

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
© Copyright by Rebecca Caroline Krueger (2002)

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

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
Bozeman, Montana

May, 2002

N378
K9395

APPROVAL

of a thesis submitted by

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.

Dr. Billie Kerans _____ (Signature) 5/16/2002 Date

Approved for the Department of Ecology

Dr. Jay Rotella _____ (Signature) 5/16/02 Date

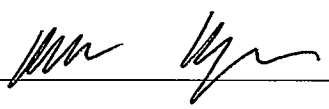
Approved for the College of Graduate Studies

Dr. Bruce McLeod _____ (Signature) 5-28-02 Date

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Montana State University, I agree that the Library shall make it available to borrowers under rules of the Library.

If I have indicated my intention to copyright this thesis by including a copyright notice page, copying is allowable only for scholarly purposes, consistent with "fair use" as prescribed in the U.S. Copyright Law. Requests for permission for extended quotation from or reproduction of this thesis in whole or in parts may be granted only by the copyright holder.

Signature 

Date 05/15/02

ACKNOWLEDGEMENTS

This research was funded by the National Partnership on Management of Wild and Native Coldwater Fisheries, and by Montana Fish, Wildlife and Parks. I am grateful for their support. I would also like to thank Lusha Alzner, Dan Downing, Cal Fraser, Amy Herrera, Jody Hupka, Jason Jannot, Jay Lemmon, Carter Maden, James Munro, Matt Norberg, Charlotte Rasmussen, Denine Schmitz, Leah Steinbach, Dick Vincent, and Julie Zickovich for their field and laboratory assistance and contributions to this research. I am also appreciative of Chelsea Cada, Bob Garrott, Rose Jaffe, Mandy Hardy, Steve Hess, Brett Marshall, and Jennifer Sands for their additional assistance in editing. I am grateful for my committee members Tom McMahon and Al Zale for their assistance in the study design and editorial comments. I would especially like to thank my advisor Billie Kerans for her continual guidance and support.

TABLE OF CONTENTS

Page

1. INTRODUCTION.....1

2. STUDY AREA.....11

3. METHODS.....16

Physical and Chemical Environment.....16
Oligochaete Densities.....20
Myxobolus cerebralis Infection in *Tubifex tubifex*.....23
Comparisons between Features of the Environment and Densities of Infected *Tubifex tubifex*.....28
Comparisons of Oligochaete Taxa with Features of the Environment and *Tubifex tubifex*.....28
Myxobolus cerebralis Infection in Sentinel Rainbow Trout.....29
Comparisons between Features of the Environment and Infection in Sentinel Rainbow Trout.....32
Comparisons between Infections of *Tubifex tubifex* and Sentinel Rainbow Trout.....32

4. RESULTS.....34

Physical and Chemical Environment.....34
Oligochaete Densities.....40
Myxobolus cerebralis Infection in *Tubifex tubifex*.....42
Comparisons between Features of the Environment and Densities of Infected *Tubifex tubifex*.....47
Comparisons of Oligochaete Taxa with Features of the Environment and *Tubifex tubifex*.....52
Myxobolus cerebralis Infection in Sentinel Rainbow Trout.....53
Comparisons between Features of the Environment and Infection in Sentinel Rainbow Trout.....55
Comparisons between Infections of *Tubifex tubifex* and Sentinel Rainbow Trout.....57

5. DISCUSSION.....62

REFERENCES CITED.....74

TABLE OF CONTENTS– CONTINUED Page

APPENDICES.....83

APPENDIX A.....84
Oligochaete Densities.....85
APPENDIX B.....87
Tubificid Triactinomyxon Release and Identification.....88
APPENDIX C.....90
Estimated Densities of Infected *Tubifex tubifex*.....91
APPENDIX D.....93
Differential Infection in Sentinel Rainbow Trout.....94

LIST OF TABLES

Table Page

1. Characteristics for Selection of Side Channels.....14

2. Composition of Side Channel Surface Sediments.....35

3. Chemical and Physical Measurements of Side Channels 36

4. Principal Components Analysis and Coefficient of Variation of Features of the Environment.....37

5. Densities of *Tubifex tubifex*.....43

6. Triactinomyxon Measurements 47

LIST OF FIGURES

Figure Page

1. Life Cycle of *Myxobolus cerebralis*.....2

2. Conceptual Diagram of Possible Linkages of Factors Relating to Whirling Disease.....6

3. Study Side Channels.....12

4. Features of Side Channels on Principal Component One and Principal Component Two Continua.....39

5. Densities of Mature *Tubifex tubifex*.....41

6. Percent *Tubifex tubifex* releasing Triactinomyxons.....44

7. Density of *Tubifex tubifex* releasing Triactinomyxons.....46

8. Relationships among Estimates of *Tubifex. tubifex* Infection and Principal Component One.....49

9. Relationships among Estimates of *Tubifex tubifex* Infection and Principal Component Two.....50

10. Correlations among Features of the Environment and the Density of Infected *Tubifex tubifex*.....51

11. Differential Mean Grade of Sentinel Rainbow Trout.....55

12. Relationships among Differential Mean Rainbow Trout Infection Grade, Principal Component One, and Principal Component Two.....58

13. Correlations among Features of the Environment and Differential Mean Rainbow Trout Infection Grade.....59

14. Relationships among *Tubifex tubifex* Infection Estimates and Differential Mean Rainbow Trout Infection Grade.....61

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

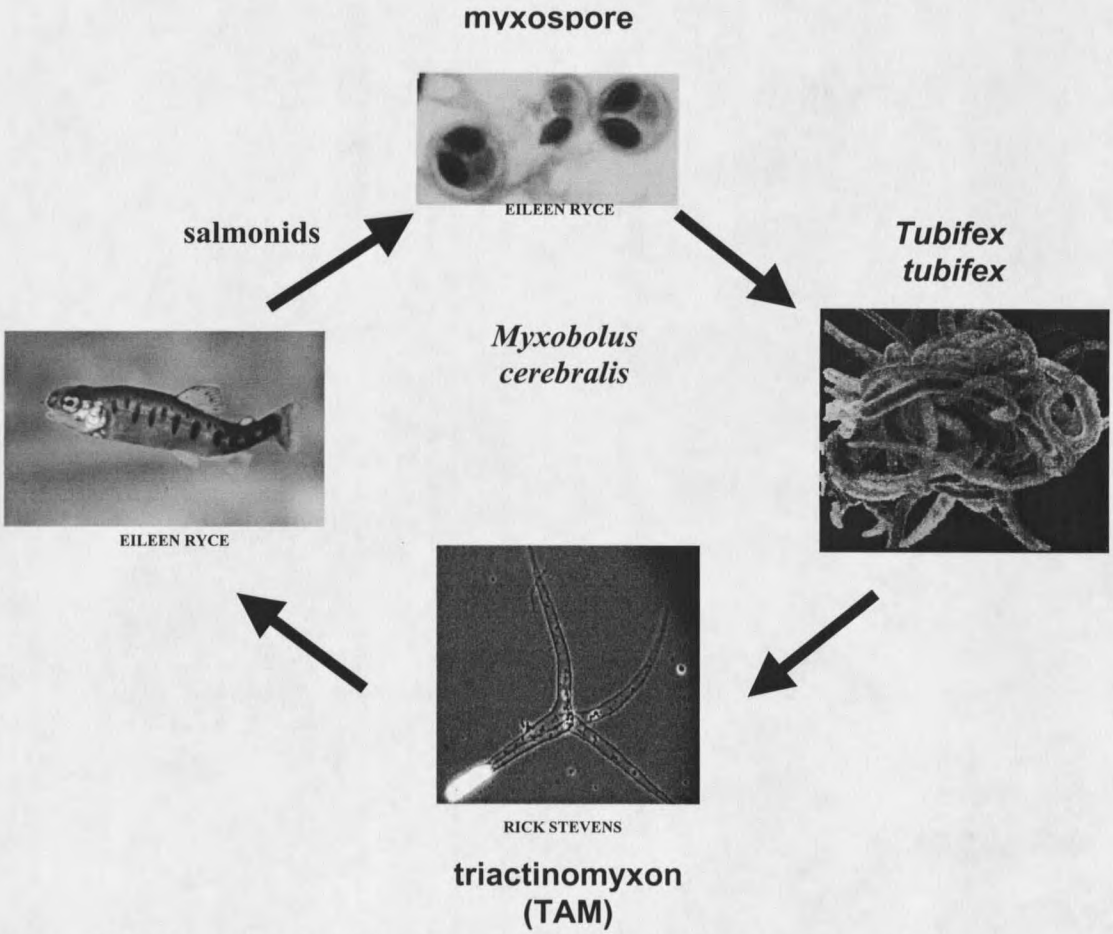


Figure 1. The life cycle of *Myxobolus cerebralis* is dependent upon both a salmonid and a *Tubifex tubifex* host. *Myxobolus cerebralis* has two distinctive stages within its life cycle: the myxospore stage and the triactinomyxon stage that are infective to the worm and fish respectively.

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

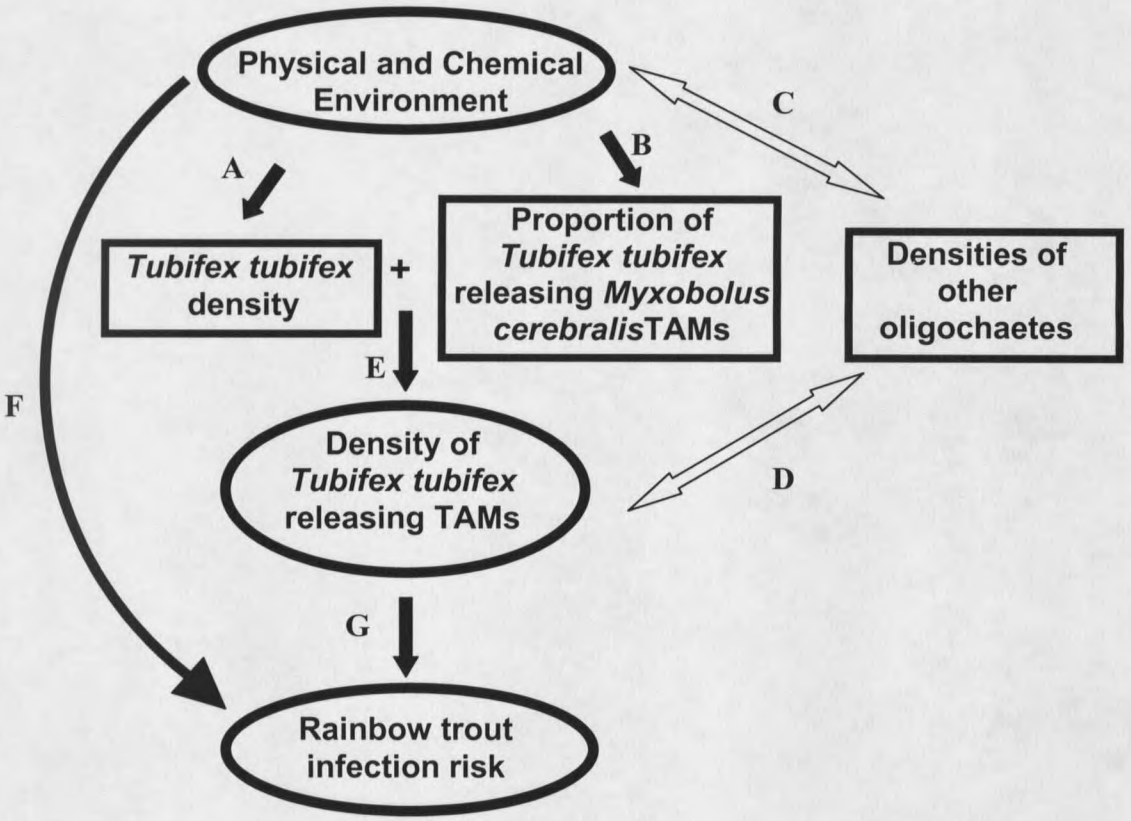


Figure 2. Conceptual diagram of the possible linkages among the physical and chemical environment, density of *T. tubifex* releasing *Myxobolus cerebralis*, other oligochaetes, and rainbow trout infection risk as they relate to salmonid whirling disease. Hollow arrows represent indirect relationships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

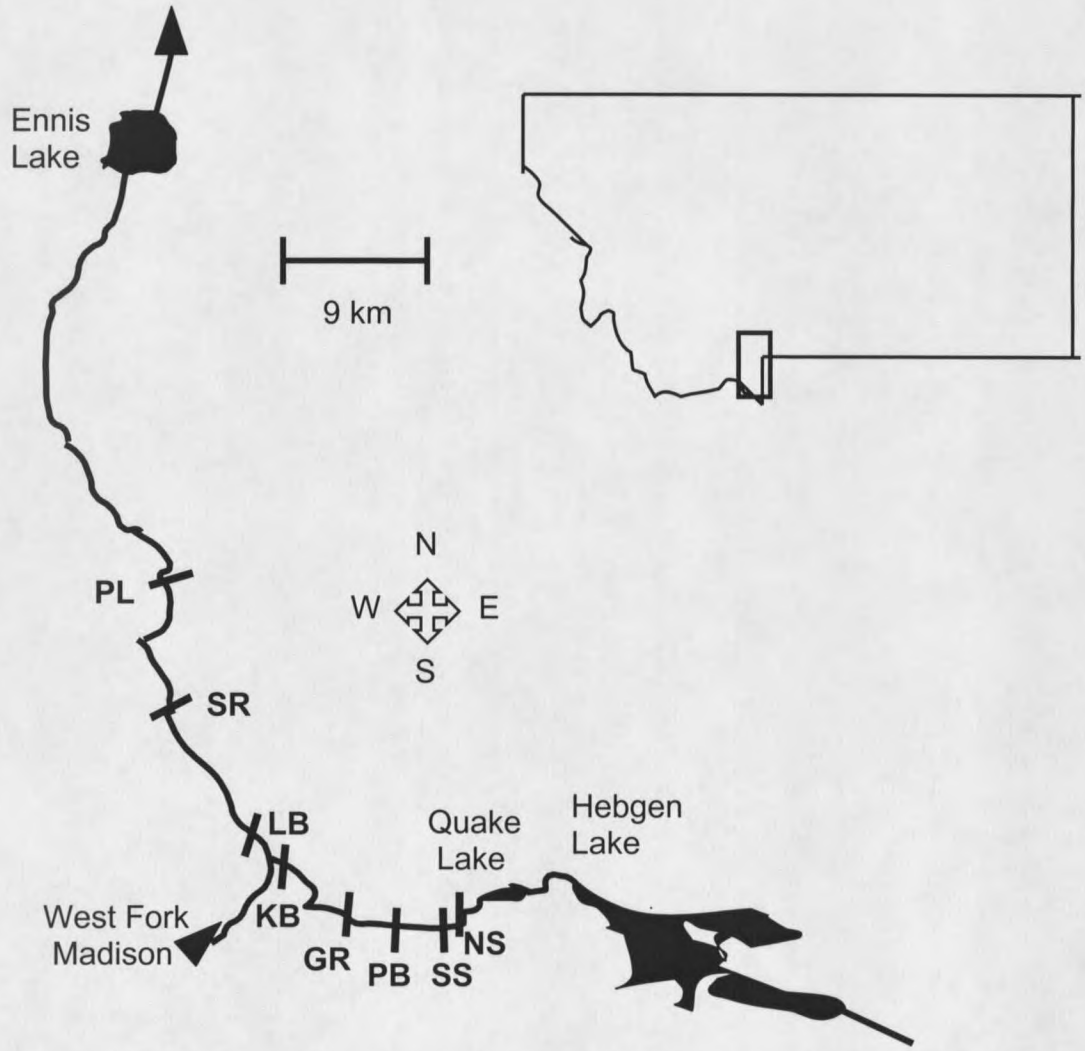


Figure 3. Side channel locations in the upper Madison River, Montana. Side channels shown from upstream to downstream are: North Slide (NS), South Slide (SS), Pine Butte (PB), Grizzly (GR), Kirby Bridge (KB), Lyons Bridge (LB), Sun Ranch (SR), Palisades (PL).

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

sample weight after it was burned in a muffle furnace for 2 hr at 550 °C, divided by the original dry weight (Wetzel and Likens 1991). Means and standard errors were calculated for the proportions of organic material in the sediments in each side channel. Estimates of CPOM were made by recording the presence or absence of CPOM at each step during the pebble count procedure. One CPOM estimate was made for each side channel by dividing the total number of CPOM particles encountered while walking the pebble count transects by the total number of transect observations.

Temperature (°C), dissolved oxygen concentrations (DO, mg/l), conductivity (mS/cm), and pH of the water in each side channel were measured with a Yellow Springs Instrument (YSI) meter. These measurements were taken once with every oligochaete core sample (see Oligochaete Densities), oligochaete kicknet sample (see *Myxobolus cerebralis* Infection in *Tubifex tubifex*), and sediment sample, for a total of 10 to 14 measurements for most side channels across seasons (see Table 3). The different sample sizes were a result of the YSI meter malfunctioning on some dates. Two side channels (Palisades, Grizzly) had much smaller sample sizes (n = 3) because the oligochate kicknet sampling was not conducted there (see Table 3). The means and standard errors of each chemical characteristic were calculated for each side channel.

Depth and current velocity (at 0.6 of water depth) were measured with a Model 3000 Swoffer Instrument flow meter at three equidistant points along three randomly selected transects within each side channel in July 1999, and additionally

at three equidistant points along one randomly selected transect with each core sample (see Oligochaete Densities), for a total of 18 measurements along six transects in each side channel. The means of all depth measurements were determined, and each velocity measurement was averaged across each transect and transects were averaged for each side channel.

Side channel widths were measured in July 1999. Multiple random transects were taken across each side channel to the water edges to determine the side channel width. The number of width measurements varied among side channels according to their length. One width measurement was taken about every 9 m of length. The width measurements were averaged into one mean and standard error per side channel.

The characteristics of each side channel were described using the means and coefficient of variation (CV). Data were normally distributed and therefore nontransformed values were used in analyses. A principal component analysis (PCA) using the correlation matrix was conducted on all of the means of the physical and chemical characteristics except for the study section lengths using SAS (PROC PRINCOMP, SAS Institute 1999 – 2000), to assess which characteristics of the environment best described differences among the eight side channels. The features that had eigenvectors larger than 0.25 were considered the most influential features of PC1 and PC2 (see Table 4).

Oligochaete Densities

Invertebrates were collected from the sediment of each of the eight side channels using a 42 mm diameter core sampler (Brinkhurst and Kennedy 1965) to assess oligochaete densities. The core sampler was inserted about 10 cm into the soft sediment because most invertebrates live in the top 10 cm of the sediment (Krezoski et al. 1978; Takada et al. 1992). The core sampler was lifted out of the sediment, with one hand on the vacuum top and the other hand on the bottom, and sediments were transferred to a one-liter container. Kahle's solution (Pennak 1978) was used to wash the excess sediment in the core sleeve and preserve the sample. Eight core samples were taken in soft sediment in each side channel during the summer 1999, fall 1999, and spring 2000 for a total of 24 samples per side channel. The core samples were preserved in Kahle's solution for one week and then sieved with a 500 μm sieve. The fraction larger than 500 μm was rinsed and stored in 70% ethanol until sorting. The fraction smaller than 500 μm was discarded. Sorting involved separating all invertebrates from the sediment, and counting and identifying the oligochaetes to family or species (Kathman and Brinkhurst 1998).

