Vol. 59: 225–233, 2004 | DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org | Published June 11

Effects of fish age and parasite dose on the development of whirling disease in rainbow trout

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ABSTRACT: We determined the ages at which juvenile rainbow trout *Oncorhynchus mykiss* became resistant to the effects of whirling disease following exposure to a range of parasite doses. Heretofore, the development and severity of whirling disease in salmonids was known to be generally dependent on the age or size of fish when first exposed to the triactinomyxon spores of *Myxobolus cerebralis*; larger, older individuals tended to be less diseased. However, no systematic determination had been made of the exact age at which fish become resistant to the development of the disease. We exposed rainbow trout at 9 ages (1 to 17 wk post-hatch) to 4 parasite dose levels (0, 100, 1000 and 10 000 triactinomyxons per fish). Disease severity was measured using mortality, clinical signs, microscopic pathology, and myxospore counts. Disease and mortality were substantially reduced when exposure to the parasite occurred for the first time at 9 wk post-hatch (756 degree-days at 12°C) or older. High doses elicited more disease among the younger age groups, but the effect was dampened in groups exposed at about 9 to 11 wk post-hatch and absent thereafter. Rainbow trout reared in *M. cerebralis*-free waters for 9 wk post-hatch or longer, whether in the wild or in a hatchery situation, should experience greater survival and less disease than fish first exposed to the parasite at younger ages.

KEY WORDS: *Myxobolus cerebralis* · Whirling disease · Resistance · Age · Dose · Rainbow trout

INTRODUCTION

The development and severity of whirling disease in salmonids is known to be generally dependent on the age or size of fish when first exposed to the triactinomyxon spores of *Myxobolus cerebralis* (O'Grodnick 1979, Markiw 1991, 1992a, Thompson et al. 1999). The intensity of disease decreases with increasing size (O'Grodnick 1979), or age (Markiw 1992a), and trout exposed to the parasite at larger mean weights exhibit better survival (Thompson et al. 1999). Fish older than about 4 to 8 mo, or longer than about 5 to 13 cm, were believed to be disease-resistant (Hoffman 1961, 1976, Halliday 1976, Wolf 1986, Lom 1987, Schaperclaus 1991, Garden 1992). Such resistance is generally assumed to be conferred by increased ossification of the skeleton (El-Matbouli et al. 1992). Younger fish are also more vulnerable than older fish to nerve damage caused by the parasite (Rose et al. 2000). Whirling disease severity is also known to be dependent on the density of triactinomyxons, i.e. the parasite dose to which the fish are exposed (Hoffman 1974, O'Grodnick 1979, Markiw 1992a,b, Thompson et al. 1999). Disease intensity increases with increased triactinomyxon dose. Despite such general understanding of age and dose relations, no systematic determination of the age at which rainbow trout *Oncorhynchus mykiss* become resistant to the development of whirling disease had been conducted heretofore, nor had the interaction between age of fish at exposure and parasite dose been investigated. Consequently, it was unknown if an age of resistance existed and if it varied with parasite dose. This uncertainty hindered understanding of the disease, as well as its management. Fishery managers did not know for how long young trout must be reared in *M. cerebralis*-free waters to

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reduce or eliminate the effects of the disease. Similarly, the control of whirling disease in aquaculture facilities relied on rearing juveniles in parasite-free water for as long as possible before transfer to earthen ponds or *M. cerebralis*-positive waters. Narrowing the range of recommended rearing times for rainbow trout in parasite-free water would enhance management of rainbow trout in *M. cerebralis*-positive areas, whether in the wild or in hatcheries. Documentation of the age of resistance could also provide insight about the underlying mechanisms of the disease.

We addressed these information deficiencies by assessing the disease susceptibility of rainbow trout exposed to a range of parasite doses at different ages in a replicated factorial experiment under standardized laboratory conditions. Our objective was to determine the effect of both age of rainbow trout at time of exposure to *Myxobolus cerebralis*, and of dose rate of the parasite, on the development of the disease in rainbow trout. Fish were exposed at the same age to different doses and to the same dose at different ages. Effect of dose at a particular age could thereby be determined, as could effect of age at a particular dose and the interaction between the 2 factors.

