR, HL, GRXHL and GRXCRR strains, FRH personnel produced over 33 different lots during the spawn take. Surprisingly the overall egg quality remained quite good with 1st egg pick-off of only 17% overall. FRH personnel were able to fill all GR, HL, GRXHL and GRXCRR egg requests for Colorado, both production and research directed projects in 2009-2010.

Table 1.1. 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.

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 the following section, Job 2: Whirling Disease Resistance Laboratory Experiments.

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.

Evaluation of Infection in Hofer and Harrison Strains of Rainbow Trout

Much of the laboratory work in the last two years has 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.

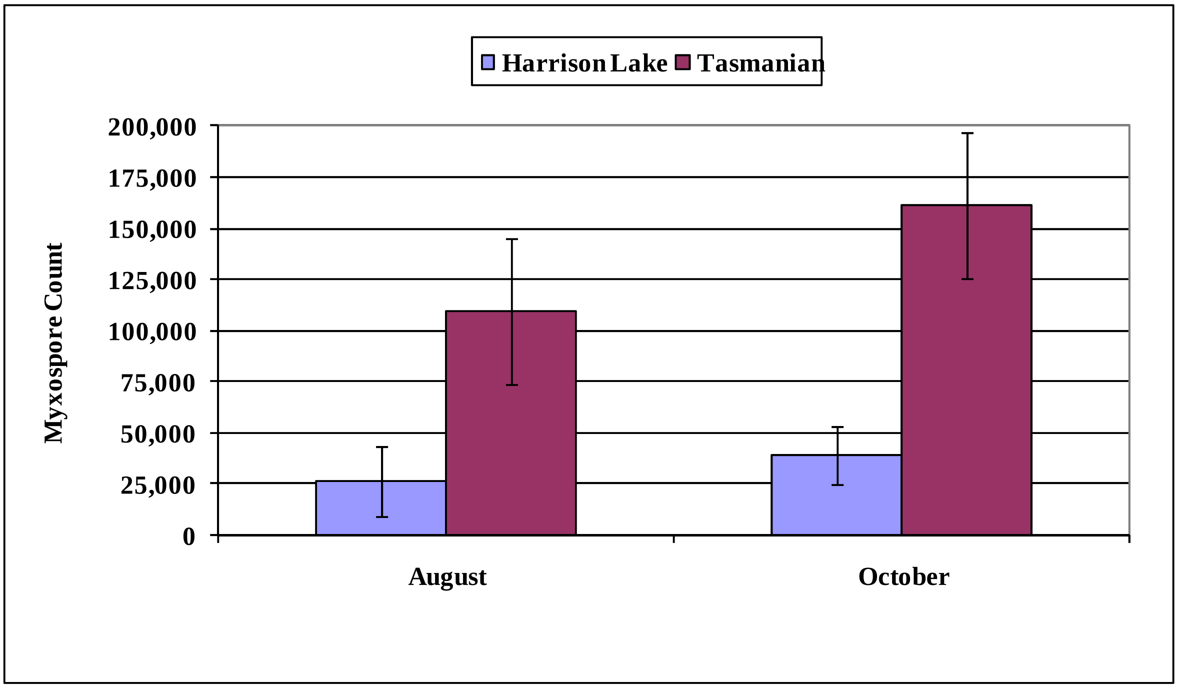
Evaluations of the pure Harrison and GR-Harrison varieties for resistance to *M. cerebralis* had been previously limited to experiments conducted in 2003 and 2004. In 2003, the Harrison Lake strain was compared with Big Thompson rainbow trout, Colorado River cutthroat trout, and Colorado River rainbow 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 2.1). 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 2.1. 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 2.1).

Figure 2.1. Myxospore counts for Harrison Lake and Tasmanian rainbow trout reared at the Poudre Rearing Unit for 10 and 12 months.

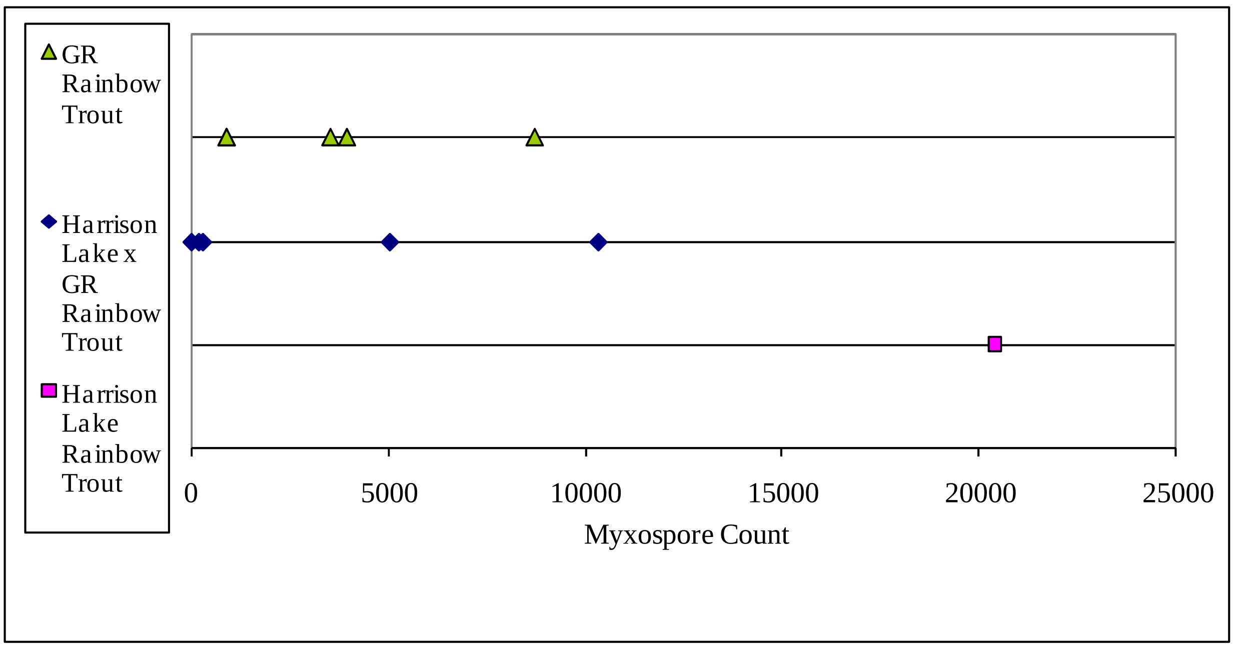


In 2004, a laboratory experiment was conducted to test not only the pure Harrison Lake strain, but also the GR and Harrison Lake (50:50) cross, and pure Hofer strain rainbow trout. In this experiment, individual families (single male/female matings) were used as replicates, with 30 fish per family. Fish 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 2.2). 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 2.2).

Table 2.2. 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 2.2. 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 (HXN), 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

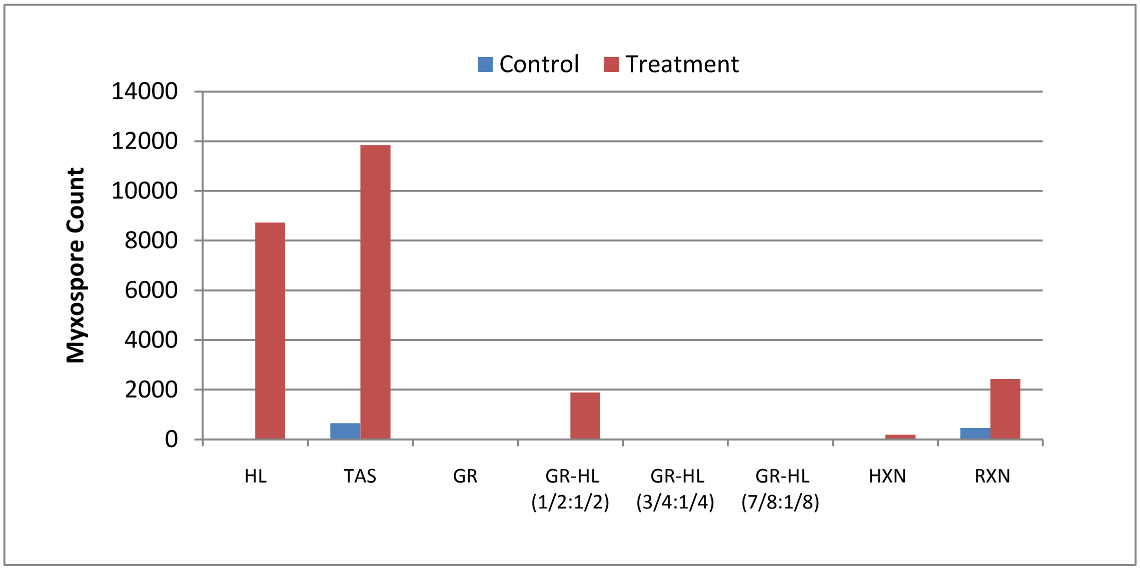
Mortality was low for most of the test lots. In the aquarium holding the GR-Harrison (75:25) fish, the standpipe was dislodged, which resulted in the death of 13 of the 20 fish. However, the remaining seven survived until the end of the experiment and were evaluated. The single lot that experienced other mortality was the pure Harrison Lake lot, in which three individual fish died over the course of the 6 month rearing period. Growth of the eight varieties (both control and treatment) was variable (Table 2.3). 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 2.3 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 ½ : ½	GR:HL ¾ : ¼	GR:HL 7/8 : 1/8	HXN	RXN
Control July 2009	**Length (mm)** **Weight (g)**	92 8.2	114 17.6	120 21.0	116 17.8	117 18.5	111 16.5	105 12.0	98 10.1
Treatment July 2009	**Length (mm)** **Weight (g)**	91 7.6	113 17.6	114 17.5	115 17.6	112 15.4	114 17.6	104 12.2	96 9.9
Average	**Length (mm)** **Weight (g)**	91.5 7.9	113.5 17.6	117.0 19.3	115.5 17.7	114.5 17.0	112.5 17.0	104.5 12.1	97.0 10.0
Control April 2010	**Length (mm)** **Weight (g)**	163 46.4	191 85.1	222 134.9	201 89.3	205 98.3	199 93.4	198 85.4	187 72.5
Treatment April 2010	**Length (mm)** **Weight (g)**	162 47.6	197 92.4	237 150.5	213 113.8	206 94.7	207 99.4	200 94.3	178 65.9
Average	**Length (mm)** **Weight (g)**	162.5 47.0	194.0 88.8	229.5 142.7	207.0 101.6	205.5 96.5	203.0 96.4	199.0 89.9	182.5 69.2
Net Growth	**Length (mm)** **Weight (g)**	71.0 39.1	80.5 71.2	112.5 123.4	91.5 83.9	91.0 79.5	90.5 79.4	94.5 77.8	85.5 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 2.3). 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 HXN 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 2.3. 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

Beginning weights and lengths are reported in Table 2.4. The fish collected at the end of this experiment have not yet been processed.

Table 2.4. Weights and lengths for eight varieties of rainbow and rainbow-cutthroat crosses at the beginning of Experiment 2.

