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The Effects of *Myxobolus cerebralis* on the Physiological Performance of Whirling Disease Resistant and Susceptible Strains of Rainbow Trout

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ARTICLE

The Effects of *Myxobolus cerebralis* on the Physiological Performance of Whirling Disease Resistant and Susceptible Strains of Rainbow Trout

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Abstract

The development of rainbow trout *Oncorhynchus mykiss* strains that are resistant to whirling disease has shown promise as a management tool for populations in areas where *Myxobolus cerebralis* is present. However, the physiological effects of the disease on characteristics necessary for fish survival in natural river conditions have not been tested in many of these strains. Five rainbow trout strains were evaluated for their swimming ability and growth characteristics in relation to *M. cerebralis* exposure: the resistant German rainbow trout (GR) strain (Hofer strain), the susceptible Colorado River rainbow trout (CRR) strain, and three intermediate (hybrid) strains (F_1 = GR × CRR; F_2 = F_1 × F_1; B_2 = backcross of F_1 × CRR). Three broad response patterns among strain and exposure were evident in our study. First, exposure metrics, growth performance, and swimming ability differed among strains. Second, exposure to the parasite did not necessarily produce differences in growth or swimming ability. Exposure to *M. cerebralis* did not affect batch weight for any strain, and critical swimming velocity did not differ between exposed and unexposed families. Third, although exposure did not necessarily affect growth or swimming ability, individuals that exhibited clinical deformities did show reduced growth and swimming performance; fish with clinical deformities were significantly smaller and had lower critical swimming velocities than exposed fish without clinical deformities. Research and management have focused on GR × CRR hybrid strains; however, given the performance of the GR strain in our study, it should not be discounted as a potential broodstock. Additional field trials comparing the GR and F_1 strains should be conducted before wholesale adoption of the GR strain to reestablish rainbow trout populations in Colorado.

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Myxobolus cerebralis, the parasite responsible for salmonid whirling disease, has caused widespread population collapses in wild rainbow trout *Oncorhynchus mykiss* throughout the intermountain western USA (Nehring and Walker 1996; Vincent 1996; Schisler et al. 1999a, 1999b; Nehring and Thompson 2001). Due to the economic and recreational importance of rainbow trout, researchers and managers have focused on developing disease-resistant rainbow trout strains to reestablish populations in rivers that are affected by the parasite (Schisler et al. 2006). Most of this research has focused on a strain that was raised for decades in Bavarian hatcheries in Germany (Hedrick et al. 2003). Although the German rainbow trout (GR) strain (also known as the Hofer strain) can be infected with the parasite, the spore burdens are usually low (Hedrick et al. 2003; Schisler et al. 2006) and the GR strain is known to survive and reproduce in the presence of the *M. cerebralis*. Low spore burdens and the strain's ability to persist when exposed have been termed "resistance," and this resistance is presumed to be a result of long-term exposure to the parasite (Hedrick et al. 2003).

In laboratory exposure experiments, the GR strain was found to be more resistant to whirling disease than either the North American Trout Lodge strain or the Colorado River rainbow trout (CRR) strain (Hedrick et al. 2003; Schisler et al. 2006). The CRR strain was widely stocked in Colorado and comprised many of the naturally reproducing wild rainbow trout fisheries before the introduction of whirling disease (Walker and Nehring 1995). Despite the resistance seen in the GR strain, it was thought that survival in the wild might be low due to the strain's hatchery origins. Therefore, the GR strain was crossed with the susceptible CRR strain to create several intermediate strains with varying levels of susceptibility to whirling disease (Schisler et al. 2006) and presumably better physiological characteristics for survival in the wild. However, the GR and intermediate strains have not been rigorously evaluated for their potential use in reestablishing rainbow trout in the rivers of Colorado.

Disease influences fish population dynamics through long-term impacts on physiological processes, such as growth and swimming performance (Wedemeyer 1970; Arkoosh et al. 1998; Hedrick 1998). Schisler et al. (2006) observed reduced growth and body size as a result of exposure in more susceptible strains of rainbow trout. Exposure to *M. cerebralis* can also differentially affect swimming performance in various rainbow trout strains—in part by destroying the structural framework needed for healthy bone formation, resulting in skeletal deformities (El-Matbouli et al. 1992). Parasite exposure can reduce critical swimming velocities (U_{crit}; DuBey et al. 2007) and can decrease time to fatigue for rainbow trout that are held at a constant water velocity (Ryce et al. 2005).

Our objectives were to evaluate growth and swimming performance of *M. cerebralis*-exposed and unexposed rainbow trout representing several strains. Our overall goal was to identify the strain with the best combination of *M. cerebralis* resistance and swimming and growth characteristics for use in reestablishing rainbow trout populations in Colorado.

METHODS

Spawning and rearing.—The five strains of rainbow trout used in the physiological experiments were spawned at the Bellvue Fish Research Hatchery (Colorado Division of Wildlife [CDOW]). Within each strain, each family consisted of a unique male–female pairing, and offspring were kept separate throughout the rearing process. We used pure families of the GR or CRR strain. The F_1 families were created by spawning a GR individual with a CRR individual. The F_2 families were created by spawning two F_1 individuals from different families. Finally, the B_2 families were created by backcrossing an F_1 individual with a CRR individual.