METHODS

Experimental procedures. Fish were exposed to a range of parasite doses (0, 100, 1000, or 10 000 triactinomyxons per fish) over a range of ages (1, 3, 5, 7, 9, 11, 13, 15, or 17 wk post-hatch) in a replicated factorial experiment. Erwin strain rainbow trout were hatched from eggs supplied by the Ennis National Fish Hatchery, United States Fish and Wildlife Service, Ennis, Montana, and maintained at 12°C at the Bozeman Fish Technology Center, Bozeman, Montana, until exposure (Table 1). Three replicate lots of 55 fish were exposed to each dose level at each age for a total of 12 lots at each age. Exposures were conducted at the Wild Trout Research Laboratory, Montana State University, Bozeman, Montana. *Myxobolus cerebralis* triactinomyxons were produced in the laboratory from cultures of infected *Tubifex tubifex* (Hedrick et al. 1999b). Each lot of fish was exposed to triactinomyxons in aerated 5 l exposure chambers for 2 h (Hedrick et al. 1999a). Following exposure, lots were maintained separately at 13°C in 38 l glass aquaria supplied with filtered and oxygenated water by a recirculating process system at the Wild Trout Research Laboratory. The fish were fed a commercial trout diet at 2 to 3% body weight d^{-1}. Mortalities were counted and removed daily.

Table 1. *Myxobolus cerebralis* infecting *Oncorhynchus mykiss*. Mean weights and degree-days of development of rainbow trout at ages of exposure

Age at exposure (wk post-hatch)	Degree-days at exposure	Mean weight at exposure (g)
1	84	0.05
3	252	0.10
5	420	0.23
7	588	0.43
9	756	0.86
11	924	1.30
13	1092	1.72
15	1260	2.59
17	1428	3.33

Whirling disease severity was characterized using mortality, clinical signs of disease, microscopic pathology categories, and myxospore burdens. Cumulative mortality and microscopic pathology of each treatment group were assessed twice during the experiment, first at 17 wk post-exposure and again when the fish reached 33 wk post-hatch. Disease severity attributes at an equal time after exposure (17 wk), and also when the fish were all at the same age (33 wk post-hatch), were thereby estimated. Clinical signs (i.e. blacktail, skeletal deformities, and whirling behavior) were only estimated at 33 wk post-hatch, as this was the only time that all the fish could be handled and visually inspected adequately for all signs of disease without affecting results. Myxospore burdens were estimated only at 33 wk post-hatch as well. Disease responses not significantly different from those of unexposed controls were judged to be indicative of resistance.

Nine fish were randomly selected and euthanatized from each replicate at 17 wk post-exposure and at 33 wk post-hatch. The heads and tails of the euthanatized fish were removed, preserved in Davidson's fixative, and prepared for microscopic examination using standard histological techniques. One head half and the caudal peduncle from each fish were prepared for histology. Head and caudal sections from each fish were stained with hematoxylin and eosin or Giemsa, i.e. 2 head sections and 2 caudal sections were evaluated histologically for each fish collected. Microscopic pathology was categorized according to the MacConnell-Baldwin Scale (Hedrick et al. 1999b), whereby cartilaginous tissues (cranium, gill arches, jaw, vertebrae, and nares) were examined for the presence of the parasite and associated lesions. The abundance of parasites, cartilage damage, inflammation, extent of lesions, involvement of other tissues, and bone distortion were evaluated and categorized into 1 of 6 qualitative categories: no infection, minimal, mild, moderate, high, or severe. Microscopic pathology was not determined at 17 wk post-exposure for fish exposed at 1 wk post-hatch to 1000 or 10 000 triactinomyxons per fish because of poor survival.

The remaining halves of the heads of the fish sacrificed at 33 wk post-hatch were used to obtain myxospore counts using the standard plankton-centrifuge extraction method (O'Grodnick 1975). After extraction, myxospores were resuspended in a known volume of deionized water, and 1 ml aliquots were placed on both sides of a standard 1 ml hemocytometer counting chamber. Total myxospores per whole head were calculated as follows: (2 × total number of myxospores counted × 10^4 × volume of suspension)/(number of 1 mm^2 areas counted). Three counts of myxospores were made from each suspended sample; the mean of the 3 was used in analyses.

Bias was reduced throughout the experiment. Fish were randomly assigned to lots and lots were assigned to tanks randomly. Mortalities and clinical signs were recorded blindly for each replicate. Sampled fish were selected randomly from the tanks for histology and myxospore counts. Histology and myxospore count samples were examined blindly and in random order. The exposure designation of each sample was not determined until all samples had been examined and recorded.