		Pure HAR	Pure TAS	Pure GR	GR:HL ½ : ½	GR:HL ¾ : ¼	GR:HL 7/8 : 1/8	HXN	RXN
Replicate 1 Treatment	**Length (mm)** **Weight (g)**	89 7.8	111 17.6	116 18.9	106 13.9	111 17.6	116 19.5	100 10.9	97 10.0
Replicate 1 Control	**Length (mm)** **Weight (g)**	92 8.6	110 15.8	115 19.0	110 15.4	110 14.9	115 18.4	100.2 11.1	97 8.9
Replicate 2 Treatment	**Length (mm)** **Weight (g)**	93 8.1	110 16.4	113 17.8	104 13.5	114 17.7	111 16.8	101 10.6	96 8.9
Replicate 2 Control	**Length (mm)** **Weight (g)**	91 9.5	110 18.6	118 20.8	114 17.8	116 17.7	106 14.7	104 12.3	95 8.7
Average	**Length (mm)** **Weight (g)**	91 8.5	110 17.1	116 19.1	109 15.1	113 17.0	112 17.4	101 11.3	96 9.2

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 and 66 fish were collected from each. 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, and submitted for testing with PTD.

Results

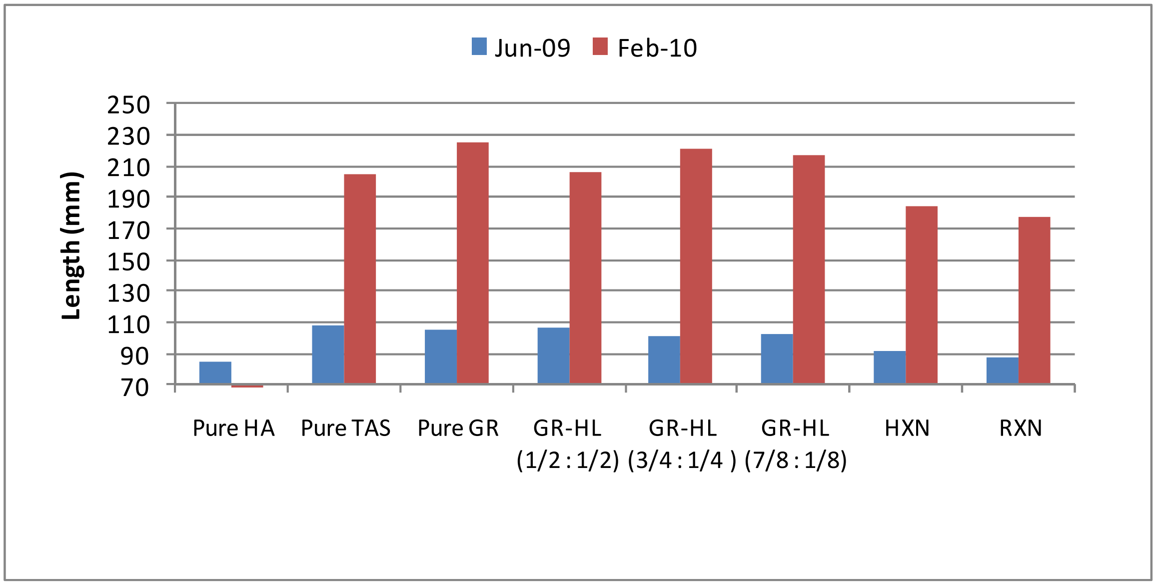
Catch results for the eight-month sample by gear type are summarized in Table 2.5. No Harrison Lake rainbow trout were found during the eight-month post-release sample among the 125 fish collected. Only five pure Tasmanian strain fish were found, and six GR-Harrison (50:50) crosses. The other strains were relatively uniform in catch, ranging from 18 (14.4%) to 26 (20.8%).

Table 2.5. Total catch for the eight-month post-release sample at Poudre Ponds.

	Pure HAR	Pure TAS	Pure GR	GR:HL ½ : ½	GR:HL ¾ : ¼	GR:HL 7/8 : 1/8	HXN	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%)

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 2.4). 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 2.5). At the time of this writing, the 12-month samples had not been processed. These results will be incorporated into the next report.

Figure 2.4. Lengths of eight rainbow and rainbow-cutthroat trout cross varieties upon release, and eight months post-release at the Poudre Rearing Ponds.



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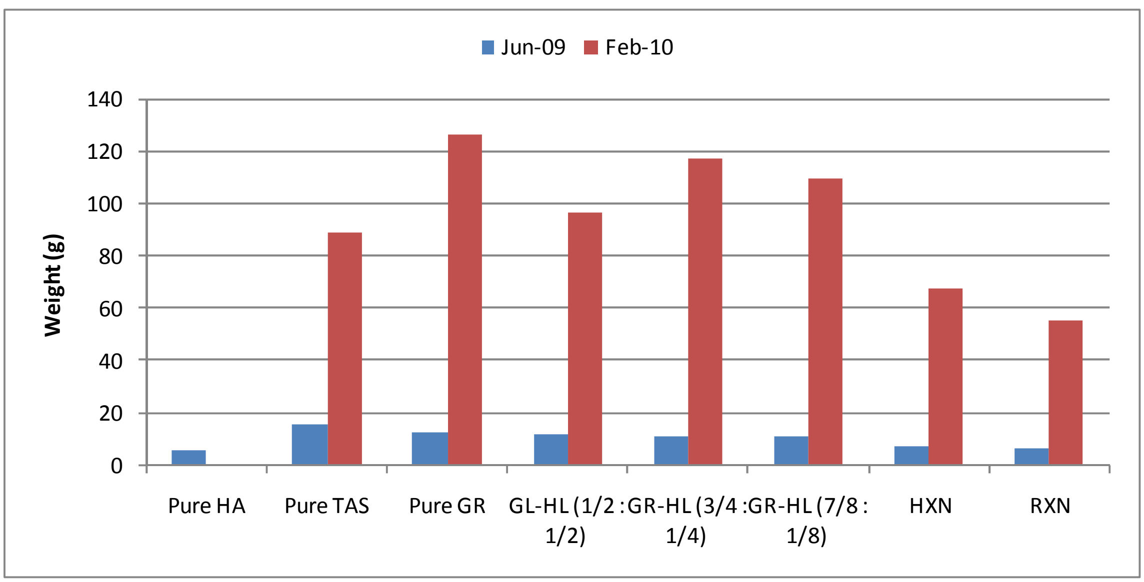


Job No. 3. Whirling Disease Resistant Domestic Brood Stock Development and Evaluation

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.

Parvin Lake Fingerling Stocking Experiment

Earlier experiments have demonstrated that the GR and GRxHL crosses have excellent growth and return-to-creel when stocked as catchable-sized fish (Schisler et al. 2008). 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 3.1) located 45 miles northwest of Fort Collins, Colorado, was used as the test site for this evaluation. The reservoir is stocked annually with fingerling brown trout (*Salmo trutta*), splake (*Salvelinus namaycush x Salvelinus fontinalis*), and rainbow trout (*Oncorhynchus mykiss*). The reservoir was also stocked in 2000 through 2003 with tiger muskies (*Esox masquinongy x Esox lucius*) to control the abundant white sucker (*Catostomus commersoni*) population. An inlet trap that was historically used for rainbow trout spawning operations has also been operated more recently to remove white suckers from the reservoir in the months of May-July during their annual spawning run up the inlet stream. Numbers of suckers and trout captured in the trap vary from year to year, but appear to have been greatly reduced in recent years (Figure 3.2). In 2009, 539 white suckers, and 67 salmonids were captured in the inlet trap.

A fall electrofishing survey has been conducted annually since 2002 to monitor species composition and growth in Parvin Lake. A shift from a population dominated by white suckers to one dominated by rainbow trout has occurred since 2006 (Figure 3.3). In 2009, a record 69.7% of the total catch was rainbow trout, compared with only 14.4% white suckers. This compares well with the figures from 2006, when over 60% of the total catch was white suckers. Average size of white sucker, rainbow trout, splake, and brown trout has remained fairly stable, while average tiger musky length has steadily increased since 2002 (Figure 3.4).

Figure 3.1. Parvin Lake, Colorado.

Figure 3.2. Number of catostomids and salmonids caught at Parvin Lake inlet trap (May-July) for years where data are available.

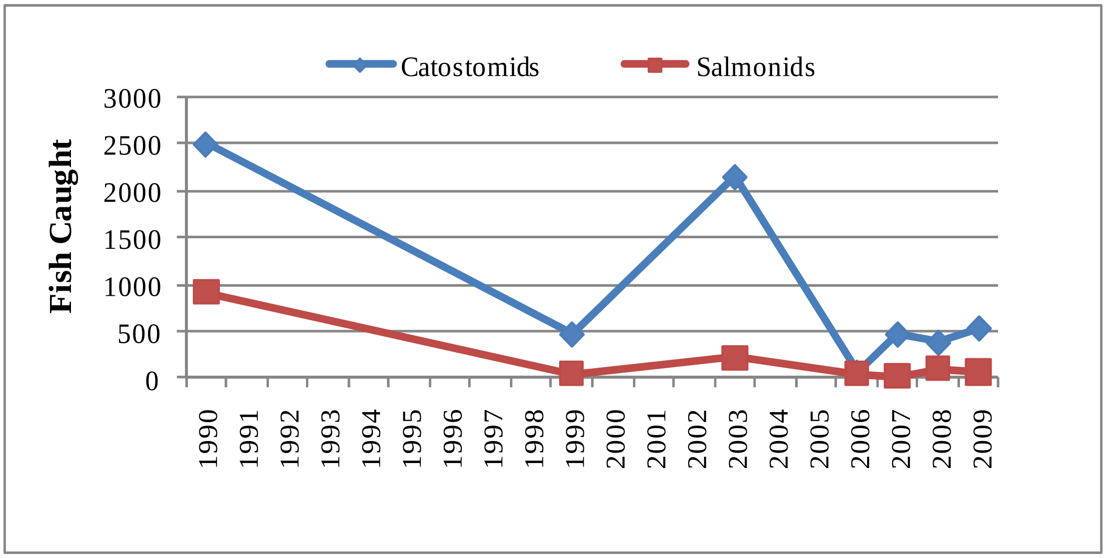


Figure 3.3. Percent of catch by species during fall electroshocking surveys for the years 2002 - 2009.