Oligochaetes were slide-mounted with CMCP-10 (Polysciences, Incorporated, Warrington, Pennsylvania) and identified to family with a Nikon Optiphot-2 compound microscope with phase contrast. Worms in the family Tubificidae were further identified to species when possible (Kathman and Brinkhurst 1998). Immature tubificids cannot be identified to species, as identification is based on reproductive parts. However, immatures can be divided into categories based on characteristics of their chaetae to more accurately estimate

the proportions of immatures of each species in a sample. The proportions of adults in samples can be estimated and immature tubificids can then be assigned to species accordingly (Brinkhurst 1986). Immature tubificids were divided into two categories, those with hair and pectinate chaetae, such as *T. tubifex*, and those with bifid chaetae. Adult worm identifications were pooled across dates for each side channel, and the proportions of adults of each species were calculated by dividing the total number of adults of each species by the total number of adults in the appropriate category (hair and pectinate or bifed chaetae) for each side channel. The immatures were allocated to species according to the adult proportions. The total abundance of a tubificid species was estimated by summing the identified adults of that species and the allocated immatures. Tubificids were pooled across dates to characterize the tubificid assemblages of the side channels across the year and to minimize bias due to seasonal variability in life histories. Pooling in this way assumes that all of the tubificid populations and species have similar age-class demographics (Brinkhurst 1986).

Oligochaete densities were calculated by dividing the number of individuals in a taxon, including both matures and allocated immatures, by the surface area of the core (Lazim and Learner 1986). Density estimates were averaged for all samples to characterize each side channel throughout the year. The oligochaete density estimates were ln (x + 1) transformed to achieve normality. A two-way analysis of variance (ANOVA) with side channels and time of the year as factors, followed by

the Tukey procedure for pairwise comparisons, was used to determine if mean densities of mature *T. tubifex* differed among side channels.

Oligochaetes were categorized into a higher taxon when low numbers of individuals in a species were found. *Limnodrilus hoffmeisteri* and *L. udekemianus* were combined into the taxon of *Limnodrilus* species, and *R. coccineus*, *R. falciformis*, and *R. sodalis* were combined into the taxon of *Rhyacodrilus* species. Additional groupings included the category of "Lumbricidae and Lumbriculidae" and the category of "all oligochaete species except for *T. tubifex*". The remaining groups included Naididae, Enchytraeidae, and *I. templetoni*.

Myxobolus cerebralis Infection in *Tubifex tubifex*

Tubificids were collected monthly from six of the eight side channels (North Slide, South Slide, Pine Butte, Kirby Bridge, Lyons Bridge, Sun Ranch) to estimate the proportions of tubificids with hair and pectinate chaetae releasing TAMs (hereafter referred to as "infected tubificids") and of *T. tubifex* releasing TAMs (hereafter referred to as "infected *T. tubifex*"). The proportion of infected *T. tubifex* was estimated because this is the more direct measurement of *M. cerebralis* in the oligochaete host, whereas the proportion of infected tubificids was measured to compare infection in Madison River worms with the infected tubificids measured in previous studies and to compare the accuracy of both estimates for future studies. Only six of the eight side channels were sampled because of the intensity of the work, and these were selected based on differing severities of infection in sentinel

rainbow trout (Vincent 2000), and densities of *T. tubifex* (B. L. Kerans, unpublished data).

Tubificids were collected using a kick net (mesh size of 200 μm) in each side channel about monthly from June through October in 1999 and March through June in 2000, except in June 2000 when two collections were made. Some side channels were not sampled every month due to limited access or time constraints (South Slide and Lyons Bridge in March 2000; Sun Ranch in June 2000) (see Appendix B). If no worms were found during a collection, we erred conservatively and treated the data as not sampled rather than assuming that no worms were producing TAMs. Two side channels (South Slide and Lyons Bridge) were additionally sampled on June 3, 2000, but because data from this date were only available for two side channels, they were not used in statistical analyses.

About 300 oligochaetes were collected from each side channel during each collection. Oligochaetes were picked and counted, then placed in a 500-ml container on ice in a cooler and oxygenated during transportation to the laboratory. The following day, the oligochaetes were separated under a light microscope according to the presence or absence of hair chaetae, thereby enabling the removal of worms without hair chaetae that were known not to be *T. tubifex*. Oligochaete worms cannot be positively identified to species while alive, but selecting worms with hair chaetae increases the probability that the worms being observed are *T. tubifex*.

Two hundred worms with hair chaetae (like *T. tubifex*), or as many as were found, were separated from the original 300 (see Appendix B). Once separated, they

were individually held in 12-well plates with 4000 µl of filtered water per well and maintained at 15 to 18 °C. Worms were observed for TAM release with a base illuminated Leica stereo dissecting microscope every other day over the following four days. A worm was considered infected if TAMs were observed in its well.

Triactinomyxon-positive worms were confirmed for *M. cerebralis* infection using the polymerase chain reaction (PCR) analysis and TAM measurements. First, the worms were cut in half and the anterior sections were preserved in 70% ethanol for worm identification. The posterior section of each infected worm was dissolved in 180 µl of Clontech T1 buffer and 25 µl of Bioline proteinase K solution in an ISC Bioexpress eppendorf tube, and sent to the Western Fisheries Research Center, Seattle, Washington, where PCR methods using the 18S rRNA gene were used to confirm the parasite as *M. cerebralis* (Andree et al. 1997; Andree et al. 1998). Wells holding TAM-positive tubificids were also randomly selected for measuring the TAMs. Forty µl of water was pipetted from each selected well and observed under a compound microscope for TAMs. The process length, sporoplast length, style length, and style width of 200 TAMs from 41 worms were measured (Lom and Dykova 1992; Lom et al. 1997; El-Matbouli and Hoffman 1998). Means and standard errors were calculated for each characteristic.

Worms that released TAMs were identified to determine the proportions of *T. tubifex* that were observed for each side channel. All TAM-positive worms and random samples of TAM-negative worms from the 200 that were observed were slide mounted and identified to calculate the relative abundance of *T. tubifex* in each

sample. The anterior sections of all TAM-positive worms (in front of segment 12), forty randomly selected TAM-negative worms without clitella, and all TAM-negative worms (up to 40) with clitella were slide mounted and identified to species if mature for each side channel and collection (Kathman and Brinkhurst 1998). Worms with clitella visible to the naked eye were selected for identification because they were more likely to be reproductively mature, and thus identifiable (Charlotte Rasmussen, Western Fisheries Research Center, personal communication).

The relative abundance of *T. tubifex* in each side channel was determined by pooling the relative abundance of *T. tubifex* for all sampling dates to obtain one estimate for each side channel. The cumulative sums of all identified *T. tubifex* from each side channel were divided by the cumulative sums of all identified tubificids with hair chaetae from each side channel to determine the proportions of *T. tubifex* per side channel. Pooling minimized the bias due to seasonal variability in oligochaete species life cycles.

The proportion of infected tubificids was calculated for each collection period and side channel by dividing the number of tubificids with hair chaetae observed releasing TAMs by the total number of tubificids with hair chaetae observed during that collection period in that side channel. The proportion of infected *T. tubifex* was then calculated for each collection by dividing the proportion of infected tubificids at each collection period by the proportion of *T. tubifex* found in each side channel. Mean proportions and standard errors were calculated for all estimates for each side channel. The tubificid infection estimates and the *T. tubifex*

infection estimates were not distributed normally, and were normalized by ln (x + 1) transformations.

Estimates of the density of infected *T. tubifex* were then calculated by multiplying the estimates of the proportion of infected *T. tubifex* (from the kicknet samples) with the *T. tubifex* density estimates (from the core samples) for each side channel.

An additional study was conducted in June 2001 to examine the possible influence of fluctuating temperatures during transport and laboratory rearing on the subsequent release of TAMs. The proportion of infected tubificids was examined from one side channel (Lyons Bridge) with two samples using the methods used in this study, and two modified samples where temperatures were maintained constant while worms were taken from the field and while held in the laboratory. The modified sampling regime maintained more constant temperatures by placing the oligochaetes from the river directly into a cooler instead of maintaining the oligochaetes for an hour at the side channel before bringing them back to the cooler in the vehicle. Furthermore, tubificids were held in an incubator at a constant 15 °C in the laboratory instead of in the main laboratory room where temperatures ranged from 15 – 18 °C. These modified techniques were not normally used because of the difficulty and time constraints of bringing a cooler full of worms to more than one side channel, and not having access to the incubators on a regular basis. The proportions of infected tubificids from the two samples using our standard methods were compared to the proportions of infected tubificids from the two samples using

the modified methods with a two-sample non-parametric Wilcoxon test (PROC NPAR1WAY, SAS Institute, 1999 -- 2000).

Comparisons between Features of the Environment and Densities of Infected *Tubifex tubifex*

Linear regression analyses were used to investigate correlations among the results of the principal component analysis of the features of the environment and the estimates of *T. tubifex* density, proportion of infected *T. tubifex*, and density of infected *T. tubifex* (Arrows A, B, and E, Figure 2). Additionally, similar comparisons were made among the principal components and the estimates of the density of tubificids with hair chaetae, the proportion of infected tubificids, and the density of infected tubificids with hair chaetae. The density estimate from Palisades was not used in regression analyses involving *T. tubifex* because no *T. tubifex* were found at Palisades. Linear regressions were also used to examine specific relationships between each influential factor of PC1 and PC2, and the density of infected *T. tubifex*. Residuals were checked for normality and variance in all comparisons and no further transformations were needed.

Comparisons of Oligochaete Taxa with Features of the Environment and *Tubifex tubifex*

Linear regression analyses were used to investigate relationships among the transformed densities of oligochaete taxa, including both matures and allocated immatures, and the principal components of the environment, transformed densities

of *T. tubifex*, and the transformed densities of infected *T. tubifex* (Arrows C and D, Figure 2). Only oligochaete taxa that were found in every side channel were used in comparisons with principal components and estimates of the densities of infected *T. tubifex* because all calculations needed to be on the same scale so that estimates and correlations were comparable, and the manipulations that were used to estimate the density of infected *T. tubifex* did not allow zeros in the data. Regressions were conducted with the oligochaete taxa of "*Limnodrilus* species", Naididae, Enchytraeidae, and "all oligochaetes except *T. tubifex*". Furthermore, all of the oligochaete taxa were used independently in comparisons with the estimates of *T. tubifex* densities because the manipulations necessary for the density estimates allowed for zeros in the data. Residuals were examined for normality and variance in all comparisons, and no further transformations were needed.

Myxobolus cerebralis Infection in Sentinel Rainbow Trout

The Eagle Lake strain of sentinel rainbow trout was used to assess the contribution of each side channel to the risk of whirling disease infection in the Madison River. A Montana Fish Wildlife and Parks (MFWP) state hatchery provided the rainbow trout fry used for this study and raised them until 65 days of age, when they were transferred to a holding facility. The facility used a single passage water system with 13 °C, pathogen-free water, and fed the rainbow trout commercial trout feed.

Rainbow trout fry were between 40 and 60 mm in length and 60 to 80 days old when 50 were placed in each sentinel cage at the upstream and downstream ends of each side channel during two periods beginning on May 25, 1999 and June 24, 1999, which is the time of peak infection in the Madison River according to previous investigations on sentinel rainbow trout (Vincent 2000). The sentinel cages were placed at each end of the study reaches within side channels to determine the contribution of *M. cerebralis* produced within each side channel by calculating the differences between the two cages.

Sentinel cages consisted of a circular wire mesh 0.32 cm in diameter and 46 cm by 51 cm in depth, and were held in place in slower moving areas of the side channels with reinforcement bars. The fish were exposed for 10 days, removed, and returned to the hatchery for a subsequent 85 to 100 days. They were then sacrificed by exposure to 5 % tricaine methanesulfonate. The gill arches and heads were removed and fixed in 10 % neutral buffered formalin for a minimum of 24 hours. Preserved fish heads were sent to Washington State Veterinary School in Pullman, Washington, for histological examination (Baldwin et al. 2000). Each sentinel fish that survived was graded on the modified McConnell-Baldwin scale of zero to five (Hedrick et al. 1999a) according to the severity of *M. cerebralis* infection in an attempt to describe the probability of long-term survival (Baldwin et al. 2000). Histology scores were quantified based on the amount and severity of degeneration to the cartilage and the infiltration of leukocytes. The number of fish that survived and

were examined histologically varied from 20 to 50 among cages, although over 40 fish survived in most cages.

The mean and median infection grade and the proportion of rainbow trout infected were determined for each cage. The data were distributed normally without transformation. The specific disease exposure within each side channel was assessed by subtracting the mean or median infection grades and proportions of infected fish of the upstream from the downstream cages for each time period. Estimates of the difference in infection severity between sentinel fish placed upstream and downstream of a specific area (hereafter referred to as differential infection risk) can be used to predict the influence of that area on infection risk to salmonids. In other words, the difference between the amount of *M. cerebralis* entering and leaving an area, as evaluated by infection severities in sentinel rainbow trout, could indicate the contribution of that area to *M. cerebralis* levels in a river system. However, the estimate of the differential risk of rainbow trout infection held assumptions. Subtracting the grades of sentinel rainbow trout infection of the upper from the lower cages and the histological scale itself assumes that the histological scale is linear although no evidence exists to support the linearity or non-linearity of the histological scale. Additionally, the differential equation assumes that the TAMs that were produced within a side channel were able to travel the entire distance of the side channel to reach the bottom cage. Furthermore, the equation assumes that the same water flowed through both upstream and downstream cages of each side channel. Grand mean, median, and proportion differences were calculated for each side channel using the means, medians, and proportions from both time periods.

The grand mean differences will subsequently be referred to as the "differential risk of rainbow trout".

Comparisons between Features of the Environment and Infection in Sentinel Rainbow Trout

Linear regression analyses were used to investigate the relationships among the results of the principal component analysis of the features of the environment and the differential risk of rainbow trout infection (Arrow F, Figure 2). Linear regressions were also used to further examine specific relationships between each influential environmental factor of PC1 and PC2 and the differential risk of rainbow trout infection. The means instead of the medians were used for all detailed comparisons because an underlying assumption of this study was that the histology grades are based on a linear scale because the upstream cages were subtracted from the downstream cages. Furthermore, means and medians should be similar in normally distributed populations according to the central limit theorem. Residuals were examined for normality and variance in all comparisons, and no transformations were necessary.

Comparisons between Infections of *Tubifex tubifex* and Sentinel Rainbow Trout

Linear regression analyses were used to investigate the relationships among the estimates of *M. cerebralis* infection in tubificids with hair chaetae and in *T. tubifex* with estimates of the differential risk of rainbow trout infection (Arrow G,

Figure 2). The estimates of the density of *T. tubifex*, proportion of *T. tubifex* infected, and density of infected *T. tubifex* were compared with estimates of the difference in the mean and median grades of infection of sentinel rainbow trout and the proportion of sentinel rainbow trout infected between downstream and upstream cages. The estimates of *T. tubifex* densities from the Palisades side channel were not used in regression analyses involving *T. tubifex* because no *T. tubifex* were found in Palisades. Estimates of tubificids with hair chaetae were also compared with estimates of the relative risk of rainbow trout infection. Residuals were examined for normality and variance in all comparisons, and no further transformations were needed.

RESULTS

Physical and Chemical Environment

Features of the physical and chemical environment varied among the side channels (Tables 2 and 3). Sand was the dominant substrate in all side channels except South Slide, where both silt and sand were most abundant (Table 2). Side channels ranged in mean temperatures from 10.15 to 13.55 °C, in conductivity from 0.17 to 0.21 mS/cm, in DO from 10.46 to 13.29 mg/l, and in pH from 8.33 to 8.61 (Table 3). Certain physical and chemical characteristics co-varied. For example, side channels with higher velocities (e.g., Pine Butte) tended to have smaller proportions of fine sediments, whereas side channels with lower velocities (e.g., South Slide, Lyons Bridge) had greater proportions of fine sediments. Side channels with faster velocities and larger sediments also tended to be wider than those with slower velocities and smaller sediments (Tables 2 and 3).

Both the coefficient of variation (CV) and the principal components analysis suggested that the side channels were similar in certain physical and chemical attributes such as conductivity, whereas other attributes, such as the dominance of silt and boulders in the sediments, varied widely (Table 4). Principal components analysis of the physical and chemical data resulted in four major components that cumulatively accounted for 91% of the variation among side channels. Principal component one (PC1), which explained 40 % of the total physical and chemical variation among side channels, and

Table 2. The composition of the riverbed surface sediment in eight side channels in the Madison River, Montana, USA in 1999 -- 2000. SE = ± 1 standard error.

Side Channel	Inorganic sediment composition (%)							CPOM (%)	Mean % Sediment Organic Material (± 1 SE) n = 2
	Boulder	Cobble	Pebble	Gravel	Sand	Silt	Clay		
North Slide	0.85	14.80	13.76	6.02	53.26	8.72	2.58	0	1.10 (± 0.07)
South Slide	1.63	11.33	9.04	5.73	31.83	35.44	5.06	4.11	1.80 (± 0.52)
Grizzly	3.34	16.16	16.95	3.74	53.52	5.08	1.20	0.48	4.38 (± 1.0)
Pine Butte	5.58	12.67	22.17	15.35	41.12	2.21	0.88	5.67	2.54 (± 0.97)
Kirby Bridge	0.24	4.05	12.62	2.83	74.65	3.21	2.41	2.95	2.97 (± 0.96)
Lyons Bridge	0.90	7.61	1.28	0.21	65.25	21.15	3.60	13.95	4.76 (± 0.67)
Sun Ranch	0.08	1.15	13.50	6.22	73.52	3.95	1.58	1.87	1.51 (± 0.21)
Palisades	7.46	23.60	24.19	10.75	31.61	2.38	0	2.07	1.88 (± 0.03)

Table 3. Chemical and physical measurements of the environment of eight side channels in the Madison River, Montana, USA from 1999 -- 2000. SE = ± 1 standard error.

