Resistant Rainbow Trout in Colorado: Current Status and Uses

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Salmonid whirling disease, caused by the myxozoan parasite, *Myxobolus cerebralis*, has caused considerable difficulty for fisheries managers and fish rearing facilities in Colorado since its introduction in 1987. Loss of year-classes of wild rainbow trout, leading to population collapse, has occurred in many previously robust rainbow trout fisheries in the state, including the Gunnison, Colorado, Rio Grande, Cache la Poudre, South Platte, Dolores, Fryingpan, Fraser, Roaring Fork, and others. Over a decade of stocking large numbers of fingerling rainbow trout in these locations has failed to remedy the problem. Even with the stocking measures in place, the rainbow trout component of these fisheries has remained at less than 10% of historical densities and biomass. *M. cerebralis* 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. Presently, five state-operated facilities are considered *M. cerebralis*-positive. Due to the surface-water influences at these facilities, complete eradication of the parasite is not feasible. Fish reared at these facilities are currently subjected to the Colorado Division of Wildlife D-9 policy, which restricts the stocking of fish from these facilities to non-salmonid habitat.

One reason for the severe problems with whirling disease in Colorado is the vulnerability of rainbow trout to *M. cerebralis* infection. Laboratory and field trials have demonstrated that Colorado River rainbow (CRR) trout, which are used as a wild riverine strain in Colorado, are extremely vulnerable to the effects of the parasite. Similarly, varieties such as the Tasmanian and Bellaire strains, used for put-and-take or put-grow-and-take fisheries in Colorado are also very susceptible to infection and can develop very high parasite loads. Until recently, it was thought that all rainbow trout strains were equally vulnerable to the effects of the parasite. In 2000, Richard Vincent of the Montana Department of Fish, Wildlife and Parks evaluated ten separate varieties of rainbow trout including the Arlee, DeSmet, DeChutes, Erwin, Eagle Lake, Finger Lake, Firehole River, Madison River, Missouri River, and Randolf strains. He found that only the DeSmet variety showed some reduced infection severity compared to the others (Vincent 2001). A wild rainbow trout spawning run at Willow Creek, a tributary of Harrison Lake in Montana, is comprised of a stock originating in part from the DeSmet strain. Subsequent testing determined that this "Harrison Lake" strain exhibited increased resistance to the parasite. In 2001, Dr. Mansour El-Matbouli of the University of Munch, while evaluating infection in various varieties of domestic strains of rainbow trout in Germany, also found that fish from the Hofer Trout Farm in Bavaria appeared to have a strong resistance to infection (El-Matbouli et al. 2002). Laboratory experiments conducted concurrently by Dr. El-Matbouli and Dr. Ron Hedrick in Germany and at the University of California-Davis determined that the infection prevalence and severity in the "Hofer" rainbow trout strain was significantly lower than in the Mt. Lassen and Trout Lodge rainbow trout strains (Hedrick et al. 2003). The identification of rainbow trout strains with potential resistance to the parasite in both Montana and Germany were promising findings, and the Colorado Division of Wildlife immediately began the process of importing both strains for follow-up evaluations. Eyed eggs of the Harrison Lake variety were imported to the Colorado Division of Wildlife Fish Research Hatchery (FRH) in Bellvue in the spring of 2002. These fish were obtained from a wild rainbow trout spawning operation at Harrison Lake. Hofer rainbow trout were transported to the FRH as 1-year old fish from the University of California-Davis in the spring of 2003. These fish were originally imported to the University of California-Davis from the Hofer Trout Farm in Germany as eyed eggs.

Two separate objectives were set for the use of the resistant strains. The first was to establish brood stocks of domestic strains to be used in put-and-take, and put-grow-and-take fisheries that would be potential replacements for more susceptible varieties in use by the Colorado Division of Wildlife. The second objective was to develop brood stocks for use in wild rainbow trout fisheries that would be capable of reproducing in the wild and have a sufficient amount of resistance to *M. cerebralis* to re-establish recruitment in these locations. Verification of the testing conducted by previous investigators was an important component of this work. Another important consideration is the evaluation of the strains in various management situations to determine which varieties are best suited for the desired applications.

Three strains of rainbow trout have been used in these evaluations as components in the overarching goal of identifying functional varieties for the aforementioned purposes. These include the Colorado River rainbow (CRR) trout, the Harrison Lake rainbow trout, and the Hofer rainbow trout strains. The Colorado River rainbow trout has a long history, prior to the introduction of *M. cerebralis*, as a highly successful "wild" rainbow trout strain in Colorado. The CRR strain has characteristics typical of other wild varieties, such as exhibiting slow growth, long lifespan, and natural spawning behavior. Another characteristic that has been considered beneficial is the tendency of the fish to take up permanent residency near their natal spawning areas. Rainbow trout are not native to Colorado, and the Colorado River rainbow trout, therefore, is not a native strain. The strain was derived from a combination of stocking events by private, State, and Federal hatcheries in the late 1800's and early 1900's. However, the excellent reproduction and recruitment success of the strain suggest that it was quite well adapted to rivers in Colorado, and it is considered a naturalized strain. As a result, an effort has been made to integrate the resistant strains into the wild rainbow trout recovery effort without completely abandoning the Colorado River rainbow trout strain, in order to maintain some of the desired characteristics that made it successful in Colorado.

The Harrison Lake rainbow trout strain is considered a "wild" variety best suited for lake and reservoir environments. Typical of most other wild varieties, the Harrison Lake strain is slow-growing, long-lived, has a fusiform body conformation, and is a prolific spawner in natural environments. More specifically, the strain is an inlet spawner, and the fry tend to migrate downstream out of spawning areas and into downstream lakes or reservoirs very soon after emergence. The strain is reported to feed primarily on zooplankton and tends to occupy open water areas rather than the shoreline of these water bodies.

The Hofer rainbow trout strain is a highly domesticated variety that has been reared in a hatchery environment for over 100 years, principally as a food fish. As a result, the strain is extremely fast-growing and early maturing. The ability of the strain to survive and reproduce in the wild is unknown. Cross-breeding of the Hofer strain with wild-type strains, such as the Colorado River and the Harrison Lake rainbow trout strains would presumably make this strain better adapted to reproduction and survival in natural systems.

Several laboratory, hatchery, and field studies have been conducted by the Colorado Division of Wildlife on these strains and their crosses over the course of the last few years. There are also ongoing projects for which results are not yet available. The following descriptions of these experiments are short summaries of more detailed narratives available in the Federal Aid in Fish Restoration Project F-394, Salmonid Disease Studies, for the years 2005-2008, and published laboratory experiments in Schisler et al. (2006).

Pure Harrison Lake Strain

Laboratory and hatchery experiments conducted in Colorado have substantiated the earlier work by the Montana Department of Fish, Wildlife and Parks, indicating that the Harrison Lake strain is more resistant to infection and develops lower parasite loads than other strains of rainbow trout. The observed resistance is not as dramatic as that observed in the Hofer strain, but the Harrison Lake strain does demonstrate a marked advantage over other strains.

In one laboratory experiment, the Harrison Lake strain was compared to Colorado River cutthroat trout, and the Colorado River and Big Thompson River rainbow trout strains for susceptibility to *M. cerebralis*, exposed as two-month old fingerlings. Three replicates of thirty fish from each strain were exposed to 2,358 triactinomyxons (TAMs) per individual. TAMs are the stage of the parasite that infects the fish, and an exposure of 2,000 - 3,000 TAMs per individual at this age is considered a relatively high exposure level. This level of exposure results in infections similar to that seen in the wild where population-level impacts would be observed. The fish were reared for five months and then evaluated for infection prevalence and severity using the pepsin-trypsin digest (PTD) method. Fifteen fish from each of three replicate groups for each strain were evaluated with PTD. The Harrison Lake strain had the lowest infection prevalence, with 77.7% of fish found to be infected (Table 1). Severity of infection is determined by the enumeration of mature parasites (myxospores) present in the head cartilage of a fish. In this experiment, an average of 137,523 myxospores was found in the Harrison Lake strain, which was the lowest of the strains tested. Growth in the unexposed Harrison Lake strain individuals was similar to the other unexposed "wild" varieties tested in this experiment. However, growth in the Harrison Lake strain, as measured by weight, was significantly better than the other strains when exposed to *M. cerebralis* (Table 2).

