

Comparison of Susceptibility of Five Cutthroat Trout Strains to *Myxobolus cerebralis* Infection

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Abstract.—Susceptibility to infection by the myxosporean parasite *Myxobolus cerebralis* was compared among strains of cutthroat trout *Oncorhynchus clarki* in two separate exposure tests in the laboratory. In both tests, each strain was exposed to 1,000 triactinomyxons/fish for 2 h in 8.0 L of water. In the first test, three strains of 10-week-old cutthroat trout were compared: two strains of Bonneville cutthroat trout *O. c. utah* (Bear Lake and southern Bonneville strains) and Yellowstone cutthroat trout *O. c. bouvieri*. In the second test, these strains plus Snake River fine-spotted cutthroat trout *O. c. subsp.* and Colorado River cutthroat trout *O. c. pleuriticus* were exposed at either 5 or 10 weeks of age. The prevalence of the *M. cerebralis* infection was determined by single-round polymerase chain reaction (PCR) assay 5 weeks after exposure. In the first test, the prevalence was significantly lower in the Bear Lake strain of Bonneville cutthroat trout (78.5%) than in the Yellowstone (97.8%) or southern Bonneville (100%) strains when exposed at 10 weeks of age. In the second test, the Bear Lake strain also had significantly lower infection rates after exposure at 5 (54%) or 10 weeks (82%) of age than the other four strains, which did not differ from each other (94–100%). The severity of the infection was also significantly reduced in Bear Lake Bonneville cutthroat trout, as suggested by the strength of the product of the single-round PCR assay. These results suggest that intraspecific differences in susceptibility to *M. cerebralis* infection exist, further supporting the need to maintain the genetic diversity among subspecies and geographic variants of cutthroat trout.

Whirling disease is caused by the myxosporean parasite *Myxobolus cerebralis*, which is found only in fish of the family Salmonidae (Markiw 1992a). Susceptibility to infection varies with age, dose, and species (Markiw 1992a, 1992b). For example, for species naturally exposed in a contaminated stream, O'Grodnick (1979) reported that rainbow trout *Oncorhynchus mykiss* was the most susceptible, followed in order of increasing resistance by sockeye salmon *O. nerka*, brook trout *Salvelinus*

fontinalis, chinook salmon *O. tshawytscha*, brown trout *Salmo trutta*, coho salmon *O. kisutch*, and lake trout *Salvelinus namaycush*. These fish were ranked based on the development of clinical signs, presence of developmental stages in histological sections, and the number of spores isolated by the plankton centrifuge method 160 d after exposure (O'Grodnick 1975). In another field exposure test in which mortality, clinical signs, histology, and polymerase chain reaction (PCR) assay (Andree et al. 1998) were used to assess susceptibility, rainbow trout survived better than Rio Grande cutthroat trout *O. clarki virginalis* or Colorado River cutthroat trout *O. c. pleuriticus* (Thompson et al. 1999). In the same study, Thompson et al. (1999) also noted a greater resistance of brown trout to *M. cerebralis* than noted for rainbow, brook, or cutthroat trout.

Laboratory trials have also been used to examine the susceptibility of fish exposed to known doses of triactinomyxons. For example, greenback cutthroat trout *O. c. stomias* were more resistant to infection by *M. cerebralis* than were rainbow trout (Markiw 1992a). Hedrick et al. (1999) compared rainbow trout and westslope cutthroat trout *O. c. lewisi* and found that rainbow trout had more severe histological lesions and higher prevalence of clinical signs of the disease, but the prevalence of infection did not differ between the two species. Also, when exposed to low doses of triactinomyxons (100–200/fish), rainbow trout became infected but cutthroat trout did not (Hedrick et al. 1999). Similarly, these authors found fewer myxospores in Yellowstone cutthroat trout *O. c. bouvieri* than in rainbow trout. The prevalence of infection did not differ between the two species and low doses resulted in no infection in either species. Bull trout *Salvelinus confluentus* had an infection prevalence similar to rainbow trout but produced fewer myxospores (Hedrick et al. 1999). Hedrick et al. (1999) found that Arctic grayling *Thymallus*

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arcticus exposed to 1,000–2,000 triactinomyxons/fish did not become infected.

