Correlations among environmental features, Myxobolus cerebralis infection prevalence in oligochaetes, and salmonid infection risk in the Madison River, Montana
by Rebecca Caroline Krueger

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biological Sciences
Montana State University

Abstract:
Myxobolus cerebralis, the causative agent of whirling disease in salmonids, has had detrimental effects on several salmonid populations in the Intermountain West, including the rainbow trout in the Madison River, Montana. Myxobolus cerebralis is dependent upon both a salmonid and an oligochaete host, Tubifex tubifex, to complete its life cycle. The goal of this study was to determine the influence of features of the physical and chemical environment, the density of infected T. tubifex, and the densities of other oligochaetes on rainbow trout infection in side channels of the Madison River. I hypothesized that the interactions among the environment, infection in T. tubifex, and densities of other oligochaetes would affect the risk of M. cerebralis infection to rainbow trout and that side channels would differ in their production of the parasite. Features of the environment were measured in side channels of the Madison River and differences were described with a principal components analysis. The densities of infected T. tubifex, densities of other oligochaetes, and rainbow trout infection risk (using sentinel rainbow trout) were also measured in the side channels and compared with regression analyses. Fine sediments, slow mean water velocities, narrow side channel widths, and low maximum, high minimum, and low mean water temperatures were the most influential factors of the principal components analysis that exhibited positive relationships to infection in T. tubifex and rainbow trout infection risk. The density of infected T. tubifex exhibited the strongest relationship with rainbow trout infection risk and was better predicted by the physical environment compared to other estimates of T. tubifex and tubificid populations. Side channels differed in rainbow trout infection risk and in their contributions to M. cerebralis in the Madison River. Furthermore, certain features of the environment predicted infection in T. tubifex and infection risk in rainbow trout. The ability to predict and locate areas of high infection is essential for management of whirling disease in the Madison River and essential for improving our understanding of the dynamics of M cerebralis.

CORRELATIONS AMONG ENVIRONMENTAL FEATURES, *MYXOBOLUS CEREBRALIS* INFECTION PREVALENCE IN OLIGOCHAETES, AND SALMONID INFECTION RISK IN THE MADISON RIVER, MONTANA

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Rebecca Caroline Krueger

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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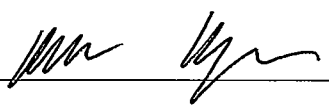
Signature 

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TABLE OF CONTENTS

TABLE OF CONTENTS– CONTINUED Page

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Myxobolus cerebralis, the causative agent of whirling disease in salmonids, has had detrimental effects on several salmonid populations in the Intermountain West, including the rainbow trout in the Madison River, Montana. *Myxobolus cerebralis* is dependent upon both a salmonid and an oligochaete host, *Tubifex tubifex*, to complete its life cycle. The goal of this study was to determine the influence of features of the physical and chemical environment, the density of infected *T. tubifex*, and the densities of other oligochaetes on rainbow trout infection in side channels of the Madison River. I hypothesized that the interactions among the environment, infection in *T. tubifex*, and densities of other oligochaetes would affect the risk of *M. cerebralis* infection to rainbow trout and that side channels would differ in their production of the parasite. Features of the environment were measured in side channels of the Madison River and differences were described with a principal components analysis. The densities of infected *T. tubifex*, densities of other oligochaetes, and rainbow trout infection risk (using sentinel rainbow trout) were also measured in the side channels and compared with regression analyses. Fine sediments, slow mean water velocities, narrow side channel widths, and low maximum, high minimum, and low mean water temperatures were the most influential factors of the principal components analysis that exhibited positive relationships to infection in *T. tubifex* and rainbow trout infection risk. The density of infected *T. tubifex* exhibited the strongest relationship with rainbow trout infection risk and was better predicted by the physical environment compared to other estimates of *T. tubifex* and tubificid populations. Side channels differed in rainbow trout infection risk and in their contributions to *M. cerebralis* in the Madison River. Furthermore, certain features of the environment predicted infection in *T. tubifex* and infection risk in rainbow trout. The ability to predict and locate areas of high infection is essential for management of whirling disease in the Madison River and essential for improving our understanding of the dynamics of *M. cerebralis*.

INTRODUCTION

Parasite-host relations play important roles in most ecosystems, including parasite regulation of host population growth and host evolution (Anderson and May 1979; May and Anderson 1979). Parasites can indirectly influence predation and competition of host populations by affecting host behavior and physiology. Parasites can also directly influence host demography by affecting host survival and fecundity. All of these indirect and direct effects influence the diversity and abundance of organisms (Minchella and Scott 1991). *Myxobolus cerebralis* (Myxozoa: Myxosporea), the causative agent of whirling disease in salmonids, strongly influences and relies upon both oligochaete and salmonid hosts to complete its life cycle (Markiw and Wolf 1983; Wolf and Markiw 1984). *Myxobolus cerebralis* was discovered in Europe in 1893 and appeared in the United States in 1958 (Hoffman 1990). Although it is currently found in 23 states, major declines in wild salmonids have thus far only been found in the Intermountain West (Nehring and Walker 1996; Vincent 1996).

