

ARTICLE

Survival of Whirling-Disease-Resistant Rainbow Trout Fry in the Wild: A Comparison of Two Strains

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Abstract

Introduced pathogens can affect fish populations, and three main factors affect disease occurrence: the environment, host, and pathogen. Manipulating at least one of these factors is necessary for controlling disease. *Myxobolus cerebralis*, the parasite responsible for salmonid whirling disease, became established in Colorado during the 1990s and caused significant declines in wild Rainbow Trout *Oncorhynchus mykiss* populations. Attempts to re-establish Rainbow Trout have focused on manipulating salmonid host resistance. A Rainbow Trout strain known as GR × CRR was developed for stocking in Colorado by crossing a whirling-disease-resistant strain known as the German Rainbow Trout (GR) with the Colorado River Rainbow Trout (CRR). The GR × CRR fish exhibit resistance similar to that shown by GR, and survival and reproduction were expected to be similar to those of CRR. One disadvantage of stocking GR × CRR is that outcrossing and backcrossing could decrease resistance, and laboratory studies have indicated that this can occur. A potential disadvantage of stocking pure GR is lower survival due to domestication. To compare fry survival between the strains, a field experiment was conducted in 1.6-km reaches of nine Colorado streams. Each stream was stocked in August 2014 with 5,000 GR × CRR and 5,000 GR individuals. In October 2014, April 2015, and August 2015, apparent survival was assessed. Two laboratory predation experiments were also conducted. The field experiment revealed that short-term apparent survival was influenced by stream, and growth rate was influenced by strain and stream. However, after 12 months, there was no difference in apparent survival or growth rate between the GR and GR × CRR strains. Laboratory experiments showed that survival did not differ between the strains when confronted with Brown Trout *Salmo trutta* predation. Our results indicate that the GR strain is a viable option for stocking in streams where *M. cerebralis* is enzootic. Further evaluation is needed to determine whether GR fish will survive to maturity and reproduce.

Myxobolus cerebralis, the parasite responsible for salmonid whirling disease, caused a near-complete loss of wild Rainbow Trout *Oncorhynchus mykiss* populations in

several Intermountain West states. Significant declines in Rainbow Trout were documented after the establishment of *M. cerebralis* in the Colorado River, Colorado

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(Nehring and Walker 1996), and the Madison River, Montana (Vincent 1996). Subsequently, the parasite became established in many coldwater systems in Colorado, with similar negative effects on Rainbow Trout populations (Nehring and Thompson 2001; Nehring 2006). Age-0 salmonids are particularly susceptible to infection and mortality because skeletal ossification has not occurred (El-Matbouli et al. 1992, 1995), and declines in Rainbow Trout are primarily due to recruitment failure (Nehring and Thompson 2001).

The complex multistage life cycle of *M. cerebralis* requires two hosts, a salmonid and the oligochaete *Tubifex tubifex*. *Myxobolus cerebralis* has two free-living stages: myxospores and triactinomyxons. The complexity of the life cycle makes it difficult to eradicate *M. cerebralis* once it has become established. Control strategies used in the hatchery environment, such as dewatering, disinfection, and pond substrate management (Hoffman and Putz 1969; Hoffman and Hoffman 1972; Schaperclaus 1986; Wagner 2002), are clearly not viable in wild fisheries. Therefore, manipulating or using existing host resistance was thought to be the most realistic option to disrupt the parasite's life cycle in wild populations (Beauchamp et al. 2002; Schisler et al. 2006; Wagner et al. 2006; Fetherman et al. 2011, 2012; Nehring et al. 2013, 2016). Resistant *T. tubifex* lineages were associated with reductions in parasite production (Beauchamp et al. 2005; Nehring et al. 2013), and attempts were made to manipulate *T. tubifex* community composition by introducing whirling-disease-resistant *T. tubifex* lineages. However, those efforts did not dramatically reduce parasite prevalence (Clapp 2009; Winkelman and Gigliotti 2014). Due to the limited success with using resistant *T. tubifex*, additional management and research efforts became focused on stocking Rainbow Trout that are genetically resistant to the parasite (Fetherman et al. 2014).

Historically, the Colorado River Rainbow Trout (CRR) strain was used to stock and establish Rainbow Trout populations in Colorado (Nehring 1987, 1988, 1992). However, CRR were highly susceptible to *M. cerebralis*, which allowed high parasite production within the host, and the continued stocking of this strain was unsuccessful in re-establishing wild Rainbow Trout fisheries. The need for new Rainbow Trout management options led Colorado Parks and Wildlife (CPW) to research the efficacy of using whirling-disease-resistant Rainbow Trout (Schisler et al. 2006). Resistant Rainbow Trout were discovered at a hatchery in Germany, and whirling disease resistance presumably developed because those Rainbow Trout were continuously in contact with *M. cerebralis* for over a century. The German Rainbow Trout (GR) strain is more resistant to whirling disease than many other Rainbow Trout strains found in North America (Hedrick et al. 2003).