Side Channel	Dissolved Oxygen (mg/l) n = 10 unless indicated	Conductivity (mS/cm) n = 11 unless indicated	Temperature (°C) Mean (± 1 SE) n = 14 unless indicated	Temperature (°C) Minimum	Temperature (°C) Maximum	Mean pH (± 1 SE) n = 10 unless indicated	Mean Velocity (m/s) (± 1 SE) n = 6	Mean Depth (m) (± 1 SE) n = 18	Mean Width (m) (± 1 SE)
North Slide	10.89 (± 0.83)	0.19 (± 0.01)	11.32 (± 1.24)	2.45	17.50	8.34 (± 0.09)	0.67 (± 0.14)	0.26 (± 0.03)	8.78 (± 1.12) n = 14
South Slide	10.98 (± 0.82)	0.21 (± 0.01)	10.15 (± 0.73)	5.73	15.00	8.47 (± 0.10)	0.32 (± 0.05)	0.17 (± 0.03)	5.99 (± 0.45) n = 25
Grizzly	13.29 (± 1.28) n = 3	0.19 (± 0.00) n = 3	13.55 (± 2.93) n = 4	7.04	19.50	8.54 (± 0.35) n = 2	0.64 (± 0.13)	0.35 (± 0.03)	9.32 (± 0.42) n = 12
Pine Butte	10.84 (± 0.82)	0.19 (± 0.01)	11.93 (± 1.14)	3.13	18.50	8.42 (± 0.06)	1.34 (± 0.16)	0.36 (± 0.02)	17.61 (± 0.43) n = 10
Kirby Bridge	10.46 (± 0.69)	0.19 (± 0.01)	12.10 (± 1.17)	3.71	19.50	8.39 (± 0.12)	0.75 (± 0.07)	0.25 (± 0.03)	11.44 (± 1.44) n = 14
Lyons Bridge	10.78 (± 0.79)	0.18 (± 0.01)	10.96 (± 0.91)	5.39	16.29	8.36 (± 0.10)	0.41 (± 0.09)	0.40 (± 0.04)	7.1 (± 0.12) n = 23
Sun Ranch	11.60 (± 0.32) n = 8	0.18 (± 0.01) n = 9	11.05 (± 1.27) n = 12	4.87	19.50	8.33 (± 0.10) n = 8	0.79 (± 0.08)	0.23 (± 0.02)	12.76 (± 1.34) n = 10
Palisades	12.35 (± 0.71) n = 3	0.17 (± 0.01) n = 3	12.74 (± 1.95) n = 4	8.12	17.53	8.61 (± 0.21) n = 2	0.77 (± 0.11)	0.32 (± 0.02)	8.78 (± 0.50) n = 10

principal component two (PC2), which explained 23 % of the variation among side channels (Table 4), were the only principal components that were significantly associated with infection in *T. tubifex* and the differential risk of infection in rainbow trout in regression analyses.

Table 4. Features of the physical and chemical environment of eight side channels in the Madison River, Montana, USA in 1999 and 2000. The features were described with principal components analysis and coefficient of variation (CV). Principal components one and two (PC1, PC2) accounted for about 63 % of the variation among side channels.

	Physical and chemical environment feature	CV	PC1 eigenvectors	PC2 eigenvectors
Size Composition Of Sediment	% Boulder	108.37	0.3083	0.2020
	% Cobble	62.63	0.2292	0.3272
	% Pebble	51.07	0.3485	-0.0151
	% Gravel	74.69	0.2532	-0.0435
	% Sand	32.52	-0.1270	-0.3496
	% Silt	116.31	-0.2887	0.2753
	% Clay	74.69	-0.3544	0.1112
	% Organic matter	51.19	-0.0269	0.0669
	Mean CPOM	114.82	-0.1661	0.0349
	Mean Depth	26.47	0.1498	0.0036
	Mean Velocity	43.08	0.2581	-0.2873
	Mean DO	8.47	0.2267	0.2139
	Mean Conductivity	6.21	-0.1914	0.0718
Temperature	Mean Temperature	9.25	0.3045	0.0219
	Minimum Temperature	38.30	0.1201	0.3660
	Maximum Temperature	9.28	0.2002	-0.3267
	Mean PH	1.19	0.2315	0.3585
	Mean Width	36.06	0.1888	-0.3618

Features of the physical and chemical environment were arrayed differently on the PC1 and PC2 continua (Table 4, Figure 4). Side channels were differentiated on PC1 primarily according to substrate composition, water temperature, and water velocity.

Side channels with low values on PC1 had high proportions of silt and clay, and low proportions of gravel, pebbles, and boulders in the sediment; low water velocities, and low mean water temperatures. Principal component two differentiated side channels based primarily on water temperatures, pH, side channel width and sediment composition. Side channels with low values on PC2 had high mean water velocities, high maximum water temperatures, high mean side channel widths, low minimum temperatures, low mean pH, and high proportions of sand and low proportions of cobble and silt in the sediment. The percentages of organic matter in the sediment and CPOM, mean water DO, mean depth, and mean conductivity were not strongly associated with either PC1 or PC2 (Table 4).

Side channels formed four distinct groups when arrayed on the PC1 and PC2 continua (Figure 4). The first group included South Slide and Lyons Bridge, which had positive values on PC2 and negative values on PC1. The proportions of clay, silt, and cobble in sediments were high compared to other side channels, whereas the percentages of sand, pebble, gravel, and boulder sediments were generally lower than other side channels (Table 2). Additionally, they exhibited higher mean pH, higher minimum water temperatures, lower mean and maximum water temperatures, lower mean water velocities, and smaller side channel widths compared to other side channels (Table 3). The second group, which only included Pine Butte, exhibited characteristics opposite those of South Slide and Lyons Bridge. The third group, which included Grizzly and Palisades, had positive values on PC1 and PC2. They were characterized by larger proportions of gravel, pebble, cobble, and boulder sediments; higher pH, smaller side

channel widths and higher mean and minimum water temperatures compared to other side channels. The fourth group, which included North Slide, Kirby Bridge and Sun

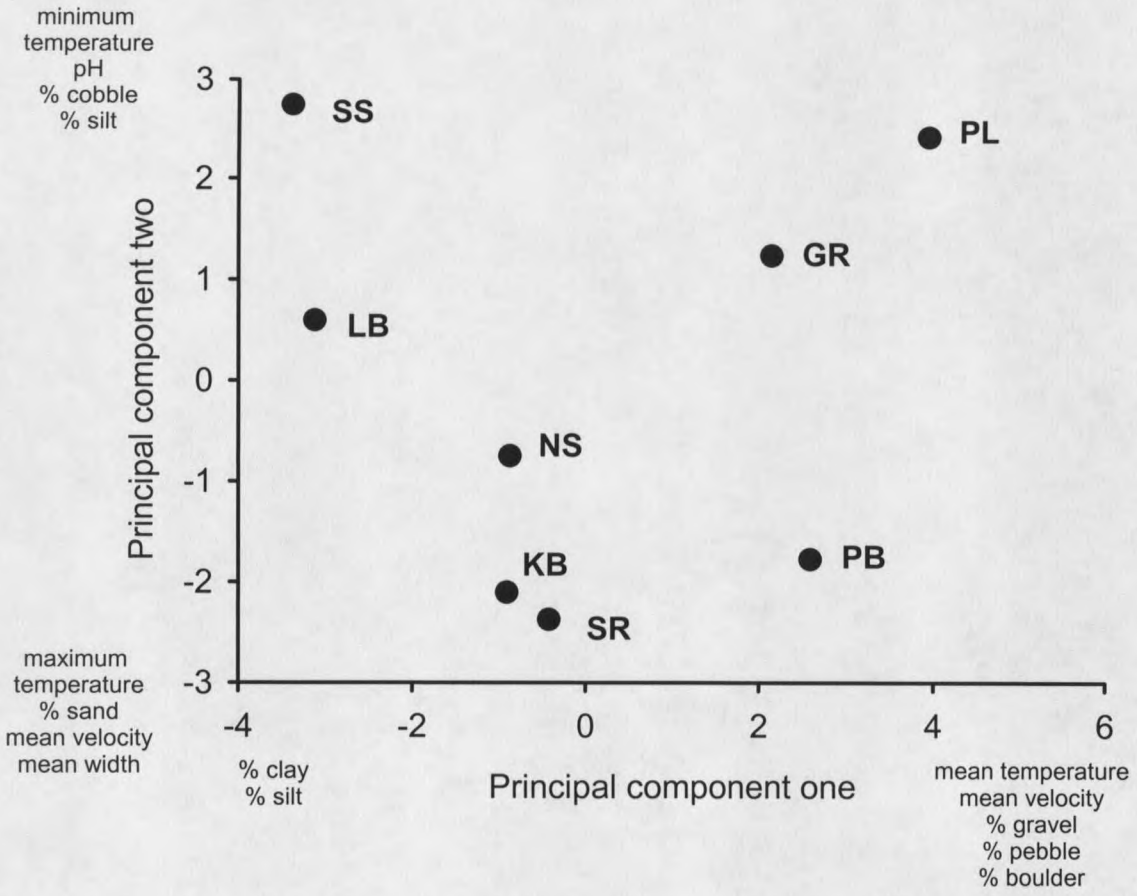


Figure 4. Features of the physical and chemical environment in eight side channels [North Slide (NS), South Slide (SS), Grizzly (GR), Pine Butte (PB), Kirby Bridge (KB), Lyons Bridge (LB), Sun Ranch (SR), and Palisades (PL)] in the Madison River, Montana, USA in 1999 -- 2000. Principal component one and principal component two from a principal components analysis differentiated side channels according to the variation among features of the environment. High values of the physical features that were most influential in the principal components are indicated at the ends of the axes.

Ranch, exhibited characteristics opposite those of Grizzly, Palisades, South Slide, and Lyons Bridge on the PC2 axis, but were intermediate on the PC1 axis. They exhibited high proportions of sand and low proportions of cobble and silt in the sediment; high

water velocities, larger side channel widths, lower pH, higher maximum water temperatures, and lower minimum water temperatures compared to other side channels (Tables 2 and 3).

Oligochaete Densities

Densities of *T. tubifex* differed spatially and temporally among side channels. The densities of mature *T. tubifex* differed by as much as four orders of magnitude among side channels. Whereas the mean densities of mature *T. tubifex* were moderate in most side channels, ranging between 120/m^2 and 529/m^2, we detected no mature *T. tubifex* in the Palisades side channel, and about 3100/m^2 in the Lyons Bridge side channel (Figure 5). Densities of mature *T. tubifex* also varied across seasons. Four side channels (North Slide, South Slide, Kirby Bridge, Lyons Bridge) had highest densities of mature *T. tubifex* occurring in the spring, and three side channels (Grizzly, Pine Butte, Sun Ranch) had highest mature *T. tubifex* densities in the summer (Appendix A). The densities of mature *T. tubifex* ranged from about 0 to 1000 individuals/m^2 among seasons within side channels, with the exception of Lyons Bridge, which ranged seasonally from 0 to 8723 *T. tubifex*/m^2.

The densities of mature *T. tubifex* differed across times of the year ($F_{2,168} = 6.81$, $p = 0.001$) and among side channels ($F_{7,168} = 2.34$, $p = 0.03$), but the interaction term was not significant ($F_{14,168} = 1.53$, $p = 0.11$) with a two-way ANOVA analysis. Densities of mature *T. tubifex* were higher in Lyons Bridge than Palisades; however, the other six side channels were not significantly different from each other or from Lyons Bridge or

Palisades with a Tukey's pairwise comparison test.

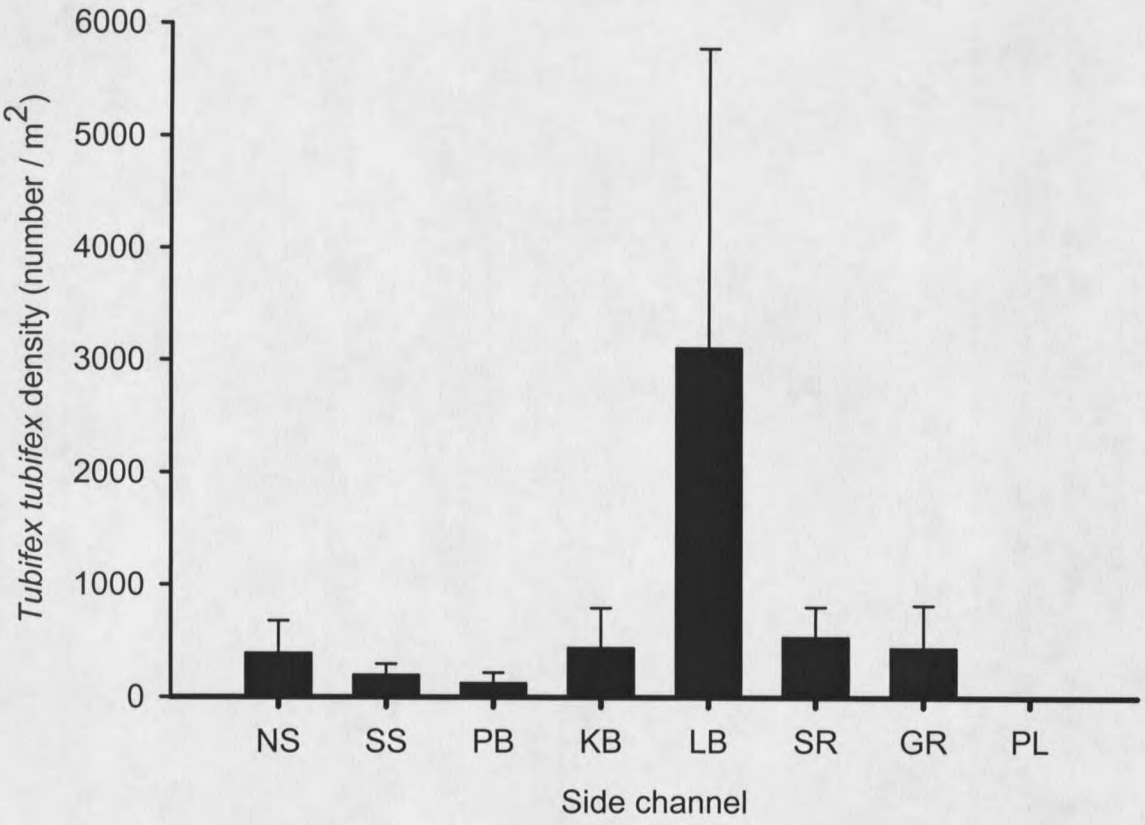


Figure 5. The densities of mature *Tubifex tubifex* in eight side channels [North Slide (NS), South Slide (SS), Pine Butte (PB), Kirby Bridge (KB), Lyons Bridge (LB), Sun Ranch (SR), Grizzly (GR), and Palisades (PL)] of the Madison River, Montana, USA in 1999 -- 2000.

Densities of other oligochaete taxa also differed spatially and temporally (Appendix A). The most abundant mature oligochaetes in the samples other than *T. tubifex* were *Rhyacodrilus* species (*R. coccineus* + *R. sodalis* + *R. falciformis), Ilyodrilus templetoni, Limnodrilus* species *(L. hoffmeisteri* + *L. udekemianus)*, "Lumbricidae and Lumbriculidae", Enchytraeidae, and Naididae. Mature *Rhyacodrilus* species, Lumbriculidae and Lumbricidae, and Enchytraeidae were most common at the upstream

side channels (South Slide and North Slide), and less common downstream. Mature *I. templetoni* were only found in four of the eight side channels, whereas the other oligochaete taxa were found in most side channels. Densities of oligochaete taxa also varied across seasons. Densities of mature *Limnodrilus* were highest in the spring in all side channels except Grizzly, and densities of naidids were highest in the summer in five out of the eight side channels (North Slide, South Slide, Grizzly, Pine Butte, Sun Ranch). Other oligochaete taxa differed in their timing of highest densities among side channels. The proportions of adults to immatures also varied across seasons and among side channels (Appendix A).

The proportions of mature *T. tubifex* among other tubificids with hair chaetae also differed among side channels, ranging from 0 to 100% of the tubificid assemblage (Table 5). When the immature *T. tubifex* were added to mature *T. tubifex* densities, the final estimates of mean densities of *T. tubifex* increased by an order of magnitude. The final estimates of mean densities of *T. tubifex* including matures and immatures ranged from 0 to 31214/m^2 among side channels (Table 5).

Myxobolus cerebralis Infection in *Tubifex tubifex*

The number of tubificids with hair chaetae that were observed and the number that were observed releasing TAMs varied among side channels (Appendix B). The number of tubificids with hair chaetae observed ranged from fewer than 10 to 204 per collection. The total number of tubificids with hair chaetae observed for a side channel

ranged from 436 at Sun Ranch to 1796 at Lyons Bridge; however, Sun Ranch was the only side channel with fewer than 1000 observations (Appendix B).

Table 5. The densities of mature *Tubifex tubifex* in eight side channels of the Madison River, Montana, USA. Twenty-four samples were taken in each side channel. The mean densities of *T. tubifex* were estimated for each side channel by adding the mean densities of mature *T. tubifex* to the estimated mean densities of immature *T. tubifex*.
SE = standard error.

Side channel	Mean density of mature *T. tubifex* number / m^2 (± 1 SE)	Proportion of immatures estimated to be *T. tubifex*	Estimated mean density of immature *T. tubifex* number / m^2 (± 1 SE)	Estimated mean density of *T. tubifex* number / m^2 (± 1 SE)
North Slide	384.47 (± 291.81)	0.24	2699.2 (± 551.07)	3083.67 (± 744.93)
South Slide	192.23 (± 102.19)	0.21	2014.73 (± 754.61)	2206.96 (± 822.42)
Grizzly	432.53 (± 385.38)	0.78	5682.09 (± 2550.47)	6114.62 (± 2904.84)
Pine Butte	120.15 (± 98.06)	0.56	1549.79 (± 602.87)	1669.93 (± 604.08)
Kirby Bridge	432.53 (± 359.50)	0.72	2439.45 (± 1005.14)	2871.97 (± 1214)
Lyons Bridge	3099.77 (± 2678.06)	1	28114.19 (± 17606.69)	31213.96 (± 17950.23)
Sun Ranch	528.64 (± 275.35)	0.73	2307.36 (± 865.12)	2836 (± 918.96)
Palisades	0	0	0	0

The mean percentages of infected *T. tubifex* varied between 0.5 and 29 % among side channels (Figure 6). Five of the six side channels, however, had mean estimates of infected *T. tubifex* below 5 %. The South Slide side channel had the highest percentage

of infected *T. tubifex*, followed by North Slide, Kirby Bridge, Lyons Bridge, Sun Ranch, and finally Pine Butte.