Myxobolus cerebralis has a complex life cycle with two distinct spore stages, the myxospore and the triactinomyxon (Figure 1). The triactinomyxon (TAM) stage of *M. cerebralis* is released into the water column by infected *Tubifex tubifex* (Oligochaeta: Tubificidae), the only known oligochaete host (Wolf et al. 1986; Kerans et al. in preparation). The TAM drifts in the water column and can be viable for at least 15 days at temperatures between 6 and 15 °C (El-Matbouli et al. 1999). Upon contact with a fish, the sporoplasm cells of the TAM penetrate the epidermis and enter the peripheral and central nervous system, where they multiply (El-Matbouli et al. 1995). The

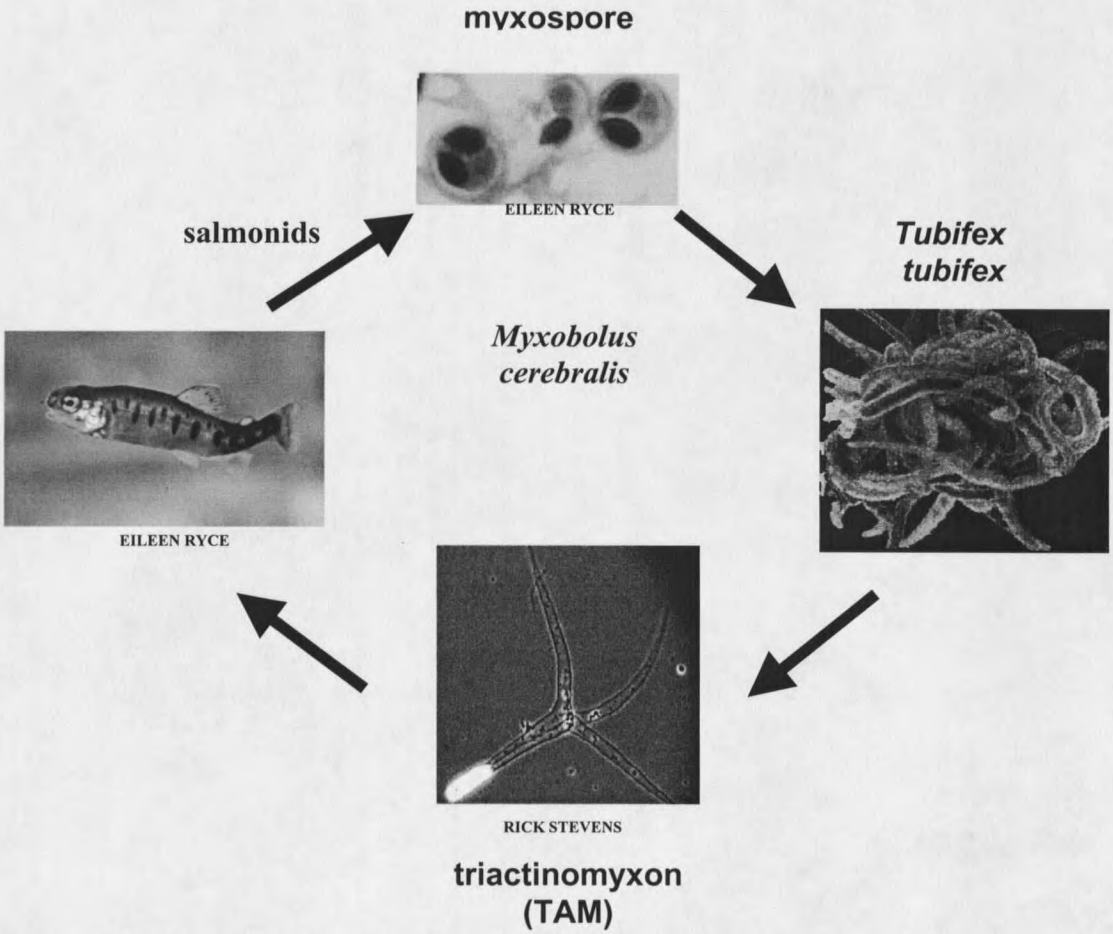

Figure 1. The life cycle of *Myxobolus cerebralis* is dependent upon both a salmonid and a *Tubifex tubifex* host. *Myxobolus cerebralis* has two distinctive stages within its life cycle: the myxospore stage and the triactinomyxon stage that are infective to the worm and fish respectively.