Fertilized eggs were incubated at the Bellvue Fish Research Hatchery or in the Quonset hut wet laboratory at the Colorado Cooperative Fish and Wildlife Research Unit and were held until they reached the eyed stage. Eggs were then transferred to the wet laboratory and placed in 76-L tanks, where they were hatched. The tanks received air-saturated water at a rate of 0.5 L/min.

One-hundred tanks were used for the experiment, and each tank contained one family. Eighty families were exposed to *M. cerebralis*: 10 GR families, 10 CRR families, 20 F_1 families, 20 F_2 families, and 20 B_2 families. The 20 F_1 families were created by using 10 CRR males mated with 10 GR females and, reciprocally, 10 GR males mated with 10 CRR females. The 20 B_2 families were composed of 10 CRR males mated with 10 F_1 females and, reciprocally, 10 F_1 males mated with 10 CRR females. Reciprocal families were included to test whether fish performance after *M. cerebralis* exposure differed based on the directionality of spawning. Because reciprocal families cannot be created in the F_2 strain, 20 F_2 families were used to provide an equal number of families in each of the generational strains. A sample size of 10 exposed families/strain was required for the calculation of heritability (Fetherman 2008). Space constrained the number of tanks that could be accommodated; therefore, only four families from each strain were used as unexposed controls (i.e., there was a total of 20 unexposed families). Unexposed families were placed together on the upper level of a two-tier shelving unit to avoid potential contamination from overflow or spills from exposed tanks; exposed and unexposed families from the five strains were otherwise randomly assigned to tanks.

Myxobolus cerebralis *exposure*.—We standardized exposure date by degree-day (°C) in an attempt to expose all families at 650 degree-days (following Schisler et al. 2006). In addition to degree-days, age and size at exposure have been shown to affect the development of whirling disease in rainbow trout (Ryce et al. 2004), so fish weight and age at exposure were also recorded.

Triactinomyxons (TAMs) were supplied by T. McDowell and R. Hedrick (University of California–Davis) and R. Barry Nehring (CDOW, Montrose). Both TAM cultures were produced from laboratory cultures of Mount Whitney-strain *Tubifex tubifex* worms (origin: Mount Whitney Fish Hatchery, Lone Pine, California). Viable TAMs from both sources were counted

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upon arrival by mixing 1,000 μL of filtrate containing the TAMs with 60 μL of crystal violet; 84.6 μL of this mixture were then placed on a slide, and the number of TAMs per slide was counted. Ten counts were conducted in this fashion to account for a possible uneven distribution of the TAMs within the filtrate. An average of the 10 counts was taken, and this number was used to calculate the number of TAMs per milliliter. Fish were exposed to 2,000 TAMs/individual, resulting in a total of 50,000 TAMs/tank (~715 TAMs/L).

Prior to the addition of TAMs, water flow to each aquarium was stopped for 1 h, and each aquarium received aeration to ensure the mixing of TAMs and the equal exposure of all fish. The amount of filtrate needed to deliver 50,000 TAMs was placed in each aquarium in two doses, each dose containing half of the necessary filtrate. The use of two doses helped to ensure equal distribution of TAMs in the tank and accounted for a possible unequal distribution of TAMs within the filtrate. Twenty tanks (i.e., four tanks for each of the five strains) were not exposed with whirling disease but were treated in the same manner as the exposed tanks. After infection, fish were reared for approximately 6 months (2,240 ± 38 degree-days, °C [mean ± SD]) to ensure the full development of myxospores.

Exposure metrics.—Exposure evaluations began when the fish reached 2,240 ± 38 degree-days postexposure (mean ± SD). Ten individuals were removed from each tank, measured (total length, mm), weighed (g), and sacrificed for myxospore enumeration (O'Grodnick 1975) by the pepsin–trypsin digest method (Markiw and Wolf 1974). The head of each fish was severed from the body just behind the operculum and pectoral fins and was placed into an individually labeled bag. Heads that were used for myxospore enumeration were sent to the Brush Fish Health Laboratory (CDOW, Brush).

Clinical deformities typical of exposure to *M. cerebralis* (e.g., blacktail; cranial, opercular, spinal, and lower jaw deformities; and exophthalmia) were recorded for each individual. Percent mortality and the percentage of exposed fish exhibiting clinical signs were calculated for each strain. Mortality was unusually high in one of the unexposed GR families for unknown reasons, and this family was excluded from analyses.

Statistical analyses were conducted by using the GLM procedure in the Statistical Analysis System (SAS; SAS Institute 2007). Mean age, degree-days, and weight per strain at exposure were compared via a single-factor analysis of variance (ANOVA). Myxospore counts were analyzed by using a two-factor ANOVA with exposure and strain as the factors. Mortality percentages were arcsine–square root transformed prior to analysis with a two-factor ANOVA. Values for all analyses were reported from the type III sum of squares to account for the unbalanced design of the experiment. If significant main effects were identified ($P < 0.05$), the least-squares means method with a Bonferroni adjustment was used to determine which strains were significantly different.