A second experiment was conducted at the Poudre Rearing Unit (a facility known to harbor *M. cerebralis*) to evaluate the effects of chronic long-term exposure to the parasite on the Harrison Lake strain compared with a commonly used hatchery strain, the Tasmanian rainbow trout strain. Seven hundred-fifty fish of each variety, approximately 3-inches in length and five months of age, were transported to the facility and reared together in a single raceway for one year. The Harrison Lake strain was adipose-clipped to distinguish between the two strains. Sixty-fish samples were collected from each strain once the fish had been at the facility for four months, and at subsequent two month intervals, to test for infection due to *M. cerebralis*. No myxospores were found in either strain during the first three collections. On the fourth collection (at 10 months), an average of 26,104 myxospores were found in the Harrison Lake strain, and 109,402 were found in the Tasmanian strain. On the fifth collection (at 12 months), an average of 38,857 myxospores were found in the Harrison Lake strain, and 161,276 were found in the Tasmanian strain. The differences were highly significant for both sampling events (Figure 1). Growth of the Harrison Lake strain was much slower than the Tasmanian strain throughout the rearing period (Figure 2). The Harrison Lake strain did have the potential to produce much lower parasite loads than other strains currently used in Colorado. The downside of the Harrison Lake strain from a production standpoint was the slow growth that was evident for this strain of rainbow trout. Use of the Harrison Lake strain in some capacity, either as a wild strain or crossed with other varieties remained a possibility.

Table 1. PTD and PCR results, at five months post-exposure, of Colorado River cutthroat, Colorado River rainbow, Harrison Lake rainbow, and Big Thompson River rainbow trout exposed to *M. cerebralis* at a dose of 2,358 TAMS per individual as two month-old fry.

			PTD Results	
Strain	Replicates	N	Myxospore counts (Infection Severity)	Percent positive (Infection Prevalence)
Colorado River Cutthroat	3	45	204,572	100.0
Colorado River Rainbow	3	45	335,327	95.5
Harrison Lake Rainbow	3	45	137,523	77.7
Big Thompson Rainbow	3	45	675,633	100.0

Table 2. Weight and length information, at five months post-exposure, for Colorado River cutthroat, Colorado River rainbow, Harrison Lake rainbow, and Big Thompson River rainbow trout, both exposed and not exposed to *M. cerebralis*. Subscripts ‘a’, ‘b’ and ‘c’ indicate significant differences.

	Not Exposed to *M. cerebralis*		Exposed to *M. cerebralis*	
Strain	Weight (grams)	Length (cm)	Weight (grams)	Length (cm)
Colorado River Cutthroat	8.0 a	9.6 a	6.5 b	8.8 a
Colorado River Rainbow	7.5 a	9.1 a	7.0 b	9.0 a
Harrison Lake Rainbow	7.3 a	9.2 a	7.7 a	9.1 a
Big Thompson Rainbow	5.7 b	8.1 b	5.6 c	8.0 b

Figure 1. Myxospore counts found in pure Harrison Lake and Tasmanian rainbow trout strains reared at the Poudre Rearing Unit for 10 and 12 months.

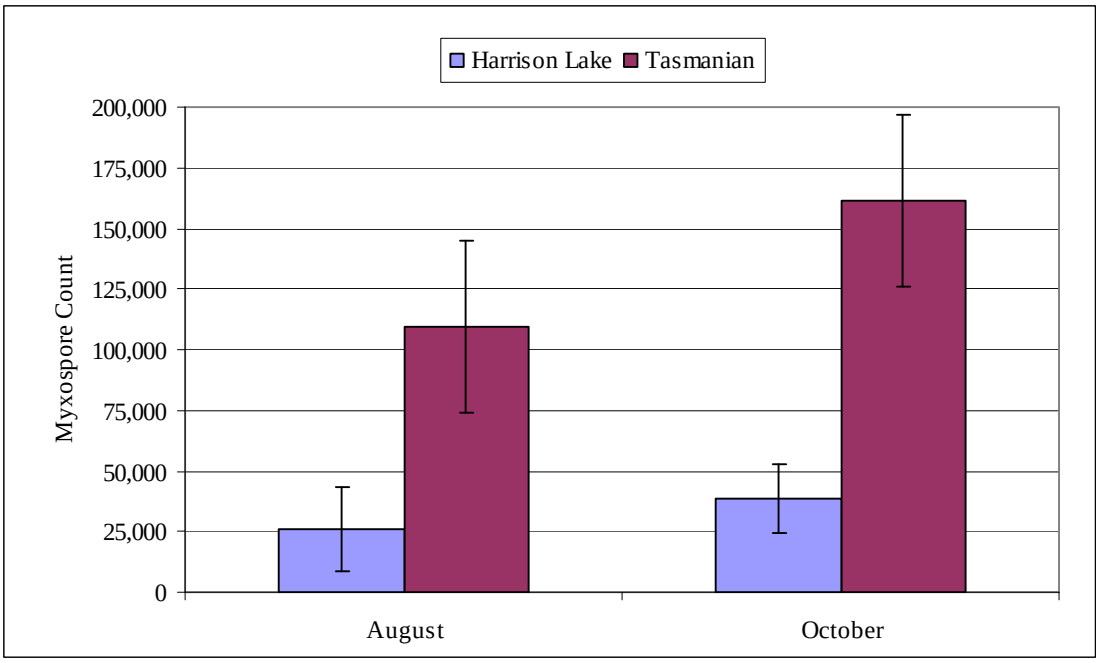
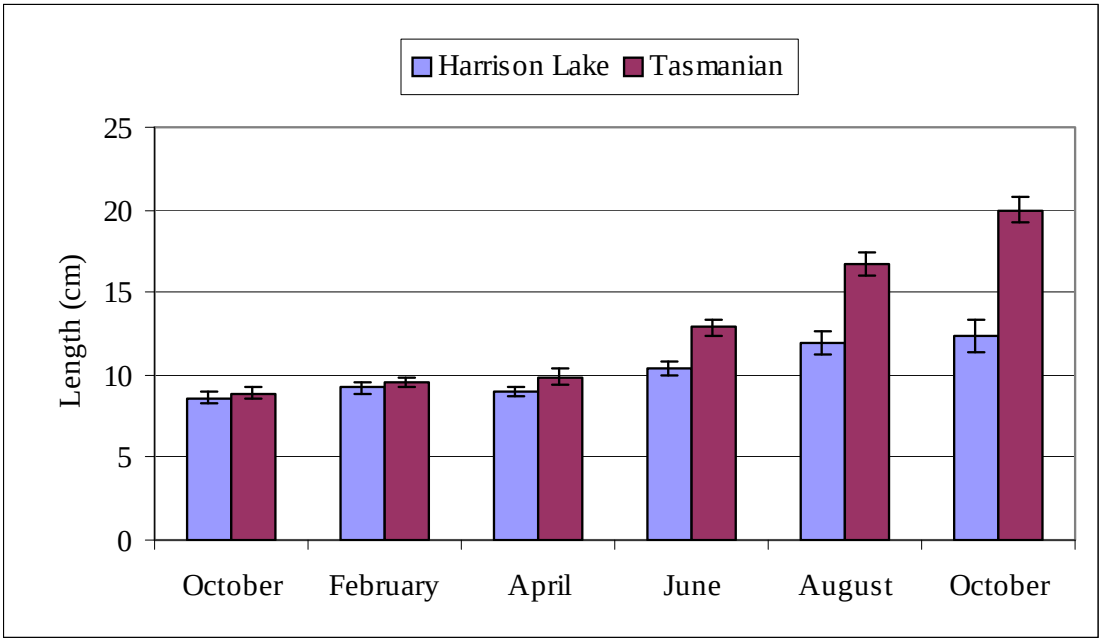


Figure 2. Average lengths of Harrison Lake and Tasmanian rainbow trout strains reared over the course of one year, in the Poudre Rearing Unit raceways.