resulting sporoplasms then enter the vertebral and cranial cartilage of the fish host where they cause lesions of head and other tissue. These lesions induce symptoms including whirling behavior and darkening of the tail, and may result in mortality (Hoffman 1990). *Myxobolus cerebralis* myxospores develop about three months after sporoplasm penetration of the salmonid host (Halliday 1976; Markiw 1992). Myxospores, which are infectious to *T. tubifex* (Markiw and Wolf 1983; Wolf and Markiw 1984), are released into the sediment upon death of the salmonid and subsequent decomposition of host

tissue (El-Matbouli et al. 1992). The myxospore stage can resist degradation through a variety of adverse conditions for over 5 months when held at 13°C (El-Matbouli and Hoffman 1991), and possibly up to 30 years (Hoffman and Putz 1969). *Tubifex tubifex* ingest the myxospores, which then penetrate between the epithelial cells of the intestine of the oligochaete and multiply. Mature TAMs are released into the gut and subsequently into the water about 80 to 110 days after initial ingestion of myxospores (Markiw 1986; El-Matbouli and Hoffman 1998; Stevens et al. 2001).

Salmonids differ widely in their susceptibility to *M. cerebralis*. Rainbow trout (*Oncorhynchus mykiss*) are the most susceptible species and as a result have undergone severe population declines from whirling disease (Hedrick et al. 1998). In contrast, arctic grayling (*Thymallus arcticus*) and bull trout (*Salvelinus confluentus*) are not susceptible to infection by *M. cerebralis*. Additionally, rainbow trout less than nine weeks post-hatch are the most susceptible to the development of whirling disease in part because of their abundance of cartilage relative to body size, whereas older fish are relatively immune to whirling disease induced mortality (Hoffman and Byrne 1974; Markiw 1991; E. K. N. Ryce, Montana State University, personal communication)

The effects of *M. cerebralis* on rainbow trout populations appear to vary in the environment. Some areas where *M. cerebralis* has been introduced, including certain drainages in Montana (Vincent 1996; Baldwin et al. 1998) and Colorado (Nehring and Walker 1996), have suffered declines in wild rainbow trout populations. In other areas where *M. cerebralis* has been introduced, such as drainages in California (Moden 1998) and Oregon (Sandell et al. 2001), rainbow trout populations have not declined.

On smaller spatial scales, the risk of the exposure of wild rainbow trout to *M. cerebralis* can vary among locations within a river where *M. cerebralis* is present. Exposure risk to wild rainbow trout is often examined using the prevalence of infection and the severity of the disease in juvenile, hatchery born rainbow trout of known size, age, and strain held in sentinel cages. Using sentinel fish eliminates confounding factors involved with studying infection in a natural population, including fish migration (Baldwin et al. 1998), predation of weaker, diseased fish (Hoffman 1990), and other causes of fish mortality. Infection severity in fish is typically ranked from zero to four on the MacConnell-Baldwin scale (Baldwin et al. 2000), or the modified zero to five scale (Hedrick et al. 1999a), based on histological examination of the number and severity of lesions in the head region. The prevalence of infection in sentinel rainbow trout varied between 0 to 100% in the Lostine River in Oregon (Sandell et al. 2001). Moreover, the severity of infection in sentinel rainbow trout varied from nonexistent to severe across several locations within Willow Creek, Montana (Baldwin et al. 2000), within the Madison River, Montana (Vincent 2000), and within the South Fork of the Boise River, Idaho (Hiner and Moffitt 2001).

The prevalence of infection in oligochaetes can also vary spatially within a river. The prevalence of infection in worms also provides information on infection risk to rainbow trout because whirling disease severity in rainbow trout is correlated positively to the number of TAMs to which a fish is exposed (Markiw 1992). The prevalence of infection in wild tubificid populations [assessed with genetic analyses that detected *M. cerebralis* DNA (Andree et al. 1997; 1998)] varied between 0 and 4.6% in one stream

in Montana (Rognlie and Knapp 1998) and between 1.1 and 6.8% in one stream in Colorado (Zendt and Bergerson 2000).

The factors causing such spatial variation in infection remain unclear. Variation and interactions among features of the physical and chemical environment likely play important roles in determining the abundance of *T. tubifex* and the prevalence of *M. cerebralis* infection in *T. tubifex*. Furthermore, environmental features and densities of infected *T. tubifex* probably influence the risk of infection in sentinel rainbow trout. Figure 2 shows the potential interactions between the physical and chemical environment, *T. tubifex*, other oligochaetes, and sentinel rainbow trout as they relate to the *M. cerebralis* infections.

Certain features of the physical and chemical environment may encourage high densities of *T. tubifex*, thereby providing host habitat for *M. cerebralis* (Figure 2, Arrow A) and leading to high risks of whirling disease in rainbow trout. *Tubifex tubifex* are commonly found in areas with high amounts of clay and silt sediments (Sauter and Gude 1996; Zendt and Bergerson 2000), high amounts of bacteria commonly found in organic matter (Lazim and Learner 1987), and slow water velocities (Brinkhurst 1996). Though *T. tubifex* are found in a wide variety of habitats, including oligotrophic areas (Brinkhurst 1964), they are often associated with degraded habitats that have low dissolved oxygen concentrations and high amounts of siltation (Reynoldson 1987; Casellato and Caneva 1994; Finogenova 1996; Zendt and Bergerson 2000).