Statistical analyses. The experiment was designed and analysed as a 2-way factorial. The 2 factors, or treatments, were age of fish at exposure (1, 3, 5, 7, 9, 11, 13, 15, and 17 wk post-hatch) and parasite dose (0, 100, 1000, or 10 000 triactinomyxons per fish). The number of mortalities, percent of fish with clinical signs, microscopic pathology categories, and myxospore burdens were compared among the 36 treatment groups (i.e. 9 ages and 4 parasite dose levels). All responses were treated parametrically with the exception of microscopic pathology. Microscopic pathology categories are ordinal but thresholds across categories (i.e. the differences between categories) cannot be assumed to be equal. For example, the differences in pathology between the minimal and mild categories and high and severe categories are slight but the difference in pathology between the mild and moderate categories is appreciable. In other words, the difference between a low mild and a high moderate is greater than the difference between a low minimal and a high mild. Therefore, parametric analysis of differences between means of numerical codes assigned to the categories, as has commonly been performed, is inappropriate and may lead to distorted results. We therefore used nonparametric tests based on ranks to analyse microscopic pathology categorizations.

Myxospore counts were analyzed by including random factors in the model for tank and fish, and the fish were treated as the experimental unit. Mortality and clinical signs were analyzed in the same way as myxospore counts, with the exceptions that tanks were the experimental units and no factor for fish was included in the model. A mixed linear model was used that combined both the fixed (age of fish at exposure and parasite dose) and random (tank and fish) effects. Type III *F*-statistics were used (Montgomery 1997). The important assumptions supporting this analysis are that the data are normally distributed and that they are independent with constant variance. Visual inspection of residual plots of data for all responses confirmed that these assumptions were met. The units of measure (fish or tank) were not independent; however, this assumption could be dropped by modeling statistical correlation into the analysis, which assumes constant variance and constant covariance (SAS PROC MIXED, compound symmetry covariance option; Littell et al. 1996). The model used for the analysis was the following:

$$y_{ijklm} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_{k(ij)} + \varepsilon_{ijklm}$$

where $i = 1,\ldots,a$; $j = 1,\ldots,b$; $k = 1,\ldots,c$; $l = 1,\ldots,d$; $m = 1,\ldots,e$; and μ = the overall mean, α_i = the effect of the ith level of the fixed factor A (age at exposure); β_j = the effect of the jth level of the fixed factor B (parasite dose); $(\alpha\beta)_{ij}$ = the interaction effect between the ith level of factor A and the jth level of factor B; $\gamma_{k(ij)}$ = the effect of the kth level of the random factor C (tank nested in factors A and B); $\delta_{l(ijk)}$ = the effect of the lth level of the random factor D (fish nested in factors A to C; this effect is not included in the model when analyzing the mortality or clinical signs response); and ε_{ijklm} = a random error caused by sampling.

Bonferroni's multiple comparison procedure was used to compare all pairwise differences of the least-square means. For each significance test, $\alpha = 0.05$. The non-parametric chi-square test of homogeneity was used to determine whether age at exposure significantly affected microscopic pathology within each dose and to determine whether parasite dose significantly affected microscopic pathology within each age group (Daniel 1990). All statistical analyses were conducted with the statistical software program SAS/STAT (SAS Institute 1996).

RESULTS

Mortality

Cumulative mortalities at 33 wk post-hatch decreased significantly with increasing fish age at exposure ($p < 0.0001$; Fig. 1). The response at 17 wk post-exposure was similar ($p < 0.0001$). Mortality of fish exposed at 1 wk post-hatch was significantly greater than those of all other age groups at all triactinomyxon doses. Fish exposed at 3 to 7 wk post-hatch had signif-

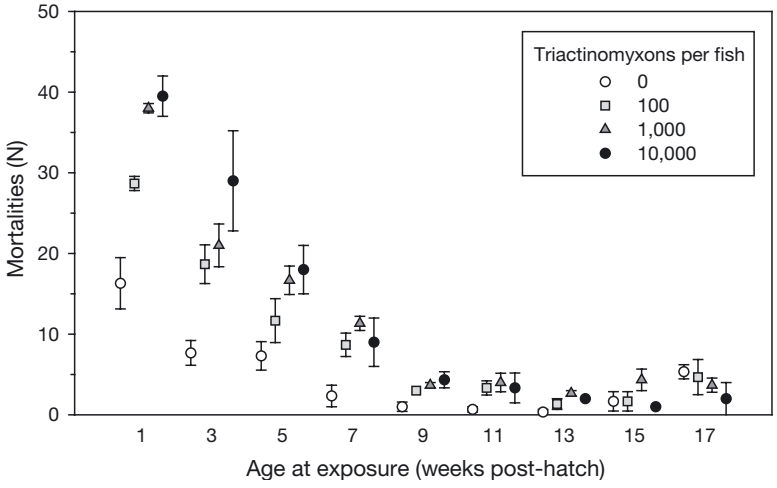

Fig. 1. *Myxobolus cerebralis* infecting *Oncorhynchus mykiss*. Mean (±SE) number of cumulative mortalities of rainbow trout at 33 wk post-hatch exposed to 0, 100, 1000 or 10 000 triactinomyxons of *M. cerebralis* per fish at 1 to 17 wk post-hatch

icantly less mortality than those exposed at 1 wk post-hatch and significantly more mortality than those exposed at older ages.