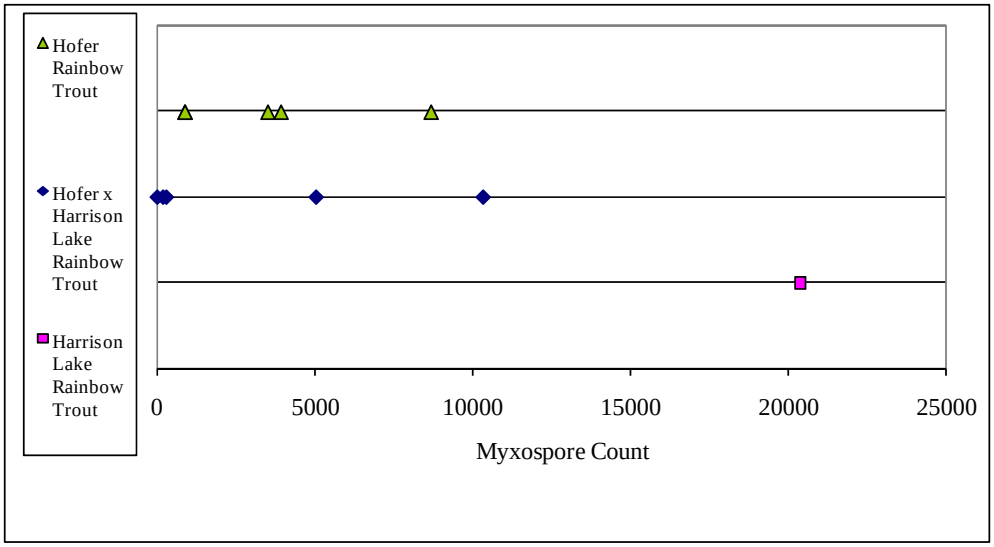
Hofer and Hofer-Harrison Lake Crosses

While the Harrison Lake strain appeared to have some promise as a resistant strain, the Hofer strain was reported to be much more resistant to the effects of *M. cerebralis*. An experiment was conducted in a laboratory setting, in which the Harrison Lake strain was evaluated against the Hofer strain. In addition, a 50:50 cross of the two strains was created by fertilizing Hofer eggs with Harrison Lake strain milt, which was evaluated in conjunction with the two pure strains. The objective was two-fold; to determine if the Hofer strain was substantially more resistant to *M. cerebralis* than the Harrison Lake strain, and to determine how a cross of the two strains would perform when exposed to the parasite. Five replicate groups of the pure Hofer, one replicate of the pure Harrison Lake, and five replicates of the Hofer-Harrison (50:50) cross were used in this experiment. Thirty fish per each replicate group were exposed to 2,000 TAMs per individual as two-month old fingerlings. The fish were reared for five months post-exposure. At the end of the rearing period, ten fish from each family were evaluated for infection using the PTD method. The Harrison Lake strain performed fairly well again in this experiment, producing an average of only 20,398 myxospores per fish (Table 3). However, the pure Hofer strain was even more resistant to the parasite, developing an average of 3,593 myxospores per fish. The Hofer-Harrison (50:50) cross developed a very low myxospore count as well, with an average of 3,168 per fish. These results indicated that out-crossing the Hofer strain with the Harrison Lake strain would not significantly dilute the resistance. The resistance found in the two strains may actually be somewhat enhanced in the Hofer-Harrison (50:50) cross.

Table 3. Overall myxospore counts and prevalence of infection in Hofer, Harrison Lake, and Hofer-Harrison (50:50) crosses exposed to 2,000 TAMs per individual.

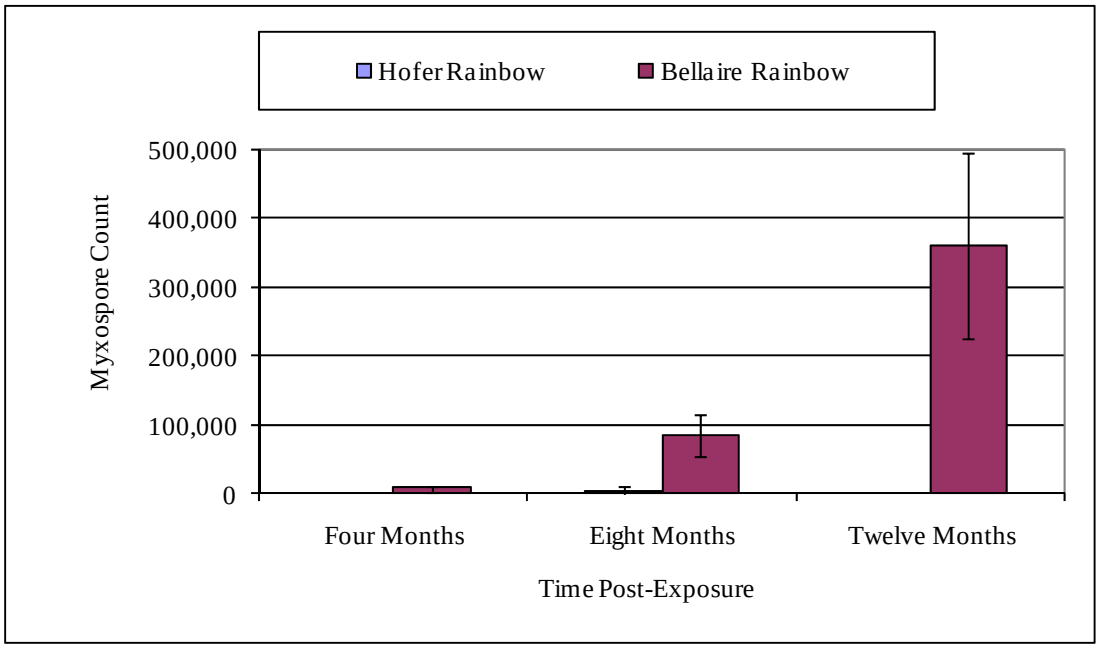
Strain	Families	N	Spore Count Mean	PTD Infected (%)
Hofer Rainbow	5	50	3,593	30.0
Hofer- Harrison Lake (50:50) Cross	5	50	3,168	30.0
Harrison Lake Rainbow	1	10	20,398	40.0

Figure 3. Average myxospore counts for pure Hofer, Harrison Lake, and Hofer-Harrison (50:50) crosses. Each point represents the average myxospore count for each individual family.



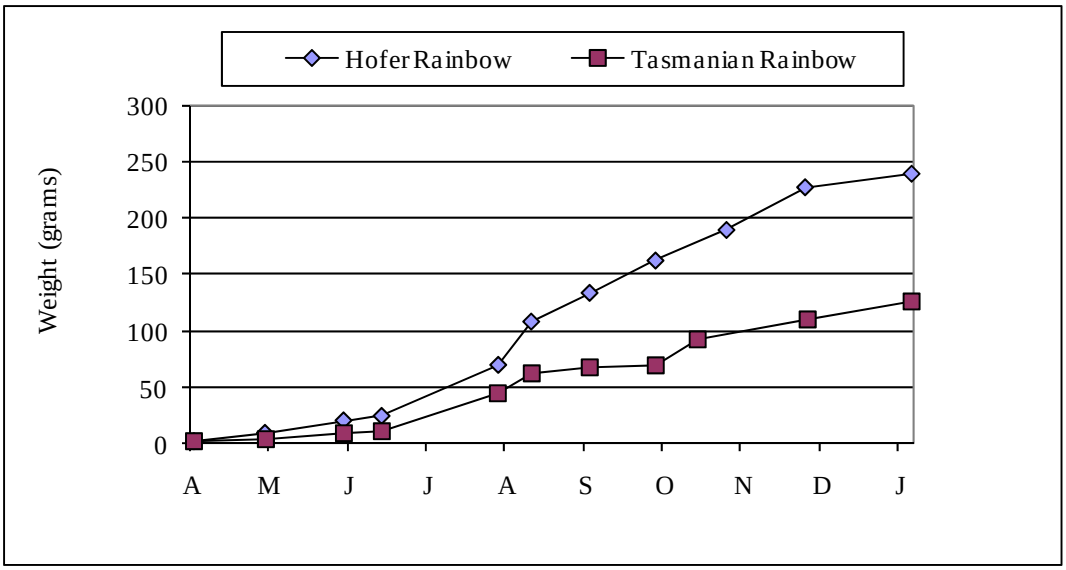
A follow-up evaluation of the Hofer strain was then conducted in a hatchery setting. An experiment similar to the earlier Harrison Lake and Tasmanian rainbow trout experiment was performed at the Colorado Division of Wildlife Poudre Rearing Unit, using pure Hofer rainbow trout. In this experiment, another commonly used hatchery strain, the Bellaire rainbow trout strain, was used for comparison. Seven month-old Hofer and 9.5 month-old Bellaire rainbows were brought to the facility as 5-inch long fingerlings. The difference in age was due to the faster growth of the Hofer strain, making it necessary to use younger Hofer strain fish to size-match with the Bellaire strain fish. This situation provided an advantage to the Bellaire strain with regard to infection, since rainbow trout become more resistant as they get older and larger. The Hofer strain fish were adipose clipped for identification purposes, and the fish were reared in the same raceway for one year. Thirty fish per strain were sampled at four, eight, and twelve months after being brought to the facility. At four months, no myxospores were found in any of the Hofer strain rainbow trout, while the Bellaire strain rainbow trout had an average of 7,314 myxospores (Figure 4). At eight months, the Hofer strain had an average myxospore count of 3,440, with only three individuals in the sample identified as infected. The Bellaire strain had an average myxospore count of 84,993, and every fish was identified as infected. At 12 months, no infected fish were found in the sample from the Hofer strain, while the average myxospore count among Bellaire strain was 361,099.

