

THE EFFECTS OF *MYXOBOLUS CEREBRALIS* MYXOSPORE DOSE ON TRIACTINOMYXON PRODUCTION AND BIOLOGY OF *TUBIFEX TUBIFEX* FROM TWO GEOGRAPHIC REGIONS

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ABSTRACT: The aquatic oligochaete *Tubifex tubifex* is an obligate host of *Myxobolus cerebralis*, the causative agent of salmonid whirling disease. *Tubifex tubifex* can become infected by ingesting myxospores of *M. cerebralis* that have been released into sediments upon death and decomposition of infected salmonids. Infected worms release triactinomyxons into the water column that then infect salmonids. How the dose of myxospores ingested by *T. tubifex* influences parasite proliferation and the worm host are not well understood. Using replicated laboratory experiments, we examined how differing doses of myxospores (50, 500, 1,000 per worm) influenced triactinomyxon production and biomass, abundance, and individual weight of 2 geographically distinct populations of *T. tubifex*. Worm populations produced differing numbers of triactinomyxons, but, within a population, the production did not differ among myxospore doses. At the lowest myxospore dose, 1 worm population produced 45 times more triactinomyxons than myxospores received, whereas the other produced only 6 times more triactinomyxons than myxospores. Moreover, total *T. tubifex* biomass, abundance, and individual weight were lower among worms receiving myxospores than in myxospore-free controls. Thus, *T. tubifex* populations differ in ability to support the replication of *M. cerebralis*, and infection has measurable consequences on fitness of the worm host. These results suggest that variability in whirling disease severity observed in wild salmonid populations may partially be attributed to differences in *T. tubifex* populations.

Whirling disease (WD) among salmonid fish is caused by *Myxobolus cerebralis* (Myxozoa: Myxosporae) (Hoffman, 1990) a parasite that spread from central Europe and northern Asia to the United States by 1958. Although WD was initially thought to be only a problem for salmonid aquaculture, it has recently been implicated as the cause of severe declines in some wild rainbow trout (*Oncorhynchus mykiss*) populations in the intermountain west (Nehring and Walker, 1996; Vincent, 1996). Other wild rainbow trout populations appear little affected by WD (Baldwin et al., 1998). Such variability is to be expected given the dynamic nature of a disease that depends on interactions among the hosts, pathogen, and the environment (e.g., Anderson and May, 1979; May and Anderson, 1979; Hedrick, 1998; Reno, 1998). Understanding the causes of this variability should provide greater insight into the dynamics of WD in natural ecosystems.

The life cycle of *M. cerebralis* alternates between salmonids and aquatic oligochaetes (Markiw and Wolf, 1983; Wolf and Markiw, 1984; El-Matbouli and Hoffmann, 1989). Myxospores are released into sediments when *M. cerebralis*-infected salmonids die and decompose or perhaps through the feces of predators that have eaten infected fish. Oligochaetes are infected after they consume myxospores; however, other modes of parasite entry into oligochaetes have not been explored. Myxospores attach to the gut lining of oligochaetes using extruded polar filaments and the sporoplasms penetrate between the epithelial cells where they proliferate (El-Matbouli and Hoffmann, 1998). After an incubation period of about 3 mo (at 15 C), worms release the triactinomyxon form of the parasite that is infective to salmonids into the water column. Upon contact with fish, the triactinomyxons inject their sporoplasms into the epidermis. Sporoplasms migrate through the epidermis and nervous tissue to the cartilage (El-Matbouli et al., 1995). Although

many salmonid species can be infected by *M. cerebralis* (Hedrick et al., 1998, 1999), *Tubifex tubifex* (Oligochaeta: Tubificidae) is the only worm species that has been shown to support replication of *M. cerebralis* in studies performed to date (Wolf et al., 1986).

Host ecology influences the spread and impact of infectious disease in the wild (Reno, 1998). Mathematical models show that the disease transmission rate (the efficiency of pathogen transfer from a single infectious individual) affects disease progress in the short term by influencing characteristics such as the incidence of disease and the duration of infection in a host population (e.g., Anderson and May, 1979; May and Anderson, 1979; Reno, 1998). Transmission depends on factors relating to host resistance, the pathogen, and the environment. *Myxobolus cerebralis* must be transferred between 2 hosts, and thus, the rate of indirect transmission from fish to fish (via worms) will be influenced by the ability of *T. tubifex* to support *M. cerebralis* infections. The ability of *T. tubifex* to support infections over a wide range of myxospore doses has not been adequately examined using replicated laboratory experiments. Moreover, *T. tubifex* collected from different geographic regions are often genetically distinct and differ in the conditions that favor growth, development, reproduction, and tolerance to heavy metals (e.g., Anlauf, 1994, 1997; Anlauf and Neumann, 1997; Sturmbauer et al., 1999). Consequently, the proliferation of *M. cerebralis* may also vary among *T. tubifex* populations.