Parasite dose significantly affected cumulative mortality at both endpoints ($p = 0.0007$ for 33 wk post-hatch, Fig. 1, and $p < 0.0001$ for 17 wk post-exposure). Mortality increased significantly with increasing parasite dose in groups exposed at 1, 3, 5 and 7 wk post-hatch. Fish exposed at 9 wk post-hatch or older to any dose level did not suffer significantly more mortality than controls (Fig. 1). Significant interaction effects on mortality were present between the age of fish at exposure and parasite dose at both endpoints (both $p < 0.0001$) because increased parasite dose significantly increased the number of mortalities only among fish exposed at 7 wk post-hatch or younger (Fig. 1). Parasite dose did not significantly affect the number of mortalities among fish exposed at 9 wk post-hatch or older.

Clinical signs

The proportion of fish exhibiting any clinical sign (i.e. any combination of blacktail, skeletal deformities, and whirling behavior) at 33 wk post-hatch decreased significantly with increased age at exposure ($p < 0.0001$; Fig. 2) and increased with parasite dose ($p < 0.0001$), albeit only among fish exposed at 5 and 7 wk post-hatch. A significant interaction was present between parasite dose and age of fish ($p < 0.0001$). All fish exposed at 1 and 3 wk post-hatch exhibited clinical signs regardless of dose, whereas no clinical signs were observed among any of the fish exposed at 13 wk post-hatch or older regardless of dose. Within dose levels, proportions of fish with clinical signs decreased significantly with increasing age among fish exposed at 9 wk post-hatch or younger. Fish exposed at 1 and 3 wk post-hatch to 100 triactinomyxons per fish had significantly higher incidences of clinical signs than all older age groups exposed to this dose. Prevalences of clinical signs in fish exposed at 5 and 7 wk post-hatch to 100 triactinomyxons were not significantly different and were significantly higher than in all older groups exposed to this dose. No significant difference in the prevalence of clinical signs occurred among fish exposed from 1 through 7 wk post-hatch to either 1000 or 10 000 triactinomyxons per fish, but all had significantly higher incidences of clinical signs than fish exposed at 9 wk post-hatch or older. No significant difference in clinical sign incidence occurred among the age groups exposed at 9 wk post-hatch or older, regardless of parasite dose level.

Major skeletal deformities (scoliosis, lordosis, or major cranial deformities) were common only among

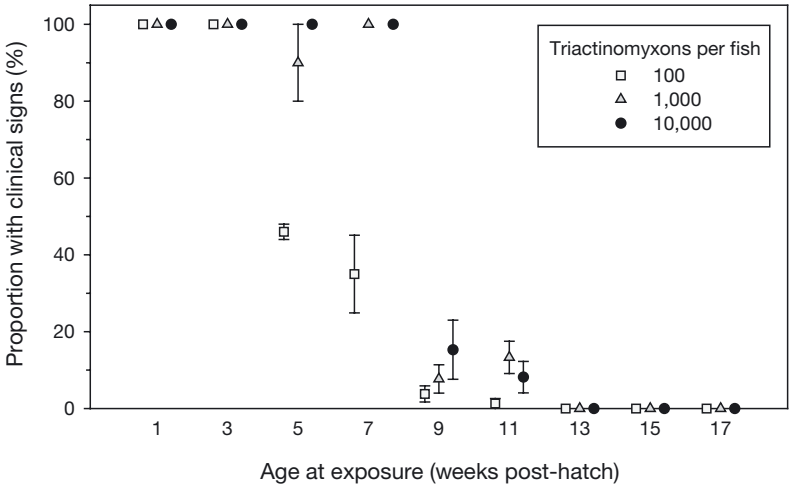

Fig. 2. *Myxobolus cerebralis* infecting *Oncorhynchus mykiss*. Mean (±SE) percent of rainbow trout exhibiting any clinical sign of whirling disease (i.e. any combination of blacktail, major and minor skeletal deformities, and whirling behavior) at 33 wk post-hatch exposed to 100, 1000 or 10 000 triactinomyxons of *M. cerebralis* per fish at 1 to 17 wk post-hatch