One concern over using GR to re-establish Rainbow Trout fisheries was the strain's long history of domestication (>100 years; Hedrick et al. 2003). Due to the domestication of the GR strain, it was thought that the survival of GR in the wild might be lower than that of other historically stocked Rainbow Trout strains (Schisler et al. 2006). Specifically, it was thought that GR would be predator naïve and particularly susceptible to predation in the wild (Suboski and Templeton 1989; Brown and Laland 2001; Brown et al. 2003). As a strategy to increase survival and recruitment of stocked Rainbow Trout, CPW started a breeding program using resistant GR and susceptible CRR. The objective of the breeding program was to produce a Rainbow Trout strain that retained whirling disease resistance while gaining the survival and reproductive characteristics of CRR (Schisler et al. 2006). The cross was referred to as the GR × CRR strain.

Since 2008, CPW has stocked GR × CRR fish into all major Colorado coldwater drainages to re-establish Rainbow Trout populations. Larger (≥150 mm TL) GR × CRR individuals were initially stocked for two reasons: (1) they are less susceptible to *M. cerebralis* because the skeleton is largely ossified at this size (Ryce et al. 2005) and (2) they exceed the gape limit of most natural aquatic predators (Fetherman et al. 2014). However, survival of larger GR × CRR was low, and there was little evidence of recruitment (Fetherman et al. 2014). More recently, CPW biologists began stocking GR × CRR fry (<100 mm TL) into many river systems. Stocking of GR × CRR fry was thought to increase survival by reducing hatchery-related behavioral conditioning (Olla et al. 1998; Jackson and Brown 2011). Stocked fry have shown increased survival and have started to recruit to older age-classes in the Colorado and Gunnison rivers (Fetherman and Schisler 2016).

Observed survival of stocked GR × CRR fry is promising. However, studies show that outcrossing and backcrossing of GR × CRR can produce lower resistance and increased variability in resistance (Fetherman et al. 2011, 2012). If wild reproduction occurs, the potential for reduced resistance could slow recovery efforts. Outcrosses and backcrosses are still more resistant than pure CRR (Fetherman et al. 2011), but their resistance is more variable, resulting in higher average myxospore counts per fish, which could reduce survival (Fetherman et al. 2012). One option to overcome the loss of resistance associated with GR × CRR reproduction is to stock pure GR. Although GR stocked as fingerlings exhibited low survival in reservoir plants (Fetherman and Schisler 2013), GR fry and fingerlings in small-pond studies have been shown to survive, grow to maturity, and spawn successfully (Nehring 2014). However, GR survival in stream situations has not been extensively evaluated. Additionally, laboratory results have shown that exposure to *M. cerebralis* did not

result in differences in growth or swimming ability between the GR and GR \times CRR (Fetherman et al. 2011), indicating that the stocking of pure GR could be an option for establishing whirling-disease-resistant Rainbow Trout populations in certain situations.

The goal of our research was to determine whether there were differences in fry survival between the GR and GR \times CRR strains in tributary streams occupied by potential predators. To achieve this goal, we designed two experiments. First, a field experiment was used to evaluate potential differences in apparent survival between GR and GR \times CRR fry when stocked into Colorado's headwater streams. Second, two laboratory experiments compared predation susceptibility between GR and GR \times CRR fry in the presence of a Brown Trout *Salmo trutta*, the dominant predator in most of the systems where Rainbow Trout are being re-established in Colorado.

METHODS

Field experiment.—Stream survival evaluations were conducted in 1.6-km reaches of nine streams in Colorado between August 2014 and August 2015. The nine streams consisted of three streams in each of three separate drainages (Table 1). As a result of restricted stream access due to property ownership, the reach in Jefferson Creek was limited to 0.7 km. We selected streams based on accessibility for stocking, fish community structure, and a qualitative visual assessment of habitat, choosing to use streams of similar size and with similar physical characteristics (Table 1). We assumed that *M. cerebralis* was enzootic in most if not all of the nine streams based on the proximity to positive stream sources, although the presence of *M. cerebralis* was not confirmed in this study.

Prior to the introduction of Rainbow Trout fry, two sampling sites (average length = 66 m) were established in

each stream reach, and fish population estimates were conducted in July 2014 utilizing three-pass removal backpack electrofishing techniques (Temple and Pearsons 2007). We used these estimates to confirm that no Rainbow Trout were present prior to stocking and to provide baseline data on initial fish assemblage composition, density, and biomass. Streams were primarily dominated by Brown Trout (Table 2). The Brook Trout *Salvelinus fontinalis*, another potential predator, was present in four of the study streams and was common in three. Five other non-salmonid species were commonly found, and four others were less common. The estimated total biomass of fish species in each stream prior to the stocking of Rainbow Trout ranged from 358 to 3,723 kg/ha (Table 2). However, in the North Fork Poudre River and Jefferson Creek, the second electrofishing pass yielded more fish than the first pass, resulting in imprecise estimates.