In the study reported here, we evaluated innate differences in susceptibility to infection by *M. cerebralis* among strains of cutthroat trout available in the state of Utah for fisheries management. Susceptibility tests were conducted with five strains of cutthroat trout exposed at 5 and 10 weeks of age to known concentrations of triactinomyxons. Susceptibility to infection was assessed by PCR analyses developed by Andree et al. (1998) and modified by Schisler et al. (2001). The PCR assay can detect *M. cerebralis* at lower concentrations than the pepsin-trypsin digest method and can detect the pathogen in all known stages of its life cycle and in both hosts (Andree et al. 1998).

Methods

Fish origin.—There were two susceptibility tests conducted, the first of which used three cutthroat trout strains: two strains of Bonneville cutthroat trout *O. c. utah* (the Bear Lake Bonneville [BL] and southern Bonneville [BV] strains) and the Yellowstone cutthroat trout *O. c. bouvieri* (YL). In the second test, these three strains were also compared with Snake River fine-spotted cutthroat trout *O. c.* subsp. (SN) and Colorado River cutthroat trout *O. c. pleuriticus* (CR). The BL strain was from the Mantua State Fish Hatchery and was one generation removed from the wild. The BV strain is a stream-adapted form from the southern Bonneville Basin (Hepworth et al. 1999), and eggs were taken directly from broodstock in Manning Meadow Reservoir, Monroe Mountains, Utah. The CR strain eggs were obtained from wild broodstock in Dougherty Basin Lake, Utah, that had been transferred there from Boulder Creek, Boulder Mountains, Utah. The SN strain eggs were obtained from the Jackson Hole National Fish Hatchery and the YL strain eggs originated from Electric Lake, Utah. All egg sources and rearing facilities were certified free of prohibited pathogens, including *M. cerebralis*.

Test 1.—Three strains of cutthroat trout (BL, BV, and YL) were raised at the Fisheries Experiment Station, Logan, Utah, from eggs until the experiment began at 10 weeks after hatching. Temperatures at the hatchery were constant (13°C well water) and fish were reared in the same location, so the total degree-days of fish development before exposure were similar among strains. Mean weights a week before exposure were 1.01 g for BL, 1.07 g for BV, and 0.94 g for YL. The experiment was conducted at a field station in Par-

adise, Utah. Each strain was exposed to 1,000 triactinomyxons/fish for 2 h in 8.0 L of water oxygenated via airstones. Triactinomyxons were harvested the day of the test from worm cultures held at Utah State University, Logan. The total harvest was estimated by counting the number of triactinomyxons on three slides of 50 µL each and extrapolating the average to the total volume of triactinomyxon stock. Viability was also verified at the same time using the vital stains propidium iodide and fluorescein diacetate (Markiw 1992c).

The three strains were exposed to triactinomyxons 10 weeks after hatching. The BL were exposed on a different date and paired BV and YL exposures were conducted three separate times (8–14 fish/exposure/strain) during 1 week. This was necessary due to the different dates of hatching for the strains and a lack of sufficient triactinomyxons. After the 2-h exposure, the fish were transferred to plastic tanks, 30 fish/tank. For each of the three strains, two tanks held exposed fish and two additional tanks held controls that had not been exposed to triactinomyxons. Automatic feeders dispensed commercial pelleted feed once a day. Mortalities were recorded during maintenance visits (3 times/week). Occasionally fish were lost due to human error during tank cleaning, but these mortalities were not included in the statistical analysis of mortality. After 8 weeks, all the surviving fry (18–30/replicate) were collected following administration of a lethal dose of tricaine methanesulfonate, and whole fish were individually packaged into plastic bags. The samples were kept on ice and later frozen at –45°C until they were shipped for analysis. Total length of each fish was also measured at the time of collection. Prevalence of infection was determined by single-round PCR using the whole fry. The PCR results were scored into one of five categories based on a visual assessment of the intensity of the DNA banding pattern as negative (–), weakly positive (w+), positive (+), strongly positive (++), or very strongly positive (+++; Schisler et al. 2001).