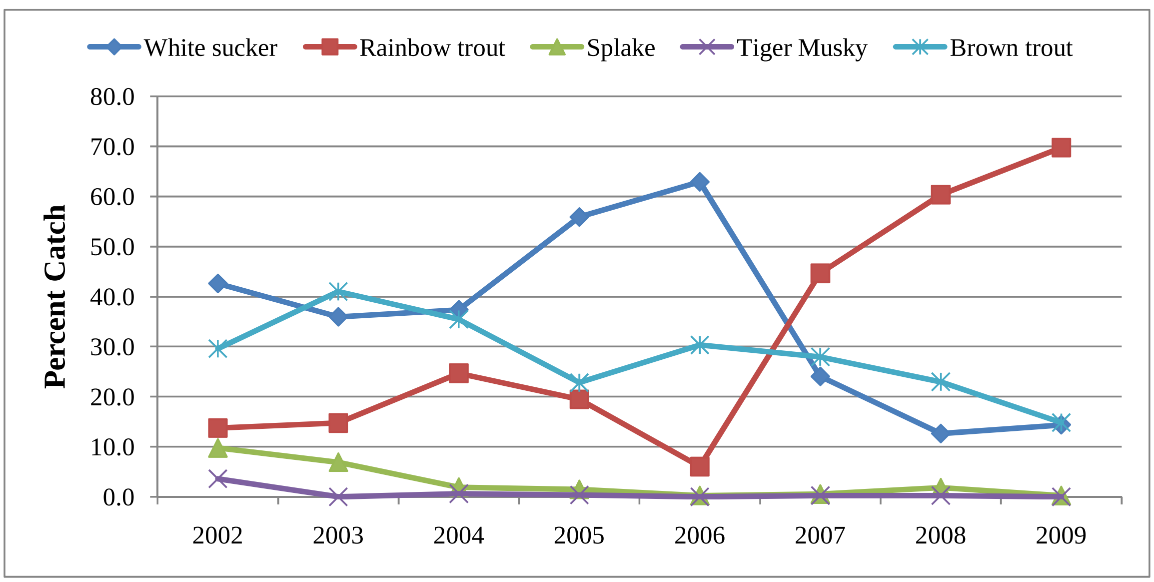
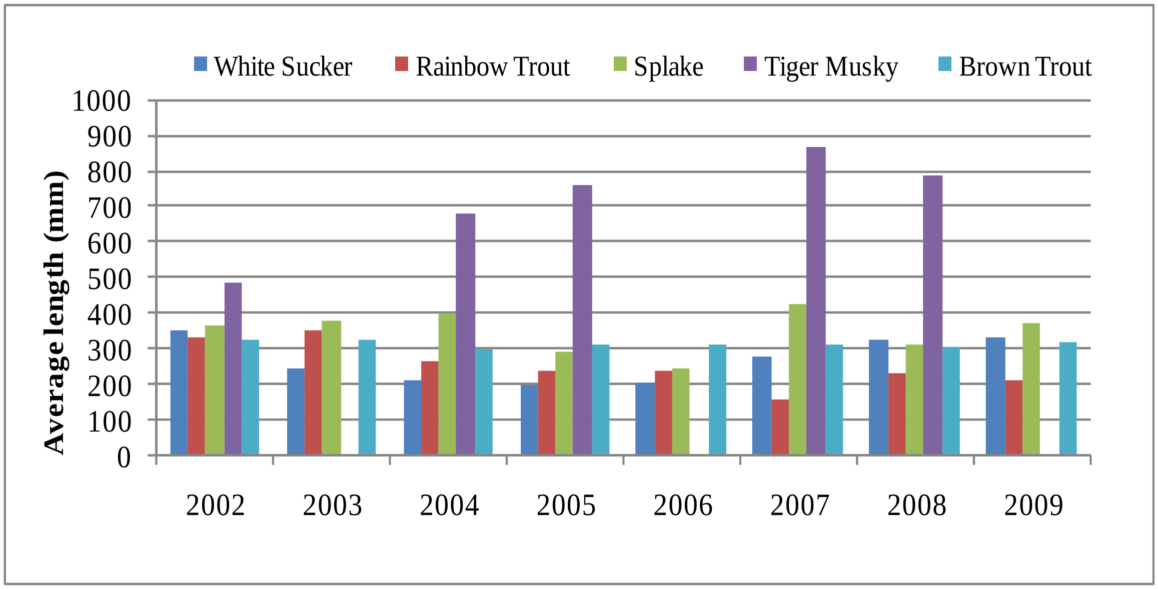


Figure 3.4. Average length of fish by species during fall electroshocking surveys for the years 2002 – 2009.



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 3.1). 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 previously described in Job 2 for the laboratory and Poudre Pond experiments (Table 3.2). This included all the varieties stocked in 2007 and 2008, along with the addition of the pure Tasmanian rainbow trout, the GRxHL (87.5:12.5) cross and the HXN cross. The fish were stocked on August 12, 2009, and as in previous years, released in the lake inlet. As in previous years, fish collections were made every two months during the open-water period.

Collections of coded-wire tagged fish were made using electroshocking and gill net sets every two months during the open-water season in 2007 and 2008. In 2009, all fish were collected by evening boat electroshocking. Marked fish from the 2007 plant were collected beginning in August, 2007, and marked fish from the 2008 plant were collected beginning in August, 2008. Marked fish from the 2009 plant were collected beginning in August, 2009. An attempt was made to collect 30 fish per event for each age class of marked fish.

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

2007 Plants				**2008 Plants**			
Strain	**Lbs**	**Number**	**Length (mm)**	**Strain**	**Lbs**	**Number**	**Length (mm)**
GR	225	2800	147	GR	103	2050	127
HL	64.2	2800	97	HL	38.4	2050	91
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 *3.2*. 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)	HXN (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

Figure 3.5. Percent of catch for each of the five varieties of fingerling rainbow trout stocked in Parvin Lake in August, 2007. January-March samples are ice-fishing angler returns.

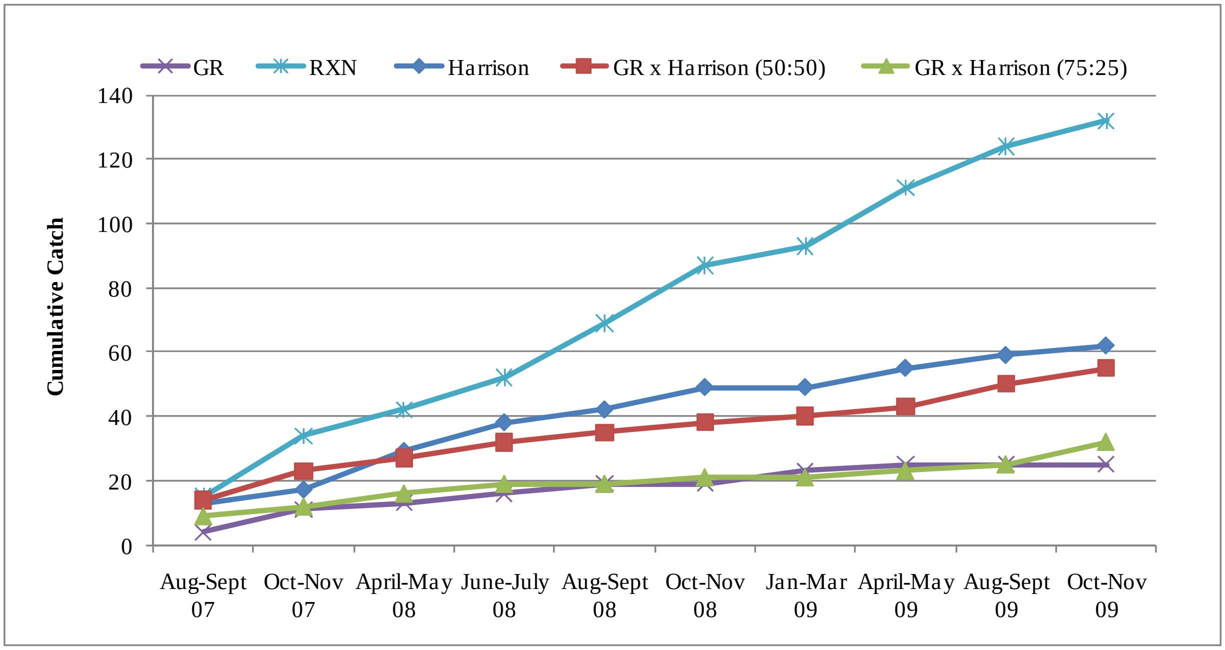


Figure 3.6. Percent of catch for each of the five varieties of fingerling rainbow trout stocked in Parvin Lake in July, 2008.

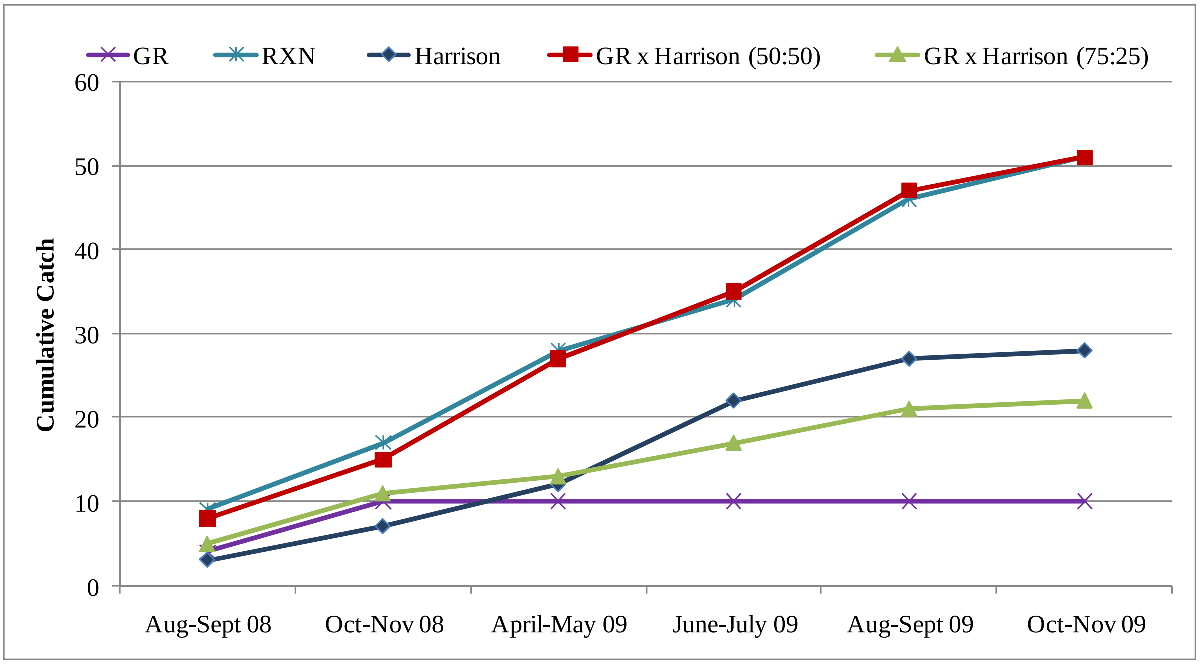


Figure 3.7. Lengths of fish from 2007 through 2009 for each of the five varieties stocked in Parvin Lake in 2007.