fish exposed at 1 and 3 wk post-hatch. Minor skeletal deformities (minor cranial indentations or swelling of the caudal peduncle suggestive of minor spinal deformation) and blacktail were largely restricted to fish exposed at 7 wk post-hatch or younger. The few clinical signs observed among fish exposed at 9 and 11 wk post-hatch (Fig. 2) were minor skeletal deformities. No blacktail was observed among fish exposed at 9 wk post-hatch or older regardless of dose. Whirling behavior was the least frequent clinical sign observed, occurring at rates less than 10% in all treatments; no whirling behavior was observed in groups exposed at 9 wk post-hatch or older.

Microscopic pathology

Microscopic pathology attributes at both 17 wk post-exposure and 33 wk post-hatch decreased as age at exposure increased. Age-group specific frequency distributions of pathology category were significantly different ($p < 0.0001$) within the same parasite dose level. The modal pathology category within all dose levels decreased with increasing age (Table 2). An example (100 triactinomyxons per fish at 33 wk post-hatch) is shown graphically in Fig. 3. At a given age of exposure (e.g. 5 wk post-hatch, Fig. 4), increased parasite dose levels generally elevated pathology category distribu-

Table 2. *Myxobolus cerebralis* infecting *Oncorhynchus mykiss*. Frequency distributions of microscopic pathology categories of rainbow trout at 33 wk post-hatch to specific doses of *M. cerebralis* triactinomyxons at different ages

Age at exposure (wk post-hatch)	Dose (triactinomyxons per fish)	Microscopic pathology category (%)					
		None	Minimal	Mild	Moderate	High	Severe
1	0	100	0	0	0	0	0
	100	0	0	0	50	50	0
	1000	0	0	0	67	33	0
	10000	0	0	0	100	0	0
3	0	100	0	0	0	0	0
	100	0	0	0	70	30	0
	1000	0	0	9	30	61	0
	10000	0	0	7	53	40	0
5	0	100	0	0	0	0	0
	100	0	0	31	42	27	0
	1000	0	0	0	37	56	7
	10000	0	0	11	44	45	0
7	0	100	0	0	0	0	0
	100	0	0	7	67	26	0
	1000	0	0	7	48	45	0
	10000	0	0	7	71	22	0
9	0	100	0	0	0	0	0
	100	0	0	41	55	4	0
	1000	0	4	26	63	7	0
	10000	0	0	8	88	4	0
11	0	100	0	0	0	0	0
	100	0	0	67	33	0	0
	1000	0	0	37	59	4	0
	10000	0	0	16	68	16	0
13	0	100	0	0	0	0	0
	100	7	71	22	0	0	0
	1000	4	52	26	18	0	0
	10000	0	34	44	22	0	0
15	0	100	0	0	0	0	0
	100	11	60	22	7	0	0
	1000	15	37	41	7	0	0
	10000	0	44	44	12	0	0
17	0	100	0	0	0	0	0
	100	7	74	19	0	0	0
	1000	0	48	48	4	0	0
	10000	0	46	38	16	0	0