Long-term effects of disease on host populations also depend on the negative effects of disease on hosts (Reno, 1998). Negative effects of WD on salmonids, including tail-chasing behavior (from which the disease gets its name), skeletal deformities, blackened tails, and death, have been widely documented (e.g., Hedrick et al., 1998, 1999). However, little is known about the negative effects of the disease on *T. tubifex*. In the laboratory, infected *T. tubifex* can live for almost 2 yr (Gilbert and Granath, 2001), indicating that WD does not cause direct mortality of worms. However, the intestines of infected worms contain masses of *M. cerebralis* cells that cause compression and hypotrophy of epithelial cells (El-Matbouli and Hoffmann, 1998). Consequently, infection may interfere with

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TABLE I. Mean (± 1 SE) of total number of triactinomyxons produced and the mean (± 1 SE) number of days postexposure needed to produce 50% of total triactinomyxon production (T_{50}) for the Mount Whitney and Madison River *Tubifex tubifex* populations.

Myxospore dose		Mount Whitney		Madison River	
		Per worm	Total	Numbers of triactinomyxons	T_{50}
50	5,000	228,333.3	149.0	31,416.7	149.7
		($\pm 52,718.1$)	(± 0)	($\pm 3,873.9$)	(± 12.7)
500	50,000	383,416.7	113.3	29,250.0	142.7
		($\pm 107,864.7$)	(± 4.3)	($\pm 11,861.2$)	(± 15.2)
1,000	100,000	233,250.0	145.0	34,750.0	149.0
		($\pm 15,712.9$)	(± 14.7)	($\pm 9,148.1$)	(± 0)

the absorption of nutrients and affect worm growth, reproduction, and ability to compete with other worms.

We examined the hypothesis that triactinomyxon production was a function of *M. cerebralis* myxospore dose using 2 geographically distinct populations of *T. tubifex*. We also examined the hypothesis that infection by *M. cerebralis* negatively affected the success of *T. tubifex* by comparing worm biomass, abundance, and individual growth among *M. cerebralis*-infected and -uninfected *T. tubifex* populations.

MATERIALS AND METHODS

The effect of myxospore dose on triactinomyxon production of *T. tubifex* was tested using worms collected from 2 geographic regions where WD is enzootic, the Madison River (MR) in Montana, and the California Department of Fish and Game Mount Whitney fish hatchery (MW) in Lone Pine, California. The MR worms were collected from the upper Madison River below Quake Lake just north of the Montana-Wyoming border. The MW worms were derived from an established laboratory culture that was originally collected from settling ponds of the water supply to the hatchery. Worms from the MW population have been used successfully in laboratory experiments to produce triactinomyxons for fish infection studies (e.g., Hedrick et al., 1998). In 1997, laboratory cultures of each population were established from offspring of worms that were positively identified as *T. tubifex*. Worms from these cultures were checked periodically for the presence of *M. cerebralis* using molecular markers (Andree et al., 1997). Infection was never detected in these cultures.

Myxospores of *M. cerebralis* were extracted from rainbow trout (mixed sources: experimentally exposed fish from Willow Creek, Montana and the Wild Trout Laboratory, Montana State University and wild rainbow trout from the Missouri River, Montana) using the plankton centrifuge method (O'Grodnick, 1975). Andree et al. (1999) found little geographic variation in the genetic makeup of *M. cerebralis*. The resulting emulsion of myxospores was thoroughly mixed and myxospore numbers were quantified using duplicate hemacytometer counts (Markiw and Wolf, 1974). We determined that 85–95% of the myxospores were viable using the methylene blue staining technique (Hoffman and Markiw, 1977). Moreover, myxospores were positively identified as *M. cerebralis* using morphological characters and molecular techniques (Andree et al., 1997).