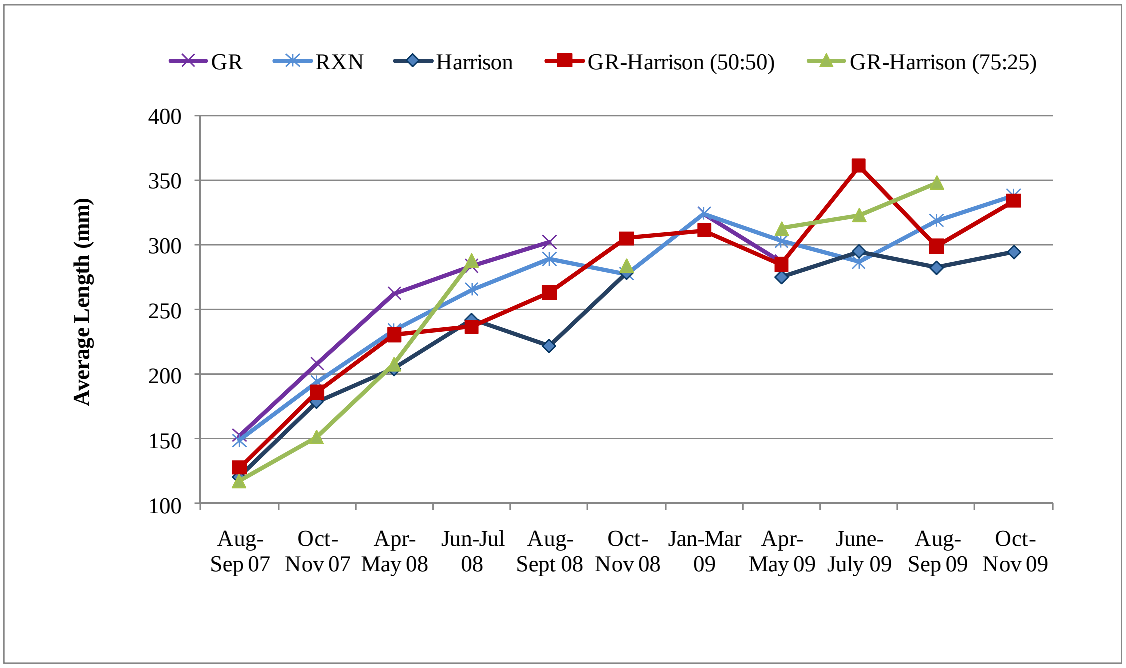
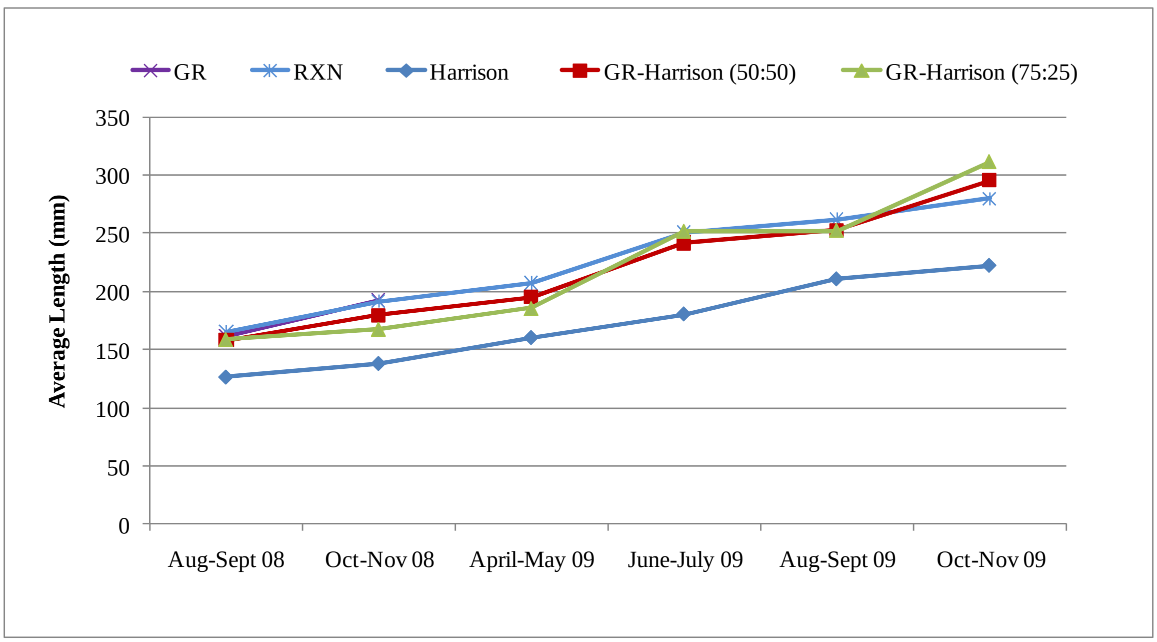


Figure 3.8. Lengths of fish from 2008 through 2009 for each of the five varieties stocked in Parvin Lake in 2008.



Collections of fish from the 2007 plant (Figure 3.5) resulted in the RXN strain being most consistently more abundant in the samples than the other strains, contributing to 43.1% (132 fish) of the overall catch of 306 fish. The Harrison Lake strain contributed to 20.3% (62 fish) of the overall catch. The GRxHL (50:50 cross) contributed to 17.9% (55 fish) of the overall catch. The GRxHL (72:25 cross) contributed to 10.4% (32 fish) of the overall catch, and the pure GR strain contributed to 8.1% (25 fish) of the overall catch.

Collections of fish from the 2008 plant have thus far resulted in the RXN and GRXHL (50:50) cross being more abundant in the samples than the other strains (Figure 3.6). The RXN strain contributed to 31.5% (51 fish) of the overall catch of 162 fish. The Harrison Lake strain contributed to 17.3% (28 fish) of the overall catch. The GRxHL (50:50 cross) contributed to 31.5% (51 fish) of the overall catch. The GRxHL (72:25 cross) contributed to 13.6% (22 fish) of the overall catch, and the pure GR strain contributed to 6.2% (10 fish) of the overall catch.

Growth of the five strains was relatively equal for all strains for both the 2007 and 2008 plants (Figures 3.6 and 3.7). The exception was the Harrison Lake strain, which grew slower than the other varieties in both 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.

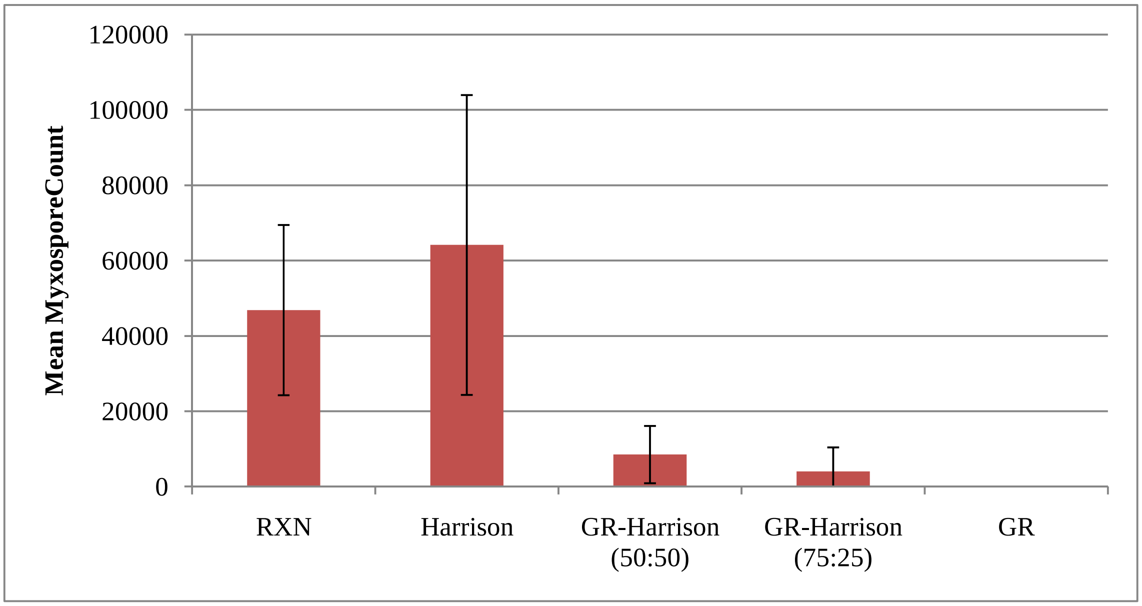
Collections of the 2009 plant consist only of the August and October, 2009 samples thus far. Percent of total catch is still uniform among strains, ranging from 5.4% to 18.4% for each strain as of October, 2009. Similarly, growth is still relatively uniform, with length averages for each of the strains ranging from 167 to 199 mm at that time.

A sub-set of fish from the 2007 and 2008 plants that were collected during the open-water season in 2009 were submitted for *M. cerebralis* testing. In April, 2009, samples were only submitted from the 2007 plant. In June, August, and October, samples 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 3.3). Figure 3.9 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 3.3. 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
Overall Averages	**37,362**	**69,487**	**62,459**	**66,458**	**14,331**	**4,104**	**632**	**8,170**	**0**	**NC**

Figure 3.9. Myxospore counts for the 2007 and 2008 plants of five strains of trout during 2009.



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.

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. Further evaluations of the RXN and HXN crosses will continue in 2010 to determine how well these two rainbow-cutthroat crosses compare with each other.

References

Schisler, G. J., E. R. Fetherman, and P. J. Schler. 2008. Salmonid Disease Investigations. Federal Aid Project F-394-R7 Job Progress Report. Colorado Division of Wildlife Fish Research Section, Fort Collins, Colorado.

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

Job Objective: These experiments are designed to 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

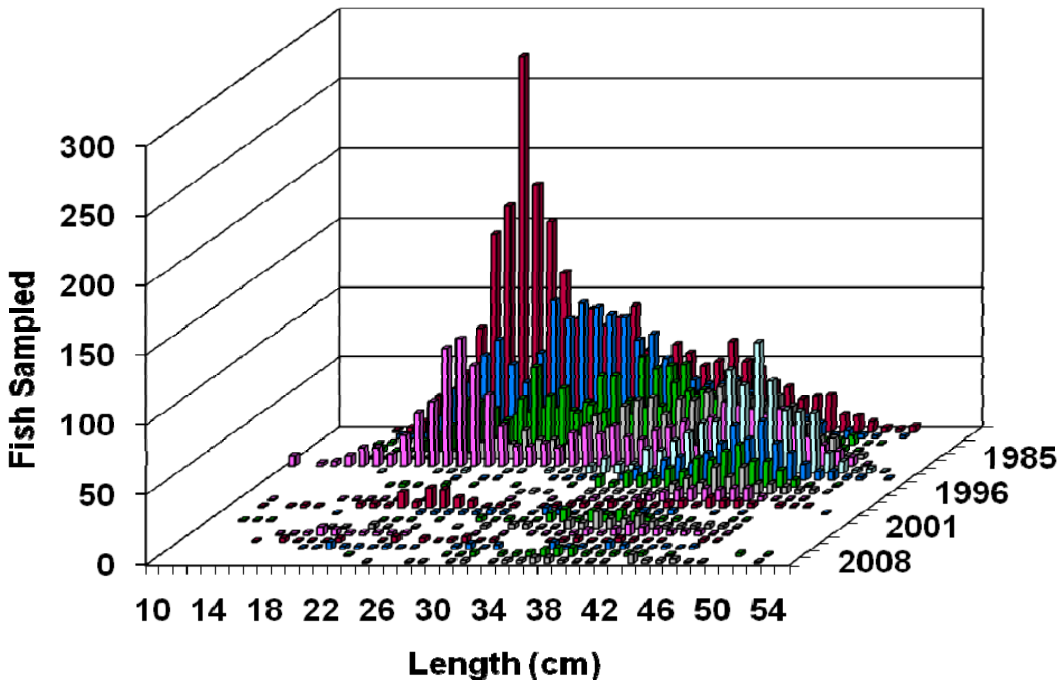
A substantial effort has been exerted in the last several years to incorporate the resistant strains into both domestic and wild rainbow trout programs. An overview of those efforts is summarized in the 2009 Federal Aid Report, Appendix II- *Resistant Rainbow Trout in Colorado: Current Status and Uses* (Schisler et al. 2009). Specific work conducted during the 2008-2010 field seasons is presented below.