Features of the environment may also influence the prevalence of *M. cerebralis* infection in *T. tubifex* populations by influencing the prevalence of myxospores in the

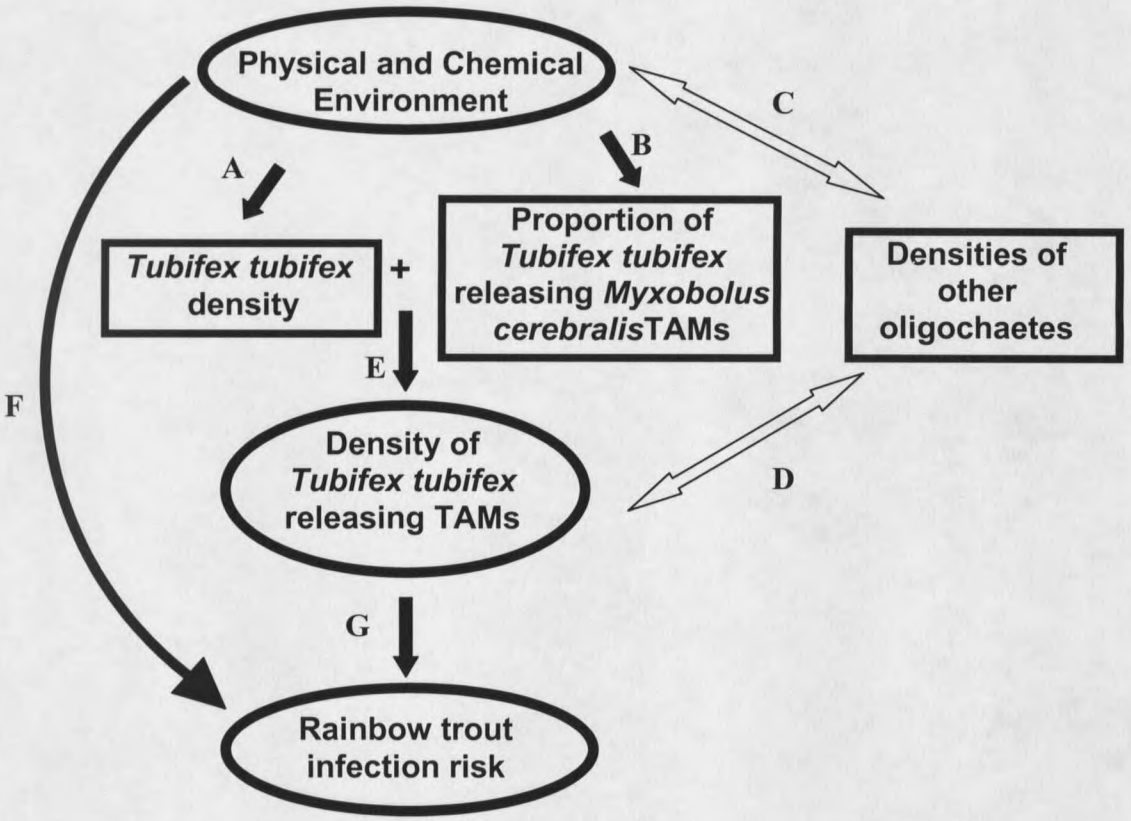

Figure 2. Conceptual diagram of the possible linkages among the physical and chemical environment, density of *T. tubifex* releasing *Myxobolus cerebralis*, other oligochaetes, and rainbow trout infection risk as they relate to salmonid whirling disease. Hollow arrows represent indirect relationships with *M. cerebralis*.

environment or the development of infection in *T. tubifex* (Figure 2, Arrow B). Riverbed sediment type may have different capacities for holding myxospores. Smaller, more adhesive sediments may hold myxospores better than larger sediments, thereby increasing the likelihood that *T. tubifex* contacts and becomes infected with *M. cerebralis* (Lemmon and Kerans 2001). Areas with slow water velocities may also

contain higher densities of *M. cerebralis* myxospores due to increased deposition. Temperature may influence the development of *M. cerebralis* in *T. tubifex*. For example, when infected tubificids were transferred from temperatures of 15 °C to higher temperatures of 25 °C and 30 °C they immediately released more TAMs, but for a shorter period of time, than tubificids that were transferred to lower temperatures of 5 – 15 °C (El-Matbouli et al. 1999). Features of the environment could therefore affect infection of *T. tubifex* by influencing the densities of *T. tubifex*, the densities of *M. cerebralis* myxospores, and the interactions between *M. cerebralis* and *T. tubifex* (Figure 2, Arrow E).