Three replicates of 100 worms from each geographically distinct population of *T. tubifex* were randomly assigned to 1 of 4 treatments (0, 50, 500, or 1,000 *M. cerebralis* myxospores per worm) in a completely cross-classified design. The age and developmental stages of the worms were unknown. Myxospore densities encountered by worms in nature are unknown; however, the doses used in the experiment spanned the range used in other laboratory studies (e.g., El-Matbouli et al., 1998, 1999). The groups of 100 worms in each replicate were measured (wet weight) to the nearest mg and held for 24 hr without food in dechlorinated tap water. Worms were placed in 250-ml plastic food containers ($9 \times 9 \times 5$ cm) with 40 ml of autoclaved sand and about 200 ml of dechlorinated tap water. Worm densities of this magnitude ($\approx 13,000$ individuals/m²) are commonly found in streams (e.g., Lazim and Learn-

er, 1986, 1987). Spore-positive treatments were inoculated with an aliquot of spore extract of the appropriate dose on 7 December 1998. The spore extract was placed into the water about 5 mm above the sediment. Negative controls were inoculated with an equivalent aliquot of an emulsion obtained by processing *M. cerebralis*-free fish. Replicates were aerated and held in dechlorinated tap water under ambient light and temperature conditions (13–17 C). Most of the water (about 150 ml) was changed weekly, and worms were fed dehydrated *Spirulina* at the same time. The treatment group of each replicate was not revealed to the person responsible for maintaining the experiment (Stevens; i.e., a double-blind study).

Beginning approximately 60 days postexposure, all water drawn from weekly water changes was filtered through 20- μ m Nitex mesh to retain triactinomyxons. Filters were back-flushed with deionized water to remove triactinomyxons. The triactinomyxon-containing solution was diluted to 10 ml. We counted all triactinomyxons in 2, 20- μ l aliquots of the filtrate (100 \times magnification, phase contrast or darkfield). The mean of the 2 counts was extrapolated to calculate the total number of triactinomyxons produced by each replicate. On 20 May 1999 (164 days postexposure), all worms in each replicate were counted and collectively wet-weighed. Small aliquots of sediments (about 5 ml) from each replicate were placed in a small white collecting pan and covered with dechlorinated tap water. Each aliquot was searched and worms were counted, removed, and placed in a container with dechlorinated tap water and sediments were placed in another container. When the entire 40 ml had been searched the mass of worms was weighed and the sediments and worms were returned to the original experimental container. On 25 June 1999 (199 days postexposure), after triactinomyxon production dropped to low levels, the experiment was concluded and worms were counted and weighed.

Two-way analysis of variance (ANOVA) was used to determine whether the total numbers of triactinomyxons produced (logarithmically transformed to stabilize variances) differed between the *T. tubifex* populations and among myxospore doses. The number of days (counted from the day of first release) required for each replicate to produce 50% of the total triactinomyxon numbers (T_{50}) was determined to examine temporal patterns. The Kruskal-Wallis test was used to determine if T_{50} differed among populations and myxospore doses. Separate, multiple analyses of variance (MANOVA) for each sampling date were used to determine whether total worm biomass, average individual worm weight (calculated from biomass and worm numbers), and worm numbers (all logarithmically transformed to stabilize variances) differed between *T. tubifex* populations and among myxospore doses. Comparisons across dates, i.e., include date as a treatment in MANOVA, were not used to simplify analyses. If MANOVAs were significant, individual 2-way ANOVAs and Tukey's pairwise comparisons tests were done to elucidate treatment effects. Two-way ANOVA was also used to determine if initial worm biomass (and thus, individual weights) differed among treatments. In all statistical tests a critical α -value of 0.05 was used to determine significant differences.

RESULTS

Both the MR and MW populations of *T. tubifex* started producing triactinomyxons 87 days postexposure at all 3 dosing