Upper Colorado River

The upper Colorado River downstream of Windy Gap Reservoir is known to be one of the most heavily infected rivers with whirling disease in the state of Colorado. The 26 km reach, downstream of the reservoir to the Kemp-Breeze State Wildlife area has been an area of particular interest with respect to whirling disease investigations (Figure 4.1). Historically, before 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.



In 2006, a single lot of GR-CRR 50:50 cross (F1) rainbow trout were stocked in to the upper Colorado River at 23.5 cm (9.4 inches) in length to evaluate the survival of these larger fish in an area dominated by brown trout, and with an extremely high prevalence of *M. cerebralis*. This plant of fish has been monitored during annual population estimates. An extensive population estimate was conducted in spring, 2008. This 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 event over a distance of 6.28 river km (3.9 river miles). 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 1,307.5 fish per kilometer (2,092 fish per mile). Colorado River rainbow trout (residual wild fish and fish present due to repeated stocking of Colorado River rainbow fingerlings) were estimated to exist at a density of 109.4 fish per kilometer (175 fish per mile). The F1 rainbow trout from the 2006 plant were present at a density of 92.5 fish per kilometer (148 fish per mile). They averaged 34.3 cm (13.5 inches) in length, ranging from 30.0 cm to 40.9 cm (11.8 to 16.1 inches). The fish from this single plant of 3,000 F1 fish comprise almost 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 is 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 is favorable with respect to re-establishing a wild rainbow trout population. Fingerling fish were collected in 2007 and 2008 and tested for the presence of markers for GR rainbow trout genes using the Amplified

Length Frequency Polymorphisms (AFLP) technique. None of the fish in the 2007 samples contained significant Hofer genetic backgrounds, and only a few individuals from the 2008 collections exhibited high proportions of Hofer markers (Figures 4.4 and 4.5).

Figure 4.3. Hofer-CRR rainbow cross (F1) fish sampled during the spring, 2008 mark-recapture event on the upper Colorado River, compared with pure Colorado River rainbow trout in the same reach.

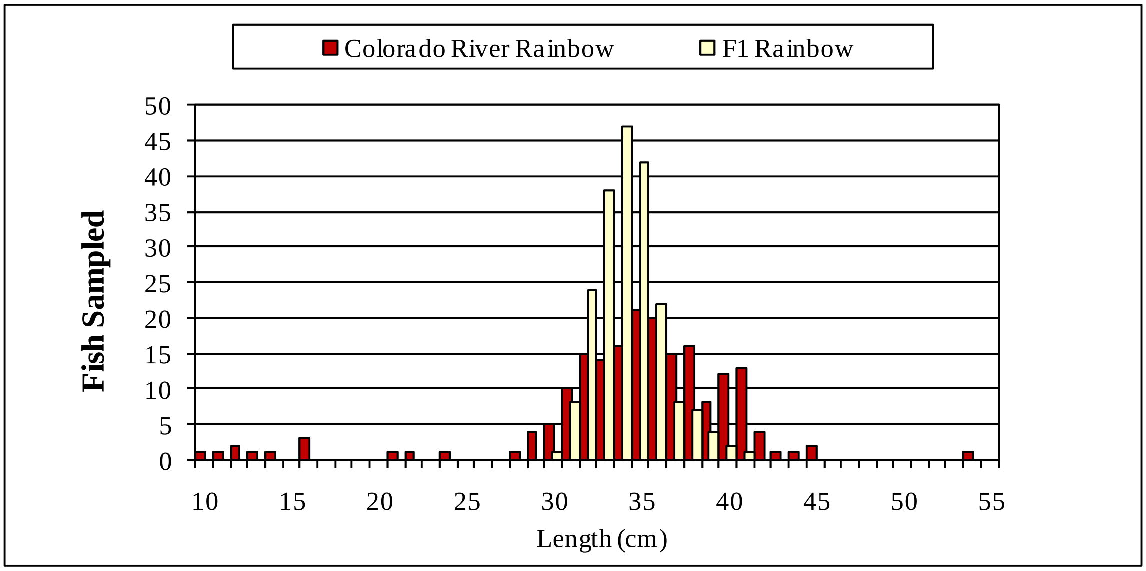


Figure 4.4. AFLP markers for Colorado River rainbow trout (CRR) and Hofer (GR) among rainbow trout fry collected in the upper Colorado River in 2007.

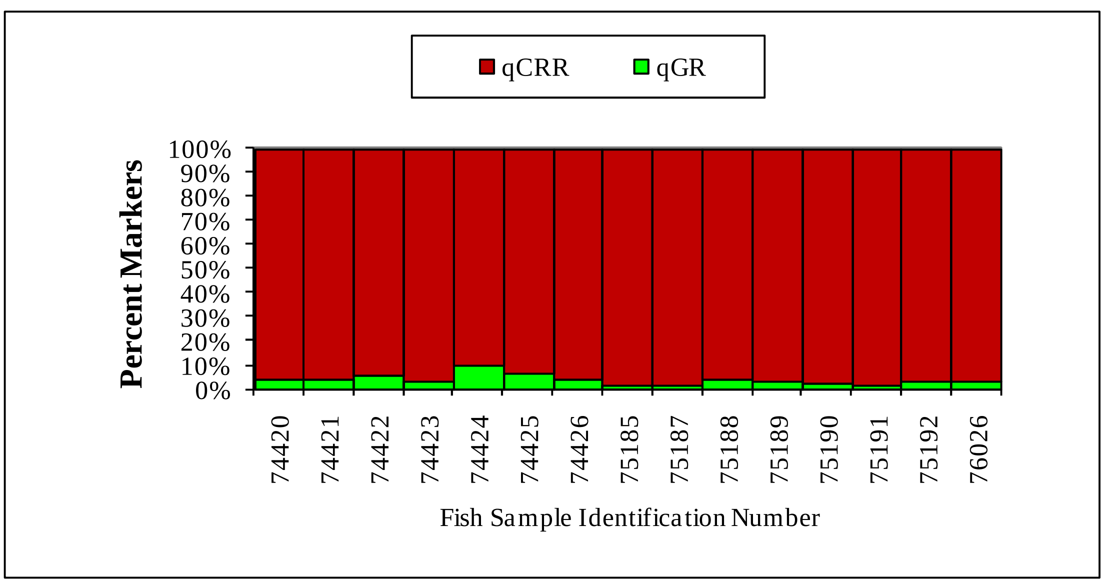
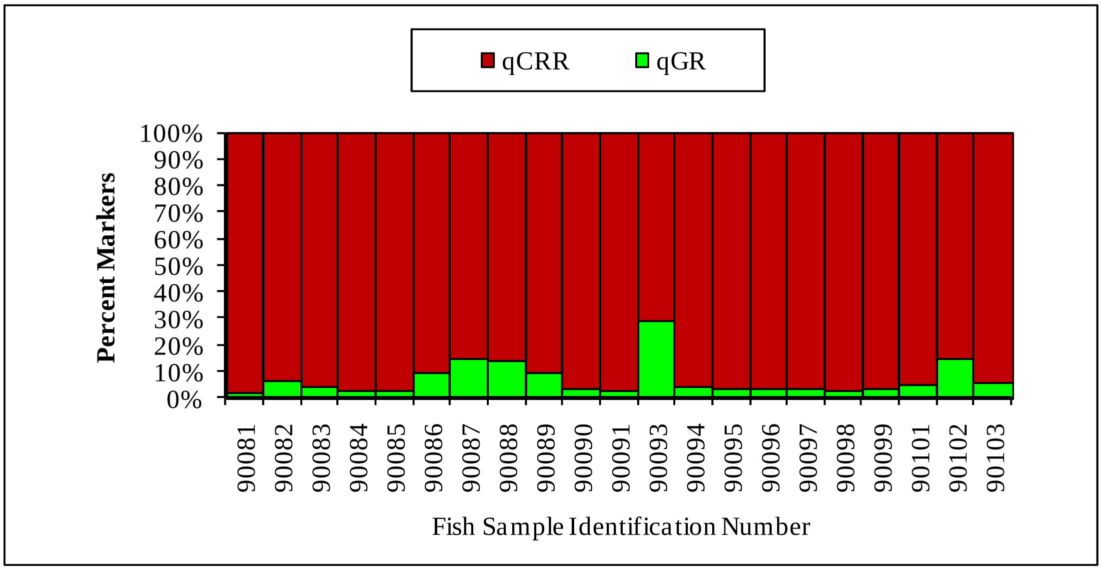


Figure 4.5. AFLP markers for Colorado River rainbow trout (CRR) and Hofer (GR) among rainbow trout fry collected in the upper Colorado River in 2008.



On April 28 and 30, 2009, a population estimate was conducted on the same 6.28 km reach as in 2008. 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 from 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 the 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.

The population estimate was calculated using the Petersen estimator (with the Bailey modification). The brown trout were present in the reach at a density of 1,208.8 fish per km (1,934 fish per mile). Colorado River rainbow trout, including residual wild fish and fish present due to repeated stocking of Colorado River rainbow fingerlings, were estimated to exist at a density of 29.5 per km (48 fish per mile). The F1 rainbow trout, from the 2006 plant, were present at a density of 40.9 per km (66 fish per mile). Other fish species encountered during the population 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 captured was 327 mm, ranging from 70 to 537 mm. The 92 F1 rainbow trout captured averaged 368 mm in length, ranging from 327 to 440 mm. The 84 CRR trout captured averaged 365 mm, ranging from 140 to 495 mm (Figure 4.6). The F1 rainbows averaged 532 g in weight, ranging from 290 to 1030 g, and the CRR trout averaged 520 g in weight, and ranged from 124 to 1254 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 4.7).

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 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 were females, eight of which were already spent, and eight were males). An additional 16 green females and 39 green fish of unknown sexual status were present. Six sexually immature CRR individuals were also captured.

Figure 4.6. Length-frequency distribution for brown trout, Colorado River Rainbow trout, and F1 (2006 plant) rainbow trout in the upper Colorado River from the Hitchin' Post Bridge, downstream to the Sheriff Ranch, April 2009.