Test 2.—An extensive effort was made to get eggs from each of the five tested strains (BL, BV, YL, SN, and CR) at the same time to ensure equal ages and sizes of fish at the time of exposure. The strains hatched within a few days of each other: July 9 (CR), 11 (BL, BV, SN), or 12 (YL) in 1999. Fish were reared from the eyed egg stage in the same location and under similar conditions at the Fisheries Experiment Station until the experiment began at the field station in Paradise, Utah.

Sixty fish from each of the five strains were

exposed to 1,000 triactinomyxons/fish in 8.0 L of hatchery well water for 2 h. Fish were exposed either at 5 or 10 weeks of age (posthatch). Mean weights at exposure for BL, BV, YL, SN, and CR at 5 weeks were 0.46, 0.23, 0.33, 0.27, and 0.24 g, respectively, and at 10 weeks were 1.4, 1.0, 1.3, 1.0 and 1.0 g. Triactinomyxons were harvested from worm cultures the same day as the exposure. All five strains were exposed to the same batch of triactinomyxons on the same day. Supplemental oxygen was supplied by airstones to each cooler during the exposure. Fish were then dipnetted into 114-L tanks, 30 fish/tank. Two replicate tanks were used for each strain. Unexposed controls were adipose-fin-clipped, and 15 fish were put into the same tanks as the exposed fish (i.e., total of 45 fish/tank). This was necessary because of limitations of water and tank space. Fish were fed by automatic feeders once a day. Tanks were cleaned and mortalities counted three times per week. Water for the tanks was supplied by a spring-fed pond. Temperatures ranged from 10°C to 14°C (mean, 12.0°C) during the 5-week incubation period for the 5-week-old group and from 5°C to 14°C (mean, 10.5°C) for the 10-week-old group.

After 5 weeks in the tanks, 25 exposed fish and 5 controls were sampled from each of the two replicates for each period. The 5-week incubation period was designed so that the tanks for the 10-week-old group would be available. Fish from the 10-week-old group were similarly collected after 5 weeks for consistency in the incubation period between the two age groups. Whole heads (10-week-old group) or whole fry (5-week-old group) were sampled and stored individually. Head samples were taken individually, disinfecting the saw between individuals by brushing with full strength chlorine bleach solution (5.25% sodium hypochlorite) and wiping the blade with a clean paper towel. Prevalence of *M. cerebralis* was determined by single-round PCR. Total length of each fish was also measured at the time of collection.

Statistical analysis.—A significance level of $\alpha = 0.05$ was used for all tests. The normality assumption was tested for total length using the Kolmogorov–Smirnov statistic (SPSS 1993). To achieve homogeneity of error variance, mortality percentages for each tank were arcsine-transformed before using a *t*-test. A hierarchical log-linear model using backward elimination (SPSS 1993) and species, tank replicate, and PCR code as factors was used to determine the most parsimonious model to fit the data. The model was run separately for fish exposed to triactinomyxons and

unexposed fish. Data for the frequency tables were tested using all five PCR signal strength categories or collapsed to reduce the number of cells with few or no cases (Fienberg 1980). In the PCR data set for 5-week-old fish, there were significant differences among replicates in the full PCR frequency table for BV, but this was ameliorated by combining the strongly positive and very strongly positive categories together. Similarly, replicates in the full frequency table were significantly different for CR exposed at 10 weeks, so positive and strongly positive categories were combined. Replicates for both data sets were subsequently combined for further chi-square tests. Additional tests (one-way analysis of variance [ANOVA]) in which prevalence percentages for each tank were arcsine-transformed were conducted to compare differences among strains. Subsequent mean comparisons among strains were tested with Tukey's least-significant-difference test. For comparing differences in mortality among strains and exposure categories (control and exposed), a two-way ANOVA model was applied using arcsine-transformed data. For comparing PCR results between 5- and 10-week-old groups, PCR data (classified as positive or negative) were pooled across replicates and chi-square analysis was used separately for each species.