The GR and GR \times CRR fry were reared at the CPW Rifle Falls Fish Hatchery. To make field identification possible, we marked GR \times CRR fry with coded wire tags (Northwest Marine Technology, Inc., Shaw Island, Washington) and left the GR fry untagged. We decided not to tag the GR fish because we expected them to exhibit lower survival in the wild due to their history of domestication, and we wanted to avoid potential reductions in survival due to tagging. We assumed that untagged Rainbow Trout captured after stocking were pure GR and that tagged Rainbow Trout were GR \times CRR. Although tag loss might bias our results, tag retention is generally above 90% when using coded wire tags (Ostergaard 1982; Elrod and Schneider 1986; Hale and Gray 1998; Munro et al. 2003). Coded wire tags were injected into the nose of anesthetized GR \times CRR (tricaine methanesulfonate [MS-222]) by using two Mark IV automatic tag injectors (Northwest Marine Technology). Fish were placed into a holding raceway to recover and were monitored for

TABLE 1. General overview of each study stream in Colorado where fry of two Rainbow Trout strains were released (*M. cerebralis* = *Myxobolus cerebralis*).

Stream	Drainage	Average elevation (m)	Average width (m)	Average annual temperature (°C)	<i>M. cerebralis</i> presence
Lone Pine Creek	Poudre River	1,789	4.8	13.3	Negative ^a
North Fork Poudre River	Poudre River	2,172	8.6	10.6	Positive
Sheep Creek	Poudre River	2,549	5.0	11.5	Negative
Willow Creek	Colorado River	2,608	8.8	9.9	Unknown
Spielberg Creek	Colorado River	2,485	7.7	11.7	Unknown
Rock Creek	Colorado River	2,635	5.5	10.1	Positive
Tarryall Creek	South Platte River	2,956	4.4	10.5	Unknown
Michigan Creek	South Platte River	2,975	4.2	11.3	Unknown
Jefferson Creek	South Platte River	2,891	3.0	10.8	Positive

^aSample size was one fish. Note that Lone Pine Creek is directly downstream of a positive source of *M. cerebralis*. All other sites of unknown *M. cerebralis* presence are connected to other positive streams or reservoirs.

TABLE 2. Estimated biomass (kg/ha) for each Colorado stream and each fish species in July 2014 prior to the stocking of Rainbow Trout fry (BRN = Brown Trout *Salmo trutta*; BRK = Brook Trout *Salvelinus fontinalis*; LGS = Longnose Sucker *Catostomus catostomus*; WHS = White Sucker *Catostomus commersonii*; LND = Longnose Dace *Rhinichthys cataractae*; SPD = Speckled Dace *Rhinichthys osculus*; MTS = Mottled Sculpin *Cottus bairdii*; JOD = Johnny Darter *Etheostoma nigrum*; BST = Brook Stickleback *Culaea inconstans*; CRC = Creek Chub *Semotilus atromaculatus*; FHM = Fathead Minnow *Pimephales promelas*).

Stream	Average total biomass	Species										
		BRN	BRK	LGS	WHS	LND	SPD	MTS	JOD	BST	CRC	FHM
Lone Pine Creek	3,338	2,038	0	121	1,777	54	0	0	1	5	0	1
North Fork Poudre River ^a	812	782	0	0	0	27	0	0	0	0	0	0
Sheep Creek	2,033	749	973	126	0	74	0	0	0	0	0	0
Willow Creek	358	231	26	0	0	0	11	83	0	0	0	0
Spielberg Creek	1,614	863	0	164	0	0	67	353	0	0	61	0
Rock Creek	2,581	1,325	262	677	0	0	0	0	0	0	0	0
Tarryall Creek	2,277	2,277	0	0	0	0	0	0	0	0	0	0
Michigan Creek	3,451	3,297	147	0	0	0	0	0	0	0	0	0
Jefferson Creek ^a	3,723	3,419	0	305	0	0	0	0	0	0	0	0

^aPopulation estimates are imprecise because more fish were caught during the second electrofishing pass than during the first.

mortality for a 24-h period after tagging, during which mortalities were removed and recorded, and replacement fish were tagged. Average 24-h mortality rate associated with tagging was 70 fish per d (0.7%), and average tag retention was 85% (SD = 3.8%).

Based on the suggestions of CPW biologists, 10,000 Rainbow Trout fry (5,000 of each strain) were stocked into each of the study streams between August 4 and 6, 2014. Rifle Falls Fish Hatchery personnel transported the fish to each stream in oxygenated fish transport trucks. Upon arrival, we placed fish in 19-L buckets and acclimated them to stream conditions by exchanging hatchery water with stream water. Once acclimated, we stocked fish by hand into the stream margins throughout the 1.6-km reach.