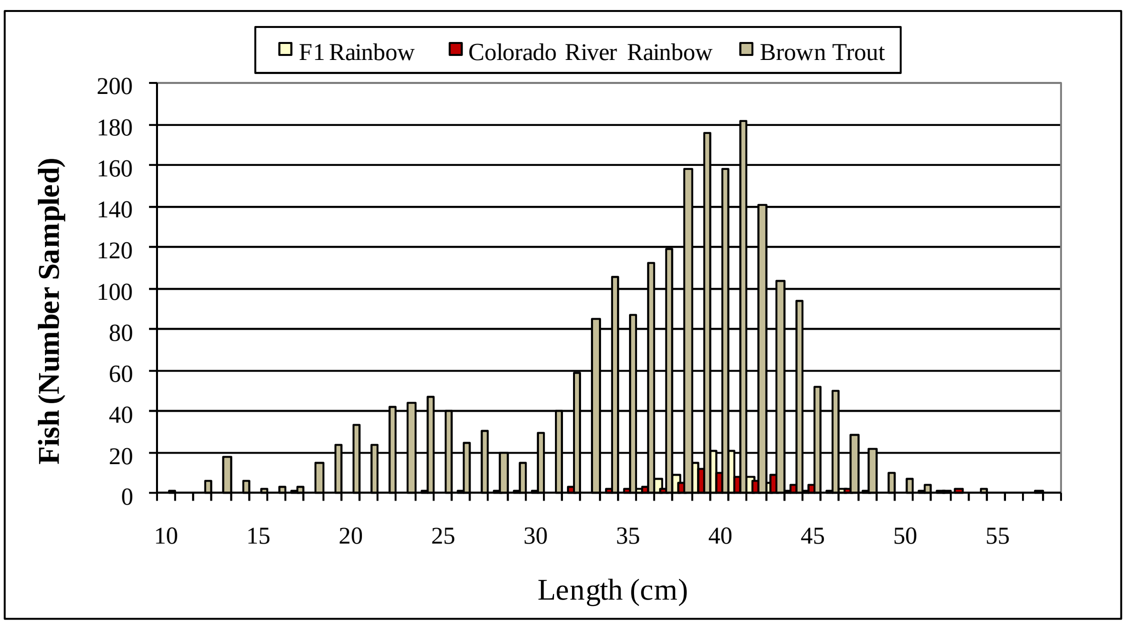
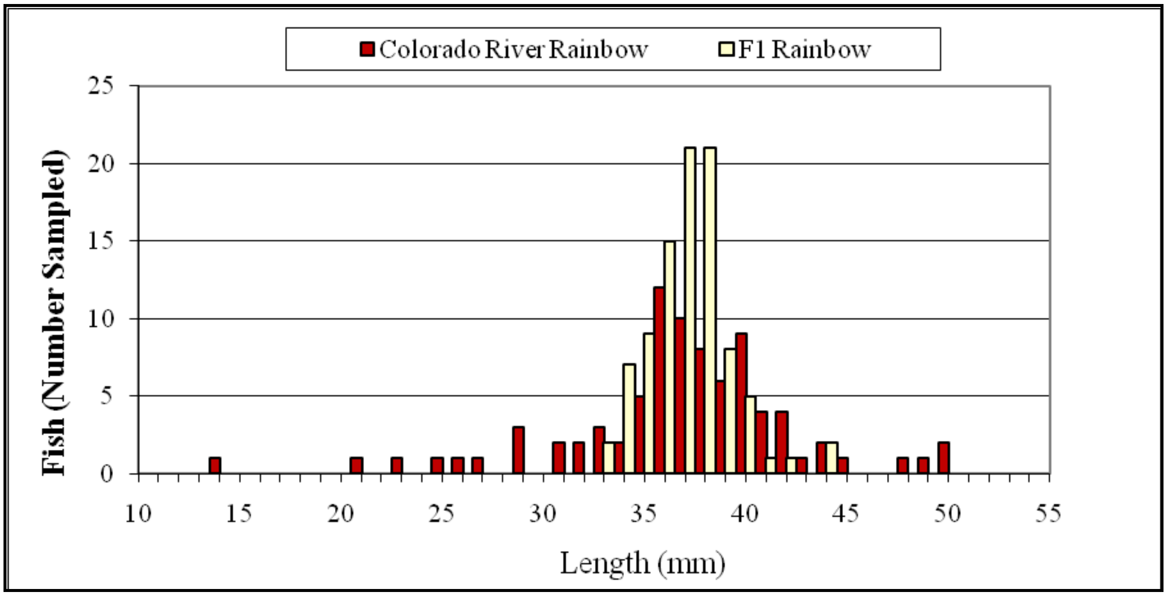


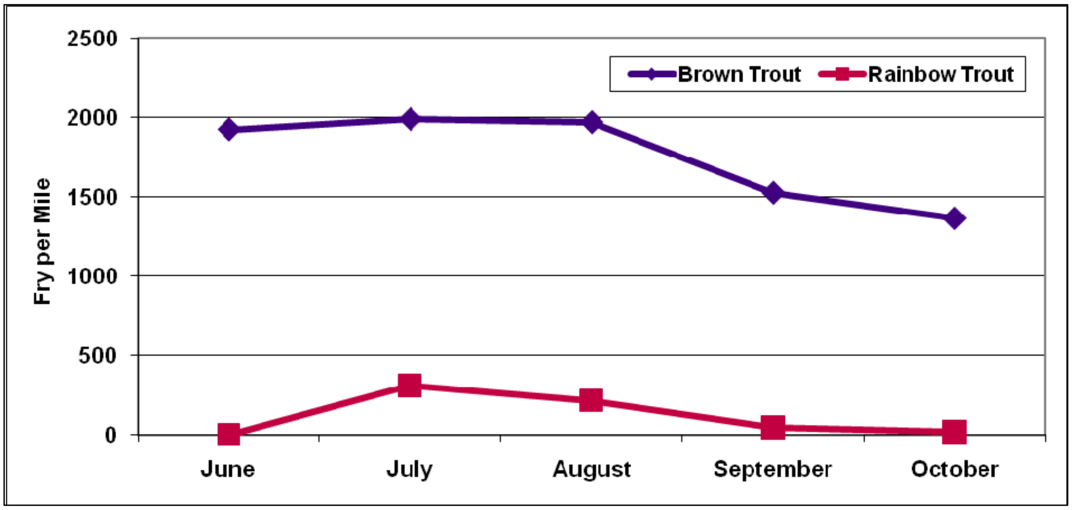
Figure 4.7. Length-frequency distribution for Colorado River Rainbow trout and F1 (2006 plant) rainbow trout in the upper Colorado River from the Hitchin' Post Bridge, downstream to the Sheriff Ranch, April 2009.



Fry estimates were conducted once a month, June through October 2009. Standard three-pass 50 foot removal estimates were conducted at seven stations throughout the upper Colorado River, with three sites downriver of Byers Canyon, and four sites within the 6.28 km study reach on the Chimney Rock and Sheriff Ranches. Two LR-24 Smith-Root backpack shockers were used to complete the fry estimates. All fry caught within the fifty-foot sections were identified as brown trout or rainbow trout fry, 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 kilometer (1,986 fry per mile), dropping to 849 fry per kilometer (1,366 fry per mile) in October. Rainbow trout fry densities also peaked in July, with an estimate of 193 fry per kilometer (310 fry per mile), dropping to 9 fry per kilometer (15 fry per mile) in October (Figure 4.8). Seven percent of the brown trout fry encountered during the fry density 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 average for the rainbow trout fry.

Figure 4.8. Upper Colorado River brown trout and rainbow trout fry density estimates for the months of June to October 2009.



On May 14 and 18, 2010, a population estimate was conducted on the same 6.28 km reach 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 modification). The brown trout were present in the reach at a density of 672 fish per km (1,081 fish per mile). Colorado River rainbow trout, including residual wild fish and fish present due to repeated stocking of Colorado River rainbow fingerlings, were estimated to exist at a density of 20 per km (33 fish per mile). The F1 rainbow trout, from the 2006 plant, were present at a density of 11 per km (17 fish per mile). White sucker were present in the reach at a density of 65.2 fish per km (105 fish per mile). Other fish species encountered during the population estimate included speckled dace and longnose sucker.

Average length of the 2,421 brown trout captured was 338 mm, ranging from 44 to 518 mm. The 78 F1 rainbow trout captured averaged 393 mm in length, ranging from 345 to 456 mm. The 91 CRR trout captured averaged 370 mm, ranging from 143 to 590 mm (Figure 4.9). The F1 rainbows averaged 582 g in weight, ranging from 351 to 880 g, and the CRR trout averaged 484 g in weight, and ranged from 29 to 930 g. As with the population estimates in 2008 and 2009, the F1 fish stocked in 2006 comprised a large proportion of the total rainbow trout population in the study area (Figure 4.10)..

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 were 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 captured.

Figure 4.9. Length-frequency distribution for brown trout, Colorado River Rainbow trout, and F1 (2006 plant) rainbow trout in the upper Colorado River from the Hitchin' Post Bridge, downstream to the Sheriff Ranch, May 2010.

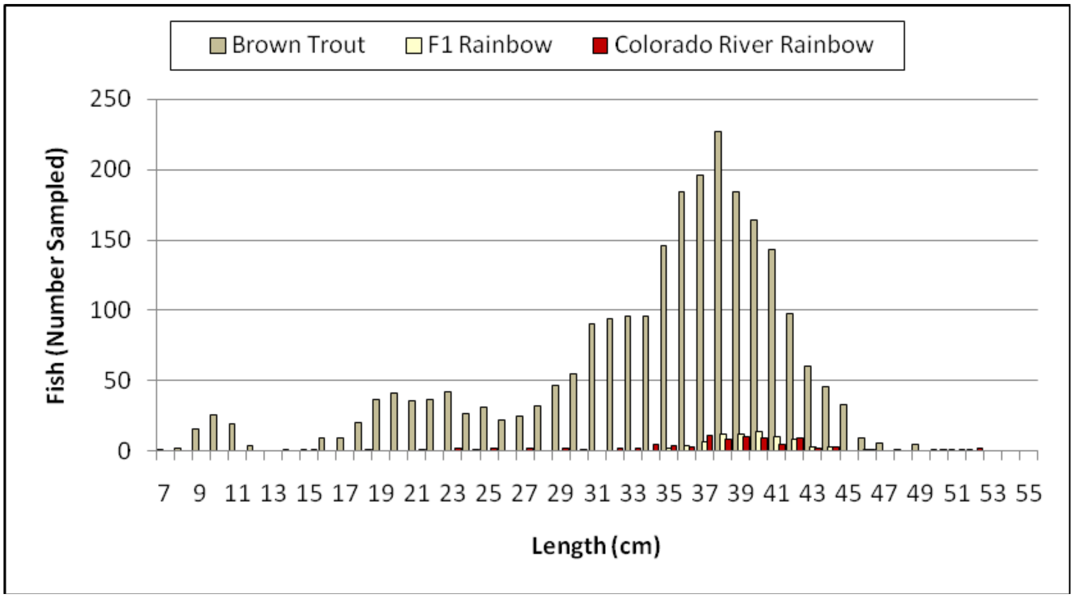
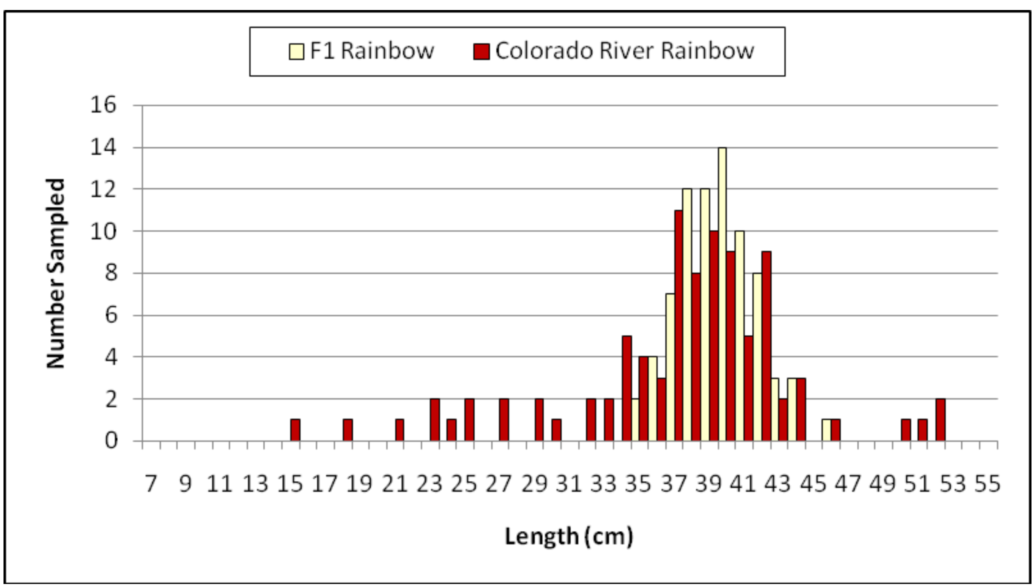


Figure 4.10. Length-frequency distribution for Colorado River Rainbow trout and F1 (2006 plant) rainbow trout in the upper Colorado River from the Hitchin' Post Bridge, downstream to the Sheriff Ranch, May 2010.