Swimming performance measurements.—The U_{crit} values of 5 fish/family (20 fish/strain) were measured at 14, 30, 74, and 134 d postexposure. Since there were only four unexposed families per strain, we evaluated swimming performance based on only four of the exposed families for each strain. The four exposed families were chosen randomly. Because individuals were randomly selected for the trials prior to developing clinical signs of the disease at 2 weeks postexposure, we could not control for the numbers of fish that developed a particular deformity; in some strains, no severe deformities developed. Therefore, we used the presence or absence of clinical signs to examine the relationship between deformities and swimming ability. In total, 735 trials were conducted over the course of 6 months, which included repeated measures on each of the individuals within a family at 14, 30, 74, and 134 d postexposure. Swimming trials were conducted at an average temperature of 12.7 ± 0.07°C (mean ± SD).

Three days prior to the first swimming trial, five fish were chosen randomly from each of the tanks that were selected for the swimming experiment. Each fish was marked with a unique visual implant elastomer tag behind the eye and along the adipose fin. All tags were present and identifiable at 14, 30, and 74 d postexposure. Twenty five (13%) of the tags were no longer visible at 134 d postexposure. In those cases, an untagged individual was randomly chosen from the same tank to be used in place of the missing individual; this was done to maintain consistent sample sizes across all four postexposure trials.

Measurements of U_{crit} were conducted in Loligo Model 32 swimming flumes. Individual fish were placed in the flume and were allowed to orient to the minimum flume velocity of 2 cm/s for 1 h. After the orientation period, water velocity was increased in 5-cm/s increments every 10 min until the fish became impinged upon the rear screen of the apparatus. Upon completion of a trial, fish weight and length were recorded; the fish was placed into an aerated bucket of water, was allowed to recover for approximately 5 min, and was returned to its tank. Absolute U_{crit} (cm/s) and relative U_{crit} (body lengths/s) were calculated for each fish.

Statistical analyses were conducted by using the GLM procedure in SAS. Swimming data were analyzed with a two-factor analysis of covariance in which exposure and strain were the two factors and individual length was the covariate ($N = 25$ fish/strain at 14, 30, 74, and 134 d postexposure). Values were reported from the type III sum of squares to account for the unbalanced design of the experiment. If significant effects were identified ($P < 0.05$), the least-squares means method (with a Bonferroni adjustment) was used to identify which strains were significantly different. Swimming data collected at 134 d postexposure were analyzed with a two-factor analysis of covariance to determine whether clinical signs of disease affected swimming ability; strain and exposure (four levels: unexposed; exposed but uninfected; exposed and infected; and exposed and exhibiting clinical deformities) were used as the two factors. Absolute U_{crit} (cm/s) was used in place of relative U_{crit}, and individual length was used as a covariate to determine whether variations in length among the strains affected U_{crit}.

TABLE 1. Mean (SD in parentheses) age (weeks), degree-days (°C), and individual weight (g) at *Myxobolus cerebralis* exposure for each rainbow trout strain (CRR = Colorado River strain; GR = German strain; F_1 = GR × CRR; F_2 = F_1 × F_1; B_2 = backcross of F_1 × CRR) and averaged across strains. Within a column, differing letters represent significant differences among strains ($P < 0.05$); age and degree-days at exposure did not differ among strains.

Strain	Age (weeks)	Degree-days (°C)	Weight (g)
CRR	9.9 (1.1)	697.3 (58.6)	0.49 (0.11) y
B_2	9.5 (0.9)	666.0 (46.7)	0.55 (0.15) y
F_2	10.1 (1.1)	675.7 (29.0)	0.51 (0.17) y
F_1	10.0 (0.6)	681.0 (37.2)	0.71 (0.15) z
GR	9.8 (1.7)	682.1 (61.8)	0.67 (0.14) zy
Average	9.8 (1.0)	678 (44)	0.59 (0.17)

Growth experiment.—Growth assessment of rainbow trout was initiated upon exposure to the *M. cerebralis* TAMs. Each family (i.e., tank; $N = 100$) was batch weighed initially and every 2 weeks thereafter to allow us to determine the appropriate food ration (i.e., 4% of body weight/d). Hatchery guidelines were used for adjusting feed pellet size: when batch weights reached 75, 162.5, and 500 g, the trout pellet was increased to size 2, 3, and 4, respectively. Adjusting the feed size at these batch weights helped to avoid starvation mortality caused by the gape limitation of small fish. The final batch weight (at 4 months) was used for growth comparisons among strains and exposure treatments. Average daily temperature during the growth experiment was 12.3 ± 0.13°C (mean ± SD), translating to an average of 1,767 ± 15.9°C degree-days (mean ± SD). After the 4-month growth experiment, the fish remained under the same culture conditions but were given a maintenance diet of 2% of body weight/d until the conclusion of the exposure experiment at 6 months. Fish were reared for 6 months to allow for the full development of myxospores. Fish were then euthanized, weighed, and evaluated for clinical signs of whirling disease, and their heads were removed for myxospore enumeration (Markiw and Wolf 1974; O'Grodnick 1975).