Differences in total length between infected and noninfected fish were tested separately for each strain by the Mann–Whitney *U*-test. Ordinary least-squares regression was used to compare the relationship between the PCR infection score and total length.

Results and Discussion

Test 1

The prevalence of *M. cerebralis* among the cut-throat trout strains exposed to the parasite varied significantly in chi-square tests, either with the data separated into the five categories of infection intensity or collapsed into tables of positive versus negative ($P < 0.001$; Figure 1). By either method, the BL strain had a significantly lower prevalence (78.5%, $N = 60$) than either the BV (100%, $N = 41$) or YL (97.8%, $N = 45$) strains, which did not differ from each other. Linear regression between fish length and the results of the PCR test indicated no significant correlation between the two for all three strains ($r^2 \leq 0.010$). This analysis was of interest to determine if size was related to infection severity. For example, larger fish may have a greater probability of encountering triactinomyxons, re-

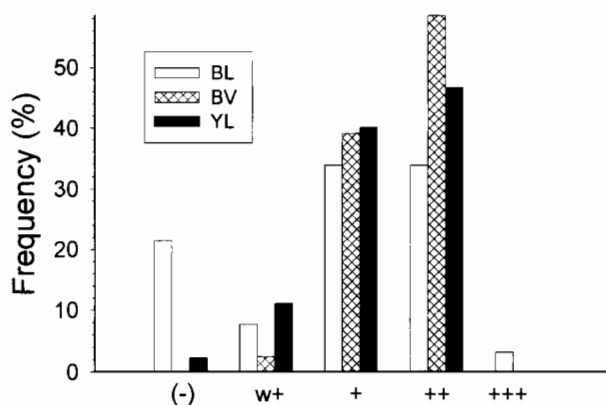


FIGURE 1.—Comparison of the frequency distribution of infection severity among three strains of cutthroat trout—Bear Lake Bonneville (BL), southern Bonneville (BV), and Yellowstone (YL)—exposed to 1,000 triactinomyxons of *M. cerebralis* at 10 weeks of age in test 1. Results were categorized during single-round polymerase chain reaction analysis as either negative (-), weakly positive (w+), positive (+), strongly positive (++), or very strongly positive (+++).

sulting in higher infection rates than smaller fish. Conversely, larger fish may have advantages in immunocompetence over smaller fish due to dominance in social hierarchies and lower associated stress responses (Peters et al. 1988), resulting in lower infection rates. All 150 control fish were negative except for a single BV strain. Tank mortality percentages did not significantly differ among the strains of cutthroat, whether exposed to triactinomyxons or not ($P = 0.62$). Mean (\pm SD) mortality percentages for exposed and unexposed fish were, respectively, $0.05 \pm 0.7\%$ and $3.3 \pm 0.0\%$ for BL, $0.0 \pm 0.0\%$ and $6.5 \pm 9.2\%$ for BV, and $2.2 \pm 3.0\%$ and $0.0 \pm 0.0\%$ for YL.

Test 2

At 5 weeks of age, exposure to 1,000 triactinomyxons/fish resulted in significant differences in infection prevalence among the five cutthroat trout strains (chi-square; $P < 0.001$, replicates combined). The BL strain had a significantly lower prevalence ($54 \pm 14\%$) than the other four strains (94–98%) of cutthroat trout (Table 1). Similar results were also obtained for the fish exposed at 10 weeks of age; the BL infection prevalence ($82 \pm 8.5\%$) was significantly lower (chi-square tests, $P < 0.001$) than for the other four strains (94–100%). The severity of infection as determined by the PCR assay rating supported the prevalence results. Fewer BL were classified as strongly positive or very strongly positive compared with the other strains for both 5- and 10-week-old groups (Figure 2).