Figure 4. Myxospore counts found in pure Hofer and Bellaire rainbow trout reared at the Poudre Rearing Unit for 4, 8, and 12 months.



A second hatchery experiment was conducted to evaluate the pure Hofer strain at the Colorado Division of Wildlife Chalk Cliffs Rearing Unit, a *M. cerebralis*-positive facility, in which Tasmanian rainbow trout were used as the comparison group. The two strains were brought to the facility as eyed eggs, hatched during the same week, and reared in parallel throughout the production cycle. Samples collected at 3 months post-hatch were identified as negative with histology for both the Hofer and Tasmanian rainbow trout strains. Samples collected at 5 months post-hatch also resulted in negative results for both histology and PTD in both strains. At 9.5 months post-hatch, infection prevalence in the Hofer strain was 73.3%, and prevalence in the Tasmanian strain was 96.7% Average myxospore count in the Hofer strain was 5,175 (N = 30, SD = 7,643), compared to 48,883 (N = 30, SD = 50,825) in the Tasmanian strain. Growth, as measured by weight, was much faster in the Hofer strain than the Tasmanian strain (Figure 5). Growth, as measured by length, was also quite different between the strains. At 9.5 months post-hatch, average length for Hofer strain was 23.6 cm (N = 60, SD = 1.5), compared to 18.5 cm (N = 60, SD = 2.4) for the Tasmanian strain. At 12 months post-hatch, the Hofer strain averaged 28.4 cm (N = 50, SD = 2.8), while the Tasmanian strain averaged 22.3 cm (N = 50, SD = 3.3).

Figure 5. Average weights of pure Hofer and Tasmanian rainbow trout reared at Chalk Cliffs for one year (data shown for last 10 months of growth).



These results for the Hofer strain, in both growth and resistance to *M. cerebralis*, suggested that the pure Hofer rainbow trout strain may be an ideal strain for hatchery production purposes. However, there were some characteristics of the pure Hofer strain, perhaps due to their long period of domestication, which we felt could eventually cause problems with the stock. For instance, the strain tends to be very surface-oriented and has been observed in raceways to swim for extended periods of time with their backs completely out of the water. The strain also has very little fright response to disturbance, and some hatchery managers have reported that the strain has a sensitivity to formalin. Finally, the strain has been shown to have low heterozygosity

(El-Matbouli et al. 2006) and therefore may lack genetic diversity. The Harrison Lake strain, on the other hand, appears to be free of these limitations. Given that the Hofer-Harrison crosses produced myxospore counts similar to the pure Hofer strain, and exhibited intermediate characteristics to the Hofer and Harrison strains in the laboratory experiment, producing a Hofer-Harrison blended stock seemed to be a logical approach for long-term domestic strain production. A higher proportion of Hofer to Harrison genetics would be desirable from a production standpoint, to maintain the high growth and superior *M. cerebralis* resistance of the Hofer strain. To test this theory, a Hofer-Harrison (75:25) cross was created by crossbreeding Hofer-Harrison (50:50) strain fish, with pure Hofer strain fish.

A second hatchery experiment was conducted at the Chalk Cliffs Rearing Unit using this Hofer-Harrison (75:25) cross. As with the previous experiment, Tasmanian rainbow trout were used as a comparison group. The two strains were brought to the facility as eyed eggs and reared in parallel throughout the production cycle. Tasmanian rainbow trout developed an average myxospore count of 5,106 (SD = 8,999) after eight months on the facility. No myxospores were found in any of the Hofer-Harrison (75:25) strain fish tested at eight months. The Tasmanian rainbow trout developed an average myxospore count of 158,437 (SD = 239,901) after 16 months of growth at the Chalk Cliffs rearing facility. Again, no myxospores could be found in any of the Hofer-Harrison (75:25) rainbow trout, even after 16 months at the facility. Growth of the Hofer-Harrison (75:25) cross was substantially greater than the Tasmanian strain. Average length was 145 mm (SD = 19.1) in the Tasmanian strain compared with 182 mm (SD = 28.9) in the Hofer-Harrison (75:25) cross after eight months. At 16 months, average length of the Tasmanian strain was 221 mm (SD = 37.0), and average length of the Hofer-Harrison (75:25) cross was 315 mm (SD = 28.6). Weight differences were even more dramatic. Average weight at eight months for the Tasmanian strain was 35.8 g (SD = 13.5) compared to 75.7 g (SD = 27.1) for the Hofer-Harrison (75:25) cross. At 16 months, average weight was 123.6 g (SD = 51.7), compared with 332.4 g (SD = 94.20) for the Hofer-Harrison (75:25) cross. These results were quite similar to the results observed in the previous hatchery experiment with the pure Hofer strain. Growth and resistance to *M. cerebralis* did not appear to be compromised by outbreeding the Hofer strain with the Harrison Lake strain in a 75:25 ratio.

At this time, brood stocks of pure Hofer strain and Hofer-Harrison crosses have been established at the Colorado Division of Wildlife Fish Research Hatchery, Poudre Rearing Unit, and the Crystal River Hatchery. Hofer and Hofer-Harrison eggs have been transported to the majority of Colorado Division of Wildlife trout rearing facilities for production purposes. The Hofer strain and Hofer-Harrison crosses have consistently demonstrated superior growth rates compared to other domestic strains of fish reared in these facilities. For instance, growth records at Bellvue-Watson from 2006 and 2007 show pure Hofer rainbow trout averaging 130 mm at 6 months of age, compared to less than 100 mm for strains such as Erwin rainbow trout and Bellaire-Snake River cutthroat crosses. At Crystal River Hatchery, Hofer-Harrison (75:25) crosses at eight months post-eye-up were nearly 180 mm, compared to strains such as Bellaire rainbow trout, Tasmanian rainbow trout, and Snake River cutthroat trout that were less than 140 mm.

Hofer and Harrison Lake Field Trials

Two separate field trials were conducted using the Hofer strain and Hofer-Harrison (75:25) cross fish produced at the Chalk Cliffs Rearing Unit. Fish of the pure Hofer strain were evaluated in 2006, and fish of the Hofer-Harrison (75:25) cross were evaluated in 2007. In both cases the Hofer or Hofer-Harrison cross rainbows were compared with the Tasmanian rainbow trout strain with respect to return-to-creel and angler satisfaction. Two front range reservoirs, Flatiron and Pinewood reservoirs, were used as study locations. Both reservoirs are typical of coolwater reservoirs on the front range of Colorado in which fish are stocked for immediate recreational angling and harvest, and managed as put-and-take fisheries.