Statistical analyses were conducted using the GLM procedure in SAS. Four-month batch weights were analyzed by using a two-factor ANOVA with exposure and strain as the factors ($N = 80$ exposed tanks and 20 unexposed tanks). Individual weights ($N = 150$ for GR and CRR strains; $N = 300$ for F_1, F_2, and B_2 strains) taken at 6 months were analyzed with ANOVA to determine whether clinical signs of disease affected individual growth across strain and exposure (four levels: unexposed; exposed but uninfected; exposed and infected; and exposed and exhibiting clinical deformities). Values for all analyses were reported from the type III sum of squares to account for the unbalanced experimental design. If significant effects were identified ($P < 0.05$), the least-squares means method with a Bonferroni adjustment was used to determine which strains were significantly different.

RESULTS

Exposure Metrics

Age (weeks) and degree-days (°C) at exposure did not differ significantly among the five strains ($F < 0.93$, $P > 0.451$). Weight at exposure differed among strains ($F = 6.51$, $P < 0.001$). The GR, F_2, B_2, and CRR strains did not differ significantly in individual weight (g) at the time of exposure ($P > 0.097$); however, the F_1 strain was significantly larger at exposure than the F_2, B_2, or CRR strain ($P < 0.017$; Table 1).

There were significant differences in myxospore count among strains ($F = 61.39$, $P < 0.001$; Table 2). Individuals from the CRR strain had a significantly higher mean myxospore count than those from any other strain ($P < 0.001$); B_2 individuals had a significantly higher mean count than the F_2, F_1, or GR strain ($P < 0.001$); and F_2 individuals had a significantly higher mean count than the F_1 or GR strain ($P < 0.001$; Table 2). Myxospore count did not differ between the F_1 and GR strains ($P \geq 0.4778$; Table 2). Myxospore counts in the F_1 and B_2 reciprocal families did not differ ($P = 0.1169$ and 0.2331, respectively), indicating that the directionality of spawning did not affect resistance to whirling disease.

Mortality did not differ between exposed and unexposed fish ($F = 2.47$, $P = 0.120$) or among the strains ($F = 1.52$, $P = 0.203$); the exposure × strain interaction was not significant ($F = 1.36$, $P = 0.252$; Table 2). Mortality averaged across strains was low: 2.6% in unexposed fish and 6.6% in exposed fish.

TABLE 2. Mean myxospore counts (with 95% confidence interval [CI]), percentage of exposed individuals that displayed clinical signs of whirling disease, and percent mortality (SD in parentheses) in the *Myxobolus cerebralis*-exposed and unexposed treatments for each strain examined (strains are defined in Table 1). Within a column, differing letters represent significant differences among strains ($P < 0.05$); mortality of unexposed and exposed fish did not differ among strains.

				Mortality	
Strain	Myxospore count	95% CI	Clinical signs (%)	Unexposed	Exposed
CRR ($N = 10$)	187,595 w	166,521–208,668	96.0	2.0 (4.0)	12.8 (18.1)
B_2 ($N = 20$)	97,865 x	83,038–112,691	55.8	3.0 (2.0)	6.2 (6.6)
F_2 ($N = 20$)	45,780 y	30,917–60,644	36.4	2.0 (4.0)	8.8 (11.6)
F_1 ($N = 20$)	9,566 z	0–24,392	14.5	2.0 (2.3)	1.8 (2.0)
GR ($N = 10$)	275 z	0–21,242	2.0	4.0 (6.9)	3.6 (5.7)

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The percentage of exposed individuals that exhibited clinical deformities was lowest in the GR strain (2.0%); increased through the F_1, F_2, and B_2 strains; and was highest in the CRR strain (96.0%; Table 2). Within all of the strains, infection was confirmed in the exposed fish that developed clinical signs of whirling disease (i.e., none of the clinically deformed fish had myxospore counts of zero).

Swimming Experiment

In all strains, U_{crit} decreased as fish length increased. There was no significant difference in U_{crit} between the exposed and unexposed treatments within a strain at any postexposure time period ($F \leq 3.01$, $P \geq 0.085$); therefore, the swimming velocity data for exposed and unexposed treatments were combined for the comparison of strains.