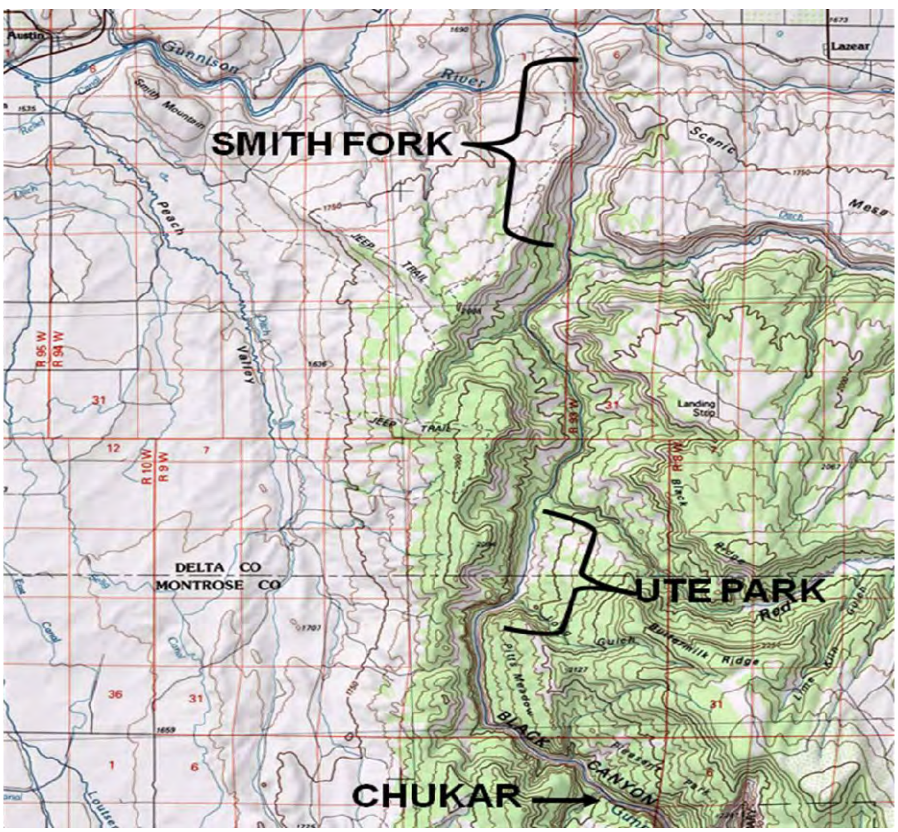


The F1 fish stocked as catchable-sized fish in 2006 are still present in the upper Colorado River in 2009. Though not as abundant as they were in 2008 and 2009, the fish from the 2006 plant continue to gain in average weight and length, and over 90% of the population is sexually mature. The much higher proportion of sexually mature F1 fish in the population could lead to higher reproductive success of these fish in 2010. The fry evaluations show that rainbow trout are reproducing in the upper Colorado River, although genetic analyses are pending to confirm that the offspring were produced by F1 adults. Rainbow trout fry densities continue to decline throughout the summer months, however, the persistence of rainbow trout fry through October is encouraging. The F1 adult population also shows a decline from 2008 to 2010. An introduction of 2,000 F1 fish to supplement the adult rainbow trout population in the upper Colorado River is scheduled for the summer of 2010. Fry evaluations will continue to determine if the F1 fish are successfully reproducing.

Gunnison River

The rainbow trout population in the Gunnison River has dramatically declined since the introduction of whirling disease. 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 grown 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 Hofer-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.

Figure 4.11. Gunnison River study area.



In 2004, Hofer-CRR 50:50 cross (F1) fish were marked with red visible implant elastomer (VIE) marks and pure CRR fish were similarly marked with green VIE marks. In this experiment, 10,104 CRR and 10,115 F1 rainbow trout were stocked as 13.6 cm and 11.9 cm fingerlings, respectively, into the Ute Park section of the Gunnison Gorge (Figure 4.11). The fish were mixed together prior to stocking to prevent bias due to handling, and then spread throughout the stream section using helicopter plants. In 2005, Hofer-CRR 25:75 cross (B2) fish were stocked, rather than F1 fish, along with pure CRR fish. The B2 fish were marked with an adipose clip and pure CRR strain fish were similarly given a right pelvic clip. Stocking was conducted using 5,000 of each variety as 15.2 cm fingerlings. In 2006, B2 fish were stocked again as 17.3 cm fingerlings to determine if the slightly larger B2 fish would perform better than the first (2005) plant of B2 fish. The pure CRR fish were not marked in this plant, while the B2 fish were given an adipose clip and a red VIE mark. In 2007, the number of fish stocked was increased to 20,000 of the pure CRR and 20,000 F1 rainbow trout stocked as 14.7 cm fingerlings. Coded wire tags were used to batch-mark the F1 and the pure CRR fish. Additionally, the F1 fish were adipose clipped to provide a second mark in case the coded wire tag was lost.

Growth, survival, and infection severity of the two strains planted each year were evaluated from samples collected during the annual population estimate conducted the following year. Estimates were conducted using mark-recapture sampling with boat-mounted electroshocking 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 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 being found in the 2,375 m sampling area. The sampling resulted in an estimate of 10 pure CRR fish per km (16 fish per mile). The estimates for F1 cross were 14 fish per km (22 fish per mile). The average total length of the CRR fish was 24.8 cm, and 28.3 cm for the F1 fish. All of the pure CRR individuals collected were found to be infected, with an average myxospore count of 124,603 (SD = 129,406). Only six of the 10 F1 individuals collected were found to be infected, with an average myxospore count of 4,055 (SD = 8,336).

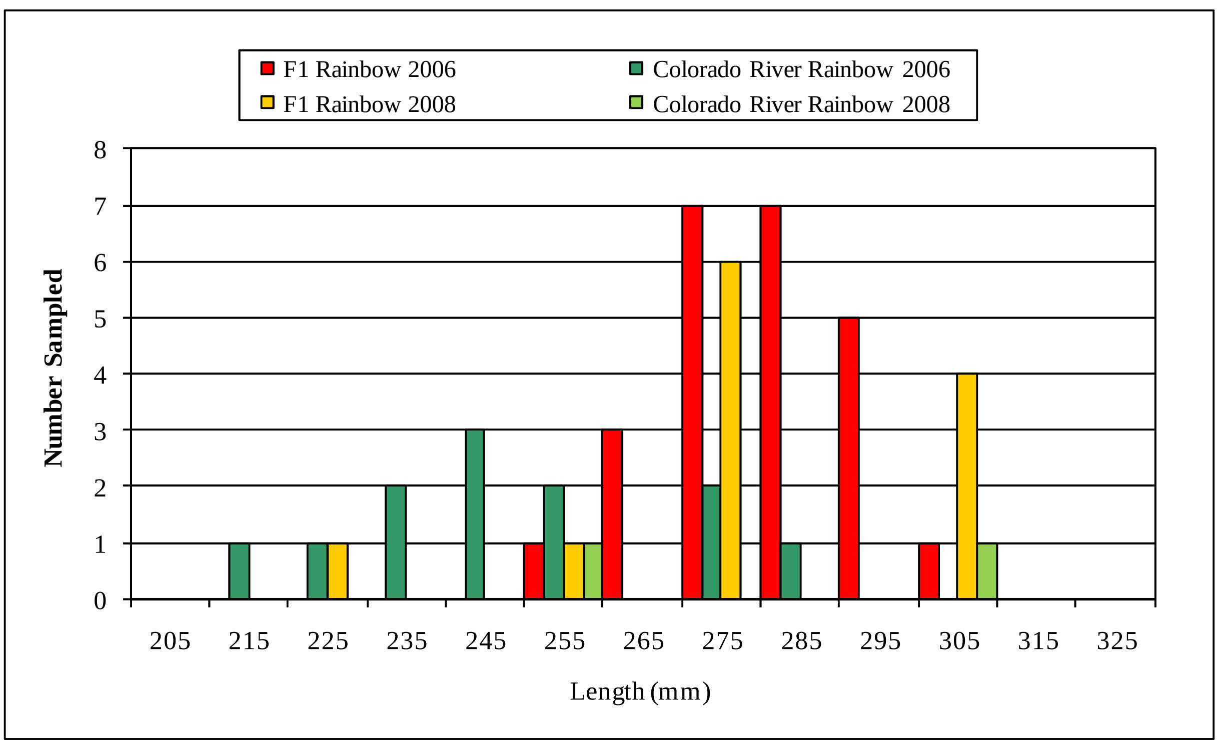
Survival and population estimates in 2006 for fish stocked in 2005 were difficult to assess directly because of mark loss (fin regeneration or poor marks) in both the CRR and B2 varieties. AFLP (Amplified Fragment Length Polymorphism) testing, a molecular technique that can help distinguish between individuals of the same species with different genetic lineages, was used to identify a subsample of unmarked fish as either B2 plants or pure CRR fish. Applying the ratio of fish identified as each variety in the subset to the overall population estimate of fish resulted in an estimate of 33 fish per km (53 fish per mile) of the pure CRR strain, and 22 fish per km (35 fish per mile) of the B2 cross. PTD testing identified an average of 83,929 myxospores (SD = 149,719) in the pure CRR fish planted in 2005. The average myxospore count among B2 fish was 40,480 (SD = 48,121).

In 2007, poor mark retention once again made estimating numbers of pure CRR and Hofer-cross fish difficult. The overall population estimate of rainbow trout (over 15 cm in length) was 135 fish per km (217 fish per mile). Of the 144 fish sampled, 16

(11.1%) were identified as either F1 or B2 fish by having either red VIE marks or adipose clips, while only three (2.1%) were identified as pure CRR fish, having green VIE marks. In 2008, the population estimate for rainbow trout (over 15 cm in length) was 111 fish per kilometer (178 fish per mile). Fish stocked in 2007 could be very clearly identified because of the coded wire tags and fin clips. Of the 157 rainbow trout that were sampled, 12 of the F1 fish and two of the pure CRR fish from the 2007 plant were positively identified, producing an estimate of seven F1 and a minimum of two pure CRR fish per kilometer (12 F1 and three CRR fish per mile), respectively. Average length of the F1 fish (27.7 cm) was similar to the pure CRR fish (27.5 cm) in 2008, after the fish had been in the river for one year. Overall, poor survival estimates were quite evident for both the pure CRR and the Hofer-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 definitively identified F1 and CRR fish could be compared directly from the stocking event in the previous year, the F1 fish were much more abundant than the pure CRR fish (Figure 4.12).