TABLE 1.—Comparison of prevalence (SD) of *Myxobolus cerebralis* among five strains of cutthroat trout exposed to 1,000 triactinomyxons/fish at either 5 or 10 weeks of age ($N = 50$) in test 2. Cutthroat trout strain abbreviations are as follows: BL = Bear Lake Bonneville, BV = southern Bonneville, CR = Colorado River, SN = Snake River fine-spotted, and YL = Yellowstone.

Strain	Prevalence (%)	
	5-week-olds	10-week-olds
BL	54 (14.1)	82 (8.5)
BV	98 (2.8)	100 (0.0)
CR	96 (5.7)	98 (2.8)
SN	94 (2.8)	94 (2.8)
YL	98 (2.8)	100 (0.0)

Data analysis using the percentage of infected fish per tank also resulted in significantly lower prevalence for BL than the other strains for both 5-week-old ($P = 0.032$) and 10-week-old ($P = 0.015$) groups. Differences in *M. cerebralis* infection rates between 5- and 10-week-old groups were significant for BL ($P = 0.002$), but not for the other strains ($P \geq 0.24$). It is not clear why BL had a higher infection rate after exposure at 10 weeks than 5 weeks. Identical numbers of fish were exposed to the same triactinomyxon dose at both times in the same coolers for the same length of time. Perhaps activity of the fry in the cooler during exposure to triactinomyxons differed between the two age groups. Alternatively, because the older fish were larger, they had a greater surface area for potential parasite attachment than did smaller fish.

Mortality rates were low ($\leq 2.2\%$) in both the 5- and 10-week-old groups. In the 5-week-old group there was only one mortality recorded (SN), and rates did not differ significantly among the five strains ($P = 0.48$). However in the 10-week-old group, CR and BL had slightly higher mortality rates (2.1% and 2.2%, respectively) than the other strains (0.0%; $P < 0.001$).

Clinical signs were noted after 5 weeks in a small number of fish. One SN fry had both a caudal and cranial deformity. One BV fish had a cranial deformity and two BL fish had mandibular deformities. In the 10-week group, deformities were also minor; spinal deformities were noted in one control CR and one exposed SN, and one BV and one BL each had a deformed isthmus. Due to the short incubation time and low number of controls, it was difficult to adequately assess differences in clinical signs among strains.

The controls for the study became infected at a low rate: four 5-week-old fish (two weakly positive