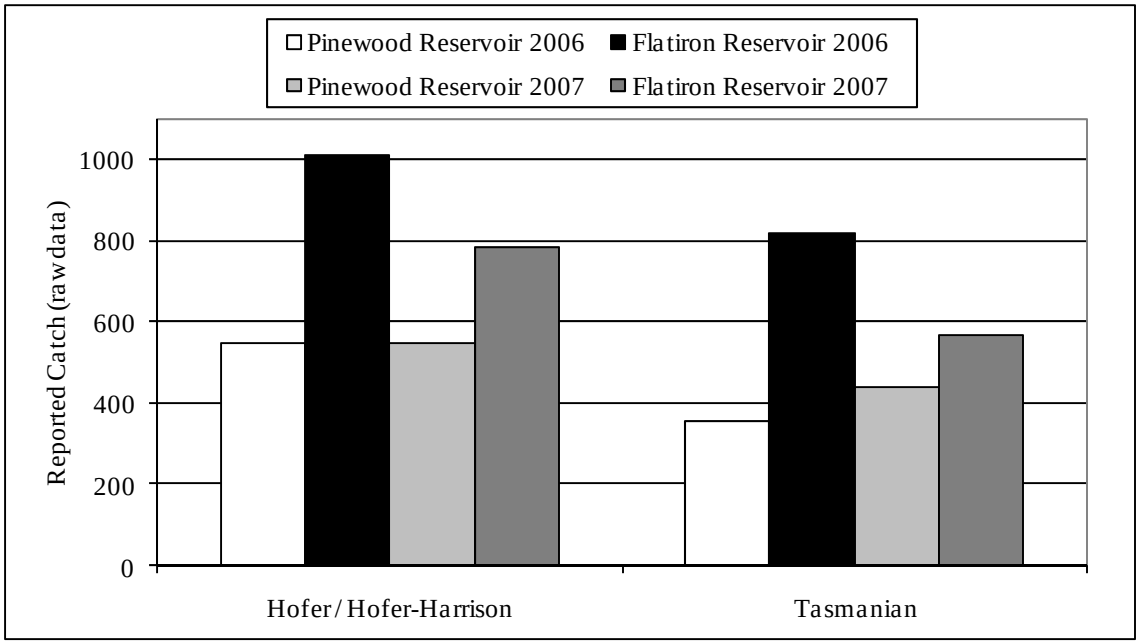
One half of the fish released on each scheduled stocking occasion were Hofer strain or Hofer-Harrison (75:25) crosses, and the other half were of the Tasmanian strain. The fish had been marked prior to stocking with fin clips to identify the fish by strain. Hofer strain or Hofer-Harrison (75:25) crosses were marked with adipose clips, and Tasmanian strain fish were marked 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 during the majority of the open water fishing season. 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, 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.

In 2006, a much higher proportion of the Hofer rainbow trout were captured than the Tasmanian rainbow trout. Total reported catch was 34.6% higher for the Hofer strain than the Tasmanian strain in Pinewood Reservoir. Total reported catch was 19.2% higher for the Hofer strain than the Tasmanian strain in Flatiron Reservoir (Figure 6). When asked about strain preference based on the fin clip marks, 22.6% of the 1,831 respondents chose the Hofer strain, compared to 3.2% that chose the Tasmanian strain. The remaining 74.2% of respondents had no preference.

In 2007, the results followed the same pattern for the Hofer-Harrison (75:25) cross as was observed with the pure Hofer strain in the previous year. At Flatiron Reservoir, 27.7% higher catch was reported for the Hofer-Harrison (75:25) cross than for the Tasmanian strain. At Pinewood Reservoir, a 24.7% higher catch was reported for the Hofer-Harrison (75:25) cross than for the Tasmanian strain. When asked about strain preference based on the fin clip marks, 9.5% of the 2,441 respondents chose the Hofer-Harrison (75:25) cross, compared with 1.1% that chose the Tasmanian strain. The remaining 89.3% had no preference. These results show that the Hofer strain and Hofer-Harrison (75:25) cross both perform better as a catchable plant than the Tasmanian strain with respect to return-to-creel. Additionally, anglers tend to have no preference with regard to strain, but slightly favor the Hofer strain and Hofer-Harrison (75:25) cross over the Tasmanian strain.

Figure 6. Reported catch by strain for Flatiron and Pinewood Reservoirs in 2006 and 2007.



Fish stocked as catchable size for immediate harvest have a different role than fish stocked as fingerlings, which are expected to have good survival in the face of predation and other natural conditions as they grow in the wild to catchable size. Presumably, fish with more Hofer background would be at a disadvantage as a fingerling plant compared to crosses containing more Harrison Lake background, because of the domesticated history of the Hofer strain. However, this theory needs to be thoroughly tested to determine which combination of the two would be best suited for fingerling plants. An ongoing evaluation is being conducted of all crosses of the Hofer and Harrison Lake strains that are currently available for testing. These include the pure Hofer, pure Harrison, Hofer-Harrison (50:50) and Hofer-Harrison (75:25) crosses. A fifth cross made from Hofer-Harrison (75:25) cross, backcrossed with pure Hofer strain rainbow trout, which is essentially 7/8 Hofer strain and 1/8 Harrison Lake strain (87.5:12.5), is also included in these evaluations. These live-release studies are currently being conducted at Parvin Lake, Northwest of Fort Collins, to determine if any particular strain or cross is better adapted as a fingerling plant in a reservoir setting. Additional work is being conducted to evaluate infection severity of all of these Hofer-Harrison varieties in combination, when exposed to high doses of *M. cerebralis* parasites as fingerlings in a pond setting at the Poudre Rearing Unit. The creation of a wild brood stock of Hofer-Harrison crosses is also being attempted at Catamount Reservoir, near Steamboat Springs, Colorado, using plants of the Hofer-Harrison crosses.

Such enthusiasm for the Hofer-Harrison crosses has been generated that suggestions have been made that they may be useful as a riverine strain. As a result, two river plant evaluations of the Hofer-Harrison (87.5:12.5) cross are in progress. Given that the Harrison Lake strain has a tendency to migrate downstream, it is quite possible that the crosses will not remain as resident

fish in these situations. The domestic background of the Hofer strain may also put the Hofer-Harrison crosses at a disadvantage in wild riverine environments. Nonetheless, in 2008, 2,200 fish of the Hofer-Harrison (87.5:12.5) cross (>200 mm) were adipose clipped, Floy tagged, and stocked in the Cache la Poudre River. Two thousand fish of this cross were similarly marked and stocked in the Middle Fork of the South Platte River, and 1,000 fish of the cross were similarly marked and stocked in the South Fork of the South Platte River upstream of Antero Reservoir. Both of these locations are known to be heavily infected with *M. cerebralis*. Survival and infection severity will be evaluated for these plants in the coming year to determine if this variety would be a possible candidate for this purpose.

Colorado River and Hofer Rainbow Crosses

Evaluations of the Colorado River rainbow (CRR) trout and their crosses have followed a different research program than the Hofer-Harrison crosses. The CRR has been a preferred strain for wild rainbow trout populations in rivers in Colorado because of the historical characteristics of the strain that have led to successful reproduction and survival. However, because the strain is extremely susceptible to *M. cerebralis*, it is now at a distinct disadvantage in rivers where it used to thrive. With the strain virtually eliminated in these waters, little hope for natural selection of resistance exists. In locations such as the Colorado River and Gunnison River, very large numbers (30,000 - 60,000) of fingerling CRR trout have been stocked annually for over a decade in an attempt to maintain the rainbow trout component of these fisheries. No natural recruitment has occurred, and the stocked fingerlings have had extremely low survival, resulting in rainbow trout biomass of less than 10% of historic levels in these locations. Increasing survival by integrating some resistance to *M. cerebralis* into these CRR populations was a possible solution to the problem. The original intent of this research was to enhance the resistance of the CRR strain through crossbreeding with the Hofer strain, while retaining as much of the CRR genetic background as possible in this stock destined for wild rainbow population recovery efforts.