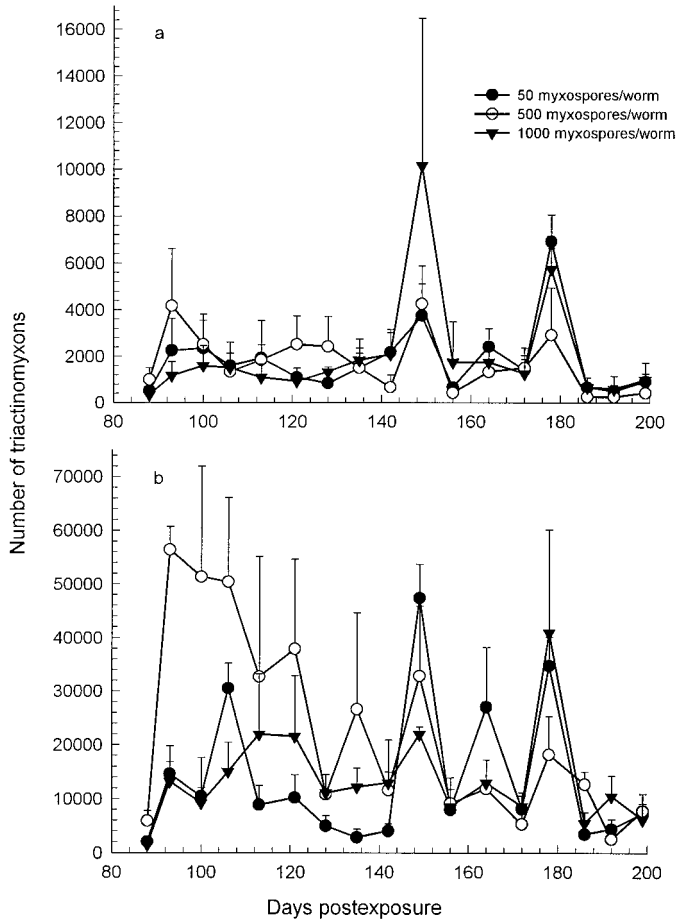


FIGURE 1. Mean triactinomyxon production beginning on day 87 postexposure for (a) the Madison River and (b) the Mount Whitney populations of *Tubifex tubifex*. Means are averages of 3 replicates of approximately weekly triactinomyxon counts. Error bars are ± 1 SE of the mean for that date.

regimes (Fig. 1). Triactinomyxons were not observed in negative controls. As shown in Figure 1, triactinomyxon production was highly variable over time. Exposed worms in all treatment groups were still producing triactinomyxons, although in low numbers, at the end of the experiment on day 199 postexposure (Fig. 1). The MW worms produced more triactinomyxons than MR worms by approximately 1 order of magnitude (Table I; ANOVA, $P = 0.0001$). Total triactinomyxon production did not differ among myxospore doses (ANOVA, $P = 0.9591$). The MW worms always produced more triactinomyxons than the MR worms in every myxospore dose treatment (interaction between myxospore dose and worm population was not significant; ANOVA, $P = 0.3394$). Although MW worms exposed to 500 spores per worm appeared to have a lower T_{50} than other treatments, the difference was not statistically significant (Table I; Kruskal–Wallis test, $P = 0.1716$).

At the beginning of the experiment, the MR worms had higher total biomass (and thus, heavier individuals) than MW worms (Figs. 2, 3; ANOVA, $P = 0.0001$) across all myxospore doses (interaction of dose and worm population was not significant; ANOVA, $P = 0.4706$). Total worm biomass did not differ among myxospore doses at the start of the experiment (Fig. 2; ANOVA, $P = 0.6426$). However, by subsequent sam-

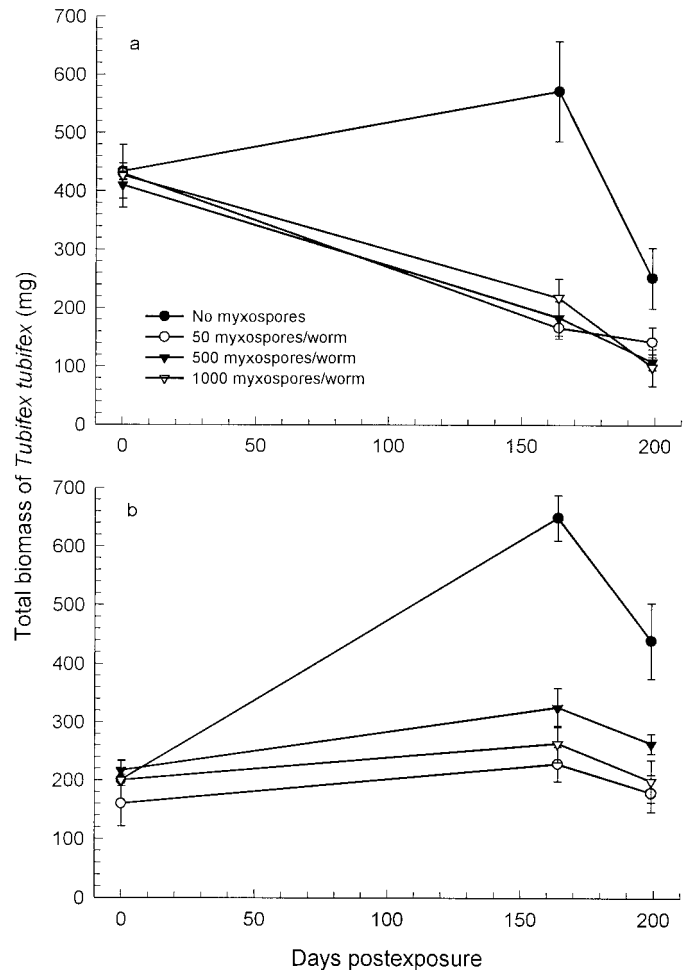


FIGURE 2. Total worm biomass of the (a) Madison River and (b) Mount Whitney populations of *Tubifex tubifex* when given varying doses of *Myxobolus cerebralis* myxospores. Tukey's pairwise comparisons indicated that total biomass was significantly higher in the negative controls than all myxospore treatments on days 164 and 199 postexposure ($P < 0.05$ for all comparisons). Error bars are ± 1 SE of the mean.