We estimated poststocking abundance of all fish species for three time periods: short term (2 months; August–October 2014), over winter (6 months; October 2014 to April 2015), and annual (12 months; August 2014 to August 2015). Within each of these periods, the apparent survival, length, and growth rate of the GR and GR × CRR strains were evaluated. Population estimates were conducted at two sampling sites (66-m average length) within the 1.6-km stocking reach in each of the study streams during October 2014 and August 2015. Due to hazardous access and unsafe sampling conditions, sampling occurred in only seven of the nine streams during April 2015. To assess apparent survival, we conducted population estimates using three-pass removal backpack electrofishing techniques (Temple and Pearsons 2007). Removals were conducted using two to three backpack electrofishing units (Smith-Root LR-24) depending on stream width. Electrofishing gear covered the wetted channel width, allowing for full coverage of all accessible trout

habitat. Captured fish were kept in separate live wells, designated by pass, until processing occurred. All Rainbow Trout captured were measured to the nearest millimeter, weighed to the nearest gram, and scanned for coded wire tags. At least 150 individuals of every other species captured were measured to the nearest millimeter and weighed to the nearest gram. After 150 lengths and weights were recorded for a species, only lengths were recorded thereafter, and a length–weight regression was later used to assign weights. All fish were returned to the sampling site after processing.

Fish species abundance was estimated within each sampling site by using three-pass removal estimates, which were calculated using the “removal” function in the R package Fisheries Stock Analysis (Ogle 2017) and then extrapolated to average abundance within each 1.6-km reach. Apparent survival for the GR and GR × CRR strains was calculated for the three different time periods. Apparent survival was defined as the estimated abundance at the end of the time period (N_{t+1}) divided by the estimated abundance at the beginning of the time period (N_t). Rainbow Trout growth rate (mm/month) was calculated as the difference in length at the end of the time period relative to the beginning of the time period.

To determine whether survival and growth rate differed between the Rainbow Trout strains or among streams, linear models were constructed for each time period to test for the effects of each factor considered separately (strain only and stream only), an additive effect of strain and stream, and an intercept-only model. Akaike’s information criterion utilizing second-order approximations (AIC_c) was used to rank models. We selected models based on AIC_c differences (ΔAIC_c) and Akaike weights (w_i), and we report parameter estimates and associated 95% confidence

intervals (CIs) from the top supported models (Burnham and Anderson 2002).

Laboratory experiments.—Two experiments were conducted at the Foothills Fisheries Laboratory (FFL), Colorado State University, to determine Rainbow Trout fry susceptibility to predation. The first experiment was conducted in September 2014, and the second was performed in May 2015. The second experiment was conducted to strengthen our inferences regarding strain-specific susceptibility to predation and because results from the first experiment suggested that Brown Trout spawning status might have affected predation results. In addition, cover was added as a factor in the second experiment to evaluate whether behavioral differences between the Rainbow Trout strains in the presence of cover potentially affected survival.

We used Brown Trout as predators because Colorado lotic ecosystems that once contained both Rainbow Trout and Brown Trout are now dominated by Brown Trout, and Rainbow Trout re-establishment must occur in the presence of Brown Trout. Therefore, it is crucial to understand whether there are strain-specific differences in survival when faced with Brown Trout predation. Brown Trout were collected from Parvin Lake (Red Feather Lakes, Colorado) using a boat-mounted electrofishing unit, transferred to the FFL in two oxygenated coolers, and placed into a holding tank. Wild Brown Trout of at least 250 mm TL were used because they exhibit piscivorous behavior at this size (L'Abée-Lund et al. 1992). Brown Trout averaged 413 g (SD = 134) and 351 mm TL (SD = 22). Rainbow Trout fry used in the experiments averaged 53 mm TL (SD = 2) in 2014 and 73 mm TL (SD = 4) in 2015, less than one-third the TL of the predators, which is the theoretical maximum prey size consumed by salmonid predators (Parkinson et al. 1989; Yule and Luecke 1993; Johnson and Martinez 2000; Ruzycski et al. 2003).

Rainbow Trout were reared at the CPW Bellevue Fish Research Hatchery. To identify the strains during the experiment, we anesthetized fish by using MS-222 and tagged each fry in the adipose tissue behind the eye with visible implant elastomer, using red for GR and green for GR × CRR. Fry were monitored for mortality for 24 h after tagging. Fish were then transferred to the FFL in two oxygenated coolers and placed into large, open-mesocosm tanks (1,136 L), one for each strain, prior to use in the experiment. Brown Trout were held without food in a separate mesocosm tank for 48 h prior to use in a trial; this was done to ensure that all previously eaten food had been evacuated.

To begin a trial, Brown Trout were placed in mesocosm tanks and allowed to acclimate for 5 min. Once acclimated, a 50:50 mix of GR and GR × CRR of known sizes were stocked into the mesocosms with the Brown

Trout. Prior to experimentation in 2014, it was estimated that a single Brown Trout predator (300 g) could consume between 5 and 12 fry in a 24-h period (40 g/d; Elliott 1975). Therefore, in the first experiment, 15 fish each of the GR and GR × CRR strains were included in a predator arena with a single Brown Trout predator. Trials ran for 24 h, and we completed 12 individual trials. At the end of a trial, all remaining fish in the tank were removed, identified to strain, measured to the nearest millimeter, and weighed to the nearest gram. Brown Trout were only used once and were euthanized after being used in a trial. After experimentation, Brown Trout were sexed to determine whether consumption rates varied by sex.