Environmental conditions may also control the distribution and abundance of other oligochaetes (Figure 2, Arrow C), which, in turn, could affect *T. tubifex* densities and prevalence of infection (Figure 2, Arrow D). Substrate composition and water flow are often considered determinants of oligochaete distributions (Birtwell and Arthur 1980; Lazim and Learner 1987; Dumnicka 1994; Juget and Lafont 1994; Sloreid 1994). Relative abundances of naidids are low and tubificids and enchytraeids are high in areas with slow water velocities and high rates of sedimentation (Robbins et al. 1989; Sloreid 1994). Naidids can adapt well to faster water velocities and different sediment compositions in part because of their asexual reproduction, lesser dependence on living in the sediment, and varied food sources (Juget and Lafont 1994; Sloreid 1994), whereas enchytraeids and tubificids, including *Limnodrilus hoffmeisteri*, *Ilyodrilus templetoni*, *Rhyacodrilus coccineus*, and *T. tubifex,* are often found in areas with slower water velocities because of their dependence on sexual reproduction and sediment

dwelling (Sloreid 1994). Dissolved oxygen concentrations and water temperatures can also influence the abundance and composition of oligochaete species (Reynoldson 1987). *Ilyodrilus templetoni*, for example, is fairly intolerant of anoxic conditions, whereas *L. hoffmeisteri* and *T. tubifex* are more tolerant of anoxia and can also withstand higher water temperatures (Chapman et al. 1982). *Limnodrilus hoffmeisteri*, like *T. tubifex*, is common in both extremely oligotrophic and extremely eutrophic habitats where benthic diversity is low (Milbrink 1983).

Interactions within an oligochaete assemblage may affect the abundance of infected *T. tubifex* (Figure 2, Arrow D). Other tubificids may decrease the number of active *M. cerebralis* myxospores in sediments, presumably through ingestion and deactivation. In laboratory experiments, mixed colonies of *T. tubifex* and non-susceptible tubificids produced fewer *M. cerebralis* TAMs than pure colonies of *T. tubifex* (M. El-Matbouli, Institute of Zoology, Munich Germany; M. Gay, T. S. McDowell, M. P. Georgiadis, R. P. Hedrick, University of California, Davis, CA, personal communication). Furthermore, resistant oligochaete species could be more likely to outcompete diseased *T. tubifex* than healthy *T. tubifex* because infected *T. tubifex* have significantly lower reproductive rates than uninfected *T. tubifex* (Stevens et al. 2001).

Environmental factors also may influence the risk of *M. cerebralis* infection to rainbow trout (Figure 2, Arrow F). Severities of infection in sentinel rainbow trout exposed in the Madison River, Montana appear to be highest at water temperatures between 9 and 17 °C, with infection peaking at 14 °C (E. R. Vincent, Montana Fish,

Wildlife and Parks, personal communication). Moreover, water temperatures between 6.2 to 11.2 °C were correlated positively to the severity of infection in sentinel rainbow trout exposed in Willow Creek, Montana (Baldwin et al. 2000). Additionally, water velocities have correlated negatively to infection severity in sentinel rainbow trout and to infection severity in rainbow trout held in controlled laboratory conditions (E. R. Vincent, personal communication). Furthermore, water conductivity has correlated positively to the prevalence of infection in sentinel rainbow trout (Sandell et al. 2001).

As a consequence of the natural variations in infection risk, where and when susceptible rainbow trout reside can influence their exposure to the parasite (Downing 2000). Spawning and rearing grounds are likely candidate areas where young fish may be exposed to *M. cerebralis*. Rainbow trout and brown trout (*Salmo trutta*), another carrier of *M. cerebralis* myxospores (Hedrick et al. 1999a), are likely to die in these areas after they spawn because of the high energy expenditure incurred (Kerans and Zale 2002), and, if infected can release millions of myxospores (Hedrick et al. 1999b; E. K. N. Ryce, personal communication). Recently hatched fry that are highly susceptible to *M. cerebralis* remain in spawning areas where they are likely at risk of encountering *M. cerebralis* (Hoffman and Byrne 1974; Markiw 1991; E. K. N. Ryce, personal communication). Therefore, salmonid spawning and rearing areas may be areas where *M. cerebralis* infections are perpetuated within systems.

Understanding the interactions among characteristics of the environment and *M. cerebralis* infection in *T. tubifex* and the resulting risk of infection in rainbow trout in spawning and rearing grounds could provide useful information for predicting future *M.*

cerebralis infections, and for planning appropriate management solutions aimed at reducing disease severity. Management techniques so far have focused on disease severity in wild rainbow trout; however, to truly understand and manage rainbow trout in infected systems, the potential explanatory variables of *M. cerebralis* infections must be investigated. Understanding these relationships in natural ecosystems is essential to continue to manage trout as a wild fishery and to maintain strong tourist-based economies upon which many local communities depend.