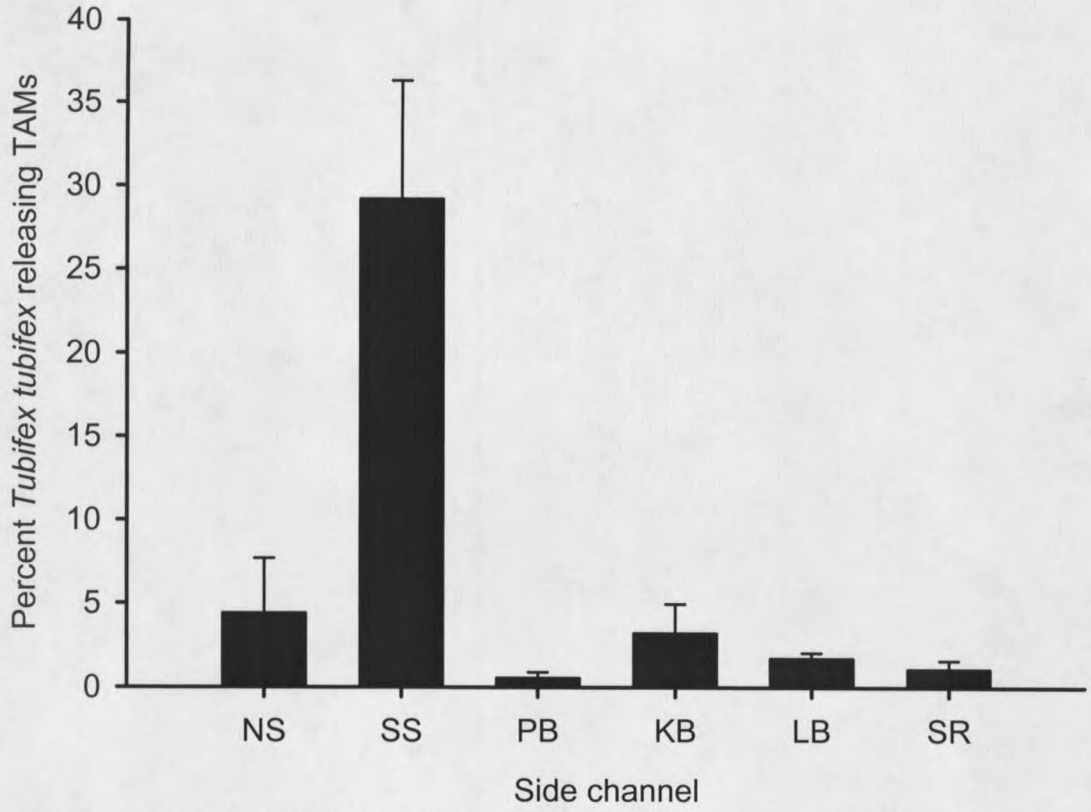


Figure 6. The percent of *Tubifex tubifex* releasing *Myxobolus cerebralis* triactinomyxons (TAMs) in six side channels [North Slide (NS), South Slide (SS), Pine Butte (PB), Kirby Bridge (KB), Lyons Bridge (LB), and Sun Ranch (SR)] in the Madison River, Montana, USA in 1999 -- 2000.

The percentages of infected *T. tubifex* also varied temporally within side channels (Appendix C). The amount of variation of infected *T. tubifex* differed among side channels, with ranges as small as 0 to 3 % at Pine Butte, or as large as 0 to 72 % at South Slide. Side channels also differed in their timing of highest infection, with Kirby Bridge

in June 1999, North Slide in July 1999, Sun Ranch in August 1999, and Pine Butte, Lyons Bridge, and South Slide in June 2000 (Appendix C).

Estimates of the prevalence of *M. cerebralis* infection of tubificids differed from infection estimates of *T. tubifex* (Appendix C). Estimates of the proportions of infected tubificids with hair chaetae followed the same general temporal and spatial patterns as the estimates of the proportions of infected *T. tubifex*, but tubificid estimates were generally lower than those of *T. tubifex*. The magnitude of the difference between the two estimates varied among side channels because the proportions of *T. tubifex* that were observed for TAM release varied among side channels (Appendix B). In some side channels (e.g., Lyons Bridge), most of the tubificids with hair chaetae were *T. tubifex*, resulting in similar estimates of the proportions of infected tubificids and of infected *T. tubifex*. In other side channels (e.g., North Slide), most of the tubificids with hair chaetae were not *T. tubifex*, resulting in larger estimates of the proportion of infected *T. tubifex* than of infected tubificids.

The estimates of the density of infected *T. tubifex*, which combined the mean density of *T. tubifex* (Figure 5) with the mean proportion of infected *T. tubifex* (Figure 6), ranged from less than one to 49 individuals/m^2 among side channels (Figure 7). The estimates of the density of infected *T. tubifex* and estimates of the prevalence of infection in *T. tubifex* placed side channels into the same order of infection with the exception of Lyons Bridge side channel (Figure 7). Side channels had different dynamics of infection, which is evident in the two most infected side channels, Lyons Bridge and South Slide. The Lyons Bridge side channel had moderate proportions of infected *T. tubifex*, but high

densities of *T. tubifex*, which resulted in a high density of infected *T. tubifex*. Conversely, the South Slide side channel had fewer *T. tubifex*, but high proportions of the *T. tubifex* were releasing TAMs, which also resulted in high densities of infected *T. tubifex*.

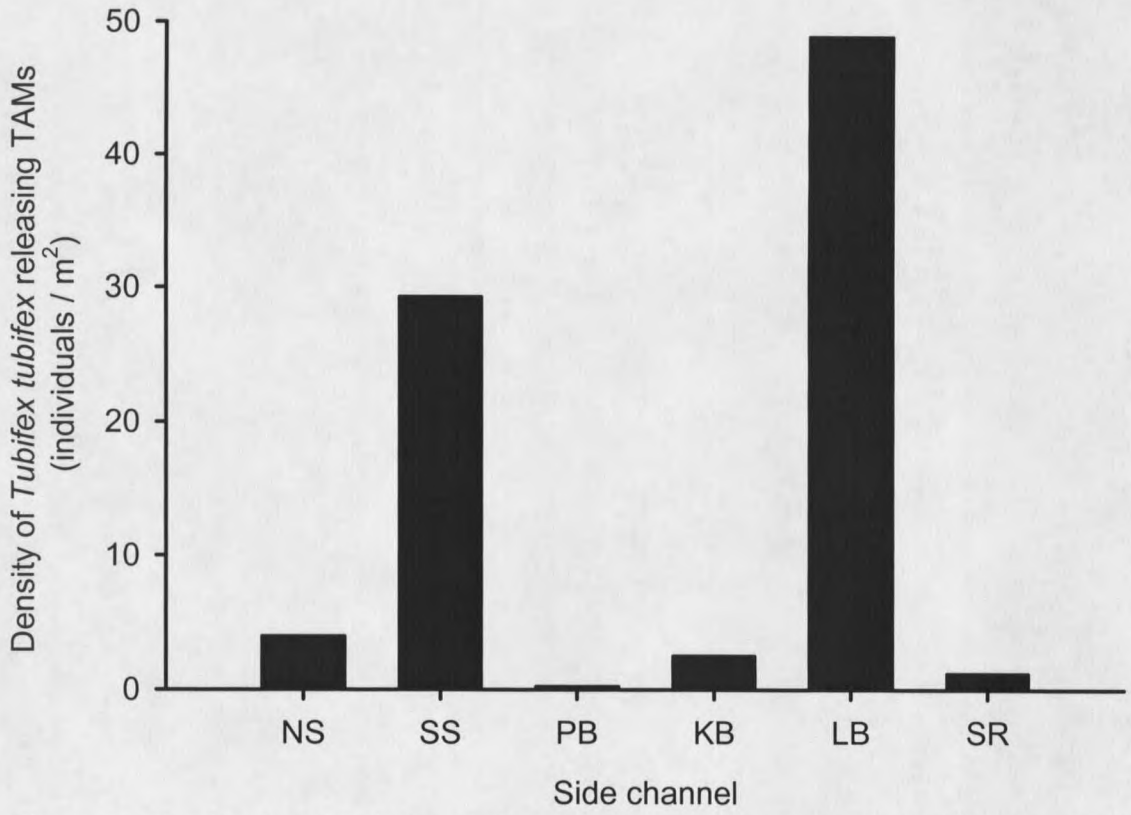


Figure 7. The density of *Tubifex tubifex* releasing *Myxobolus cerebralis* triactinomyxons (TAMs) in six side channels [North Slide (NS), South Slide (SS), Pine Butte (PB), Kirby Bridge (KB), Lyons Bridge (LB), and Sun Ranch (SR)] in the Madison River, Montana, USA in 1999 -- 2000.

The percentage of infected *T. tubifex* was marginally higher (2.0 ± 0 %) using the standard methods compared to the controlled temperature conditions (1.31 ± 0.19 %), but the difference was not significant ($p = 0.31$). Therefore, I failed to reject the null hypothesis that the percent of *T. tubifex* infected among control and non-control

populations were equal; however the power of the test was probably low because each treatment had only two replicates.

The PCR methods and structural measurements of TAMs confirmed the identity of the observed *T. tubifex* infections as *M. cerebralis*. Polymerase chain reaction results confirmed *M. cerebralis* infection in 112 out of the 119 TAM releasing worms that were tested. Measurements of TAMs were generally smaller, with the exception of sporoplast length, and ranges were larger, extending into both smaller and larger sizes, than those taken by El-Matbouli and Hoffman (1998) and Lom and Dykova (1992). However, the means of the style length and the process length did fall into the range of TAM sizes that Lom and Dykova (1992) observed (Table 6).

Table 6. *Myxobolus cerebralis* Triactinomyxons (TAMs) released from *Tubifex tubifex* that were collected in 1999 – 2000 from side channels of the Madison River, Montana, USA. Measurements were compared with previous measurements of *M. cerebralis* TAMs made by El-Matbouli and Hoffman (1998), and by Lom and Dykova (1992).

	Mean (μm)± SD (range) (n = 200)	El-Matbouli and Hoffman (1998) (n = 50)	Lom and Dykova (1992) (n = 20)
style length	128.43 ± 26.11 (35 – 195)	146.67 ± 5.09 (135.25 – 155.83)	134 (99 - 153)
style width	10.58 ± 1.89 (5 – 20)	12.42 ± 0.87 (11.5 – 14.51)	12.8 (10.9 – 16.8)
sporoplast length	59.03 ± 19.29 (15 – 120)	na	34 (27.7 – 44)
process length	162.71 ± 41.67 (30 – 240)	193.62 ± 20.18 (147.38 – 214.21)	232 (162 – 275)

Comparisons between Features of the Environment and Densities of Infected *Tubifex tubifex*

Side channels with low scores on PC1, which were defined by a sediment composition dominated by silt and clay; slow water velocities, and low mean water

temperatures, had the highest densities of *T. tubifex* and prevalence of infection in *T. tubifex* (Figure 8 a and b). Whereas the negative relationship between PC1 and the density of *T. tubifex* was relatively weak, PC1 was a better predictor of the proportion of infected *T. tubifex*. However, when the density and prevalence of infection in *T. tubifex* were combined into the estimate of the density of infected *T. tubifex*, it was strongly predicted by the attributes of the environment in PC1 (Figure 8c).

Side channels with high scores on PC2, which were defined by high minimum water temperatures, high mean pH, low maximum water temperatures, slow water velocities, narrow side channel widths, and a sediment composition dominated by silt and cobble had the highest prevalence of infection in *T. tubifex* (Figure 9). PC2 did not predict *T. tubifex* density (Figure 9a), but was positively correlated to the proportion of infected *T. tubifex* (Figure 9b) and the density of infected *T. tubifex* (Figure 9c).

The seven most influential physical and chemical features on PC1 and the eight most influential features on PC2 varied in correlation strength with the density of infected *T. tubifex* (Figure 10). Smaller sediments were strong predictors of the density of infected *T. tubifex*, with proportion of clay demonstrating the strongest positive correlation (Figure 10a). The proportion of pebbles and gravel were negatively related to the density of infected *T. tubifex* (Figure 10b). Mean water velocities (Figure 10c) and mean side channel widths (Figure 10d) negatively correlated to the density of infected *T. tubifex*, whereas the negative relationships between mean and maximum temperatures and the density of infected *T. tubifex* were only marginally significant (Figure 10e). The relationship between minimum water temperatures and the density of infected *T. tubifex*

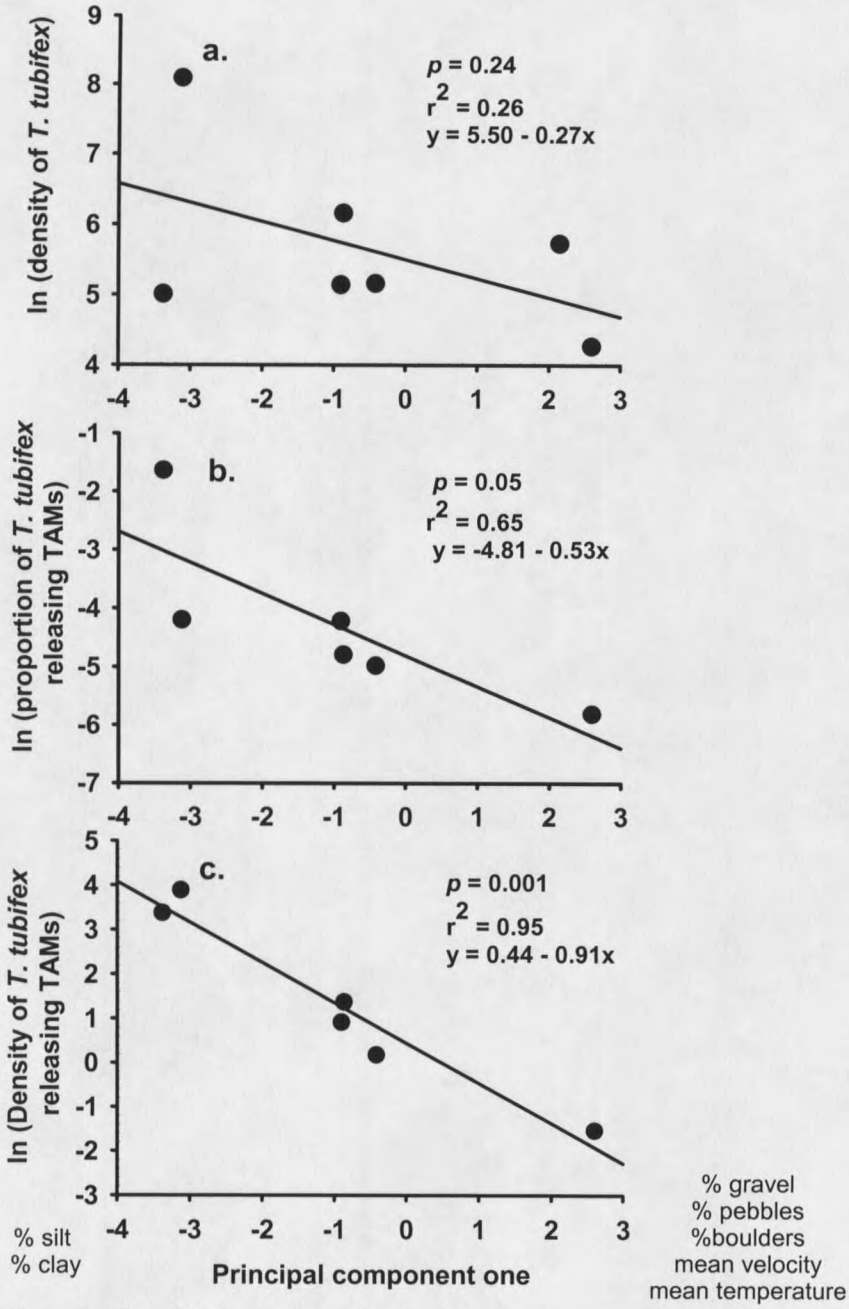


Figure 8. The relationship between principal component one of a principal components analysis which differentiated side channels based on features of the physical and chemical environment, and (a) the density of *T. tubifex*, (b) the proportion of *T. tubifex* releasing TAMs, and (c) the density of *T. tubifex* releasing TAMs in Madison River side channels, Montana, USA in 1999-2000. Each point represents one side channel mean.

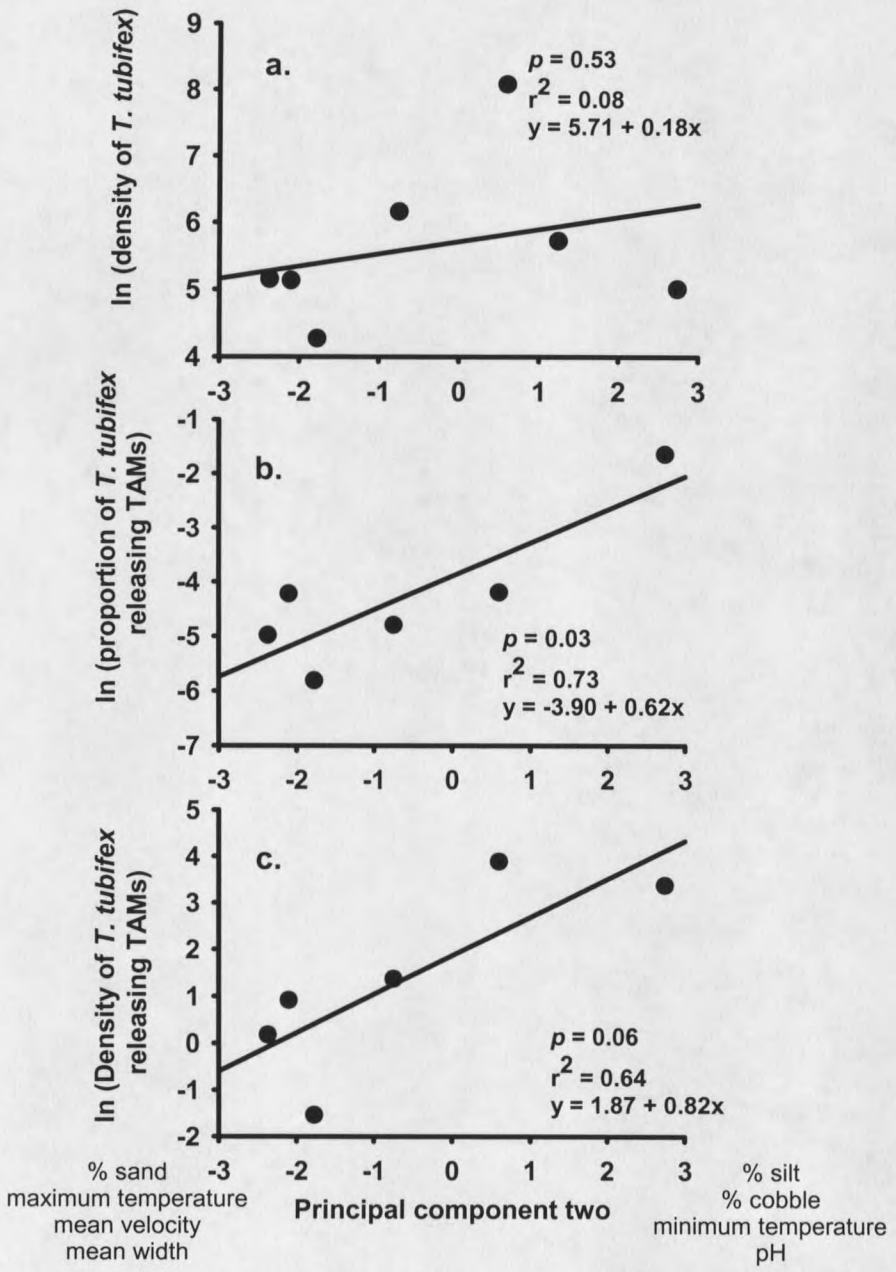


Figure 9. The relationship between principal component two of a principal components analysis that differentiated side channels based on features of the physical and chemical environment, and (a) the density of *Tubifex tubifex*, (b) the proportion of *T. tubifex* releasing triactinomyxons (TAMs), and (c) the density of *T. tubifex* releasing TAMs in Madison River side channels, Montana, USA in 1999-2000. Each point represents one side channel mean.