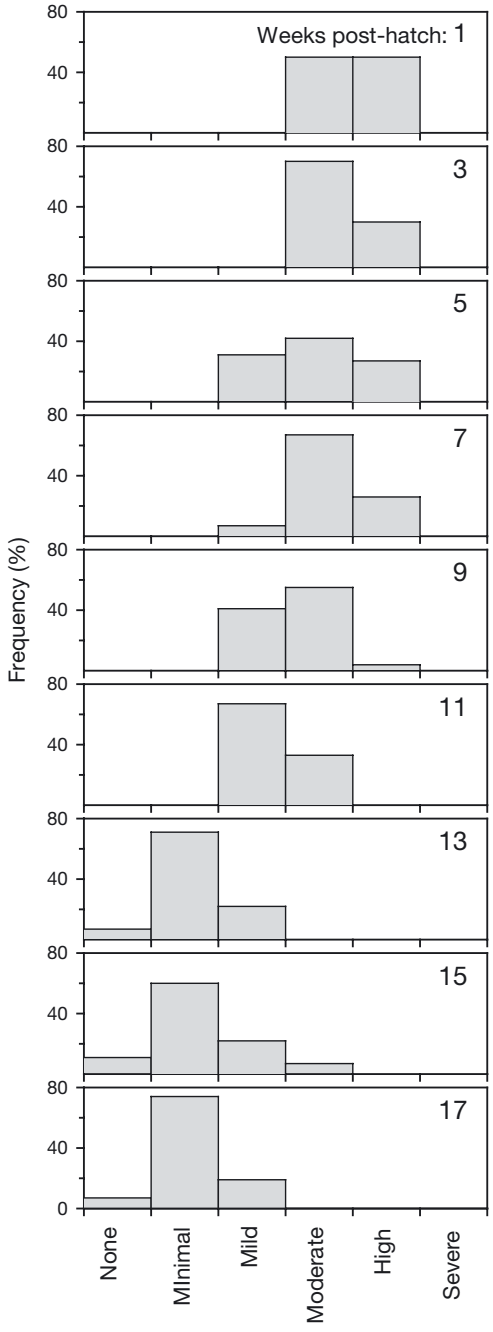

Fig. 3. *Myxobolus cerebralis* infecting *Oncorhynchus mykiss*. Frequency distributions of microscopic pathology category at 33 wk post-hatch of rainbow trout exposed to 100 triactinomyxons of *M. cerebralis* per fish at 1 to 17 wk post-hatch

tions (Fig. 4, Table 2). Frequency distributions of pathology category at different dose levels were significantly different within all age groups ($p < 0.0001$), but fish exposed at 7 wk post-hatch or older had modal pathology categories of moderate or lower, regardless of dose.

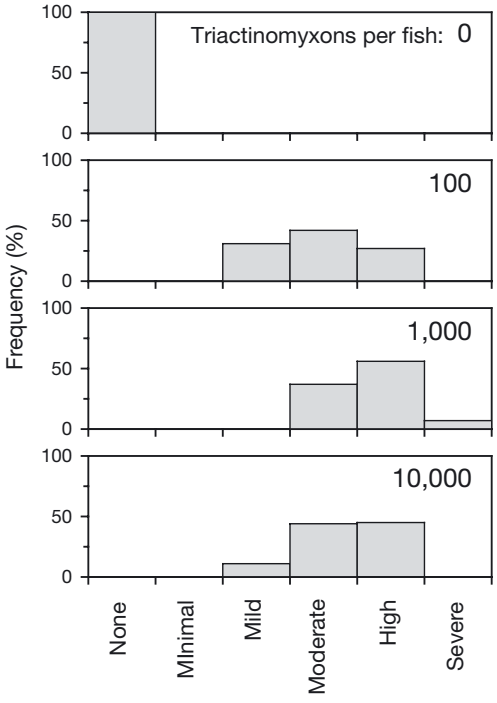

Fig. 4. *Myxobolus cerebralis* infecting *Oncorhynchus mykiss*. Frequency distributions of microscopic pathology category at 33 wk post-hatch of rainbow trout exposed to 0, 100, 1000 or 10 000 triactinomyxons of *M. cerebralis* per fish at 5 wk posthatch

Myxospore burdens

The age of rainbow trout at exposure significantly affected myxospore burdens ($p < 0.0001$; Fig. 5); myxospore counts decreased with increasing age at exposure. Fish exposed to 100 triactinomyxons per fish at 13 wk post-hatch or older had significantly lower myxospore counts than younger age groups. Fish exposed to 1000 or 10 000 triactinomyxons per fish at 9 wk post-hatch or older were burdened by significantly fewer myxospores than age groups exposed to these doses at younger ages, but all age groups (i.e. up to 17 wk post-hatch) could be infected. Triactinomyxon dose significantly increased *Myxobolus cerebralis* myxospore numbers in fish exposed at 11 wk post-hatch or younger ($p < 0.0001$; Fig. 5), but not in fish exposed at 13 wk post-hatch or older. A significant interaction effect between age of fish at exposure and parasite dose was present therefore ($p < 0.0001$). Myxospores were not found in any control fish.

DISCUSSION

The development of whirling disease in rainbow trout was dependent on both the age of fish at first