pling dates, total worm biomass, worm abundance, and individual worm weight differed among myxospore doses (MANOVAs: 164 days postexposure, $P < 0.0001$; 199 days postexposure, $P = 0.007$) and between the 2 worm populations (MANOVAs: 164 days postexposure, $P < 0.0001$; 199 days postexposure, $P = 0.0002$). The patterns were consistent across all myxospore doses and worm populations (MANOVAs: interaction effects not significant, both P -values > 0.25).

At 164 and 199 days postexposure biomasses of MW and MR worms were highest in negative controls and did not differ among treatments containing myxospores (Fig. 2; Table II, spore dose effect; Tukey's test, $P < 0.05$). Average weights of individual worms tended to be higher in the negative controls at 164 days postexposure (Fig. 3; Table II, spore dose effect; Tukey's test $P < 0.05$), but the differences were not significant at 199 days postexposure (Fig. 3; Table II, spore dose effect). Abundances of both MW and MR worms were higher in negative controls and did not differ among treatments containing myxospores at 164 days postexposure (Fig. 4; Table II, spore

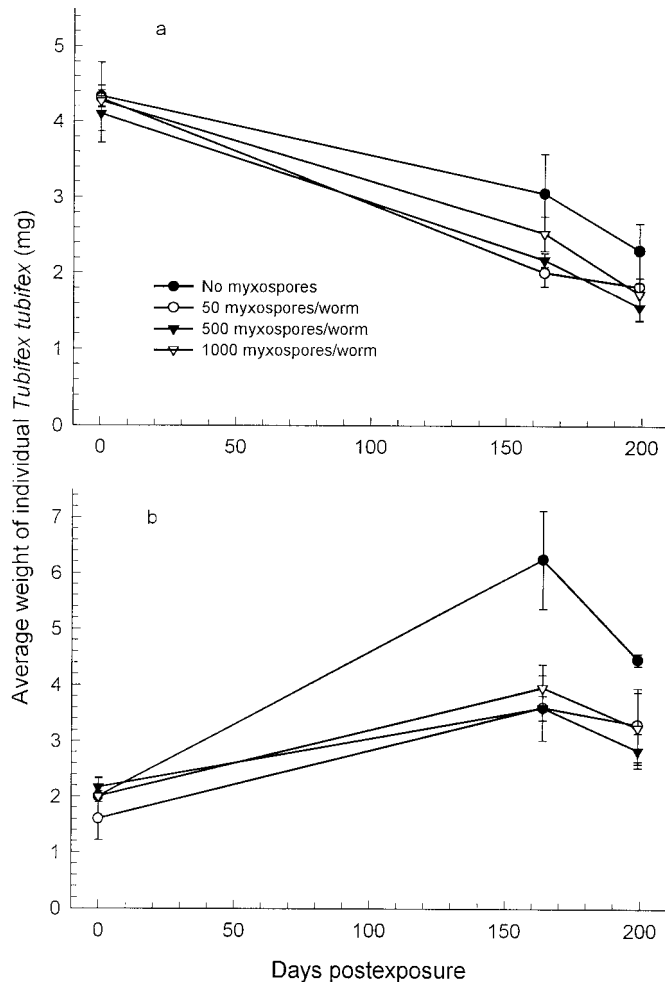


FIGURE 3. Average weight of individual worms of the (a) Madison River and (b) Mount Whitney populations of *Tubifex tubifex* when given varying doses of *Myxobolus cerebralis* myxospores. Tukey's pairwise comparisons indicated that individual worm weight was significantly higher in negative controls than in the 50 and 500 myxospores per worm treatments on day 164 postexposure ($P < 0.05$); however, on day 199 postexposure weight of individuals did not differ among any treatments. Error bars are ± 1 SE of the mean.

dose effect; Tukey's test, $P < 0.05$). The trend in abundance was similar at 199 days postexposure; however, significant differences in abundance were detected only between the negative control and the 1,000 myxospores per worm treatment (Fig. 4; Table II, spore dose effect; Tukey's test, $P < 0.05$).