The second experiment was conducted using the same protocols as the first experiment. We added cover as a factor in the second experiment to assess potential differences between the strains in using cover as a refuge from predators. Cover consisted of a polyvinyl chloride box (0.6 × 0.3 × 0.9 m) covered with plastic netting (25.4 mm). The mesh size allowed Rainbow Trout fry to enter the box but excluded Brown Trout. Aquarium plants were placed inside the box as an attractant. Trials with and without cover were run simultaneously. The number of Rainbow Trout was also increased in the second experiment to 20 of each strain (40 fish/trial) because in some trials during the first experiment, Brown Trout consumed as many as 20 fry. Brown Trout were not sexed at the end of the second experiment because it was not conducted during the Brown Trout spawning season, and sex was no longer thought to be a factor affecting Rainbow Trout consumption.

A linear model was constructed for each laboratory experiment to test for the effects of each factor considered separately (strain only, cover only, or predator sex only), an additive effect of strain and predator sex (2014 experiment) or strain and cover (2015 experiment), and an intercept-only model. Akaike's information criterion was used to rank models. Models were selected based on ΔAIC_c and w_i , and we report parameter estimates and associated 95% CIs from the top supported models (Burnham and Anderson 2002).

RESULTS

Field Experiment

Apparent survival by strain.—Model selection results showed that short-term apparent survival did not differ between the strains (Table 3); average Rainbow Trout survival was 0.10 (95% CI = 0.02, 0.2). The intercept model was ranked highest for both the overwinter and annual time periods, but the second-best model for these time periods suggested that there were differences in apparent survival between the strains (Table 3). However, the

TABLE 3. Model selection results comparing Rainbow Trout apparent survival for each time period between strains and among Colorado study streams ($\log L$ = log likelihood; AIC_c = Akaike's information criterion corrected for small sample sizes; ΔAIC_c = AIC_c difference; w_i = Akaike weight). Models with a ΔAIC_c value less than 4 were considered as contributing information to factors affecting apparent survival.

Time period	Model	$\log L$	AIC_c	ΔAIC_c	w_i
Short term (2 months)	Stream	50.82	-50.20	0.00	0.998
	Strain + Stream	51.91	-37.83	12.38	0.002
	Intercept	17.97	-31.15	19.06	0.000
	Strain	18.00	-28.29	21.92	0.000
Overwinter (6 months)	Intercept	4.95	-5.09	0.00	0.78
	Strain	5.13	-2.54	2.56	0.22
	Stream	13.83	23.77	28.86	0.00
	Strain + Stream	14.32	37.36	42.46	0.00
Annual (12 months)	Intercept	63.69	-122.58	0.00	0.71
	Strain	64.26	-120.81	1.77	0.29
	Stream	69.10	-86.77	35.81	0.00
	Strain + Stream	70.17	-74.35	48.23	0.00

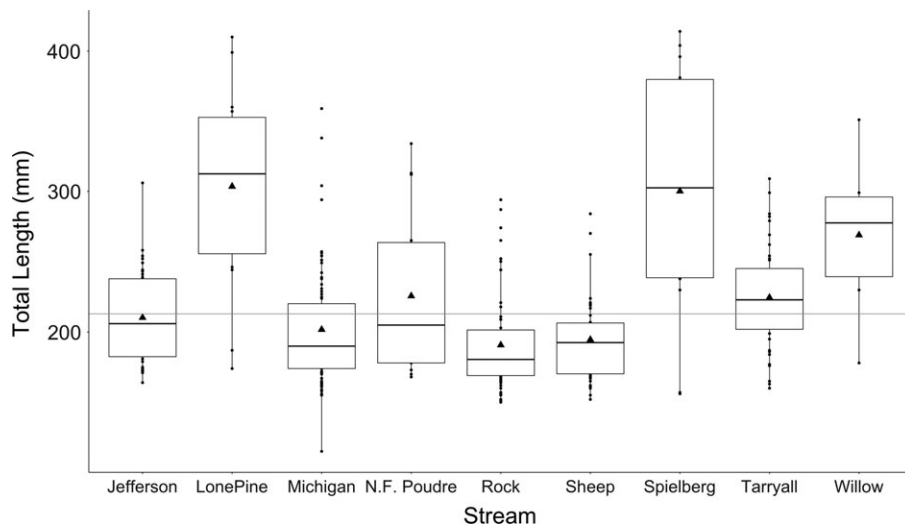


FIGURE 1. Total lengths of adult salmonids (Brown Trout and Brook Trout) within each study stream prior to stocking with two strains of Rainbow Trout (N.F. = North Fork). The solid line in each box indicates the median TL, the ends of the box denote the 25th to 75th percentiles, the whiskers indicate the lowest and highest points no greater than 1.5 times the interquartile range, and the solid circles are outliers. The solid black triangle denotes the average predator length. The solid gray line denotes a predator length that is three times the length of the German Rainbow Trout strain at stocking.