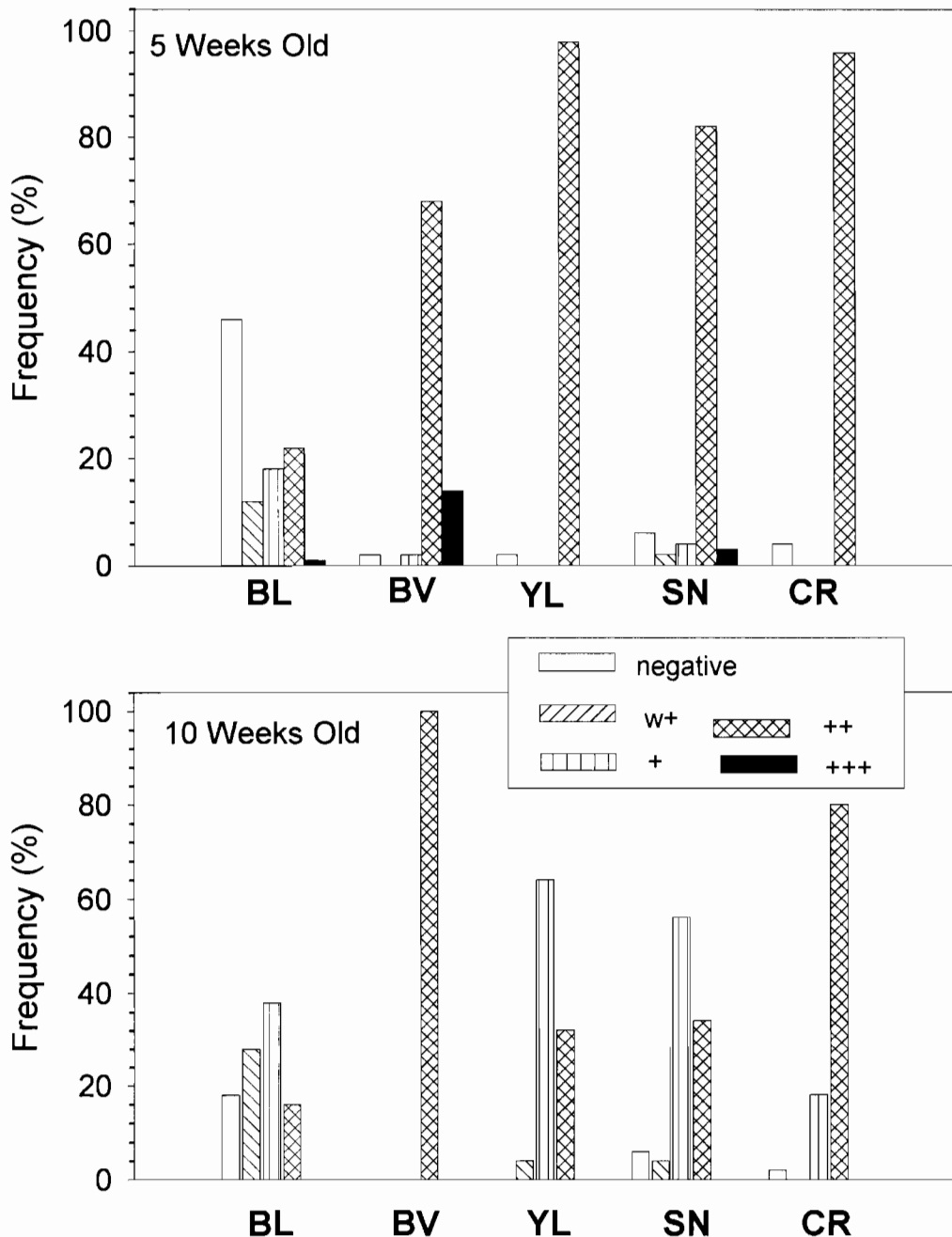


FIGURE 2.—Comparison of the frequency distribution (%) by infection category from single-round polymerase chain reaction results for five strains of cutthroat trout exposed to 1,000 *M. cerebralis* triactinomyxons at either 5 or 10 weeks of age in test 2. Strains were Bear Lake Bonneville (BL), southern Bonneville (BV), Yellowstone (YL), Snake River fine-spotted (SN), and Colorado River (CR).

and two strongly positive) of 50 tested and five 10-week-old fish (weakly positive) of 50 tested. The prevalence did not differ among strains, but was significantly lower than exposed groups. Because the PCR assay included negative controls and fin clips were double-checked, these sources of error are discounted. The field site is in an enzootic area, so contamination of the water supply

by animals is a real possibility. It is also possible that housing the controls in the same tanks as the treatments may have permitted a few stray triactinomyxons on infected fish to infect controls. Because all fish had equal probability of infection and prevalence was very low, the comparison among strains remains valid.

Mean total length at the time of sampling was

significantly different among the strains in both age groups, but there was considerable overlap in the range of values. The BL and YL strains were the longest (54 and 53 mm, respectively) and did not differ from each other in the 5-week-old group, but BL fish were significantly longer than YL fish in the 10-week-old group (67 versus 64 mm). The final mean lengths for the 5-week-old group were BV = 48 ± 4.6 mm, SN = 46 ± 3.5 mm, and CR = 45 ± 4.6 mm and for the 10-week-old group, BV = 59 ± 5.9 mm, SN = 57 ± 5.9 mm, and CR = 57 ± 4.1 mm. The YL fish were significantly longer ($P < 0.01$) than BV, SN, and CR fish for both the 5- and 10-week-old groups. There was no significant difference in total length between positive and negative fish for a given strain in either age-group ($P > 0.05$).