At 164 days postexposure the MW population had higher total worm biomass (Fig. 2), heavier individuals (Fig. 3), and lower abundance (Fig. 4) than the MR population across all myxospore doses (Table II, population effect). These trends continued at 199 days postexposure for total worm biomass and average weight of individuals, but not for worm abundance (Table II, population effect).

Over time, total worm biomass of both MR and MW worms first increased dramatically and then declined slightly in negative controls (Fig. 2), and either declined consistently (MR worms, Fig. 2a) or remained relatively constant (MW worms, Fig. 2b) in treatments containing myxospores. Interestingly, it appears that the mechanism producing the higher total worm

biomass in negative controls over treatments containing myxospores differed between the 2 worm populations. Worm abundance first increased and then declined in MR negative controls and consistently declined in treatments containing myxospores (Fig. 4a). The average weight of individual MR worms declined in all treatments including the negative controls (Fig. 3a). However, in contrast to the increase in worm abundance seen in the MR negative controls, the abundance of MW worms remained relatively constant in negative controls and declined in most treatments containing myxospores (Fig. 4b). Furthermore, the average weight of individual MW worms increased much more dramatically in negative controls than in myxospore treatments (Fig. 3b). The increase in the weight of individual MW worms in the negative controls was not seen in the uninfected MR individuals. These results suggest that *M. cerebralis* infection inhibited reproduction of MR worms and growth of MW worms. Moreover, the number of MW worms declined dramatically in 2 of the myxospore treatments, suggesting that *M. cerebralis* infection may cause significant mortality of MW worms.

DISCUSSION

The number of triactinomyxons produced by both *T. tubifex* populations did not vary with myxospore dose, suggesting that our lowest spore doses were above the threshold limits of maximum infection of the 2 *T. tubifex* populations. *Myxobolus cerebralis* proliferated in both *T. tubifex* populations when myxospore doses were low, i.e., MW worms produced about 45, and MR worms about 6 triactinomyxons for each myxospore ingested (assuming all myxospores were ingested). Thus, our results support recent laboratory experiments that have documented the existence of a proliferative phase of the parasite's life cycle within *T. tubifex* (El-Matbouli and Hoffmann, 1998). Moreover, the rate of proliferation of *M. cerebralis* in *T. tubifex* might even be higher at myxospore doses lower than the concentrations we used.

Our findings may have considerable significance for the development of the disease in river systems. In the laboratory, the apparent ability of *M. cerebralis* to proliferate during parasitism of *T. tubifex* suggests that the presence of relatively few myxospores may quickly lead to high triactinomyxon production among worm populations. In an area where *T. tubifex* is common, perhaps the death of a single infected fish that can carry over 1 million myxospores in the cranial region (Hedrick et al., 1999) is enough to create a significant disease condition. Moreover, in the laboratory, exposure to as few as 10 triactinomyxons can cause infection in rainbow trout (Hedrick et al., 1999), suggesting that the subsequent infection of susceptible salmonids could also increase rapidly.

We designed our experiments to determine if triactinomyxon production in *T. tubifex* was a function of *M. cerebralis* myxospore dose. Our laboratory results showed that within a *T. tubifex* population, worms produced similar numbers of triactinomyxons even when myxospore doses varied between 5,000 and 100,000. The similarity of triactinomyxon production across myxospore doses suggested that most worms encountered spores and were infected under all dosing regimes. However, when *T. tubifex* worms were collected from streams, infection rates were typically low ($< 5\%$), even in streams where

TABLE II. Summary of ANOVA results for differences in total biomass, weights of individual worms, and worm abundance between *Tubifex tubifex* populations, among doses of *Myxobolus cerebralis* myxospores, and with the interaction of worm population and myxospore dose.

Date	Response variable	Source of variation	P
164 days postexposure	Biomass	Population	0.0034
		Spore dose	0.0001
		Interaction of population and spore dose	0.3399
	Individual weight	Population	0.0001
		Spore dose	0.0056
		Interaction of population and spore dose	0.6387
	Abundance	Population	0.0210
		Spore dose	0.0006
		Interaction of population and spore dose	0.1413
199 days postexposure	Biomass	Population	0.0003
		Spore dose	0.0012
		Interaction of population and spore dose	0.3467
	Individual weight	Population	0.0001
		Spore dose	0.0825
		Interaction of population and spore dose	0.9900
	Abundance	Population	0.9706
		Spore dose	0.0216
		Interaction of population and spore dose	0.2500

WD has caused significant reduction in the rainbow trout population (Rognlie and Knapp, 1998). Thus, on the large spatial scale of a stream or stream reach, individual *T. tubifex* must only infrequently encounter sufficient myxospores to cause disease.