Swimming ability significantly differed among the strains at 14, 30, 74, and 134 d postexposure ($F \geq 7.91$, $P < 0.001$; Figure 1a). In general, the CRR-strain fish were significantly faster (had a higher relative U_{crit}), the GR-strain individuals were significantly slower, and fish from the other strains exhibited intermediate relative U_{crit} values (Figure 1a). The CRR-strain fish swam significantly faster than GR-strain fish at 14, 30, 74 and 134 d postexposure ($P \leq 0.001$). The CRR strain also had significantly higher U_{crit} than (1) the F_1 strain at 14, 30, and 74 d postexposure ($P < 0.001$); (2) the F_2 strain at 30 d postexposure ($P < 0.001$); and (3) the B_2 strain at 30 d postexposure ($P < 0.001$). The F_1 strain did not differ in swimming ability from the B_2 or GR strain at 14, 30, 74, or 134 d postexposure ($P > 0.066$); the F_1 strain also did not differ from the F_2 strain at 30, 74, or 134 d postexposure ($P = 1.000$). The F_2 strain had a significantly higher U_{crit} than the F_1, B_2, and GR strains at 14 d postexposure ($P < 0.044$) and a significantly higher U_{crit} than the GR strain at 74 and 134 d postexposure ($P < 0.001$). The F_2 strain did not differ in swimming ability from the F_1 and B_2 strains at 30, 74, or 134 d postexposure ($P > 0.519$). The B_2 strain had a significantly higher U_{crit} than the GR strain at 134 d postexposure ($P < 0.001$).

At 134 d postexposure, we subdivided the exposed fish into three groups: (1) fish that were not infected (i.e., those with a myxospore count of zero), (2) fish that were infected (i.e., those with a myxospore count greater than zero), and (3) fish that exhibited clinical deformities. These groups were compared with the unexposed treatment. We did not make this comparison at 14, 30, or 74 d postexposure because clinical signs had not yet appeared and because it was necessary to euthanize the fish to determine infection status. Swimming performance did not differ between the exposed, infected fish and the exposed, uninfected fish ($P > 0.90$); therefore, these two groups were combined into one group (exposed individuals without clinical deformities) for comparison with the unexposed individuals and the individuals that exhibited clinical deformities. There was a significant difference in swimming ability among the three groups ($F = 3.70$, $P = 0.026$). The absolute U_{crit} did not differ among unexposed fish, exposed fish without clinical deformities, and exposed fish with clinical deformities for the GR, F_1, or F_2 strain ($P \geq 0.897$; Figure 1b). The absolute U_{crit} of exposed individuals exhibiting clinical deformities was significantly lower than that of unexposed individuals within the B_2 and CRR strains ($P \leq 0.011$; Figure 1b). Critical swimming velocity did not differ between exposed fish without clinical deformities and unexposed fish in the B_2 and CRR strains ($P \geq 0.629$; Figure 1b).

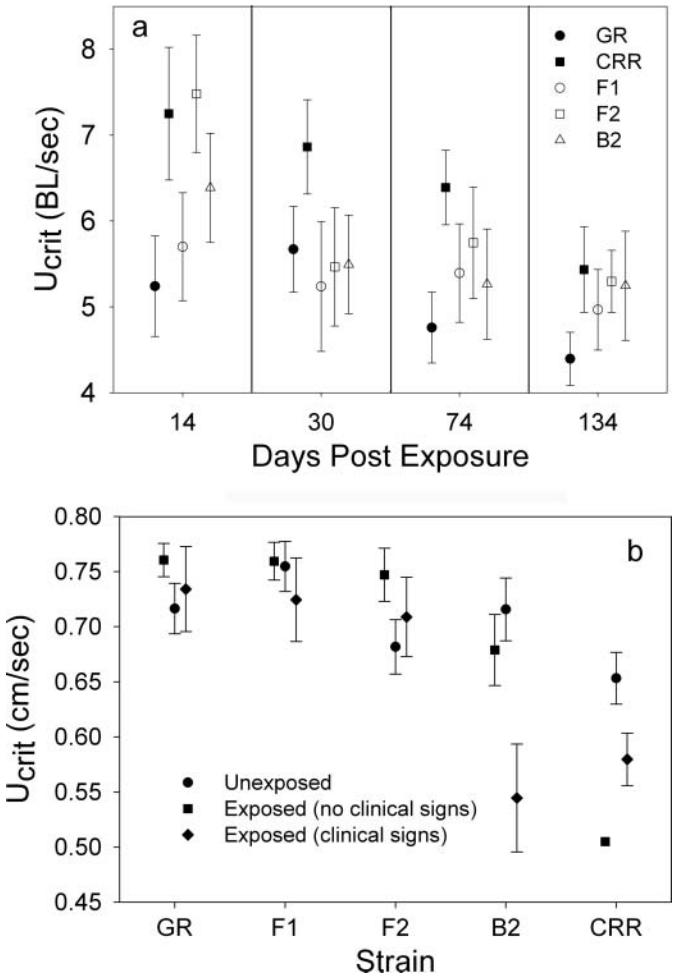

FIGURE 1. **(a)** Average (±SE) critical swimming velocity (U_{crit}; body lengths [BL]/s) of the German rainbow trout (GR), Colorado River rainbow trout (CRR), F_1 (i.e., GR × CRR), F_2 (i.e., F_1 × F_1), and B_2 (i.e., backcross of F_1 × CRR) strains at 14, 30, 74, and 134 d after exposure to *Myxobolus cerebralis*. Each point on the graph represents a combined average of the exposed and unexposed individuals because there were no significant differences between the two exposure groups within any strain at any time period. **(b)** Average (±SE) U_{crit} (cm/s) at 134 d postexposure among unexposed fish, exposed fish that showed no clinical signs of whirling disease, and exposed fish that showed clinical signs within each strain.