The goal of this study was to examine interactions among features of the environment, oligochaete assemblages, *M. cerebralis* infection in *T. tubifex*, and the resulting infection risk to salmonids (Figure 2). This study was conducted in the side channels of the Madison River, Montana, where whirling disease has severely reduced rainbow trout populations (Vincent 1996). Side channels have varying abundances of oligochaetes (B. L. Kerans, Montana State University, personal communication), rainbow trout redds and fry (Downing 2000), and risk of rainbow trout infection (Vincent 2000). The specific objectives of the study were to 1) determine whether physical and chemical features of the environment correlated to *M. cerebralis* infection in *T. tubifex* (Figure 2, Arrow E) and sentinel rainbow trout (Figure 2, Arrow F), 2) determine whether the densities of other oligochaetes related to features of the physical and chemical environment and to the densities of infected *T. tubifex* (Figure 2, Arrows C and D), and 3) determine if the densities of infected *T. tubifex* correlated to the differential risk of infection severity in sentinel rainbow trout (Figure 2, Arrow G) in the Madison River.

STUDY AREA

The Madison River originates at the confluence of the Firehole and Gibbon Rivers in Madison Junction in Yellowstone National Park, Wyoming, and flows north for 193 km through the southwestern portion of Montana until it meets the Gallatin and Jefferson Rivers to form the Missouri River near Three Forks, Montana (Figure 3). The total drainage area of the Madison River is 6475 km^2 (Vincent 1987). This study was located in the upper Madison River between Quake Lake and Ennis Lake, in Madison County, Montana (Figure 3). In this reach, the elevation is between 1665 m – 1995 m, with an average gradient of 6 m/km (Vincent 1987). Hebgen Dam controls the flow of the upper Madison River below Hebgen Lake, with an annual average discharge of 48 m^3/s and maximum discharge of 99 m^3/s (recorded in the middle of the study section), typically occurring in late May or early June. The dam is regulated by a water flow agreement that dictates an annual minimum discharge of at least 17 m^3/s (Vincent 1996).

The climate of the area varies along the river with average annual liquid precipitation of 72 cm at Hebgen Lake to 32 cm in Ennis and average total snowfall of 538 cm at Hebgen Lake and 80 cm at Ennis (NOAA 2001). Mean daily July–August water temperatures average 14 °C and rarely exceed 21 °C (Vincent 1996). The watershed of the upper Madison consists of alkaline soils in the formation of broad terraces that were caused by fault movements along the Madison Range front (Alt and Hyndman 1997) and is bounded by mountain ranges. The watershed consists of primarily field pasture and forested areas with residential and commercial areas

consisting of less than 15 % of the total landscape. About half of the riparian vegetation consists of grasses, with about one third shrubs and the rest woody trees and herbaceous plants (personal observation). Varying degrees of erosion are evident along the banks and are in part caused by ranching, recreational, and road uses (personal observation).