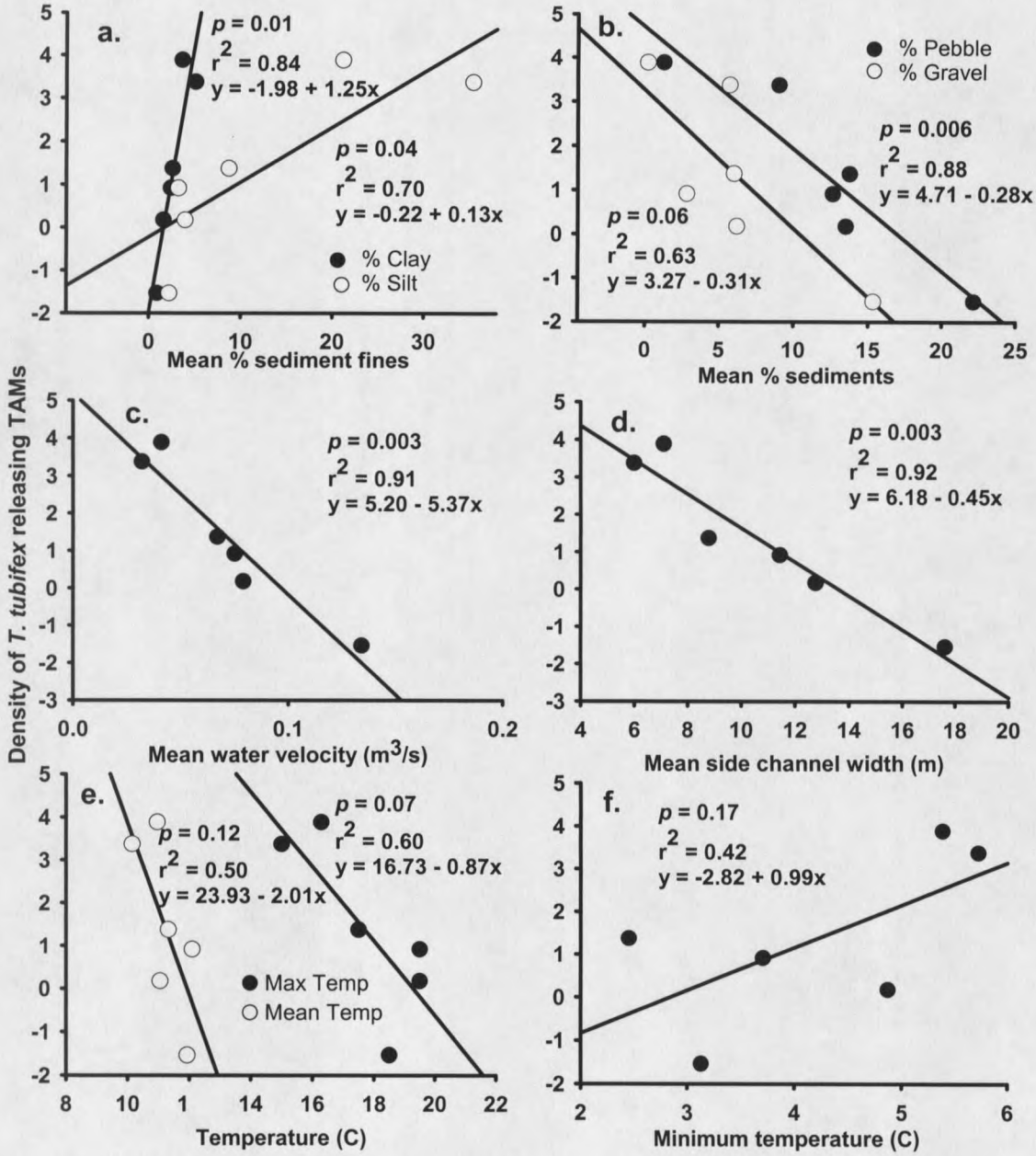


Figure 10. The relationships between features of the physical and chemical environment and the density of *Myxobolus cerebralis* infected *Tubifex tubifex* in six side channels in the Madison River, Montana, USA in 1999-2000.

was positive, but was also only marginally significant (Figure 10f). The proportions of sand, cobble, and boulder in the sediment, and the mean pH in a side channel were not related to *T. tubifex* infection (all p's > 0.29).

Estimates of the density of tubificids with hair chaetae, the proportion of infected tubificids, and the density of infected tubificids with hair chaetae related to PC1 and PC2 in ways similar to *T. tubifex* estimates, but all comparisons made with the estimates of tubificids with hair chaetae were slightly weaker, with two exceptions. Principal component one strongly predicted the density of tubificids with hair chaetae ($p = 0.01$, $r^2 = 0.70$), and PC2 showed no relationship to the percent of infected tubificids ($p = 0.30$, $r^2 = 0.26$).

Comparisons of Oligochaete Taxa with Features of the Environment and *Tubifex tubifex*

Features of the physical and chemical environment also predicted the estimated densities of certain oligochaete taxa (including matures and allocated immatures). Principal component one did not predict the densities of any of the four oligochaete taxa tested (*Limnodrilus* species, Naididae, Enchytraeidae, and "all oligochaetes except *T. tubifex*") (all p's > 0.72). Principal component two, however, was positively related to the density of Enchytraeidae ($p = 0.06$, $r^2 = 0.63$, $n = 8$) and positively correlated to the taxon of "all oligochaetes except *T. tubifex*" ($p = 0.02$, $r^2 = 0.76$, $n = 8$). Therefore, high pH and minimum water temperatures, and low mean velocities, mean widths, maximum water temperatures, and high proportions of silt and cobble and low proportions of sand in the sediment related to high densities of enchytraeids and the taxon of "all

oligochaetes except *T. tubifex*". Principal component two did not predict the densities of Naididae or *Limnodrilus* species (both p's > 0.23).

The estimated densities of certain oligochaete taxa (including matures and allocated immatures) also correlated with the estimated densities of *T. tubifex* (including matures and allocated immatures). Densities of *Rhyacodrilus* species ($p < 0.0001$, $r^2 = 0.19$, $n = 192$), *Limnodrilus* species ($p < 0.0001$, $r^2 = 0.16$, $n = 192$), and "all oligochaetes except *T. tubifex*" ($p < 0.0001$, $r^2 = 0.16$, $n = 192$), positively correlated with the densities of *T. tubifex*. Naididae ($p < 0.0001$, $r^2 = 0.09$, $n = 192$), and *I. templetoni* ($p = 0.001$, $r^2 = 0.06$, $n = 192$), also positively correlated with *T. tubifex* densities, although not as strongly. The taxa of Enchytraeidae and "Lumbricidae and Lumbriculidae" were not related to *T. tubifex* densities (both p's > 0.39). The oligochaete taxon of "all oligochaetes except *T. tubifex*" was the only category of oliogchaetes that was both predicted by a principal component and also able to predict *T. tubifex* density.

Naididae density, however, was the only oligochaete taxon of the four groups tested that correlated with the density of infected *T. tubifex* ($p = 0.04$, $r^2 = 0.71$, $n = 6$). Higher densities of naidids correlated to higher densities of infected *T. tubifex*. The other three taxa tested ("all oligochaete species except *T. tubifex*", *Limnodrilus* species, and Enchytraeidae) were not related to *T. tubifex* infection (all p's > 0.43).

Myxobolus cerebralis Infection in Sentinel Rainbow Trout

Exposure groups of sentinel rainbow trout positioned at the upstream end of side channels had detectable levels of *M. cerebralis* infection, thereby suggesting that the parasite was entering from the main stem of the river in all side channels except South Slide (Appendix D). Two side channels, South Slide and Lyons Bridge, were sources of the *M. cerebralis* parasite because sentinel rainbow trout were more severely infected at the downstream end than at the upstream end of the side channel (Figure 11). These side channels had high differential risks of infection. Alternatively, North Slide, Pine Butte, Grizzly, Kirby Bridge, Sun Ranch, and Palisades were not distinguishable as sources or non-sources for the *M. cerebralis* parasite because sentinel rainbow trout were similarly infected at the upstream end compared to the downstream end of the side channel. These side channels had lower differential risks of infection. The difference in the median grade of sentinel rainbow trout infection between upstream and downstream cages predicted the same side channels as sources (Appendix D).

The estimates of the proportion of rainbow trout infected and the estimates of the mean severity of rainbow trout infection also placed side channels into a similar order of differential infection. Both estimates placed the Lyons Bridge, North Slide, and South Slide side channels as having the greatest differential risk to wild rainbow trout, followed by Pine Butte and Palisades, and finally Kirby Bridge, Grizzly and Sun Ranch (Appendix D).

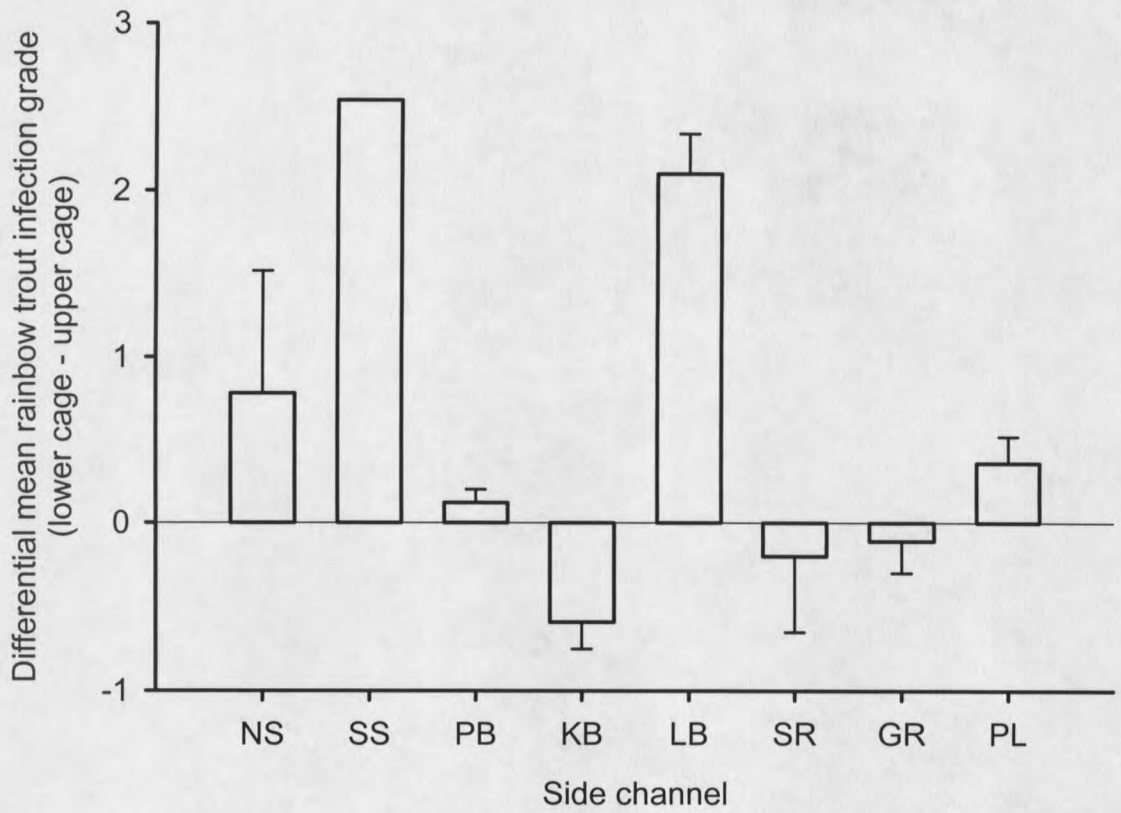


Figure 11. The differential mean grade of *Myxobolus cerebralis* infection in sentinel rainbow trout in eight side channels [North Slide (NS), South Slide (SS), Pine Butte (PB), Kirby Bridge (KB), Lyons Bridge (LB), Sun Ranch (SR), Grizzly (GR), and Palisades (PL) in the Madison River, Montana, USA in 1999. The differential risk of rainbow trout infection was assessed as the difference in mean grade of rainbow trout infection between sentinel cages at the upstream and downstream ends of side channels. Infection grade was based on the modified MacConnell-Baldwin Scale (0 -- 5) (Hedrick et al. 1999a).

Comparisons between the Features of the Environment and Infection in Sentinel Rainbow Trout

The difference in the mean grade of infection in sentinel rainbow trout between downstream and upstream cages (the differential risk of rainbow trout infection) was negatively related to PC1 (Figure 12a) and positively related to PC2 (Figure 12b).

Therefore, the features of PC1 and PC2 that predicted infection in *T. tubifex* also predicted infection in sentinel rainbow trout. The relationships between each individual physical and chemical attribute of PC1 and PC2 and the differential risk of rainbow trout infection were similar to those between the attributes and infection in *T. tubifex*, but the strength of the correlations for each attribute differed (Figure 13). Seven out of the 15 influential measurements on PC1 and PC2 were related to the differential risk of rainbow trout infection. The differential risk of rainbow trout infection was positively correlated with the mean proportions of clay and mean proportions of silt (Figure 13a), whereas it was negatively related to the mean proportions of pebble in the sediment (Figure 13b). Mean water velocity, mean side channel width, mean water temperature, and maximum water temperature were also negatively related to the differential risk of rainbow trout infection (Figure 13c – f). The proportion of silt was the best sediment predictor of differential rainbow trout infection risk (Figure 13a), and maximum water temperatures were the best non-sediment predictor of the differential risk of rainbow trout infection (Figure 13f). The proportions of boulder, cobble, gravel, and sand in the sediment; minimum water temperature, and mean pH were not related to the differential risk of rainbow trout infection (all p's > 0.33). Estimates of differences in median grades of infection in sentinel rainbow trout related to PC1 ($p = 0.13$, $r^2 = 0.48$, $n = 8$) and PC2 ($p = 0.002$, $r^2 = 0.94$, $n = 8$) in ways similar to estimates of rainbow trout mean grades of infection.

Principal component one and PC2 also predicted the difference in the proportion of sentinel rainbow trout infected between downstream and upstream cages, but

relationships were strongly influenced by one data point. Principal component one was negatively related to the difference in the proportion of rainbow trout infected between downstream and upstream cages ($p = 0.08$, $r^2 = 0.42$) until the one influential data point (South Slide side channel) was removed ($p = 0.22$, $r^2 = 0.28$). Principal component two was positively related to the difference in the proportion of rainbow trout infected between downstream and upstream cages. Principal component two also had a stronger relationship when all data points were used ($p= 0.17$, $r^2 = 0.29$), and no relationship when the influential point was removed ($p = 0.81$, $r^2 = 0.01$).

Comparisons between Infection of *Tubifex tubifex* and Sentinel Rainbow Trout

Estimates of infection in *T. tubifex* positively related to estimates of differential infection risk in sentinel rainbow trout (Figure 14). Whereas the relationship between the density of *T. tubifex* and the differential risk of rainbow trout infection was relatively weak, the proportion of infected *T. tubifex* was a better predictor of differential infection risk in rainbow trout. However, the density of infected *T. tubifex* was the strongest predictor of the differential risk of rainbow trout infection (Figure 14c).

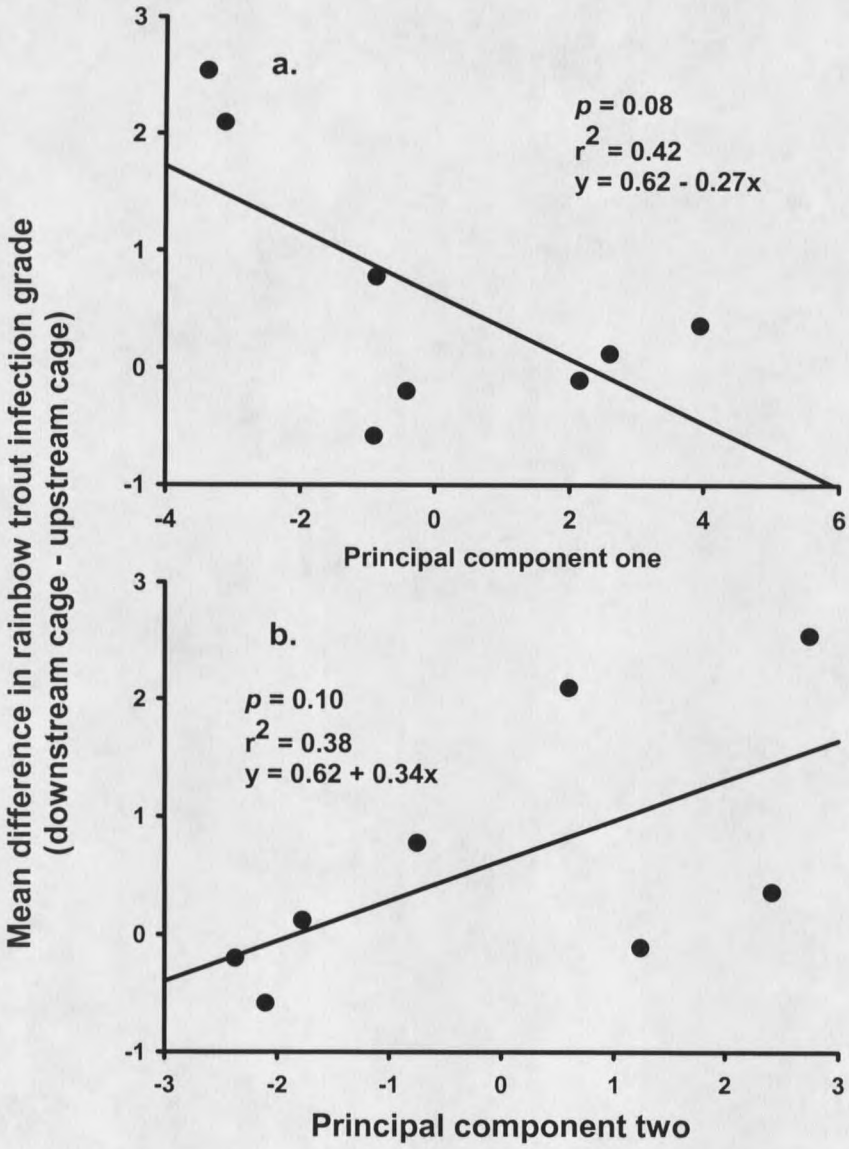


Figure 12. The relationships between principal component one and principal component two that differentiated side channels based on features of the physical and chemical environment and the differential risk of *Myxobolus cerebralis* infection in rainbow trout in eight side channels in the Madison River, Montana, USA in 1999 -- 2000. The differential risk of rainbow trout infection was assessed as the difference in mean grade of infection between sentinel cages at the upstream and downstream ends of each side channel. Infection grade was based on the modified MacConnell-Baldwin scale (0 -- 5) (Hedrick et al. 1999a). Each side channel is represented by one point.