Growth Experiment

There were significant differences in 4-month batch weights between exposed and unexposed fish ($F = 3.93$, $P = 0.050$) and among the strains ($F = 49.17$, $P < 0.001$); however, there was no significant interaction between exposure and strain ($F =$

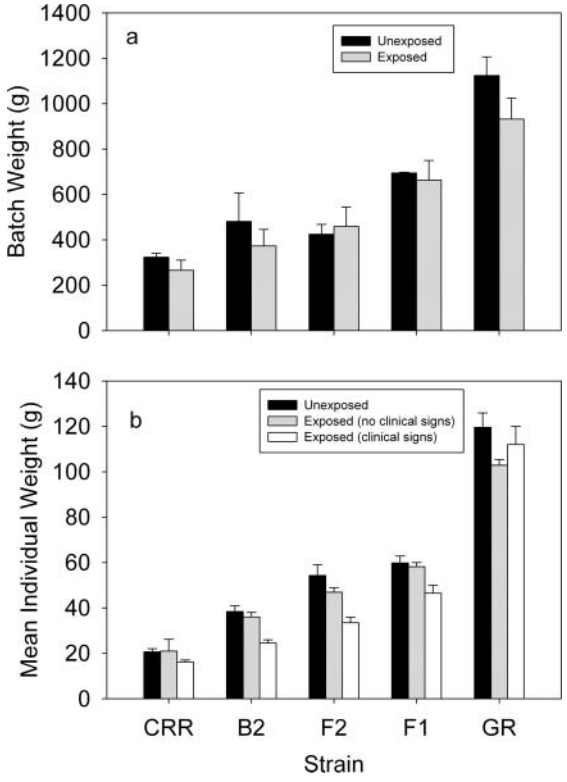

FIGURE 2. **(a)** Average (+ SE) batch weights (g; representing 25 fish/family) for *Myxobolus cerebralis*-exposed families (gray bars) and unexposed families (black bars) within each rainbow trout strain at the conclusion of the growth experiment (strains are defined in Figure 1). **(b)** Mean (+ SE) individual weight at the end of the 6-month exposure experiment among unexposed fish (black bars), exposed fish that did not exhibit clinical deformities (gray bars), and exposed fish that exhibited clinical deformities (open bars) within each strain.

1.11, $P = 0.355$; Figure 2a). Once *P*-values were corrected for pairwise comparisons, batch weight did not significantly differ between exposed and unexposed individuals within any of the strains ($P \geq 0.249$). Exposure did not significantly affect the batch weights of reciprocal families within the F_1 or B_2 strain ($F = 0.57, P = 0.461$). Therefore, the directionality of spawning did not appear to affect the growth of these strains when exposed to whirling disease.

At the end of the exposure experiment (6 months), the individuals in exposed treatments were subdivided into three groups: uninfected individuals, infected individuals, and individuals exhibiting clinical deformities. The average individual weights of these groups were compared with those of the unexposed treatment. Exposed, infected fish and exposed, uninfected fish did not differ in individual weight ($P > 0.90$); therefore, these two groups were combined into a single group (exposed individuals that did not exhibit clinical deformities), which was compared with the unexposed individuals and the individuals exhibiting clinical deformities. The 6-month individual weights significantly differed among the three groups and among strains, and there was a significant interaction ($F \geq 4.47, P < 0.001$; Figure 2b). Individuals with clinical deformities had significantly lower weights than the unexposed fish and the exposed fish without clinical deformities ($P < 0.038$). There were no differences in weight between the unexposed fish and the exposed fish that did not exhibit clinical deformities ($P = 0.118$). The GR strain was the only strain in which weight significantly differed between the unexposed fish and the exposed fish without clinical deformities: unexposed individuals were significantly heavier than the exposed individuals without clinical deformities ($P < 0.001$). In the other four strains, growth did not differ between unexposed fish and exposed fish without clinical deformities ($P > 0.100$). Within the F_2 and B_2 strains, exposed individuals with clinical deformities had significantly lower weights than exposed individuals without clinical deformities ($P < 0.026$); differences in weight were not observed between those two groups within the GR, F_1, or CRR strain ($P > 0.364$; Figure 2b).

DISCUSSION

Three broad response patterns among strain and exposure were evident in our study. First, exposure metrics, growth performance, and swimming ability differed among strains. Second, exposure to the parasite did not necessarily result in differences in growth or swimming ability. The lack of response in exposed fish was unexpected. We initially hypothesized that exposure would result in reduced growth and decreased swimming ability; however, these outcomes were not evident in our data. Third, although exposure did not necessarily produce differences in growth or swimming ability, exposed individuals that exhibited clinical deformities did show reduced growth and swimming performance. We discuss our results below relative to parasite exposure and differences among strains.