The prevalence of *M. cerebralis* was significantly lower in the BL strain of Bonneville cutthroat trout than in the YL, CR, BV, or SN strains and at two different ages. The severity of the infection was also reduced in BL as measured by the single-round PCR assay ranking. The severity rating of the single-round PCR technique has been significantly correlated with myxospore counts when both PCR and pepsin-trypsin digest methods were applied at the same time to the same samples (Schisler et al. 2001). The reason for the strain differences is unknown. The parasite was not discovered in Utah until 1991 and is not currently found in any of the waters in which cutthroat trout are used for broodstock. This precludes any natural selection for resistance to *M. cerebralis* per se. If differences in total length were responsible, one would expect the YL to have similar infection rates to BL in the 5-week-old group and have lower rates in the 10-week-old group compared with BV, SN, and CR. There was considerable overlap in total lengths among the strains in both age groups, and infection was not correlated with size. The Bear Lake strain is endemic to Bear Lake (Idaho and Utah), an oligotrophic lake in which the cutthroat trout rely heavily on other fish for prey. This strain has evolved to gain length as quickly as possible to switch over from a planktivorous diet to a piscivorous diet (Nielson and Lentsch 1988). Perhaps some physiological relationship between growth rate and immunological competence is involved. Evidence for this phenomenon has been noted for rainbow trout fry that were protected by immunization only if they were immunized at the time of or after the transition to a faster rate of weight gain (Tatner 1996). Differences in the rate of ossification may also affect resistance.

An alternative explanation for strain differences may be the degree of domestication and the natural selection against an elevated stress response. Differences in the magnitude of the stress response of rainbow trout among strains has been reported, indicating a genetic basis for differences in stress response (Pickering and Pottinger 1989). Wild fish have had higher levels of physiological stress indicators than domesticated fish (Woodward and Strange 1987), indicating that domestication can select for a reduction in the stress response. The relationship between stress and disease is well-established (Peters et al. 1988; Maule et al. 1989; Schreck 1996). Among the cutthroat trout strains tested, BL are derived from broodstock that are one generation removed from the wild, whereas the other Utah strains have been established in lakes in which the fish are trapped and progeny are from wild parents. However, the SN strain tested was also one generation removed from the wild, so it would be expected to respond similarly to BL if one generation of domestication was a factor.

Bear Lake Bonneville cutthroat trout were less susceptible to infection than four other cutthroat trout strains when exposed to a single high dose of triactinomyxons. However, fish were still infected at a high rate. Whether or not these laboratory tests apply to fish in the wild, where fish experience a chronic dose of triactinomyxons, fluctuating temperatures, and other environmental variables, remains to be seen. We attempted to conduct field studies in which the cutthroat were exposed to natural infection rates, but the data were inconclusive because of problems with predation, loss of marks, etc. However, the circumstantial evidence supported the laboratory work. Although our laboratory trials may not represent conditions in the wild, they did provide a means to evaluate susceptibility differences under controlled conditions.

Intraspecific differences in susceptibility to whirling disease have been noted previously, primarily between wild and domestic salmonid strains. For example, Densmore et al. (2001) noted differences in the frequency of clinical signs, spore counts, and severity of microscopic lesions among steelhead (anadromous rainbow trout), Cayuga Lake rainbow trout (wild strain), and Mt. Lassen rainbow trout (domesticated strain). Wild steelhead from the Deschutes River were also more resistant than the Mt. Lassen stock. J. Schachte (New York Department of Environmental Conservation, personal communication) compared two wild rainbow trout strains, the Cayuga Lake and

Eagle Lake strains, and found no significant differences in *M. cerebralis* spore counts. In our study, domestication was not an issue, but significant differences in prevalence were noted.

Whether the differences among cutthroat trout strains observed with PCR in a controlled exposure will equate to better survival in the wild remains to be studied. Genetic differences and sport fish performance differences have also been noted among Utah strains of cutthroat trout (Martin et al. 1985; Hepworth et al. 1999). These differences, as well as those observed in this study, underscore the importance of maintaining the genetic diversity among subspecies and geographic variants of cutthroat trout.

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