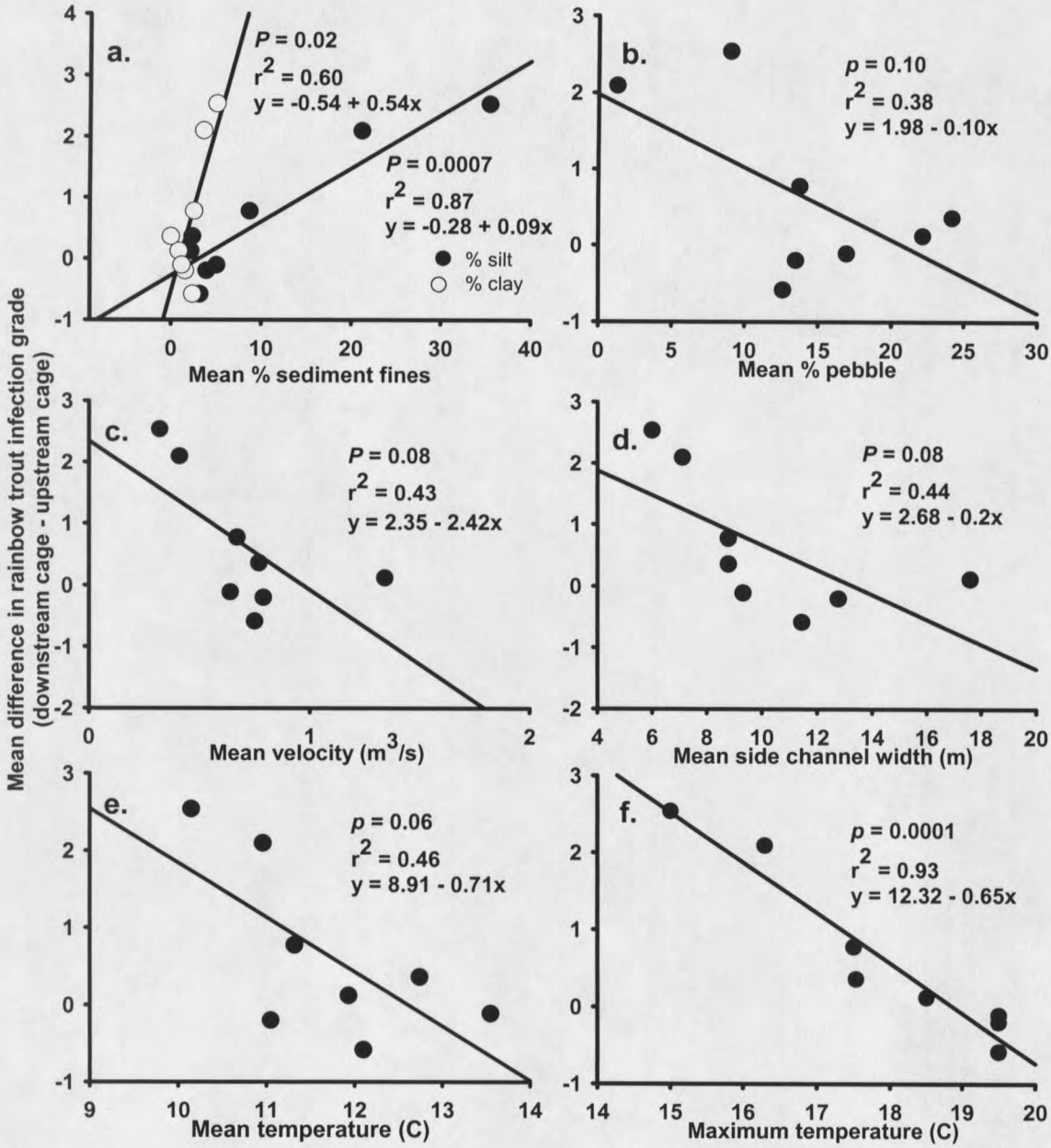


Figure 13. The releationships between features of the physical and chemical environment and the differential risk of *Myxobolus cerebralis* infection in rainbow trout in eight side channels in the Madison River, Montana, USA in 1999 -- 2000. The differential risk of rainbow trout infection was assessed as the difference in mean grade of rainbow trout infection between sentinel cages at the upstream and downstream ends of side channels. Infection grade was based on the modified MacConnell-Baldwin scale (0 -- 5) (Hedrick et al. 1999a). Each point represents a side channel.

The differential risk of rainbow trout infection more strongly related to estimates of the densities of infected *T. tubifex* than to estimates of the densities of infected tubificids with hair chaetae ($p = 0.13$, $r^2 = 0.48$, $n = 6$). However, the estimate of the density of tubificids with hair chaetae was a stronger predictor of the differential risk of rainbow trout infection than the estimate of *T. tubifex* density ($p = 0.10$, $r^2 = 0.39$; $n = 8$).

Estimates of *T. tubifex* more strongly related to estimates of the mean differences in rainbow trout infection than to estimates of median differences in rainbow trout infection and the differences in the proportion of rainbow trout infected. Estimates of the median difference in the grade of rainbow trout infection related to estimates of *T. tubifex* densities and infection in ways similar to estimates of rainbow trout means, but comparisons made with medians were slightly weaker. Estimates of *T. tubifex* also predicted the difference in the proportion of sentinel rainbow trout infected between downstream and upstream cages, although these relationships were strongly influenced by one data point, the South Slide side channel. When the influential South Slide datapoint was removed from the regressions, the densities and infection of *T. tubifex* were not significantly related to the difference in the proportion of infection in sentinel rainbow trout between upstream and downstream cages ($p > 0.14$).

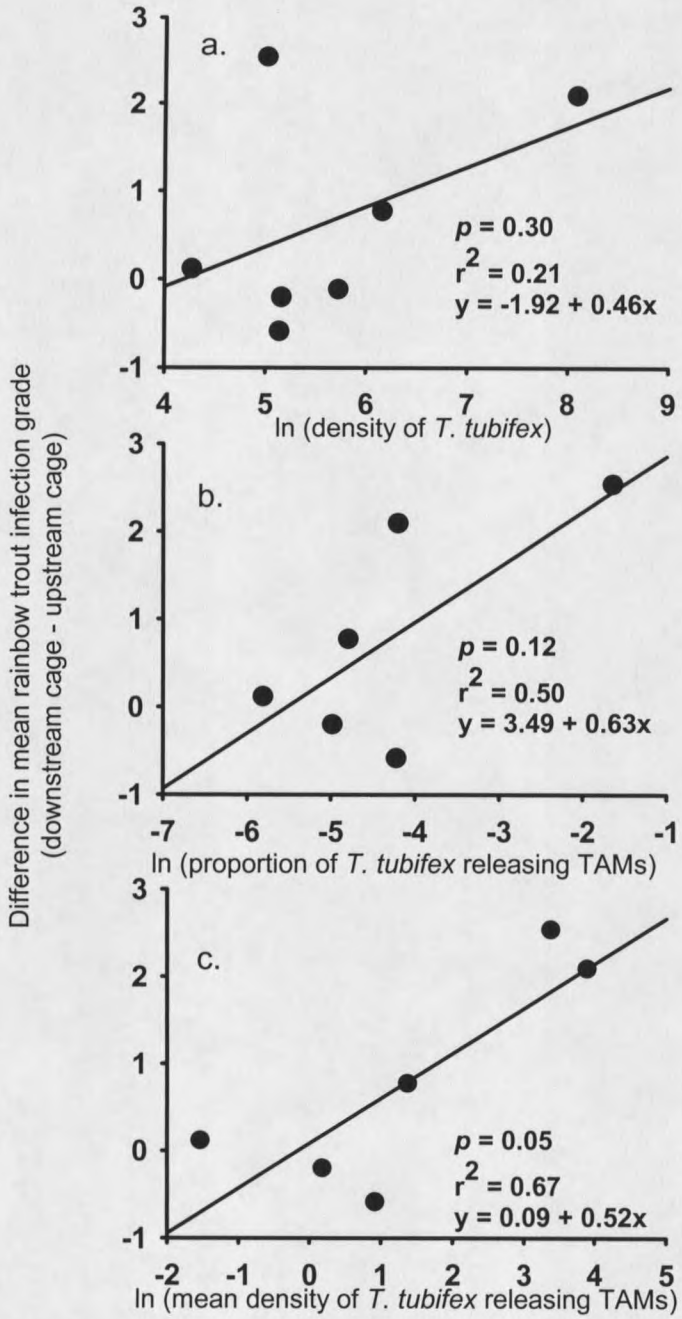


Figure 14. The relationships between the density of *Tubifex tubifex* (a), the percent of *T. tubifex* releasing TAMs (b), and the mean density of *T. tubifex* releasing TAMs (c) with the differential risk of *Myxobolus cerebralis* infection in rainbow trout, measured as the difference in mean grade of rainbow trout infection between sentinel cages at the upstream and downstream ends of side channels in the Madison River, Montana, USA in 1999 -- 2000. Infection grade was based on the modified MacConnell - Baldwin scale of 0 -- 5 (Hedrick et al. 1999a). Each point represents one side channel mean.

DISCUSSION

Myxobolus cerebralis was widespread in the Madison River. However, side channels differed widely in their contributions to the parasite load in the river. Both the differential infection risk of rainbow trout and the estimates of the density of infected *T. tubifex* varied widely among side channels. Additionally, certain features of the environment differentiated side channels and predicted the differential risk of infection in rainbow trout and infection in *T. tubifex*.

Fine sediments and slow water velocities, which positively correlated with infection in *T. tubifex* and the differential risk of infection in rainbow trout, differed among the side channels of the Madison River presumably because of geological and biological influences. Sediment composition and water velocities are known to co-vary in river systems (Allan 1995). High amounts of fine sediments in North Slide and South Slide were likely, in part, caused by a landslide that resulted from a 1959 earthquake (Alt and Hyndman 1997) that buried the original river valley directly upstream and diverted the path of the South Slide side channel. The river has gradually eroded through the landslide debris, carrying high sediment loads downstream. The South Slide side channel also likely had high proportions of clay and silt sediments as a result of a large pool that was created by a human-made dam across one of the meanders within the upper channel. In addition, water velocities were slow because it was a small side channel with a low volume of water entering from the main stem of the river. The high levels of clay and silt sediments in the Lyons Bridge side channel were likely caused by intensive riverside grazing that was evident in the extremely eroded banks and abundance of livestock

manure (personal observation). Additionally, a beaver dam located at the downstream end of Lyons Bridge slowed the water velocity, thereby facilitating the settlement of suspended silt and clay. Another beaver dam was located in a side channel directly upstream from Grizzly, and the sentinel fish held below the beaver dam (the upstream cage of Grizzly) were severely infected. Therefore, both natural and human-influenced events have probably contributed to the high amounts of sediment fines and slow water velocities in side channels where *M. cerebralis* infection in *T. tubifex* and differential risk of infection to rainbow trout were high.

Sediment and velocity affected both the infection prevalence in, and the densities of *T. tubifex*, which, when combined into the estimate of the density of infected *T. tubifex* resulted in strong correlations with the physical and chemical environment in the Madison River. A combination of factors likely accounts for the strong positive correlations between the densities of infected *T. tubifex* and the proportions of silt and clay substratum and slow water velocities. Areas with slow water velocities (Sloreid 1994) and high proportions of silt and clay can contain more *T. tubifex* than areas with higher proportions of larger substrates because *T. tubifex* prefer silt and clay substrates to coarser substrata (Birtwell 1972; Lazim and Learner 1987; Sauter and Gude 1996), and can reproduce faster in silt compared to other substrates (R. E. Arndt, E. J. Wagner, Q. Cannon, and M. Smith, Utah Division of Wildlife Resources, personal communication). High densities of *T. tubifex* can also be found in areas where human disturbances are high (Zendt and Bergerson 2000) likely because of resulting increased loads of fine sediments.

Substrate types and water velocities also likely affect the distributions and densities of *M. cerebralis* directly. Silt and clay may favor the development of *M. cerebralis* in *T. tubifex*. *Tubifex tubifex* that are raised in silt in the laboratory release more TAMs than those raised in organic debris or sand when given the same doses of *M. cerebralis* myxospores (R. E. Arndt, E. J. Wagner, Q. Cannon, and M. Smith, personal communication). Additionally, the prevalence of infection in *T. tubifex* raised in mud and sand can be higher than that of *T. tubifex* raised in leaf litter (T. Waldrop, V. Blazer, D. Smith, B. Schill, C. Densmore, National Fish Health Research Laboratory, personal communication). Finer particles like clay and silt that are found in depositional areas where water velocities are slow (Allan 1995) may hold *M. cerebralis* myxospores better than coarser particles (Lemmon and Kerans 2001). Therefore, areas with high proportions of clay and silt and slow water velocities could contain high numbers of myxospores available for *T. tubifex* to ingest, resulting in high densities of infected *T. tubifex* and a high risk of rainbow trout infection downstream. Faster flowing waters may additionally decrease the abundance of *M. cerebralis* in the water because turbulent waters may damage or destroy the fragile *M. cerebralis* TAMs. Therefore, the combined effects of the environment on *T. tubifex* densities and *M. cerebralis* distributions probably explain the relationships between fine sediments and water velocities with the densities of infected *T. tubifex*.

Side channels with high densities of *M. cerebralis* infection in *T. tubifex* and a high differential risk of infection in rainbow trout (South Slide, and Lyons Bridge) also had more constant water temperatures than other side channels (lower mean and

maximum temperatures, and higher minimum temperatures). The South Slide side channel could have lower mean and maximum water temperatures in part because of its location close to a cold water release dam. The influence of Quake Lake located directly upstream from South Slide, the beaver dam in Lyons Bridge, and cold water springs in Lyons Bridge and South Slide could also mediate water temperatures. Although cold water springs were observed in Lyons Bridge and South Slide, they were never thoroughly investigated in this study, and their abundance would need to be quantified in all side channels to substantiate if they are related to the prevalence of *M. cerebralis* in the Madison River.

Constant water temperatures could encourage longer durations of TAM release. Evidence from laboratory studies suggests that the duration of TAM release may be longer when water temperatures are constant than when water temperatures fluctuate into higher temperatures. For example, when tubificids were moved from water temperatures of 15°C to the same water temperatures once they began releasing TAMs they continued to release TAMs for at least 64 days, whereas those moved to higher water temperatures of 20 °C immediately released more TAMs but stopped after 15 days, and those transferred to 25 °C or 30 °C also immediately released more TAMs but stopped after 4 days (El-Matbouli et al. 1999). Additionally, the results of our temperature control study, in which the tubificids held at constant temperatures of 15 °C immediately released slightly fewer TAMs than those held at non-constant temperatures between 15 and 18 °C, suggests that non-constant temperatures could cause tubificids to immediately release more TAMs than those held in constant temperatures. However, existing

evidence on the relationships among the consistency of temperatures with infection in *T. tubifex* and infection risk in rainbow trout is only anecdotal in the natural environment, and needs further investigation.

Mean water temperatures could also explain infection, but their influence on infection is probably connected to other features in the environment. Although the differential risk of rainbow trout infection and infection in *T. tubifex* was negatively related to mean temperatures in the Madison River (10.2 – 13.6 °C), the severity of infection in rainbow trout was not related to mean water temperatures in Idaho (Hiner and Moffitt 2001), and was positively correlated to water temperatures (6.2 – 11.2 °C) in Willow Creek, Montana (Baldwin et al. 2000). These inconsistencies among previous studies suggest that other factors influence the effects of mean temperatures on infection. For example, the optimal temperature for the rate of *T. tubifex* infection can differ according to the type of substrate (T. Waldrop, V. Blazer, D. Smith, B. Schill, C. Densmore, personal communication).

Alternatively, the maximum temperature could be the influential factor of temperature in *M. cerebralis* infections. Lower maximum temperatures (15 °C) were the strongest temperature correlate to infection in *T. tubifex* and differential infection risk in rainbow trout in the Madison River. Furthermore, high water temperatures (25 – 30 °C) can inhibit *M. cerebralis* infections of *T. tubifex* in the laboratory (El-Matbouli et al. 1999). Maximum temperatures could therefore partially explain *M. cerebralis* infections in the Madison River.

Other features of the physical and chemical environment that were not related to differential infection risk to rainbow trout in this study may be important to whirling disease dynamics at other spatial scales in the Madison drainage and in other systems. For example, conductivity, which is typically related to stream productivity (Allan 1995), was positively correlated with *M. cerebralis* infection of sentinel rainbow trout in the Lostine River in Oregon (Sandell et al. 2001). Productivity likely predicts infection more in systems with larger variations in their range of productivity such as the Lostine River where conductivity values ranged between 13.7 – 244 μS (Sandell et al. 2001), than in systems with narrower ranges of conductivity such as the Madison River where conductivity values ranged between 170 to 210 μS. *Tubifex tubifex* may be dense in productive areas because of high reproductive rates when the amount of organic material in the sediment is high (Kaster 1980; Wachs 1967), thus providing more host habitat for *M. cerebralis* than in areas with lower *T. tubifex* densities. The attributes of the physical and chemical environment that played influential roles in infection in the Madison River, however, were those that distinguished side channels and were major influences in the principal component analysis. Unfortunately, the distances between upstream and downstream cages were not included in the principal components analysis or in any other analysis; thus, the significance of this distance to differential risk of infection to rainbow trout in the Madison River cannot be determined. However, there appeared to be little effect of distance between cages because both long and short areas had high differential risk of rainbow trout infection. In future studies, distances between cages should be kept constant.

The density of infected *T. tubifex* was the most effective oligochaete estimate at predicting the differential infection risk of rainbow trout in the side channels of the Madison River. Interestingly, the importance of the components used to estimate the density of infected *T. tubifex* (*T. tubifex* densities and the proportion of infected *T. tubifex*) differed between the two side channels with the highest densities of infected *T. tubifex* (Lyons Bridge, South Slide). Lyons Bridge had high densities of infected *T. tubifex* because of high *T. tubifex* densities, whereas South Slide had high densities of infected *T. tubifex* because of high prevalence of infection in *T. tubifex*. Therefore, the densities of *M. cerebralis* myxospores could be the more influential limiting factor to infection of *T. tubifex* in Lyons Bridge because a low proportion of the relatively dense population was infected. Alternatively, habitat that could support higher densities of *T. tubifex* could be the more influential limiting factor to infection of *T. tubifex* in South Slide because of its low densities of *T. tubifex*. Moreover, these results suggest that areas with low densities of *T. tubifex* can still produce enough TAMs to be highly infective to rainbow trout.

Although the proportions of infected tubificids found in this study were similar to those found in Colorado (Zendt and Bergerson 2000) and other areas of Montana (Rognlie and Knapp 1998), estimates of infection in *T. tubifex* were stronger predictors of differential infection risk to rainbow trout than the estimates of infection using all tubificids with hair chaetae. This suggests that the density of infected *T. tubifex* more accurately describes the disease ecology of worm hosts in an area and, therefore, is more likely to provide the necessary information for appropriate management strategies.

If future studies are limited and only worm densities and not worm infection can be examined, then differential rainbow trout infection could be more accurately predicted using estimates of the densities of tubificids with hair chaetae than by using estimates of the densities of *T. tubifex*. The densities of tubificids with hair chaetae were stronger predictors of the differential risk of rainbow trout infection ($p = 0.10$) than the densities of *T. tubifex* ($p = 0.30$) in the Madison River side channels. Tubificid densities likely positively correlated with the differential risk of rainbow trout infection because areas with high tubificid densities also had high densities of *T. tubifex*, which was suggested by the positive correlation between the densities of *T. tubifex* and all other oligochaetes. The estimates of tubificids with hair chaetae may have had better predictive powers because they were in effect larger samples than those of *T. tubifex* because of the limitations of tubificid identification. Consequently, tubificid densities were the more accurate estimates of differential rainbow trout infection risk because of their effectively larger sample sizes.