Several mechanisms could produce this apparent discrepancy between laboratory and field infections of *T. tubifex*. In streams, low encounter rates of individual worms with myxospores could occur if the abundance of *T. tubifex* was greater than the abundance of myxospores resulting in insufficient myxospores to infect a large percentage of *T. tubifex*. Alternatively, if myxospores and *T. tubifex* were unevenly distributed within a stream or stream reach, only those worms in close proximity to myxospores were likely to encounter myxospores. Little is known about myxospore abundance and distribution in infected streams; however, abundance of *T. tubifex* can vary considerably among streams and among habitats within streams. For example, mean densities of *T. tubifex* in m² sections across a stream about 8 m wide varied between about 75,000 and 375,000 individuals·m², and densities in individual samples were even more variable (Lazim and Learner, 1987). Because *T. tubifex* individuals consume only small amounts of sediment daily (0.0018–0.0269 ml·individual⁻¹·day⁻¹ at 15°C; e.g., McCall and Fisher, 1980), small-scale unevenness of worm and myxospore distributions within sediments could result in a few small areas of infected worms and many areas of uninfected worms, resulting in a low overall infection rate for *T. tubifex* within a stream reach. Moreover, *T. tubifex* is often found in association with other worm species (Brinkhurst et al., 1972; Brinkhurst and Chua, 1973) that may consume myxospores and make them unavailable to *T. tubifex* (M. El-Matbouli, pers. comm.). Thus, interactions among worm species may also influence infection in *T. tubifex* in streams. Regardless of the mechanism, our data combined with the field data of others suggest that on stream-reach or greater spatial scales, the encounter rate of *T. tubifex*

with *M. cerebralis* myxospores probably limits disease prevalence in *T. tubifex* in aquatic systems.

The 2 geographically distinct populations of *T. tubifex* produced widely different numbers of triactinomyxons when exposed to similar doses of *M. cerebralis* myxospores. The MW *T. tubifex* produced over 10 times more triactinomyxons than the MR worms. Moreover, only the MW population produced more triactinomyxons than myxospores received when myxospore doses were high. Clearly, the transmission rate of *M. cerebralis* from worm to fish would be higher for MW than MR worms. If such variation in the ability to produce triactinomyxons is widespread among populations of *T. tubifex*, differences in the ability to transmit *M. cerebralis* from worm to fish might help explain the geographic variation of effects of the disease on fish (Baldwin et al., 1998).

The mechanism causing the lower triactinomyxon production of the MR *T. tubifex* is unknown but could be related to differences in the biology of the worm populations or in the host–parasite interaction. Although we did not know the exact age or developmental stage of worms at the beginning of the experiment, MR worms were larger and probably in a different developmental stage than MW worms. However, MR worms have consistently produced fewer triactinomyxons than the MW worms in other experiments where the initial developmental state of the worms and the environment were different than the conditions outlined in this experiment (data not shown). Moreover, the fact that both *T. tubifex* populations began producing triactinomyxons at the same time makes the initial differences in developmental stage an unlikely explanation for the variation in triactinomyxon production.

Our results suggest that the host–parasite relationship may vary among populations of *T. tubifex*. Using a conserved genetic marker, the internal transcribed spacer region 1 of the ribosomal RNA gene locus (ITS-1), we have shown that the 2 populations of worms used in this experiment are genetically