Fingerling rainbow trout were collected during fry shocking events in both 2007 and 2008 to be submitted for AFLP testing to determine if offspring had been produced from the F1 and B2 stocking events. The analysis identified a high proportion of the fingerling fish collected in 2007 as having a genetic background consistent with the Hofer strain. In 2008, a lower proportion of fry were identified as having Hofer genetic background. Nonetheless, natural reproduction from the Hofer crosses stocked in the river is now occurring. There is also some evidence that Hofer-cross fry produced in 2007 survived past their first year of life evident from the large number of unmarked age-1 fish in the 2008 samples.

Figure 4.12. Length-frequency and numbers of fish by strain sampled in the Gunnison River in 2006 and 2008 where direct comparisons of pure Colorado River rainbow trout and Hofer-CRR 50:50 (F1) crosses could be made from fish stocked in the previous year.



The results of this field evaluation demonstrate that the F1 fish can survive at least as well as the pure CRR trout when planted as fingerlings. The results also demonstrate that myxospore counts developed after stocking are much lower in the F1 fish than in the pure CRR trout. The myxospore counts in B2 fish released into the wild were similar to those found in the laboratory experiments, and while lower than the spore counts from the pure CRR fish, were also higher than observed in the F1 fish. This reinforces the notion that allowing natural selection of the resistant offspring of the F1 fish to occur in the wild may be a more effective method to producing sufficient resistance and wild behaviors than creating subsequent crosses artificially.

Brown trout numbers remain high, and rainbow trout numbers low, in the Ute Park section of the Gunnison Gorge (Figure 4.13). In 2009, the brown trout population was estimated to be at a density of 4,699 fish per kilometer (7,562 fish per mile). Nine fish were positively identified as one of the F1 or B2 strain (HxC) fish stocked in past years, and were estimated to be present in the reach at a density of 3.1 fish per kilometer (5 fish per mile). Wild rainbow trout, those that could not be positively identified as HxC's, were estimated to be present in the study reach at a density of 43.5 fish per kilometer (70 fish per mile). Despite this trend, three age classes were seen in the rainbow trout population for the first time since the introduction of whirling disease (Figure 4.14).

Figure 4.13. Adult population structure for the Ute Park Section of the Gunnison Gorge, October 2009.

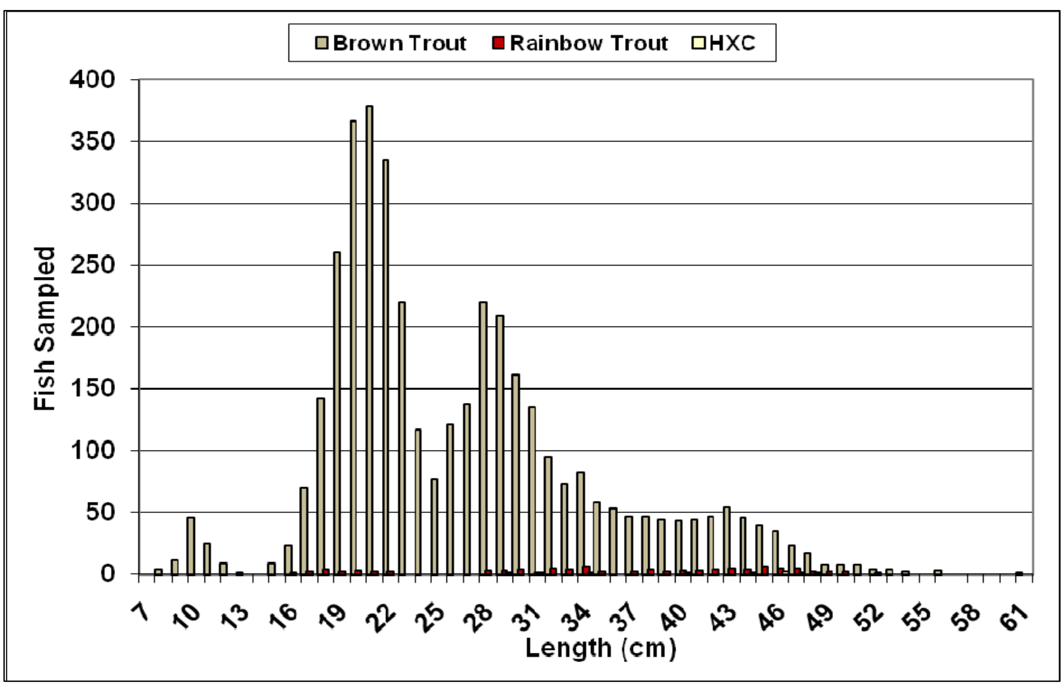
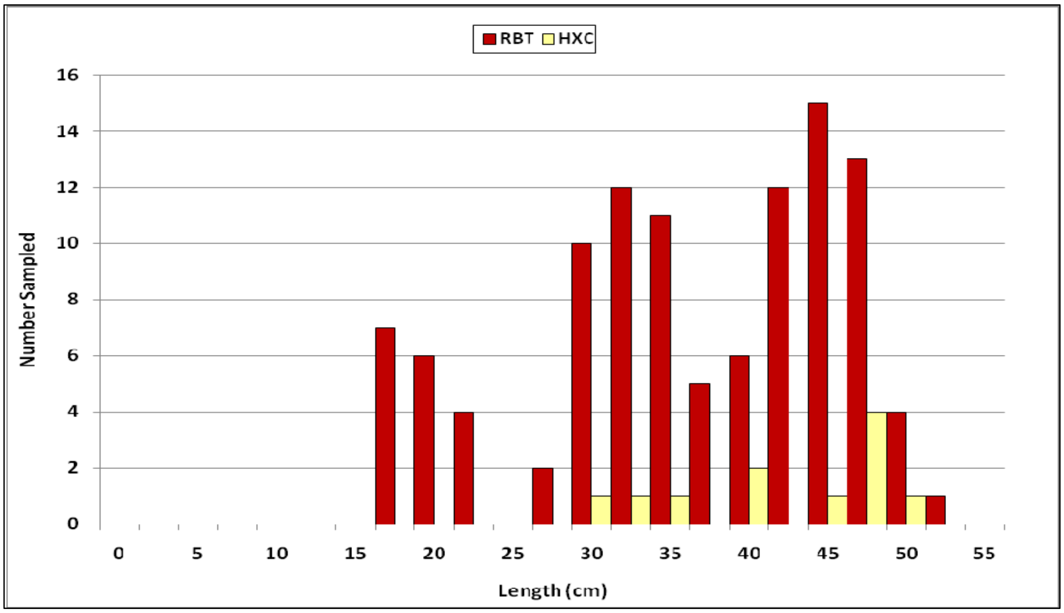


Figure 4.14. Adult rainbow trout population structure for the Ute Park section of the Gunnison Gorge, October 2009.

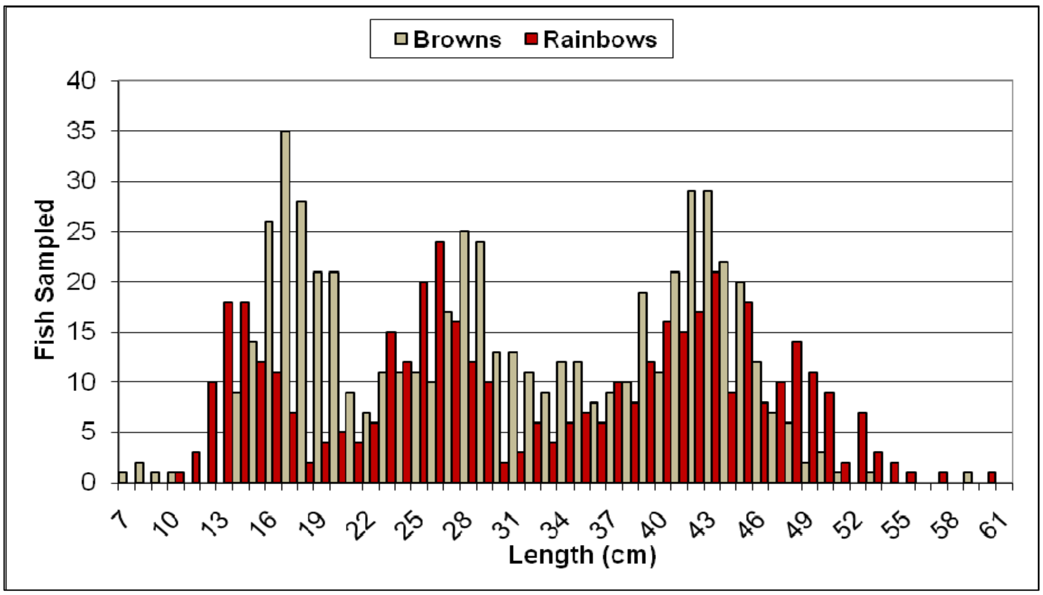


During fry evaluations conducted in July 2009, 90 rainbow trout fry were found at several sites throughout the Gunnison Gorge. Fin clips were taken from all rainbow trout fry for genetic analysis. Fry population estimates conducted in August estimated that brown trout were present in the Gunnison Gorge at a density of 803.4 fish per kilometer (1,293 fry per mile), dropping to 345.4 fish per kilometer (556 fish per kilometer) 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 had 18 rainbow trout fry when fry evaluations were conducted in October. These results suggest that brown trout fry removal may increase rainbow trout fry survival and retention in the Gunnison Gorge, and a larger scale replicate of the removal is scheduled to occur in the Ute Park section of the Gunnison Gorge summer 2010.

High densities of brown trout continue to contribute to the poor survival of the stocked rainbow trout in the Gunnison River, and poor mark retention has caused problems with producing reliable estimates of survival in B2 fish. In addition, stocked fish continue to show low survival in the Gunnison River. However, reproduction from Hofer-cross fish has been confirmed in several locations at, and downstream of, the stocking sites. These results are promising, and could lead to re-establishment of a wild rainbow trout population in the Gunnison River despite the presence *M. cerebralis*.

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 Hofer-cross wild brood stock location. In 2007, 4,100 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 inches) and 2009 (5,000 rainbow trout averaging 4.7 inches). The introduced rainbow trout have shown to have high survival in the East Portal, comprising half of the overall fish population (Figure 4.15). In September 2009, brown trout were estimated to be present in the East Portal at a density of 1,616 fish per kilometer (2,601 fish per mile), with rainbow trout estimated to be present at a density of 1,548 fish per kilometer (2,492 fish per mile). During the recapture run of the population estimate, a small-scale brown trout removal was conducted, moving the brown trout below a diversion structure 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.

Figure 4.15. East Portal of the Gunnison River adult population structure, September 2009.



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 Hofer-cross brood stock in the East Portal of the Gunnison River.

References

Schisler, G. J., R. Fetherman, and P. J. Schler. 2009. Salmonid Disease Investigations. Federal Aid Project F-394-R8 Job Progress Report. Colorado Division of Wildlife Fish Research Section, Fort Collins, Colorado.

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

Job No. 5. Job Title: 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. Continued work on the new C-SAP computer program has occurred, and assistance to area biologists in operating and conducting analysis with the program has become a routine part of this work. A summary of effects of whirling disease on whitefish (*Prosopium williamsoni)* in Colorado is in progress.

Technical Assistance Milestones

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

1) Fetherman, E. R., D. L. Winkelman, and G. J. Schisler. 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).