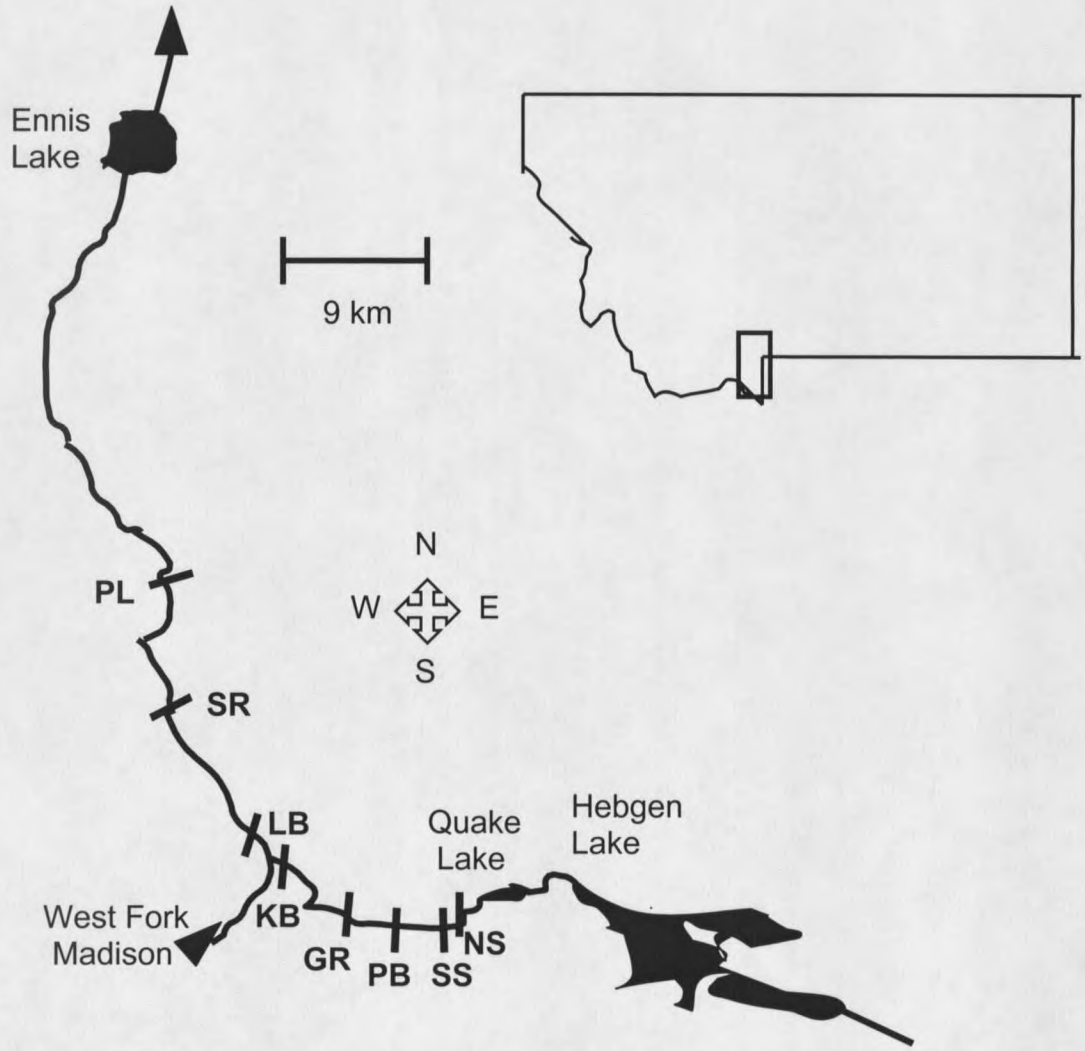

Figure 3. Side channel locations in the upper Madison River, Montana. Side channels shown from upstream to downstream are: North Slide (NS), South Slide (SS), Pine Butte (PB), Grizzly (GR), Kirby Bridge (KB), Lyons Bridge (LB), Sun Ranch (SR), Palisades (PL).

The Madison River contains numerous side channels and tributaries. Eight side channels were investigated in this study: North Slide, South Slide, Pine Butte, Grizzly, Kirby Bridge, Lyons Bridge, Sun Ranch, and Palisades. They were chosen to represent different combinations of rainbow trout redd and rearing densities (Downing 2000), whirling disease severity (Vincent 2000), and oligochaete densities (B. L. Kerans, personal communication) (Table 1). Side channels were located along a 39 km section of the upper Madison River (Figure 3) in an area where whirling disease has caused severe population declines in rainbow trout. Side channels consisted mostly of riffle habitat with some small pools. Cobble and gravel dominated the riffle habitat and finer sediments dominated the pools. The side channels ranged from 6.0 to 17.6 m in mean width and from 0.17 to 0.40 m in mean depth (see Table 3). Study section lengths within each side channel were determined as the straight-line distance between the upstream and downstream ends of the study sections and measured with Global Positioning System coordinates. Study sections lengths varied between 32 – 494 m.: four study sections were under 50 m., two were around 150 m., and two were under 500 m. Water velocities in the side channels averaged 0.32 to 1.34 m/s (see Table 3). A variety of oligochaetes inhabited the side channels, including the families of Enchytraeidae, Lumbricidae, Naididae, and Tubificidae (B. L. Kerans, personal communication).

Salmonid species in the Madison River include the nonnative rainbow trout, brown trout, and brook trout (*Salvelinus fontinalis*), and the native westslope cutthroat trout (*Oncorhynchus clarki lewisi*), arctic grayling (*Thymallus arcticus*), and mountain

whitefish *(Prosopium williamsoni)* (FERC 1997). Rainbow trout, brown trout, and brook trout were originally introduced into the Madison River in the late 1800s. Rainbow and brown trout were stocked in the Madison River from 1948 through 1974, when stocking was discontinued to encourage wild populations to increase in biomass and numbers (Vincent 1987). Montana Fish Wildlife and Parks has subsequently managed the Madison River as a wild trout fishery.

Table 1. Characteristics that were used to select the eight side channels in the Madison River, Montana, USA. Selections were based on data from 1997 and 1998 on the severity of whirling disease risk as assessed by sentinel rainbow trout (Vincent 2000), the densities of rainbow trout redds (Downing 2000), and the densities of oligochaetes (B. L. Kerans, Montana State University, unpublished data).