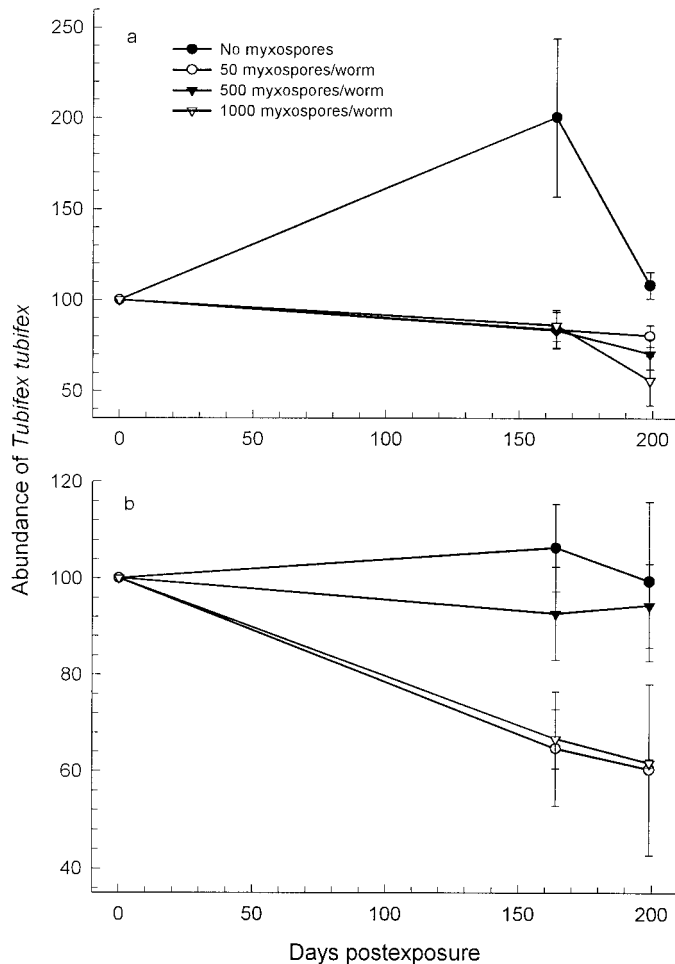


FIGURE 4. Worm abundance of the (a) Madison River and (b) Mount Whitney populations of *Tubifex tubifex* when given varying doses of *Myxobolus cerebralis* myxospores. Tukey's pairwise comparisons indicated that worm abundance was higher in negative controls than in myxospore treatments on day 164 postexposure; however, on day 199 postexposure worm abundance only differed between negative controls and the 1,000 myxospore per worm treatment ($P < 0.05$). Error bars are ± 1 SE of the mean.

distinct (data not shown). Because *M. cerebralis* is a recent invader to North America, perhaps genetically distinct populations of *T. tubifex* are differentially preadapted to infection by *M. cerebralis*. Moreover, the geographic relationships among genetically distinct populations of *T. tubifex* are complex (Sturmbauer et al., 1999; K. Beauchamp, pers. comm.), and some *T. tubifex* populations could also be recent invaders. How widespread such genetic differences in *T. tubifex* are, the generality of variation in triactinomyxon production among genetically distinct populations and how such variation in triactinomyxon production is related to spatial variation in disease in salmonids warrant future research.

Growth, reproduction, and mortality were all negatively affected when laboratory *T. tubifex* populations were infected by *M. cerebralis*. Perhaps WD infection influences the ability of *T. tubifex* to absorb nutrients that would be used for growth or reproduction depending on the developmental state of the worms. For example, the MR *T. tubifex* were large at the start

of the experiment, and worms in the replicates not exposed to myxospores reproduced (abundance of worms increased) and average weight of individual worms declined presumably because of the addition of juvenile worms. Worms in contact with myxospores did not reproduce (abundance remained relatively constant) and average weight of individual worms declined presumably because of *M. cerebralis* infection. In contrast, individual MW *T. tubifex* were small at the beginning of the experiment, and their abundance did not increase during the experiment. However, individual MW worms that were not exposed to myxospores gained more weight than those exposed to myxospores. Moreover, abundance of MW worms declined dramatically in 2 treatments with myxospores and remained relatively constant in the negative controls, suggesting that infected worms had higher mortality than uninfected ones. Why worm abundance did not decline in the 500 myxospores per worm treatment is unknown. Mortality might be an indirect effect of competition for nutrients within a population because infected *T. tubifex*, when held individually, can live almost 2 yr (Gilbert and Granath, 2001). Our results suggest that the negative effects of *M. cerebralis* infection on *T. tubifex* are complex and may depend on developmental state of worms. We will examine these aspects in future experiments. The impact of these phenomena upon wild oligochaete populations in aquatic systems is likely to be slight, because of the low infection rates typically observed in wild *T. tubifex* and the potential of other factors, such as environmental conditions, to have a greater effect on worms. However, it is possible that the disease will become self-limiting when prevalence is very high.

In conclusion, our work showed that *T. tubifex* populations produced similar numbers of triactinomyxons when challenged with widely varying doses of *M. cerebralis* myxospores. *T. tubifex tubifex* populations from different geographic regions produced different numbers of triactinomyxons when given the same dose of *M. cerebralis* myxospores. Moreover, *M. cerebralis* infection had an impact on reproduction and growth of the obligate host, *T. tubifex*. This work indicated that the differential ability of *M. cerebralis* to proliferate in *T. tubifex* populations may contribute to the variable levels of disease severity seen among stream salmonid populations.

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