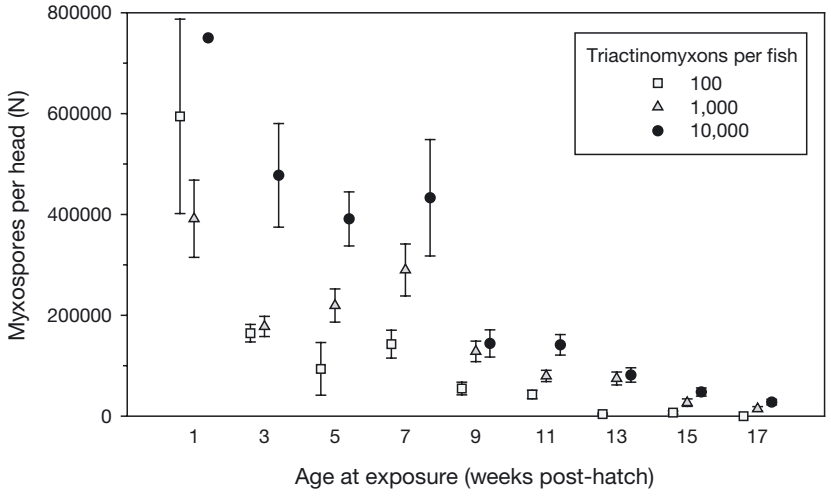

Fig. 5. *Myxobolus cerebralis* infecting *Oncorhynchus mykiss*. Mean (±SE) number of *M. cerebralis* myxospores per head of rainbow trout at 33 wk post-hatch exposed to 100, 1000 or 10 000 triactinomyxons of *M. cerebralis* per fish at 1 to 17 wk post-hatch

exposure to *Myxobolus cerebralis* triactinomyxons and on the dose of triactinomyxons to which the fish were exposed. Mortality, clinical signs, microscopic pathology, and myxospore counts decreased with increasing age of fish at exposure and decreasing level of parasite dose. Furthermore, age and dose interacted significantly such that the effect of age on the development of the disease was not the same at all levels of parasite dose, nor vice versa. High doses elicited more disease among the younger age groups, but the effect was dampened in groups exposed at about 9 to 11 wk post-hatch and absent thereafter. Our findings confirmed earlier work on the general age (O'Grodnick 1979, Markiw 1991, 1992a, Thompson et al. 1999) and dose (Hoffman 1974, O'Grodnick 1979, Markiw 1992a,b, Thompson et al. 1999) relations, but we identified the specific age thresholds at which rainbow trout became resistant to development of various manifestations of the disease. The age thresholds for mortality and clinical signs were 9 wk post-hatch. Myxospore counts in exposed fish were significantly different from controls in fish exposed at ages less than 13 wk post-hatch, but myxospore numbers decreased perceptibly between the 7 and 9 wk post-hatch exposures, and simple presence of myxospores does not denote disease. Overall, the effects of whirling disease on rainbow trout were substantially reduced, or the same as in rainbow trout not exposed to the pathogen, when exposed to the parasite at 9 wk post-hatch or older, as compared to fish exposed at younger ages. Rainbow trout reared in *M. cerebralis*-free waters for 9 wk post-hatch or longer, whether in the wild or in a hatchery situation, should therefore exhibit enhanced survival and reduced prevalence of clinical signs, myxospore counts, and severity of microscopic pathology compared to fish first exposed to the parasite at younger ages.

We did not determine the specific factor(s) responsible for the enhanced resistance conferred at 9 wk post-hatch, nor were we able to demonstrate whether the development of resistance was a factor of fish age or size, as ages and sizes at times of exposure were confounded. The development of resistance with increasing age or size at exposure has generally been thought to be a result of increasing ossification of the skeleton, but this hypothesis has never been tested. The trophozoites of *Myxobolus cerebralis* digest cartilage primarily, and the abundant cartilage in the skeletons of young trout is therefore thought to render them highly susceptible to the effects of the disease (El-Matbouli et al. 1992). Destruction of the cartilaginous structural framework for bone formation leaves fish permanently disfigured. Perhaps the rainbow trout skeleton at 9 wk post-hatch is sufficiently ossified to limit resources available to the parasite and preclude excessive disfigurement. Similarly, maturation of the nervous system may confer resistance. Significant neuropathology is associated with the presence of whirling disease in rainbow trout (Rose et al. 2000). Younger fish at first exposure would have a less mature nervous system that would be more vulnerable to neuropathological dysfunction (Rose et al. 2000). Resistance thresholds may coincide with specific development stages of the nervous system. Other possibilities include decreased penetrability of the host with increased age, induction of antibody or interferon production, or inactivation of the pathogen by serum components, phagocytic cells, acute-phase proteins, or killer cells (Chevassus & Dorson 1990). Rainbow trout develop a humoral (antibody) and cellular immune response to *M. cerebralis* (Hedrick et al. 1998). However, the cellular immune response is not evident until after significant cartilage damage has occurred (Hedrick et al. 1998) and specific anti-*M. cerebralis* antibodies are not present until 12 wk after exposure (Ryce 2003). Therefore, the induction of cellular or humoral immune responses cannot be responsible for providing the fish with progressively increased resistance against development of the disease with increasing age during the first 9 wk post-hatch. However, non-specific immune mechanisms that develop with time may confer resistance against development of the disease.