influence of strain in these models was small, and the CIs of the β estimates for strain included zero (overwinter period: $\beta = 0.05$ [95% CI = -0.14, 0.24]; annual period: $\beta = 0.003$ [95% CI = -0.004, 0.011]). Overwinter apparent survival was 0.16 (95% CI = 0.03, 0.30) for the GR strain and 0.21 (95% CI = 0.08, 0.35) for the GR \times CRR strain. Annual apparent survival was 0.005 (95% CI = 0, 0.01) for GR and 0.008 (95% CI = 0.003, 0.013) for GR \times CRR.

At stocking, GR fry (72.7 ± 0.6 mm TL [mean \pm SD]) were longer than the GR \times CRR fry (61.4 ± 0.6 mm TL) despite being reared in the hatchery for the same length of time. This size difference could have influenced survival

because larger prey may be less vulnerable to predation based on gape limitations of the predator. We conservatively assumed that Brown Trout and Brook Trout could consume Rainbow Trout fry having lengths up to one-third of the predator's length (Parkinson et al. 1989; Yule and Luecke 1993; Johnson and Martinez 2000; Ruzycski et al. 2003). Given this threshold, all streams had predators over 219 mm TL, and six of the nine streams had an average predator size that could consume both strains of Rainbow Trout (Figure 1).

Growth rate.—Strain and stream had an additive effect on Rainbow Trout short-term growth rate (Table 4). Average short-term growth rate of the GR and

TABLE 4. Model selection results for the effects of strain and stream on growth rate (mm/month) of Rainbow Trout stocked in nine Colorado streams ($\log L$ = log likelihood; AIC_c = Akaike's information criterion corrected for small sample sizes; ΔAIC_c = AIC_c difference; w_i = Akaike weight). Models with a ΔAIC_c value less than 4 were considered as contributing information to factors affecting growth rate. Note that the growth rate was only evaluated for the short-term and annual time periods (not the overwinter period).

Time period	Model	$\log L$	AIC_c	ΔAIC_c	w_i
Short term (2 months)	Strain + Stream	-5.87	77.75	0.00	0.999
	Strain	-42.05	91.82	14.07	0.001
	Intercept	-47.42	99.63	21.87	0.000
	Stream	-40.40	132.24	54.49	0.000
Annual (12 months)	Intercept	-32.30	69.61	0.00	0.51
	Strain	-30.76	69.71	0.097	0.49
	Stream	-25.19	104.79	34.77	0.00
	Strain + Stream	-18.39	111.79	42.18	0.00

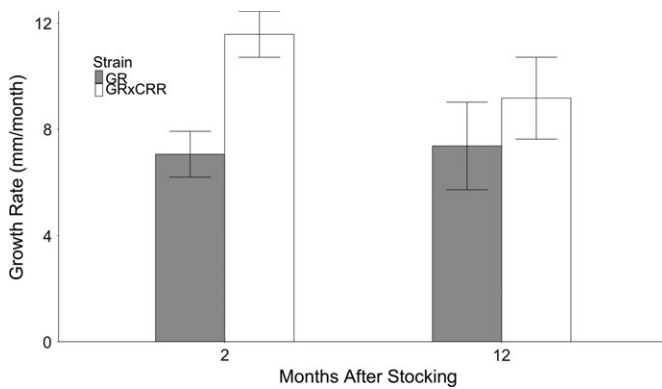


FIGURE 2. Average growth rates (mm/month; $\pm 95\%$ confidence interval) for Rainbow Trout fry of two strains at 2 or 12 months after stocking in Colorado streams (gray bars = German Rainbow Trout [GR] strain; white bars = GR \times Colorado River Rainbow Trout [GR \times CRR] strain).

GR \times CRR strains differed 2 months after stocking, with GR \times CRR fish having a higher growth rate than GR individuals (Figure 2). Annual growth rate (12 months after stocking) did not differ by stream (Table 4). Although strain appeared in the second-best model of the set (Table 4), the 95% CI of the β estimate for strain included zero ($\beta = 1.802$ [95% CI = -0.46, 4.06]), suggesting that growth rate did not differ between the strains (Figure 2).

Laboratory Experiments

In the predation susceptibility experiments, the total number of Rainbow Trout consumed during a trial was highly variable, ranging from 0 to 20 fish and averaging 6 fish (SD = 1). The intercept model was the top model with the majority of the weight for both the 2014 and 2015 experiments (Table 5), indicating that there was no difference in survival between the strains (Figure 3). The model set for the 2014 experiment suggested that the sex

of the Brown Trout predator affected Rainbow Trout consumption ($w_{2014sex} = 0.26$), and the model set for the 2015 experiment suggested that cover affected Rainbow Trout survival ($w_{2015cover} = 0.30$). However, all associated 95% CIs for the β estimates included zero ($\beta_{2014sex} = 0.11$ [95% CI = -0.099, 0.32]; $\beta_{2015cover} = 0.044$ [95% CI = -0.023, 0.11]).