Exposure

In our experiment, exposure to *M. cerebralis* did not affect batch weight for any of the strains; however, other experiments have shown that highly susceptible rainbow trout exhibit reduced feeding efficiency and growth after exposure to the parasite (Schisler et al. 2006). In our study, fish were fed a fixed ration (percentage of body weight per day) throughout the entire experiment, and this may explain the lack of differences between exposed and unexposed fish. The ration was adjusted biweekly based on batch weight, and usually some of the food was left unconsumed. This indicates that individual fish did not have to compete for food and that the exposed fish were able to grow as well as unexposed fish. Additionally, the pellet size was adjusted based on fish batch weight according to hatchery guidelines. However, high size variability within families may have meant that the pellet sizes were not ideal for larger individuals in a family, thereby reducing their growth rates. Although we believe that our approach was justified, a more realistic approach might be to restrict the ration to match natural conditions.

For the F_2 and B_2 strains, individual weights at 6 months differed between exposed fish with clinical deformities caused by whirling disease and exposed fish that did not exhibit clinical

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deformities. Exposed fish with clinical deformities were significantly smaller than unexposed fish or exposed fish without clinical deformities. Deformities may make it more difficult for fish to obtain food or to compete with individuals that lack deformities. We expected to see a similar difference between the same two groups within the CRR strain, but individual weight at 6 months did not differ between the exposed CRR-strain fish with clinical deformities and those without clinical deformities. This result is probably due to low numbers of exposed CRR-strain individuals without deformities ($N = 4$ fish), resulting in relatively high variability that hindered our ability to detect differences. Overall, it appears that exposure to *M. cerebralis* does not necessarily reduce growth, whereas development of clinical deformities does. Moreover, development of clinical deformities would be expected to compromise overall fitness and a broad spectrum of physiological functions in individual fish. Although a single exposure under laboratory conditions does not appear to reduce growth, continuous exposure to *M. cerebralis* in the wild may reduce the growth of an exposed fish over its lifetime.

At 6 months, exposed GR-strain fish were significantly smaller than unexposed fish, and clinical expression of whirling disease did not seem to affect individual weight. Only two GR fish exhibited clinical deformities, and this probably resulted in the lack of differences between GR fish with clinical deformities and those without clinical deformities. The differences in weight between exposed and unexposed GR-strain individuals were unexpected given that no differences were detected in 4-month batch weight. The differences observed at 6 months may have been due to lower variability associated with individual weights or with the partitioning of the exposed fish.

Critical swimming velocity did not differ between exposed and unexposed fish. Increases in the exposure level have been shown to reduce U_{crit} in rainbow trout and Rio Grande cutthroat trout *O. clarkii virginalis* (DuBey et al. 2007); increased exposure levels also decreased the time to fatigue for rainbow trout held at a constant water velocity (35 cm/s; Ryce et al. 2005). Our inability to detect an effect may have been due to the method we used to assess U_{crit}. Swimming endurance (as in Ryce et al. 2005) or time to recovery after being startled might have been better metrics. Whirling disease can result in an uncontrollable whirling motion when an individual is startled (Höfer 1903; Hoffman 1970; Hedrick et al. 2003), which could result in an inability to maintain position in the water column. A swimming experiment that includes an element of surprise might better represent the costs of *M. cerebralis* exposure.

Additionally, the timing of the U_{crit} tests may have affected the results. It is possible that exposure to whirling disease does not affect swimming ability until full development of the infection has occurred. Therefore, differences are not likely to be seen shortly after exposure (e.g., 14, 30, or possibly even 74 d postexposure). In U_{crit} experiments conducted by DuBey et al. (2007) and in stamina experiments conducted by Ryce et al. (2005), measurements were not made until 130 and 140 d postexposure, respectively.

Although we did not observe an effect of exposure on U_{crit}, those fish that developed clinical deformities did show significant reductions in this performance variable. The strains that were least related to GR (CRR and B_2) showed obvious clinical signs of exposure and had significantly lower U_{crit} values than unexposed fish or exposed fish without clinical deformities, indicating that abnormalities caused by the disease have an effect on swimming ability. Our results suggest that *M. cerebralis* exposure is not as critical as the parasite's effects on morphological characteristics. Similar results have been observed for the effect of deformities on the U_{crit} of juvenile sea bass *Dicentrarchus labrax* (Basaran et al. 2007).

We standardized the exposure timing by using degree-days because this protocol had been used in previous experiments (Schisler et al. 2006); thus, exposure occurred at about 9–10 weeks posthatch. Ryce et al. (2004) showed that the proportion of fish exhibiting clinical signs of whirling disease declined dramatically when fish were exposed to *M. cerebralis* at an age of 9 weeks or older. Hence, the timing of our exposure may have resulted in fewer clinical deformities and the lack of detectable physiological effects of the disease. Despite being the same age, the F_1 strain was significantly larger at exposure than the other strains. Although this may have influenced our results, the size difference was small and the GR- and F_1-strain individuals did not differ in mean myxospore count despite differing in size at exposure. Although Ryce et al. (2004) found that age influenced the clinical expression of the disease, size was confounded in the experiment and its effects on the development of infection are unknown.