To test the resistance of the Hofer-CRR crosses, the first experiment consisted of the pure CRR and Hofer strains, and an F1 (50:50) cross of the Hofer and CRR strains. Five families of Hofer rainbows, two families of pure CRR, 29 families of Hofer (female) x CRR (male), and three reciprocal cross families of CRR (female) x Hofer (male) were created. Eggs from each mated pair were kept separate during incubation. Thirty-five fish from each family were exposed to 2,000 *M. cerebralis* TAMs per individual as two-month old fingerlings and reared for five months post-exposure. Ten fish from each family were evaluated for infection from *M. cerebralis* using the PTD method. Infection was significantly more severe in the CRR strain than in the pure Hofer strain and the F1 rainbow trout families (Table 4). The myxospore counts in the reciprocal crosses were also lower than in the pure CRR families. Individual families of F1 crosses produced a wide range of myxospore counts (Figure 7). These results demonstrated that the resistance to *M. cerebralis* infection could be inherited in some individuals in the F1 cross between the pure strains. Even more interesting was the tendency of some families to inherit more resistance than others. While some individuals and families developed parasite loads similar to the pure CRR parental strain, others showed a high resistance to the parasite. Those individuals could presumably survive in the wild in areas where *M. cerebralis* had eliminated natural recruitment in previously pure CRR populations.

Because one of the original goals of this research was to maintain as much of the CRR genetic background as possible, a second laboratory experiment was conducted to determine if further out-breeding of the F1 cross with pure CRR rainbow trout would dilute the resistance in the offspring. In this experiment, three pure CRR families, three pure Hofer families, and 10 F1 families were created. In addition, 16 B2 cross families were made. The B2 (25:75) cross was an F1 cross, backcrossed with the pure CRR strain. As with the first experiment, these fish were exposed to *M. cerebralis* at a rate of 2,000 TAMs per individual at two months of age. The fish were then reared for five months to allow full development of the myxospores. The results of this experiment were very similar to the first experiment (Table 5). The pure Hofer families

developed very low myxospore counts, the pure CRR families developed very high myxospore counts, and the F1 families produced intermediate myxospore counts. The B2 families developed myxospore counts intermediate to the pure CRR and F1 families (Figure 8).

The results of these first two experiments showed that continued out-breeding of the Hofer-CRR crosses with the pure CRR strain results in a loss of resistance. Some individuals and some families in the B2 cross maintained a high level of resistance, which could still provide enough resistance in natural situations to eventually overcome the effects of *M. cerebralis*. However, the resistance is rapidly lost in most individuals and families due to dilution of the Hofer strain genetic background. This loss is substantial enough that further back-crossing of the Hofer-CRR cross with the pure CRR strain may be counterproductive towards the goal of reestablishing wild rainbow trout populations where the parasite exists.

A third experiment was conducted to validate the results of the first two experiments, and to account for another possible outcome of these crosses in the wild, the F2 (50:50) cross. This cross is a result of an F1 (50:50) cross spawning with another F1 (50:50) cross. One would expect a large proportion of offspring produced in a natural setting where F1 fish have been stocked to be of this variety. In this experiment, 10 pure Hofer families, 10 pure CRR families, 20 F1 families, 20 B2 families, and 20 F2 families were all exposed to *M. cerebralis* as 2-month old fingerlings and reared for five months. Ten fish from each family were evaluated using the PTD method, as in the previous experiments. The results determined that the resistance to *M. cerebralis* infection in the F2 cross fish was intermediate to the B2 cross and the F1 cross (Table 6). In addition, the distribution of myxospore counts, by family, was not as wide as seen in the B2 cross (Figure 9).

Because of the rapid decrease in resistance found in the B2 cross compared with the F1 cross, the laboratory results suggest that the F1 cross would be a much better candidate for reintroduction efforts in rivers where rainbow trout populations have been lost. While B2 individuals have not been crossed with other B2 individuals, or with pure CRR fish, in laboratory studies, the assumption is that an even greater loss of resistance would occur if these fish were to spawn with those strains in the wild. Some individual offspring would still retain the resistance, and heavy selection pressure would strongly favor those individuals for survival. This approach could eventually bring back wild populations in the presence of whirling disease. However, the alternative of using the F1 cross for stocking is the preferred method if more rapid re-population is the goal. This approach assumes that the loss of wild characteristics in the F1 cross does not outweigh the benefits of enhanced resistance.

The evaluation of the F2 cross shows that if exclusively F1 fish were stocked, and F2 fish were generated as offspring of those stocking events, the loss in resistance would not be overwhelming. A high proportion of the offspring in the F2 generation would retain resistance to *M. cerebralis* and would provide a relatively rapid recovery of the population if infection from the parasite was the only limiting factor.

Table 4. Average myxospore counts and prevalence of infection in the Hofer and Colorado River rainbow trout strains, and crosses of those strains (male CRR x Hofer female and reciprocal cross), exposed to 2,000 TAMs per fish.

Strain	Families	N	Spore Count Mean	PTD Infected (%)
Hofer Rainbow	5	50	3,593	30.0
Hofer (f) x Colorado River Rainbow (m)	29	290	84,400	82.4
Colorado River (f) x Hofer Rainbow (m)	3	30	42,376	86.7
Colorado River Rainbow	2	20	210,982	100.0

Figure 7. Average myxospore counts of the Hofer and Colorado River rainbow trout strains, and the F1 (50:50) cross of these strains. Each point represents the average myxospore count for each individual family.

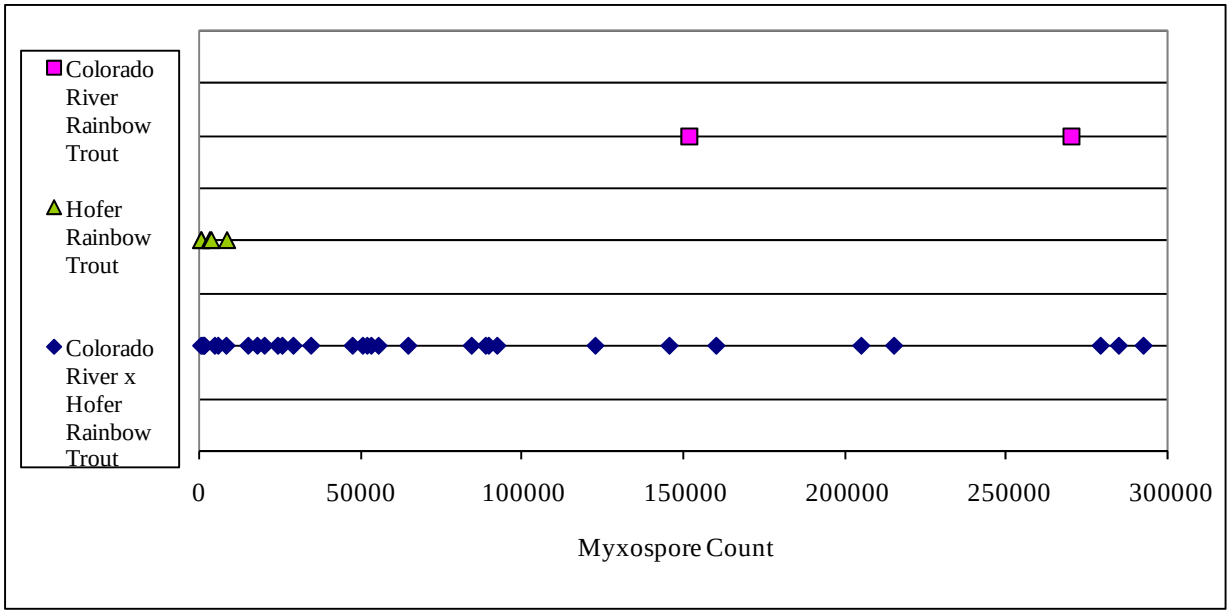


Table 5. Average myxospore counts and prevalence of infection for the Hofer and Colorado River (CRR) rainbow trout strains, and the F1 (50:50) and B2 (75:25) crosses.