DISCUSSION

Our primary goal was to evaluate potential differences in fry survival between the GR and GR \times CRR strains and to evaluate the potential for stocking GR into streams. Currently, the GR \times CRR strain is stocked because of its resistance to whirling disease and the historic advantages associated with survival and reproduction of CRR. However, resistance in GR \times CRR could be lost if natural reproduction results in backcrossing or outcrossing (Schisler et al. 2007; Fetherman et al. 2011, 2012).

TABLE 5. Model selection results for the 2014 and 2015 laboratory experiments ($\log L$ = log likelihood; AIC_c = Akaike's information criterion corrected for small sample sizes; ΔAIC_c = AIC_c difference; w_i = Akaike weight). Rainbow Trout strain and Brown Trout (predator) sex were included as factors affecting Rainbow Trout survival in the 2014 experiment; strain and cover type were included as factors affecting Rainbow Trout survival in the 2015 experiment. Models with a ΔAIC_c value less than 4 were considered as contributing information to factors affecting survival.

Year	Model	$\log L$	AIC_c	ΔAIC_c	w_i
2014	Intercept	0.451	3.67	0.0	0.53
	Sex	1.08	5.05	1.4	0.26
	Strain	0.478	6.24	2.6	0.15
	Strain + Sex	1.11	7.9	4.2	0.06
2015	Intercept	36.4	-68.5	0.0	0.38
	Cover type	37.3	-68.1	0.46	0.30
	Strain	36.8	-67.0	1.5	0.18
	Strain + Cover type	37.7	-66.5	2.0	0.14

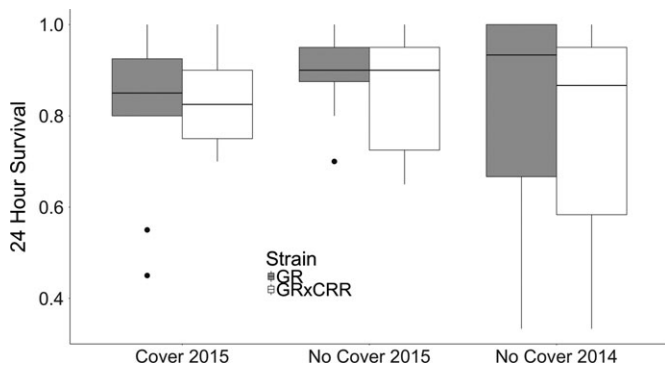


FIGURE 3. Twenty-four-hour survival of German Rainbow Trout (GR) and the GR \times Colorado River Rainbow Trout (GR \times CRR) strain, either with or without cover, when stocked with an individual Brown Trout (predator) in 2014 and 2015. The solid line in each box indicates the median survival, the ends of the box denote the 25th to 75th percentiles, the whiskers represent the lowest and highest points no greater than 1.5 times the interquartile range, and the solid circles are outliers.

The potential advantage of stocking pure GR is their high level of whirling disease resistance that would not be lost due to backcrossing or outcrossing, but they have not been used for managing or re-establishing stream populations because of concerns about potential low survival of GR fish in wild streams (Schisler et al. 2006). Field data indicated that apparent survival did not differ between the strains. Laboratory data supported our field observations and demonstrated that 24-h survival in the presence of a predator did not differ between the strains.

Our results suggest that short-term, overwinter, and annual apparent survival in our study streams did not differ greatly between the strains and that GR fry could potentially be used for re-establishment of Rainbow Trout in streams and rivers. The apparent survival rates that we observed were similar to those reported for stocked trout fry in other studies. Our short-term apparent survival estimates were similar to short-term survival estimates for stocked Brown Trout fry (Kelly-Quinn and Bracken 1989). However, short-term apparent survival in wild Rainbow Trout can be considerably higher, ranging from 70% to 100% (Mitro and Zale 2002). Our overwinter apparent survival estimates were similar to overwinter survival seen in other Rainbow Trout fry populations (Mitro and Zale 2002) and are consistent with other studies showing that variation in overwinter survival is common (Needham et al. 1945; Hunt 1969; Seelbach 1993; Ward and Slaney 1993; Quinn and Peterson 1996). Given that winter is likely the critical period that regulates population dynamics in fish (Needham et al. 1945; Hunt 1969; Quinn and Peterson 1996; Mitro and Zale 2002; Biro et al. 2004), our estimates can be used to determine future stocking rates for the GR and GR \times CRR strains.