Myxospore count and deformity data confirmed that the CRR strain was very susceptible to whirling disease—a result that had been confirmed during previous exposure experiments in the laboratory (Ryce et al. 2001; Schisler et al. 2006) and the field (Thompson et al. 1999, 2002). In addition, the more closely related a strain was to the CRR strain, the higher were the myxospore count and percentage of clinical deformities exhibited when fish were exposed *M. cerebralis*. This was apparent in the CRR, F_2, and B_2 strains, which were the only strains exhibiting blacktail, a deformity that appears in the most highly susceptible individuals within a strain (Hedrick et al. 1999). Conversely, the more closely related a strain was to the GR strain, the lower were the myxospore count and percentage of clinical deformities.

The lack of differences in mortality between the exposure treatments and among strains indicates that mortality was not necessarily attributable to whirling disease. However, other experiments have shown that exposure to whirling disease resulted in increased mortality in the CRR strain (Schisler et al. 2006). The average mortality of 6.6% observed in our study is similar to the mortality observed in fish that were exposed at a similar number of degree-days, albeit lower exposure rates (1,000 TAMs/fish; Ryce et al. 2001; DuBey et al. 2007).

Strains

As expected, the CRR strain had a significantly higher U_{crit} than the GR strain. The GR strain has been raised under

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aquaculture conditions for over a century and has been selected for performance in a hatchery; therefore, we predicted that GR-strain fish would have a lower U_{crit}. In contrast, the CRR is a wild strain and presumably has adapted to the swifter, more variable velocities that are present in natural rivers. Differences in U_{crit} between wild and hatchery-reared individuals have been documented for juvenile brown trout *Salmo trutta* (Pedersen et al. 2008), juvenile and yearling Atlantic salmon *S. salar* (McDonald et al. 1998; Pedersen et al. 2008), and juvenile coho salmon *O. kisutch* (Brauner et al. 1994).

Differences in U_{crit} observed between the CRR and GR strains may have been influenced by differences in length. In coho salmon, differences in U_{crit} between wild and hatchery-reared fish can be attributed to the larger size of the hatchery-reared fish relative to their wild counterparts (Brauner et al. 1994). Despite size differences seen at the end of our experiment, the GR-strain fish also had significantly lower U_{crit} than the CRR strain at the beginning of the experiment, when length differences were not significant. Our results seem to suggest that GR-strain fish have less aerobic capacity than CRR-strain fish and therefore may be at a disadvantage when stocked into natural systems. We also expected that the F_1, F_2, and B_2 strains would show U_{crit} values that were intermediate to those of the pure strains; however, U_{crit} for the intermediate strains did not differ from that observed for the pure strains.

Trends in our data are consistent with the results of other studies. Critical swimming velocity of all strains decreased over the course of our experiment, which is typical as fish get larger (Brett 1965; Beamish 1978). Additionally, our U_{crit} results were similar to those for similar-sized fish from the Shelton and Aberdeen strains of coastal cutthroat trout *O. clarkii clarkii* (Hawkins and Quinn 1996) and the Eagle Lake and Mt. Shasta strains of rainbow trout (Myrick and Cech 2000).

Growth differed among the strains. The GR strain had the highest batch weight at the end of the growth experiment, consistent with the suggestion that the GR strain has been selected for fast and efficient growth in a hatchery environment (Schisler et al. 2006). In contrast, the CRR-strain fish were the slowest growing, which may be an adaptation to living in natural environments. Although the CRR strain has been cultured for stocking in Colorado, broodstocks were routinely collected from wild populations and have not been kept for extended periods in hatchery environments. The other strains showed intermediate growth, and the fastest growth was shown by the F_1 strain.

Conclusions

Our overall goal was to identify the strain with the best combination of *M. cerebralis* resistance and swimming and growth characteristics for use in reestablishing rainbow trout populations in Colorado. The CDOW has been experimentally stocking the F_1 strain because it was thought to have the best combination of resistance characteristics and wild-strain survival characteristics. However, our results suggest that the F_1 strain has little advantage over the GR strain. The GR strain grows faster than the F_1 strain and has similar U_{crit} and disease resistance. Average myxospore count was not significantly different between the GR and F_1 strains because some F_1 individuals had low myxospore counts. However, the total number of myxospores produced by all GR fish (27,456 myxospores) was much lower than that produced by all F_1 fish (1,913,167 myxospores) in our experiment. Therefore, if these fish were considered for introduction into the wild, far fewer potential myxospores would be added to a stream or river by using the GR strain, thus reducing the overall myxospore load. The GR strain was not originally considered a good candidate for stocking in Colorado because of its history of domestication (Schisler et al. 2006). However, the GR strain should not be discounted as a potential broodstock for use in Colorado. Before adoption of this strain, however, we suggest additional field trials be conducted to determine whether the performance differences seen under controlled experimental conditions are paralleled by similar results in the field.

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