Side channel	Whirling disease severity	Rainbow trout redd density	Oligochaete density
North Slide	low	high	high
South Slide	high	high	--
Grizzly	--	moderate-high	--
Pine Butte	moderate	high	low
Kirby Bridge	high	low	moderate
Lyons Bridge	--	low	--
Sun Ranch	--	moderate-high	--
Palisades	high	low	low

Whirling disease has negatively affected the salmonid populations of the Madison River (Vincent 1996). During the 1970s and 1980s, rainbow trout populations were consistently estimated at about 2500 fish/km. However, the population declined to about 10 % of its former abundance in 1991 (Vincent 1996), and whirling disease is thought to be responsible. The Department of Montana Fish, Wildlife and Parks first investigated whirling disease in the Madison River in 1994, when they collected whirling disease-positive yearling and age-zero rainbow trout from several sites

(Vincent 1996). Although the abundance of the rainbow trout population dropped appreciably, it stabilized at a lower level, indicating that some rainbow trout are escaping whirling disease-induced mortality. Differing levels of infection in the rainbow trout and *T. tubifex* hosts among side channels may explain which fish are surviving whirling disease, and how.

METHODS

Physical and Chemical Environment

Physical and chemical characteristics of the eight side channels were measured to investigate their relationships to *M. cerebralis* infection in oligochaetes and infection risk to salmonids. Characteristics measured were sediment size composition, percentages of organic matter on and in the sediments, side channel width, water depth, current velocity, water temperature, dissolved oxygen concentration, conductivity, and pH.

The size composition of the sediments was assessed using three techniques that when combined partitioned sediments into seven categories: boulder, cobble, pebble, gravel, sand, silt, and clay (Cummins 1962). The first technique differentiated sediment into fine (< 2 mm diameter) and non-fine (> 2 mm diameter) categories using a surface-fines grid. The surface-fines grid consisted of a 320 mm by 320 mm Plexiglas square with a seven by seven grid drawn on it with each line 40 mm apart. (Overton et al. 1997; T. McMahon, Montana State University, personal communication). The lines of the grid were each 2 mm in diameter, the upper size limit of a surface fine. Substrates that were visible from under line intersections when the grid was placed on the surface of the water were counted and recorded as non-fines because they were visible around the 2-mm cross sections. The count of sediments visible around the 2-mm cross sections was divided by 49 (the total number of line intersections) to estimate the proportion of non-fines for that sample.

The proportion of non-fines was subtracted from 1 to calculate the proportion of fines. Ten transects perpendicular to the water flow were randomly chosen in side channels in July 1999. Three surface-fines grids were randomly placed along each transect, for a total of 30 surface-fines grid measurements in most side channels. Exceptions included Palisades, where 32 measurements were taken, and South Slide, where 30 transects were randomly chosen and one surface-fines grid measurement was taken on each transect because of the small width of the channel. The average proportions of fines and non-fines were calculated for each side channel by averaging the 30 individual measurements.

Pebble counts (Wolman 1954; Marcus et al. 1995), the second technique, were conducted to determine the composition of sediments larger than 2 mm in diameter. Randomly selected transects perpendicular to stream flow were walked toe to heel, and the intermediate axis of each mineral sediment particle greater than 2 mm was measured at each step. New transects were walked until the transect upon which the 200^{th} particle was measured was completed for each side channel. The sediments were organized into the modified Wentworth classification categories for sediment sizes of boulder (>256 mm), cobble (64 to 256 mm), pebble (16 to 64 mm), and gravel (2 to16 mm) (Cummins 1962), and the proportions of each category were calculated for each side channel.

A hydrometer gravimetric method, the third technique, was used to assess the composition of fines less than 2 mm in diameter (Day 1965). A syringe with a 2 mm opening was used to remove sand, silt, and clay sediments from 30 locations in each

side channel along randomly selected transects. The 30 samples were randomly combined into two sediment samples per side channel except at South Slide where one combined sample was lost. The samples were dried and about 50 g of each sample analyzed using gravimetric methods (Day 1965). The means and standard errors of the proportions of sand, silt, and clay from the two sediment samples were calculated for each side channel.

Surface-fines grid estimates were combined with pebble count and hydrometer estimates to obtain one measure of each sediment category per side channel. The mean proportion of non-fines estimated from surface-fines grid measurements was multiplied by the proportions of boulders, cobble, pebbles, and gravel that were estimated from pebble counts. The mean proportion of fines estimated from the surface-fines grid measurements was multiplied by the mean proportions of sand, silt, and clay that were estimated from hydrometer methods. The combinations of these estimates produced one estimate of the proportion of each sediment category for each side channel (see Table 2).

The organic contents of the sediments in each side channel were determined using dry weight analysis of benthic sediments and estimates of the abundance of coarse particulate organic material (CPOM) on the sediments. Two soil samples were taken with a trowel from the soft sediment of each side channel. All sediment samples were frozen until processed. About 30 g of wet sediment were used for each dry weight analysis. The proportion of organic material in each sample was determined as the difference between the dry weight (105 °C for 24 hr) and the