Our annual apparent survival estimates for GR (0.005 [95% CI = 0, 0.01]) and GR \times CRR (0.008 [95% CI = 0.003, 0.013]) were lower than published estimates for other salmonids, which ranged from 2% to 27% (Mortensen 1977; Kelly-Quinn and Bracken 1989). We can offer four possible explanations why our annual apparent survival estimates were lower than those observed in other studies. First, increased competition and predation could explain the low survival observed in our study. After the establishment of whirling disease, Brown Trout became the dominant salmonid species, resulting in increased competition and predation for naïve hatchery-reared Rainbow Trout fry. Avila (2016) showed that competitor biomass and predator numbers may affect at least short-term survival of stocked Rainbow Trout fry. Additionally, we stocked fry to avoid hatchery acclimatization; however, small Rainbow Trout may be more vulnerable to predation, resulting in lower survival. A second explanation for low survival may be due to the physical characteristics of the streams. However, we measured several physical factors, including temperature, pebble size, and entrenchment ratio, and none of those factors appeared to have an effect on the annual survival rate (Avila 2016). Third, it is possible that lower survival was due to the fish strains. The GR strain has a long history of domestication that could influence survival in the wild (Schisler et al. 2006), and both strains could be affected by their genetic history. Finally, we did not restrict Rainbow Trout movement, and therefore we could only estimate apparent survival. It is highly likely that Rainbow Trout moved out of our sampling reaches and were not available for capture. Hatchery-reared Rainbow Trout have been known to move away from stocking locations (Cresswell 1981; Helfrich and Kendall 1982), and Fetherman and Schisler (2013) suggested that GR crosses might move downstream. Therefore, we believe that the low numbers of fish captured at the end of our study were likely due to movement as well as mortality and that actual survival may have been higher than the apparent survival estimates indicate. Further studies will be needed to generate more precise estimates of strain-specific movement.

Our laboratory predation experiments showed that the survival of GR was similar to that of GR \times CRR, indicating that GR and GR \times CRR fish had a similar ability to avoid predation. Similarity in survival between strains in the laboratory experiments strengthened our inferences regarding similarities in apparent survival in the field. Other laboratory studies have shown that GR and GR \times CRR possess similar aerobic swimming abilities (Fetherman et al. 2011), which suggests that their predator avoidance capabilities could be similar, although there is no direct evidence linking aerobic swimming performance and survival. Our second predation experiment in the laboratory also showed that strain-specific survival did not

differ when cover was available, suggesting that cover use was consistent between the strains. Kopack et al. (2015) reported that GR exhibited appropriate antipredator behaviors when exposed to a conspecific alarm cue, indicating that GR individuals could inherently sense and respond to danger. Therefore, evidence regarding swimming performance and predator response indirectly supports our observation of no difference in predation susceptibility in our predator trials.

The GR typically grow faster and attain larger sizes in the hatchery than GR \times CRR (Fetherman et al. 2011), but our study indicated that the two strains had similar long-term growth rates in the wild. Interestingly, the short-term (2 months after stocking) growth rate of GR \times CRR was twice that of GR in every stream, indicating that the cross may be better suited to conditions in the natural environment. The mechanism underlying short-term growth differences is unknown, and a higher growth rate could have ecological implications. Body size is known to influence predation risk, and as a prey fish's body size increases, the number of predators able to consume that individual is reduced (Parkinson et al. 1989; Yule and Luecke 1993; Johnson and Martinez 2000; Ruzycski et al. 2003). Body size may also influence overwinter survival (Hunt 1969; Smith and Griffith 1994; Meyer and Griffith 1997), and increased body size is positively related to condition and overwinter survival of Rainbow Trout fry (Meyer and Griffith 1997). We were unable to detect differences in fish size or growth rate after 1 year due to low numbers of recaptured fish; thus, we are uncertain whether short-term growth differences resulted in long-term consequences for each strain.

Our study suggests that the stocking of pure GR as fry into streams and rivers is a potential alternative to stocking GR \times CRR fry. The advantage of stocking GR is their high level of whirling disease resistance, which is not lost due to the backcrossing and outcrossing that could occur with the GR \times CRR strain (Schisler et al. 2007; Fetherman et al. 2011, 2012). Studies with Chinook Salmon *O. tshawytscha* have shown that inbreeding increases the severity of *M. cerebralis* infection, indicating that resistance has a genetic component (Arkush et al. 2002). It has been estimated that 9 ± 5 independently segregating genes play a role in the GR strain's genetic resistance to *M. cerebralis*, and resistance appears to be additive (Fetherman et al. 2012), suggesting that backcrossing and outcrossing could reduce the number of associated genes working together to increase resistance. Using our results in conjunction with the results from Nehring (2014), which indicated that pure GR can survive and reproduce in a pond setting, we suggest that GR could be used in place of GR \times CRR in environments where (1) whirling disease infection levels continue to be high, (2) outcrossing and backcrossing are a concern, and (3) fish are being stocked

as fry. Ongoing evaluation of our stocking sites should provide some insight regarding long-term survival and differences in recruitment and reproduction between the strains within streams.

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