Strain	Families	N	Spore Count Mean	PTD Infected (%)
Hofer Rainbow	3	30	1,482	49.6
F1 (50:50)	10	100	47,128	77.0
B2 (25:75)	16	160	125,167	93.0
Colorado River Rainbow	3	30	232,973	100.0

Figure 8. Average myxospore counts for the three Hofer, three Colorado River rainbow (CRR) ten F1 [Hofer-CRR (50:50)] and 16 B2 [Hofer-CRR (25:75)] families. Each point represents the average myxospore count for each individual family.

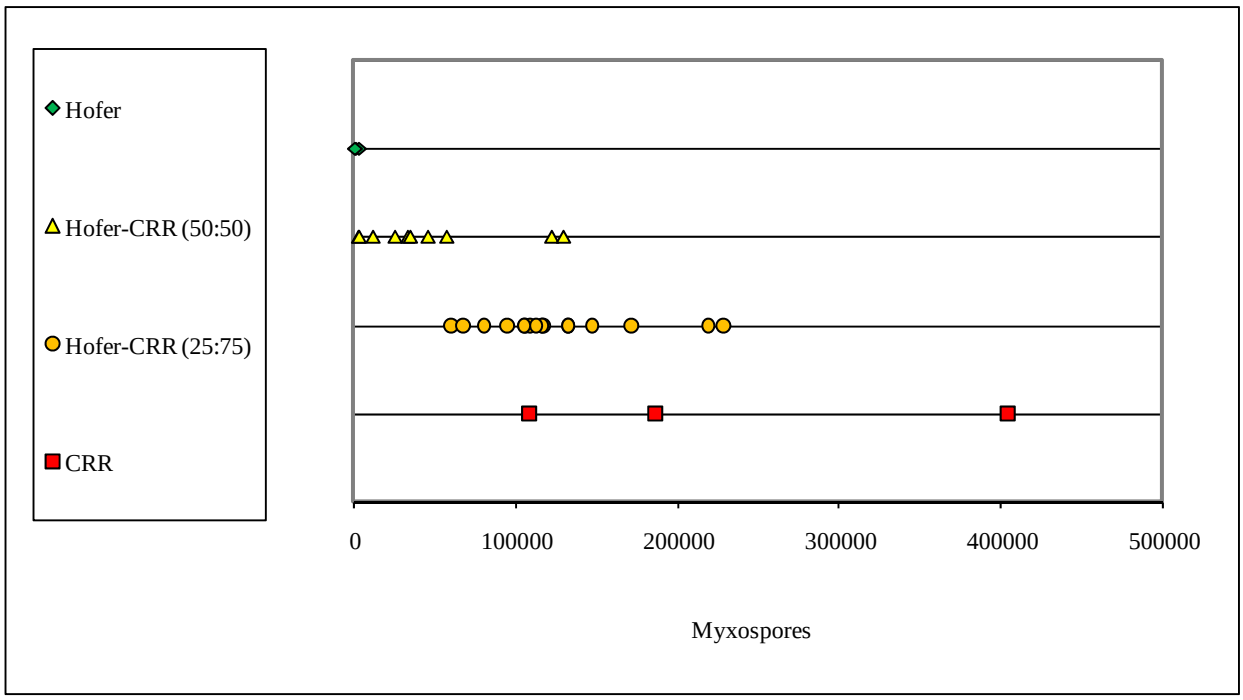
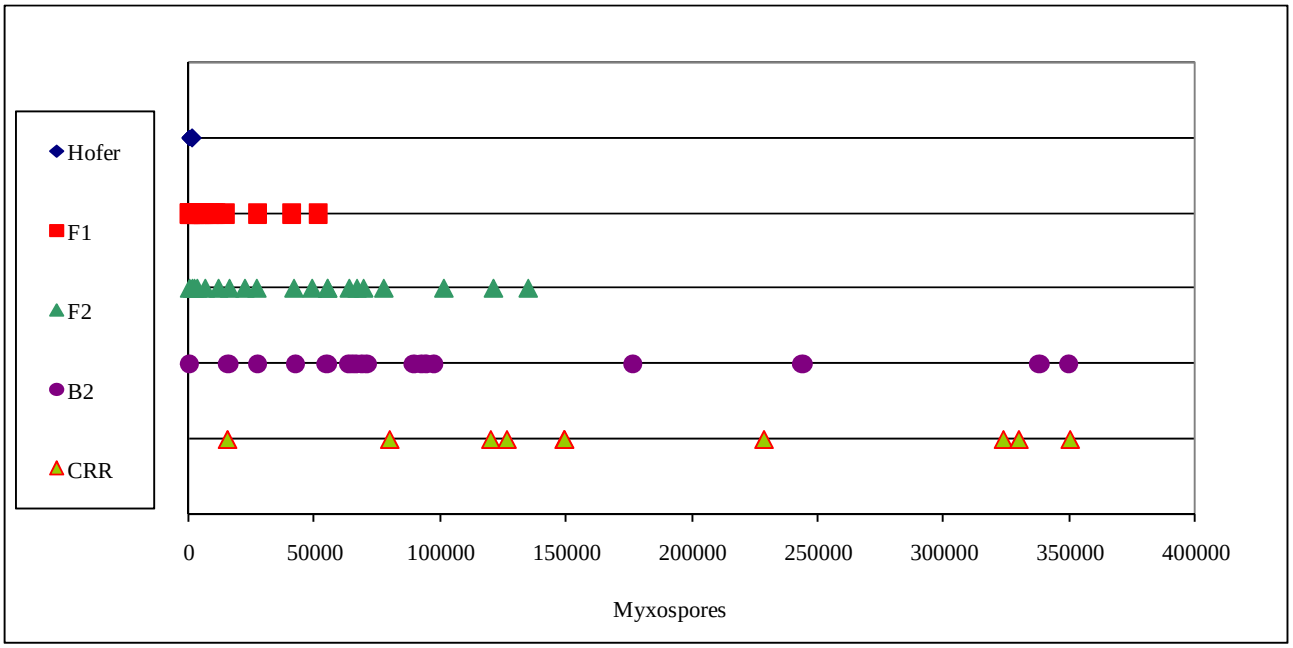


Table 6. Average myxospore counts and prevalence of infection for the Hofer, F1, F2, B2, and pure Colorado River rainbow trout.

Strain	Families	N	Spore Count Mean	PTD Infected (%)
Hofer Rainbow	10	100	275	7.0
F1 (50:50)	20	200	9,566	36.0
F2 (50:50)	20	200	46,227	52.0
B2 (25:75)	20	200	97,588	69.0
Colorado River Rainbow	10	10	187,209	100.0

Figure 9. Average myxospore counts for the10 Hofer, 10 Colorado River rainbow, 20 F1 [Hofer-CRR (50:50)], 20 B2 [Hofer-CRR (25:75)] and 20 F2 [Hofer-CRR (50:50) ^{2}] families. Each point represents the average myxospore count for each individual family.



Hofer-Colorado River Rainbow Field Trials

Formal field testing of the Hofer-CRR crosses in comparison to the pure Colorado River rainbow trout strain was conducted in a limited manner over the same time period as the laboratory evaluations. The study areas of primary focus up to this point have been the Gunnison River and Colorado River. Both of these locations had very strong wild populations of rainbow trout in the past, but have experienced complete losses of rainbow trout year-classes and lack of natural recruitment since the mid-1990's. Both locations have high ambient levels of *M. cerebralis*. Brown trout numbers have increased over the past decade in both rivers, and fingerling plants of Colorado River rainbow trout to augment the rainbow trout populations have exhibited extremely low survival.

Gunnison River

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.

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. 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).