The implications of the high spatial and temporal variations in the densities of *T. tubifex* and the prevalences of infection in *T. tubifex* complicate studies of *M. cerebralis* ecology. The prevalences of infection in *T. tubifex* were often much higher than the prevalences of infection in tubificids with hair chaetae. For example, 70% of the *T. tubifex* were infected in the SS side channel in June 2000. This is the first report of such high prevalence of infection in *T. tubifex* in the wild. Furthermore, the proportion of *T. tubifex* that were actually infected was likely even higher than these estimates, because many worms may be infected and not yet releasing TAMs (Gilbert and Granath 2001). In

addition, the high variability of *T. tubifex* densities and the different times of maturation of *T. tubifex* and other tubificids among side channels emphasize the importance of measuring densities throughout the year because measurements during only one season could misrepresent true population densities.

The positive correlation between the densities of infected *T. tubifex* and the densities of Naididae could suggest that the *T. tubifex* that live in habitats with faster water velocities and high rates of sedimentation are more susceptible to *M. cerebralis* infections because naidids are indicators of such areas (Sloreid 1994, Voshell and Simmons 1984, Barnes 1983). These areas could contain less favorable sediment types and available nutrients needed by *T. tubifex* (Juget and Lafont 1994) that could negatively affect *T. tubifex* health, thus increasing their susceptibility to infection.

The positive relationship between the densities of all other oligochaete species and the densities of *T. tubifex* suggests that competition between other species and *T. tubifex* may not play an important role in the distribution of *T. tubifex* in the Madison River. Furthermore, the lack of a relationship between densities of all oligochaetes and infection of *T. tubifex* suggests that the potential for other oligochaete species to inactivate *M. cerebralis* myxospores also may not play an important role in *M. cerebralis* infections of *T. tubifex* the Madison River. Other studies have also found no significant correlation between oligochaete densities and the risk of *M. cerebralis* infection in river systems (Hiner and Moffitt 2001).

Disease severity of sentinel rainbow trout in the Madison was highly variable and similar in range to previous studies in Colorado (Nehring and Walker 1996), Montana

(Baldwin et al. 2000), Oregon (Sandell et al. 2001), and Idaho (Hiner and Moffitt 2001). This variation in infection risk to rainbow trout among side channels of the Madison River suggests that certain side channels were more optimal locations (e.g., North Slide, Pine Butte) for rainbow trout to avoid *M. cerebralis* as fry than others (e.g., Grizzly, Lyons Bridge, Palisades). However, the ecological processes that contribute to the variation in infection risk among side channels operate both upstream and within side channels. For example, in the Grizzly side channel, rainbow trout infection risk was high; however, the differential infection risk (the difference in infection grade between upstream and downstream sentinel fish) was low. This suggests that production of *M. cerebralis* TAMs within the Grizzly side channel was low and that high rainbow trout infection risk resulted from ecological processes operating upstream. Alternatively, in the Lyons Bridge side channel, rainbow trout infection risk was high only in the sentinel rainbow trout in the downstream cage and differential infection risk was high. This suggests that Lyons Bridge was a source of *M. cerebralis* TAMs and that ecological processes operating within the side channel caused the high risk of rainbow trout infection. However, exactly how cage experiments relate to wild rainbow trout populations is still unclear.

Many questions involving the intricate dynamics of *M. cerebralis* remain and warrant further investigation. The combination of the physical and chemical features that correlated with *M. cerebralis* infections in the Madison River should be investigated further in other river systems to determine if relationships found in this study are valid across multiple systems and if these physical and chemical features could be used as

indicators of whirling disease susceptibility in other areas. For example, future studies should investigate minimum and maximum temperatures and other environmental features to determine the role of temperature and the importance of the consistency of temperature in infection in *T. tubifex* and infection risk to rainbow trout. More laboratory studies are needed to determine the underlying mechanisms upon which temperatures operate. Future studies could also examine how exposures of sentinel rainbow trout relate to wild rainbow trout populations. Relating the spatial and time constraints of cage data to wild rainbow trout populations is essential to define the scale and entire meaning of the sentinel cage data.

More recently developed technologies could provide even more information about the dynamics of whirling disease in future studies. For example, a drum filtration system with glass bedding that has been used to collect and count TAMs in the field (F. Barrows, A. Zale, T. McMahon, and R. Hedrick, Bozeman Fish Technology Center, personal communication), could augment estimates of the proportion of infected *T. tubifex* with data on TAM abundance. Using the drum filtration system in the side channels of the Madison River would offer a method to examine correlations among TAM abundance, differential disease severity in sentinel fish, and density of infected *T. tubifex*.

Whirling disease is widespread in the Madison River and the variation in the abundance of all three organisms involved provides no easy management solution. Management solutions to completely remove *M. cerebralis* from the Madison River are unlikely to be successful. Encouraging practices that reduce the amount of fine sediments entering the river could result in lower densities of *T. tubifex* releasing TAMs.

Furthermore, practices such as management of the dam to allow increased spring floods to increase the velocity of the river near times of critical rainbow trout spawning and rearing could decrease the prevalence and infectiveness of *M. cerebralis*. Increasing flows and decreasing siltation have previously been suggested as management solutions to whirling disease (Brinkhurst 1996).

The wide variation in the prevalence of *M. cerebralis* among side channels strongly suggests that side channels differed in their contributions to *M. cerebralis* in the Madison River. Furthermore, features of the physical and chemical environment predicted areas with high densities of infected *T. tubifex* and high risk of infection to rainbow trout. The ability to predict and locate such areas of high infection is essential for management of whirling disease in the Madison River and essential for improving our understanding this complex parasite.

REFERENCES CITED

Allan, J. D. 1995. Stream Ecology: Structure and Function of Running Waters. Chapman and Hall. New York, USA.

Alt, D., and D. W. Hyndman. 1997. Roadside Geology of Montana. Montana Press Publishing Company. Missoula, Montana, USA.

Anderson, R. M., and R. M. May. 1979. Population biology of infectious diseases: Part I. Nature **280**:361-367.

Andree, K. B., S. J. Gresoviac, and R. P. Hedrick. 1997. Small subunit ribosomal RNA sequences unite alternate actinosporean and myxosporean stages of *Myxobolus cerebralis* the causative agent of whirling disease in salmonid fish. Journal of Eukarotic Microbiology **44**:208-215.

Andree, K. B., E. MacConnell, and R. P. Hedrick. 1998. A nested polymerase chain reaction for the detection of genomic DNA of *Myxobolus cerebralis* in rainbow trout *Oncorhynchus mykiss*. Diseases of Aquatic Organisms **34**:145-154.

Baldwin, T.J., J.E. Peterson, G.C. McGree, K.D. Staigmiller, E.S. Motteram, C.C. Downs, and D.R. Stanek. 1998. Distribution of *Myxobolus cerebralis* in salmonid fishes in Montana. Journal of Aquatic Animal Health **10**:361-371.

Baldwin T. J., Vincent E. R., Silflow, R. M., and Stanek D. 2000. *Myxobolus cerebralis* infection in rainbow trout (*Oncorhynchus Mykiss*) and brown trout (*Salmo trutta*) exposed under natural stream conditions. Journal of Veterinary Diagnostic Investigation **12**:312-321.

Barnes, L. E. 1983. The colonization of ball-clay ponds by macroinvertebrates and macrophytes. Freshwater Biology **13**:561-578.

Birtwell, I. K. 1972. Ecophysiological aspects of tubificids in the Thames estuary. Unpublished Ph.D. Thesis, University of London.

Birtwell, I. K. and D. R. Arthur. 1980. The ecology of tubificids in the Thames Estuary with particular reference to *Tubifex costatus* (Claparede). Pages 331-382 *in* R. O. Brinkhurst and D. G. Cook, editors. Aquatic oligochaete biology. Plenum Press, New York, New York, USA.

Brinkhurst, R. O. 1964. Observations on the biology of lake-dwelling Tubificidae. Archiv Hydrobiologie **60**:385-418.

Brinkhurst, R. O. 1986. Guide to the freshwater aquatic microdrile oligochaetes of North America. Canadian Special Publication of Fisheries and Aquatic Sciences 84.

Brinkhurst, R. O. 1996. On the role of tubificid oligochaetes in relation to fish disease with special reference to the myxozoa. Annual Review of Fish Diseases **6**:29-40.

Brinkhurst, R. O., and C. R. Kennedy. 1965. Studies on the biology of the Tubificidae (Annelida, Oligochaeta) in a polluted stream. Journal of Animal Ecology **34**:429-443.

Casellato, S., and F. Caneva. 1994. Composition and distribution of bottom oligochaete fauna of a north Italian eutrophic lake (Lake Ledro). Hydrobiologia **278**:87-92.

Chapman, P. M., M. A. Farrell, and R. O. Brinkhurst. 1982. Relative tolerances of selected aquatic oligochaetes to individual pollutants and environmental factors. Aquatic Toxicology **2**:47-67.

Cummins, K. W. 1962. An evaluation of some techniques for the collection and analysis of benthic samples with special emphasis on lotic waters. American Midland Naturalist **67**:477-504.

Day, P. R. 1965. Particle fractionation and particle– size analysis. Pages *in* C. A. Black, editor. Methods of soil analysis, Part 1. Agronomy No. 9. American Society of Agronomy, Madison, Wisconsin, USA.

Downing, D. 2000. Spawning and rearing ecology of rainbow trout in relation to whirling disease infection risk. Masters Thesis, Montana State University, Bozeman, Montana, USA.

Dumnicka, E. 1994. Habitat preferences of invertebrates (especially Oligochaeta) in a stream. Acta Hydrobiologia **36**:91-101.

El-Matbouli, M., T. Fischer-Scherl, and R. W. Hoffman. 1992. Present knowledge of the life cycle, taxonomy, pathology, and therapy of some Myxosporea species important for freshwater fish. Annual Review of Fish Diseases **3**:367-402.

El-Matbouli, M., and R. W. Hoffman. 1991. Effects of freezing, aging, and passage through the alimentary canal of predatory animals on the viability of *Myxobolus cerebralis* spores. Journal of Aquatic Animal Health **3**:260-262.

El-Matbouli, M., and Hoffman. 1998. Light and electron microscopic studies on the chronological development of *Myxobolus cerebralis* to the actinosporean stage in *Tubifex tubifex*. International Journal of Parasitology **28**:195-217.

El-Matbouli, M., R. W. Hoffmann, and C. Mandok. 1995. Light and electron microscopic observations on the route of the triactinomyxon-sporoplasm of *Myxobolus cerebralis* from epidermis into rainbow trout (*Onchorhynchus mykiss*). Journal of Fish Biology **46**:919-935.

El-Matbouli, M., T. S. McDowell, D. B. Antonio, K. B. Andree, and R. P Hedrick. 1999. Effect of water temperature on the development, release and survival of the triactinomyxon stage of *Myxobolus cerebralis* in its oligochaete host. International Journal for Parasitology **29**:627-641.

Federal Energy Regulatory Commission (FERC). 1997. Draft Environmental Impact Statement Missouri-Madison Hydroelectric Project. FERC Project Number 2188, Washington D.C.

Finogenova, N. P. 1996. Oligochaete communities at the mouth of the Neva and their relationship to anthropogenic impact. Hydrobiologia **334**:185-191.

Gilbert, M. A., and W. O. Granath. 2001. Persistent infection of *Myxobolus cerebralis*, the causative agent of salmonid whirling disease, in *Tubifex tubifex*. Journal of Parasitology **87**:101-107.

Halliday, M. M. 1976. The biology of *Myxosoma cerebralis*: the causative organism of whirling disease. Journal of Fish Biology **9**:339-357.

Hedrick, R. P., M. El-Matbouli, M. A. Adkison, E. MacConnell. 1998. Whirling disease: re-emergence among wild trout. Immunological Reviews **166**:365-376.

Hedrick, R. P., T. S. McDowell, M. Gay, G. D. Marty, M. P. Georgiadis, and E. MacConnell. 1999a. Comparative susceptibility of rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) to *Myxobolus cerebralis* the cause of salmonid whirling disease. Diseases of Aquatic Organisms **37**:173-183.

Hedrick, R. P., T. S. McDowell, K. Mukkatira, and M. P. Georgiadis. 1999b. Susceptibility of selected inland salmonids to experimentally induced infection with *Myxobolus cerebralis*, the causative agent of whirling disease. Journal of Aquatic Animal Health **11**:330-339.

Hiner, M., and C. M. Moffitt. 2001. Variation in infections of *Myxobolus cerebralis* in field-exposed cutthroat and rainbow trout in Idaho. Journal of Aquatic Animal Health **13**:124-132.

Hoffman, G. L. 1990. *Myxobolus cerebralis*, a worldwide cause of salmonid whirling disease. Journal of Aquatic Animal Health **2**:30-37.

Hoffman, G. L., and C. J. Byrne. 1974. Fish age as related to susceptibility to *Myxosoma cerebralis*, cause of whirling disease. Progressive Fish-Culturist **36**:151.

Hoffman, G. L., and R. E. Putz. 1969. Host susceptibility and the effect of aging, freezing, heat, and chemicals on spores of *Myxosoma cerebralis*. Progressive Fish-Culturist **31**:35-37.

Juget, J., and M. Lafont. 1994. Theoretical habitat templets, species traits, and species richness: aquatic oligochaetes in the Upper Rhone River and its floodplain. Freshwater Biology **31**:327-340.

Kathman, R. D., and R. O. Brinkhurst. 1998. Guide to the freshwater oligochaetes of North America. Aquatic Resources Center, College Grove, Tennessee, USA.

Kaster, J. L. 1980. The reproductive biology of *Tubifex tubifex* Muller (Annelida:Tubificidae). The American Midland Naturalist 104:364-366.

Kerans, B. L., C. Rasmussen, R. Stevens, and J. R. Winton. The oligochaete host as a modulator of whirling disease in salmonids. Submitted to Molecular Ecology.

Kerans, B. L., and A. V. Zale. 2002. The Ecology of *Myxobolus cerebralis*. Pages 145-166 *in* J. L. Bartholomew and J. C. Wilson, editors. Whirling disease:reviews and current topics. American Fisheries Society, Symposium 29, Bethesda, Maryland.

Krezoski, J. R., Mozley, S. C., and Robbins, J. A. 1978. Influence of benthic macroinvertebrates on mixing of profundal sediments in southeastern Lake Huron. Limnology and Oceanography 23:1011-1016.

Lazim, M. N., and M. A. Learner. 1986. The life – cycle and productivity of *Tubifex tubifex* (Oligochaeta: Tubificidae) in the moat – feeder stream, Cardiff, South Wales. Holarctic Ecology **9**:185-192.

Lazim, M. N., and M. A. Learner. 1987. The influence of sediment composition and leaf litter on the distribution of tubificid worms (Oligochaeta). Oecologia **72**:131-136.

Lemmon, J. C. and B. L. Kerans. 2001. The extraction of whirling disease myxospores from sediments using the plankton centrifuge and sodium hexametaphosphate. In press, Intermountain Journal of Sciences.

Lom, J. and I. Dykova. 1992. Fine structure of *Triactinomyxon* early stages and sporogony: myxosporean and actinosporean features compared. Journal of Parasitology **39**:16-27.

Lom, J., J. McGeorge, S. W. Feist, D. Morris, and A. Adams. 1997. Guidelines for the uniform characterization of the actinosporean stages of parasites of the phylum Myxozoa. Diseases of Aquatic Organisms **30**:1-9.

Marcus, W. A., S. C. Ladd, and J. A. Stoughton. 1995. Pebble counts and the role of user-dependent bias in documenting sediment size distributions. Water Resources Research **31**: 2625-2631.

Markiw, M. E. 1986. Salmonid whirling disease: dynamics of experimental production of the infective stage- the triactinomyxon spore. Canadian Journal of Fisheries and Aquatic Sciences **43**:521-526.

Markiw, M. E. 1991. Earliest susceptible age of rainbow trout to the triactinomyxid of *Myxobolus cerebralis*. Aquaculture **92**:1-6.

Markiw, M. E. 1992. Experimentally induced whirling disease. I. Dose response of fry and adults of rainbow trout exposed to the triactinomyxon stage of *Myxobolus cerebralis*. Journal of Aquatic Animal Health **4**:44-47.

Markiw, M. E. and K. Wolf. 1983. *Myxosoma cerebralis* (Myxozoa: Myxosporea) etiologic agent of salmonid whirling disease requires tubificid worm (Annelida: Oligochaeta) in its life cycle. Journal of Protozoology **30**:561-564.

May, R. M., and R. M. Anderson. 1979. Population biology of infectious diseases. II. Nature **280**:455- 461.

Milbrink, G. 1983. An improved environmental index based on the relative abundance of oligochaete species. Hydrobiologia **102**:89-97.

Minchella, D. J. and M. E. Scott. 1991. Parasitism: A cryptic determinant of animal community structure. Trends in Ecology and Evolution **6**:250-254.

Moden, J. 1998. Whirling disease in California: a review of its history, distribution, and impacts, 1965-1997. Journal of Aquatic Animal Health **10**:132-142.

Nehring, B., P.G. Walker. 1996. Whirling disease in the wild: The new reality in the intermountain west. Fisheries **21** (6):28-30.

National Oceanic and Atmospheric Administration (NOAA). 2001. www.noaa.gov/climate.html.

Overton, C. K., S. P. Wollrab, B. C. Roberts, and M. A. Radko. 1997. R1/ R4/ Intermountain regions: Fish and fish habitat standard inventory procedures handbook. United States Department of Agriculture. Intermountain Research Station, Idaho.

Pennak, R. W. 1978. Fresh-water invertebrates of the United States, second edition. Johy Wiley and Sons, New York, New York, USA.

Reynoldson, T. B. 1987. The role of environmental factors in the ecology of tubificid oligochaetes-an experimental study. Holarctic Ecology **10**:241-248.

Robbins, J. A., T. Keilty, D. S. White, and D. N. Edgington. 1989. Relationships among tubificid abundances, sediment composition, and accumulation rates in Lake Erie. Canadian Journal of Fisheries and Aquatic Sciences **46**:223-231.

Rognie, M. C., and S. E. Knapp. 1998. *Myxobolus cerebralis* in *Tubifex tubifex* from a whirling disease epizootic in Montana. Journal of Parasitology **84**:711-713.

Sandell, T. A., H. V. Lorz, D. G. Stevens, and J. L. Bartholomew. 2001. Dynamics of *Myxobolus cerebralis* in the Lostine River, Oregon: Implications for resident and anadromous salmonids. Journal of Aquatic Animal Health **13**:142-150.

Sauter, G., and H. Gude. 1996. Influence of grain size on the distribution of tubificid oligochaete species. Hydrobiologia **334**:97-101.

SAS Institute. 1999 - 2000. SAS/STAT for Windows. Version 8.1, SAS Institute, Cary, North Carolina, USA.

Sloreid, S. E. 1994. Oligochaete response to changes in water flow in the Dokka Delta, Lake Randsfjorden (Norway), caused by hydroelectric power development. Hydrobiologia **278**:243-249.

Stevens, R., B. L. Kerans, J. C. Lemmon, and C. Rasmussen. 2001. The effects of *Myxobolus cerebralis* myxospore dose on triactinomyxon production and biology of *Tubifex tubifex* from two geographic regions. Journal of Parasitology **87**:315-321.