We used multiple indicators to provide a comprehensive evaluation of disease severity. Cumulative mortality and microscopic pathology were both effective indicators of the direct effects of fish age and parasite dose on disease resulting from parasitism by *Myxobolus cerebralis*. Myxospore burdens were also sensitive to variations in age and dose, but were perhaps less directly indicative of disease because low numbers of myxospores may merely denote infection, not disease. Aggregate clinical signs (blacktail, major and minor skeletal deformities, and whirling behavior) showed the same patterns in whirling disease severity as the other indicators, but individual clinical signs, particularly whirling behavior, were less effective measures. All of the indicators (except cumulative mortality) were likely compromised by disease-induced mortality. The most severely affected fish died before the indicators were measured, thereby artificially lowering the apparent frequency or intensity of each indicator. For example, we expected more high microscopic pathology category ratings than we observed among young fish exposed to high parasite doses, as compared to most other (Hedrick et al. 1999b, Sollid et al. 2002, Vincent 2002) but not all (Hedrick et al. 1999a, Sollid et al. 2002) reported findings for similar age rainbow trout and parasite doses. Differences in fish rearing conditions, triactinomyxon viability, rainbow trout strains, and grading criteria and interpretation may have contributed to such differences among studies. Nevertheless, such differences would not affect our conclusions, which were based only on comparisons among our fish, all of which were treated consistently.

Rainbow trout exposed to the whirling disease pathogen at 9 wk post-hatch or older may be resistant to the development of the disease, but will remain potential carriers of the pathogen. Some fish lacking detectable *Myxobolus cerebralis* myxospores were present in all groups exposed at 9 wk post-hatch or older, and myxospore burdens decreased with increasing age of exposure and decreasing triactinomyxon density, but we were unable to detect myxospores in any fish only in the group exposed to 100 triactinomyxons per fish at 17 wk post-hatch. We did not expose rainbow trout to high triactinomyxon densities at sufficiently old ages to determine when or if a threshold age exists beyond which myxospore production ceases entirely. Nevertheless, precluding or minimizing exposure of juvenile rainbow trout of any age to *M. cerebralis* triactinomyxons can be expected to help limit subsequent myxospore production and dissemination.

Applicability of our work to other salmonids is likely variable and unpredictable. Many other salmonids are susceptible to whirling disease, but to widely varying degrees (MacConnell & Vincent 2002). The general relationship between age at exposure and enhanced resistance is likely also present among other susceptible salmonids, but the threshold levels are almost certainly different. The same is most likely true for the relation between dose and disease severity. Specific thresholds for species of interest should be determined empirically.

The control of whirling disease in hatcheries has relied on rearing fry and fingerlings in parasite-free water for as long as possible before transfer into earthen ponds or into waters known to contain the parasite. Recommendations ranged from 4 to 8 mo after hatch or 5 to 13 cm in length (Hoffman 1961, 1976, Halliday 1976, Wolf 1986, Lom 1987, Schaperclaus 1991, Garden 1992). We recommend that cultured juvenile rainbow trout be maintained in *Myxobolus cerebralis*-free waters for a minimum of 9 wk after hatching (756 degree-days at 12°C) to reduce the severity of whirling disease and increase survival. Similarly, management of wild rainbow trout should focus on minimizing exposure of naturally produced juvenile rainbow trout to *M. cerebralis* triactinomyxons during their first 9 wk after hatching. Tactics may include discouraging colonization of spawning tributaries by *M. cerebralis* and encouraging fish to spawn and rear there, diluting triactinomyxon densities during the 9 wk period by increasing stream discharge volumes, and encouraging spawning at times that would result in rearing coincident with low triactinomyxon densities.

Acknowledgements. We thank the Whirling Disease Initiative of the National Partnership on Management of Wild and Native Coldwater Fisheries for funding this work. C. Fraser, Laboratory Manager of the Wild Trout Research Laboratory, and M. Nelson, D. Rider, T. Jentzsche, A. Gray, A. Thompson, R. Anderson, J. Knox, M. Toner, R. Stevenson, A. Herrera, and T. Wyatt provided technical assistance. J. Borkowski provided statistical advice. C. Bond, B. L. Kerans, T. E. McMahon, and C. Zabinski reviewed an earlier version of the manuscript.

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Editorial responsibility: Wolfgang Körting, Hannover, Germany

Submitted: September 5, 2003; Accepted: January 19, 2004
Proofs received from author(s): May 6, 2004