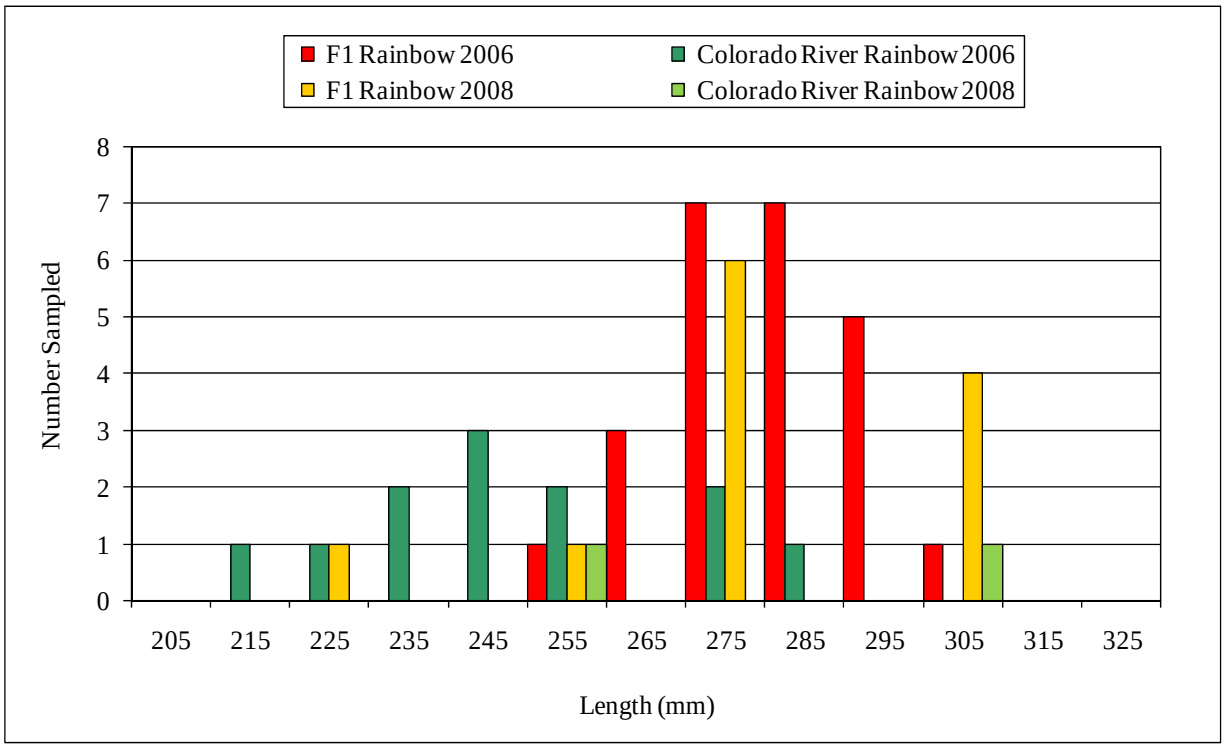
Survival and population estimates in 2006 for fish stocked in 2005 were difficult to assess directly because of mark loss (fin regeneration or poor marks) in both the CRR and B2 varieties. AFLP (Amplified Fragment Length Polymorphism) testing, a molecular technique that can help distinguish between individuals 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 10).

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 10. 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.



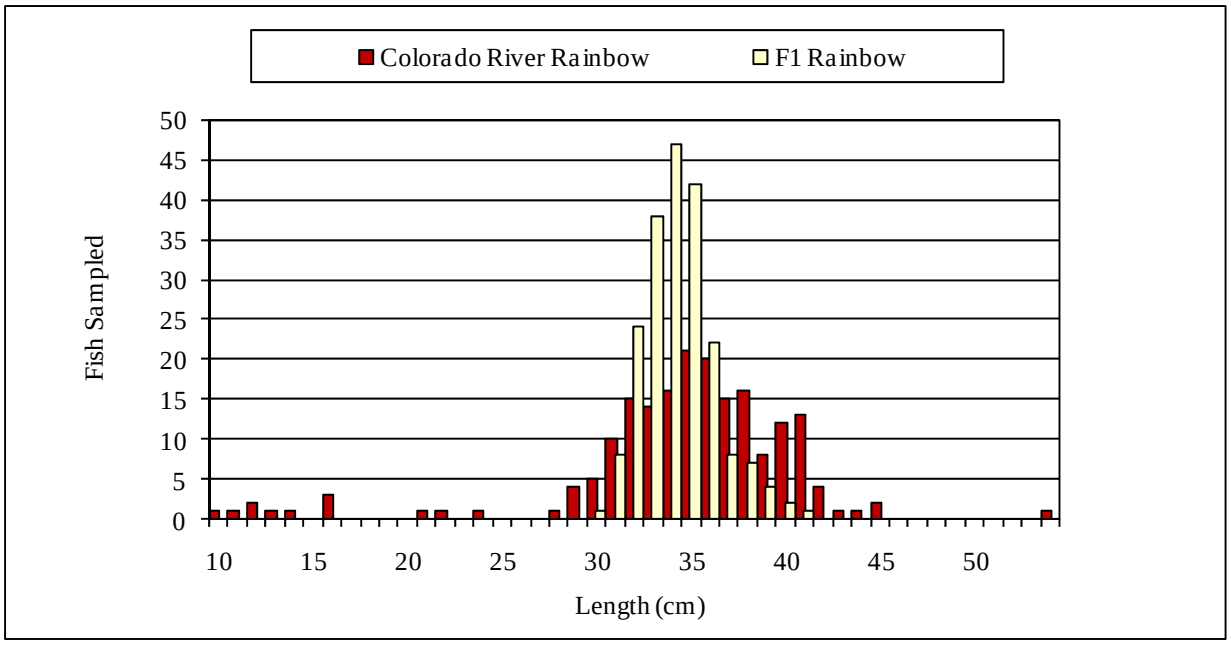
The results of this field evaluation demonstrate that the F1 fish can survive at least as well as the pure CRR trout when planted as fingerlings. The results also demonstrate that myxospore counts developed after stocking are much lower in the F1 fish than in the pure CRR trout. The myxospore counts 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.

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. 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*. More in-depth genetic analyses of the fry and age-1 fish are planned for 2009 to determine the extent of survival and recruitment from the wild-spawned rainbow trout that are now appearing in the population.

Upper Colorado River

In 2006, a single lot of Hofer-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 11).

Figure 11. 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.



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 Hofer genes using the 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. More of the F1 fish from the 2006 plant will be sexually mature in spring 2009, and have the potential to produce a large year-class of offspring. Further monitoring and evaluation of the marked fish and any new reproduction in the upper Colorado River is necessary to determine if the strategy of using the F1 cross will be successful in returning natural recruitment to locations where wild rainbow trout populations have been lost due to *M. cerebralis*.

The high survival and good post-stocking growth of the F1 fish stocked as catchable-sized fish in the upper Colorado River is particularly encouraging, as it is quite possible that these fish are capable of surviving and reproducing in large numbers when they reach sexual maturity. These results also demonstrate that stocking larger fish increases survival in the presence of predatory brown trout. Additional evaluations are planned for the upper Colorado River using marked fish. Fry evaluations using the AFLP technique will also be initiated on a large scale in 2009 to determine if the F1 fish are reproducing in this location.

The resistant strain evaluations are still in the early stages with regard to re-establishment of wild rainbow trout populations. Work conducted over the next several years will be very important in determining which combinations of the Hofer and wild strains are effective for establishing self-sustaining rainbow trout populations.

Summary

The current philosophy for use of resistant strains continues to be to use the Hofer-Harrison strain as a replacement for other varieties typically used as catchable plants in lakes and reservoirs. Pure Hofer strain fish will be maintained as a broodstock for catchable plants, and to replenish the Hofer-Harrison stock in the event that a decline in resistance is observed over time. Hofer, Harrison Lake, and several varieties of their crosses are currently being evaluated to determine which variety is best suited as a fingerling plant for lakes and reservoirs. Hofer-Colorado River 50:50 (F1) crosses appear to be a useful replacement for the Colorado River rainbow trout strain. Further dilution of the Hofer genetics by back-crossing the F1 cross with pure Colorado River rainbow trout is detrimental due to the rapid loss of resistance in the back-crosses. The increased proportion of Colorado River rainbow trout genetic background in the crosses does not appear to improve survival of the fish in the wild. Ongoing field evaluations will provide more information as to the long-term viability of the Hofer-Colorado River cross with regard to reproduction and recruitment. Additional studies to evaluate the Hofer-Harrison cross as a possible river plant will influence those decisions as well. It is unlikely that a single variety will be best suited as a catchable, subcatchable, and wild strain. Further refinement of applications for the different varieties will occur as more information becomes available from field trials in the next few years.

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