Takada, K., K. Kato, and T. Okino. 1992. Environmental parameters and estivation of Rhyacodrilus (Tubificidae, Oligochaeta) in Lake Suwa, Japan. Ecography **15**:328-333.

Vincent, E. R. 1996. Whirling Disease and wild trout: The Montana experience. Fisheries **21**:32-33.

Vincent, E. R. 1987. Effects of stocking catchable-size hatchery rainbow trout on two wild trout species in the Madison River and O'Dell Creek, Montana. North American Journal of Fisheries Management **7**:91-105.

Vincent, E. R. 2000. Whirling disease report 1997-1998. Montana Fish, Wildlife and Parks, Helena.

Voshell, J. R., and G. M. Simmons. 1984. Colonization and succession of benthic macroinvertebrates in a new reservoir. Hydrobiologia 112: 27-39.

Wachs, B. 1967. The oligochaete fauna of running waters, with special consideration of the relationships between the colonization by Tubificidae and the substrate. Archiv Hydrobiologie 63:310-386. Translation series: Fisheries and Marine service, Canada, Freshwater Resources Number 2663.

Wetzel, R. G., and G. E. Likens. 1991. Limnological Analyses, second edition. Springer-Verlag Press, New York, New York, USA.

Wolf, K., M. E. Markiw. 1984. Biology contravenes taxonomy in the Myxozoa: new discoveries show alternation of invertebrate and vertebrate hosts. Science **225**:365-368.

Wolf, K., M. E. Markiw, and J. K. Hiltunen. 1986. Salmonid whirling disease: *Tubifex tubifex* (Muller) identified as the essential oligochaete in the protozoan life cycle. Journal of Fisheries Diseases **9**:83-85.

Wolman, G. 1954. A method of sampling coarse river – bed material. Transactions, American Geophysical Union **35**: 951-956.

Zendt, J. S., and E. P. Bergersen. 2000. Distribution and abundance of the aquatic oligochaete host *Tubifex tubifex* for the salmonid whirling disease parasite *Myxobolus cerebralis* in the upper Colorado river basin. North American Journal of Fisheries Management **20**:502-512.

APPENDICES

APPENDIX A

OLIGOCHAETE DENSITIES

Mean densities (± 1 SE) of oligochaetes (individuals/m^2) were estimated in eight side channels in the Madison River, Montana, USA in 1999 – 2000. Eight samples were taken over three seasons for a total of 24 samples per side channel. Mature oligochaetes were identified to either species or family and immature oligochaetes were classified into two categories according to chaetal characteristics. Side channels were: North Slide (NS), South Slide (SS) Grizzly (GR), Pine Butte (PB), Kirby Bridge (KB), Lyons Bridge (LB), Sun Ranch (SR), and Palisades (PL).

Side channel	Date	Mature *Tubifex tubifex*	Mature *Rhyacodrilus* spp.	Mature *Ilyodrilus templetoni*	Mature *Limnodrilus* spp.	Lumbriculidae and Lumbricidae	Enchytraeidae	Naididae	Pectinate immatures	Bifed immatures	Total oligochaetes
NS	7/10/99	144.18 (± 144.18)	1946.37 (± 697.32)	0	0	1585.93 (± 863.33)	1153.40 (± 361.47)	4541.52 (± 1389.04)	4613.61 (± 1970.81)	144.18 (± 94.38)	14777.97 (± 3689.37)
	10/3/99	0	72.09 (± 72.09)	0	0	5478.66 (± 1813.89)	12110.73 (± 7252.30)	1153.40 (± 435.95)	18526.53 (± 2528.95)	792.96 (± 325.82)	38134.37 (± 8504.03)
	5/1/00	1009.23 (± 856.43)	1658.02 (± 1119.10)	0	144.18 (± 144.18)	1153.40 (± 644.77)	865.05 (± 308.26)	4181.08 (± 4018.85)	10741.06 (± 5313.50)	1153.40 (± 1073.39)	20905.42 (± 10584.44)
	mean	**384.47 (± 291.81)**	**1225.49 (± 454.35)**	**0**	**48.06 (± 48.06)**	**2739.33 (± 784.90)**	**4709.73 (± 2559.31)**	**3292.0 (± 1397.82)**	**11293.73 (± 2305.74)**	**696.85 (± 368.97)**	**24605.92 (± 4932.92)**
SS	7/10/99	216.26 (± 105.53)	648.79 (± 276.52)	0	0	792.96 (± 792.96)	1802.19 (± 811.47)	20617.07 (± 15694.69)	5406.57 (± 1836.47)	1009.23 (± 594.45)	30853.52 (± 17267.71)
	10/3/99	0	0	0	0	3604.38 (± 1424.14)	30493.08 (± 11782.34)	6343.71 (± 2910.16)	10308.54 (± 7598.96)	216.26 (± 151.70)	51254.33 (± 11763.17)
	5/1/00	360.44 (± 287.06)	1585.93 (± 1003.33)	0	72.09 (± 72.09)	5334.49 (± 2041.13)	8145.91 (± 3112.20)	4181.08 (± 3779.72)	13768.74 (± 8209.81)	1153.40 (± 871.89)	34602.08 (± 11609.47)
	mean	**192.23 (± 102.19)**	**744.91 (± 358.20)**	**0**	**24.03 (± 24.03)**	**3243.94 (± 918.96)**	**13480.39 (± 4660.1)**	**10380.62 (± 5441.47)**	**9827.95 (± 3681.0)**	**792.96 (± 350.27)**	**38903.31 (± 7834.45)**
GR	7/10/99	1297.58 (± 1141.76)	216.26 (± 216.26)	72.09 (± 72.09)	2090.54 (± 2009.42)	144.18 (± 94.38)	1297.58 (± 553.05)	12326.99 (± 3303.81)	12831.60 (± 9111.77)	1874.28 (± 1398.89)	32583.62 (± 13849.84)
	10/3/99	0	0	0	0	1585.93 (± 712.59)	792.96 (± 265.57)	2739.33 (± 1600.84)	7136.68 (± 3448.06)	9083.04 (± 6163.03)	21337.95 (± 10179.58)
	5/1/00	0	0	72.09 (± 72.09)	1081.31 (± 927.58)	576.70 (± 266.96)	937.14 (± 421.22)	72.09 (± 72.09)	1802.19 (± 989.54)	2234.72 (± 1990.12)	6776.24 (± 3784.43)
	mean	**432.53 (± 385.38)**	**72.09 (± 72.09)**	**48.06 (± 33.24)**	**1057.29 (± 727.05)**	**768.94 (± 274.80)**	**1009.23 (± 241.13)**	**5046.14 (± 1603.68)**	**7256.82 (± 3257.31)**	**4397.35 (± 2220.79)**	**20232.60 (± 6023.13)**

Side channel	Date	*Tubifex tubifex*	*Rhyacodrilus* spp.	*Ilyodrilus templetoni*	*Limnodrilus* spp.	Lumbriculidae and Lumbricidae	Enchytraeidae	Naididae	Pectinate immatures	Bifed immatures	Total oligochaetes
PB	7/10/99	288.35 (± 288.35)	0	0	144.18 (± 144.18)	0	72.09 (± 72.09)	504.61 (± 352.1)	2234.72 (± 1838.08)	360.44 (± 216.26)	3604.38 (± 2168.12)
	10/3/99	0	216.26 (± 216.26)	0	144.18 (± 144.18)	288.35 (± 217.97)	1874.28 (± 1424.14)	144.18 (± 94.38)	5334.49 (± 2520.27)	4036.91 (± 2182.45)	12254.90 (± 4579.69)
	5/1/00	72.09 (± 72.09)	0	72.09 (± 72.09)	144.18 (± 144.18)	504.61 (± 504.61)	2162.63 (± 1921.22)	216.26 (± 151.70)	792.96 (± 565.65)	216.26 (± 151.70)	4181.08 (± 2419.27)
	mean	**120.15 (± 98.06)**	**72.09 (± 72.09)**	**24.03 (± 24.03)**	**144.18 (± 79.54)**	**264.32 (± 180.31)**	**1369.67 (± 786.1)**	**288.35 (± 129.89)**	**2787.39 (± 1084.29)**	**1537.87 (± 791.33)**	**6680.12 (± 1968.87)**
KB	7/10/99	72.09 (± 72.09)	144.18 (± 144.18)	0	0	0	504.61 (± 297.23)	6343.71 (± 4435.09)	2378.89 (± 1468.03)	1153.40 (± 586.91)	12615.34 (± 8102.78)
	10/3/99	144.18 (± 94.38)	288.35 (± 188.77)	0	72.09 (± 72.09)	144.18 (± 94.38)	216.26 (± 151.70)	4253.17 (± 3339.57)	5046.14 (± 3610.97)	1369.67 (± 615.92)	12903.69 (± 8682.0)
	5/1/00	1081.31 (± 1081.31)	72.09 (± 72.09)	0	720.88 (± 418.57)	72.09 (± 72.09)	72.09 (± 72.09)	11245.67 (± 8472.24)	2739.33 (± 1848.76)	3316.03 (± 1236.65)	19319.49 (± 10572.79)
	mean	**432.53 (± 359.50)**	**168.20 (± 81.26)**	**0**	**264.32 (± 151.23)**	**72.09 (± 39.77)**	**264.32 (± 115.02)**	**7280.85 (± 3283.63)**	**3388.12 (± 1396.03)**	**1946.37 (± 519.32)**	**14946.17 (± 5105.33)**
LB	7/10/99	576.70 (± 288.35)	0	0	648.79 (± 229.58)	0	144.18 (± 144.18)	6199.54 (± 2397.08)	6055.36 (± 2627.0)	720.88 (± 357.34)	14417.53 (± 3885.48)
	10/3/99	0	0	0	0	0	0	7425.03 (± 6556.81)	71150.52 (± 51075.74)	27825.84 (± 16812.44)	106401.38 (± 59716.04)
	5/1/00	8722.61 (± 7988.36)	0	0	1946.37 (± 1705.69)	0	216.26 (± 105.53)	865.05 (± 533.92)	7136.68 (± 6562.69)	3027.68 (± 2550.72)	21914.65 (±18963.62)
	mean	**3099.77 (± 2678.06)**	**0**	**0**	**865.05 (± 573.56)**	**0**	**120.15 (± 59.92)**	**4829.87 (± 2307.80)**	**28114.19 (± 17606.69)**	**10524.80 (± 5991.17)**	**47577.85 (± 21803.96)**
SR	7/10/99	1369.67 (± 754.59)	0	0	0	0	0	3820.65 (± 1247.11)	2450.98 (± 1136.54)	72.09 (± 72.09)	8001.73 (± 2593.87)
	10/3/99	0	0	0	0	0	72.09 (± 72.09)	72.09 (± 72.09)	5262.40 (± 3244.86)	72.09 (± 72.09)	5550.75 (± 3307.40)
	5/1/00	216.26 (± 151.70)	0	576.70 (± 576.70)	0	0	216.26 (± 151.70)	72.09 (± 72.09)	1730.10 (± 956.35)	288.35 (± 108.99)	3099.77 (± 1544.91)
	mean	**528.64 (± 275.35)**	**0**	**192.23 (± 192.23)**	**0**	**0**	**96.12 (± 56.69)**	**1321.61 (± 542.77)**	**3147.83 (± 1180.24)**	**144.18 (± 52.07)**	**5550.75 (± 1486.13)**
PL	7/10/99	0	0	0	0	288.35 (± 288.35)	72.09 (± 72.09)	720.88 (± 418.57)	360.44 (± 105.53)	0	1441.75 (± 606.81)
	10/3/99	0	216.26 (± 151.70)	0	0	0	576.70 (± 188.77)	2811.42 (± 1047.84)	2450.98 (± 1722.36)	144.18 (± 144.18)	6343.71 (± 2608.85)
	5/1/00	0	72.09 (± 72.09)	72.09 (± 72.09)	216.26 (± 151.70)	288.35 (± 188.77)	0	2523.07 (± 1976.64)	865.05 (± 555.72)	144.18 (± 94.38)	4181.08 (± 2560.02)
	mean	**0**	**96.12 (± 56.69)**	**24.03 (± 24.03)**	**72.09 (± 52.79)**	**192.23 (± 113.37)**	**216.26 (± 83.69)**	**2018.45 (± 750.16)**	**1225.49 (± 606.55)**	**96.12 (± 56.69)**	**3988.85 (± 1252.05)**

APPENDIX B

TUBIFICID TRIACTINOMYXON RELEASE AND IDENTIFICATION

Tubificid identification and release of *Myxobolus cerebralis* triactinomyxons (TAMs) from six side channels [North Slide (NS), South Slide (SS), Pine Butte (PB), Kirby Bridge (KB), Lyons Bridge (LB), and Sun Ranch (SR)] in the Madison River, Montana, USA in 1999 – 2000. Dots indicate dates when no worms were found at a side channel after one hour of effort, or when a side channel was inaccessible.

		TAM release		Tubificid Identification			
Side channel	Date	Number observed	Number TAM +	Total Identified	*T. tubifex*	Other tubificids with hair chaetae	Immatures
	6/24/1999	179	0	18	0	11	7
	7/16/1999	71	1	53	7	9	37
	8/7/1999	87	0	42	2	8	32
	9/14/1999	203	0	29	0	3	26
	10/21/1999	102	0	37	0	2	35
NS	3/24/2000	187	0	37	0	5	32
	4/16/2000	103	0	42	0	10	32
	5/18/2000	198	0	68	0	42	26
	6/14/2000	195	1	93	0	52	41
	6/22/2000	137	0	96	0	54	42
	TOTAL	**1462**	**2**	**515**	**9**	**196**	**310**
	6/24/1999	193	0	32	0	15	17
	7/16/1999	65	1	43	0	7	36
	8/7/1999	104	8	60	3	16	41
	9/14/1999	191	9	54	5	10	39
	10/21/1999	199	3	48	0	5	43
SS	3/24/2000	.	.	.	.	.	.
	4/16/2000	80	2	46	1	0	45
	5/18/2000	200	8	80	0	28	52
	6/3/2000*	200	18	116	7	27	82
	6/14/2000	200	5	102	7	29	66
	6/22/2000	199	6	116	2	45	69
	TOTAL	**1431**	**42**	**581**	**18**	**155**	**408**
	6/24/1999	.	.	.	.	.	.
	7/16/1999	14	0	11	1	1	9
	8/7/1999	199	0	35	1	0	34
	9/14/1999	204	0	40	0	0	40
	10/21/1999	204	1	46	7	1	38
PB	3/24/2000	110	0	37	1	1	35
	4/16/2000	87	0	52	0	3	49
	5/18/2000	84	0	58	1	10	47
	6/14/2000	169	0	54	2	9	43
	6/22/2000	199	2	65	1	5	59
	TOTAL	**1270**	**3**	**398**	**14**	**30**	**354**

Side channel	Date	TAM release		Tubificid Identification			
		Number observed	Number TAM +	Total Identified	*T. tubifex*	Other tubificids with hair chaetae	Immatures
	6/24/1999	6	1	3	0	0	3
	7/16/1999	8	0	0	0	0	0
	8/7/1999	58	0	50	3	5	42
	9/14/1999	204	3	52	2	0	50
	10/21/1999	155	4	51	0	0	51
KB	3/24/2000	195	1	52	5	0	47
	4/16/2000	103	0	26	5	0	21
	5/18/2000	86	0	44	11	1	32
	6/14/2000	120	4	77	14	0	63
	6/22/2000	126	6	104	26	0	78
	TOTAL	**1061**	**19**	**459**	**66**	**6**	**387**
	6/24/1999	200	1	42	12	0	30
	7/16/1999	196	1	27	0	0	27
	8/7/1999	200	3	52	0	0	52
	9/14/1999	204	3	46	1	0	45
	10/21/1999	202	2	51	2	0	49
LB	3/24/2000	.	.	.	.	.	.
	4/16/2000	193	2	118	31	2	85
	5/18/2000	202	3	113	47	4	62
	6/3/2000*	200	4	77	36	0	41
	6/14/2000	199	4	102	12	5	85
	6/22/2000	200	8	123	13	4	106
	TOTAL	**1796**	**27**	**674**	**118**	**15**	**541**
	6/24/1999	3	0	3	1	1	1
	7/16/1999	9	0	3	0	0	3
	8/7/1999	159	5	59	3	0	56
	9/14/1999	.	.	.	.	.	.
	10/21/1999	182	4	44	0	0	44
SR	3/24/2000	7	0	5	1	0	4
	4/16/2000	13	0	9	4	0	5
	5/18/2000	63	1	11	6	0	5
	6/14/2000	.	.	.	.	.	.
	6/22/2000	.	.	.	.	.	.
	TOTAL	**436**	**10**	**134**	**15**	**1**	**118**

* Collections made on 6/3/2000 were not used in the totals or regression analyses because they were only made in two side channels.

APPENDIX C

ESTIMATED DENSITIES OF INFECTED *TUBIFEX TUBIFEX*

The percentages and densities of *Tubifex tubifex* and tubificids with hair chaetae releasing *Myxobolus cerebralis* triactinomyxons (TAMs) in six side channels on the Madison River, Montana, USA in 1999-2000. The combination of the estimates of the percent of *T. tubifex* releasing TAMs and the densities of *T. tubifex* produced the estimates of the densities of *T. tubifex* releasing TAMs.

Side channel	Date	% Tubificids releasing TAMs	Estimated % *T. tubifex* releasing TAMs	Estimated density of *T. tubifex* releasing TAMs (individuals/m^2)
North Slide	6/24/1999	0	0	3.94
	7/16/1999	1.41	32.08	
	8/7/1999	0	0	
	9/14/1999	0	0	
	10/21/1999	0	0	
	3/24/2000	0	0	
	4/16/2000	0	0	
	5/18/2000	0	0	
	6/14/2000	0.51	11.68	
	6/22/2000	0	0	
	Mean	**0.19 ± 0.14**	**4.38 ± 3.29**	
South Slide	6/24/1999	0	0	29.26
	7/16/1999	1.54	12.31	
	8/7/1999	7.69	61.54	
	9/14/1999	4.71	37.7	
	10/21/1999	1.51	12.1	
	3/24/2000	.	.	
	4/16/2000	2.5	20	
	5/18/2000	4	32	
	6/14/2000	2.5	20	
	6/22/2000	3.02	24.12	
	Mean	**3.65 ± 0.89**	**29.17 ± 7.14**	
Pine Butte	6/24/1999	.	.	0.22
	7/16/1999	0	0	
	8/7/1999	0	0	
	9/14/1999	0	0	
	10/21/1999	0.49	1.54	
	3/24/2000	0	0	
	4/16/2000	0	0	
	5/18/2000	0	0	
	6/14/2000	0	0	
	6/22/2000	1.01	3.16	
	Mean	**0.17 ± 0.12**	**0.52 ± 0.37**	

Side channel	Date	% Tubificids releasing TAMs	Estimated % *T. tubifex* releasing TAMs	Estimated density of *T. tubifex* releasing TAMs (individuals/m^2)
Kirby Bridge	6/24/1999	16.67	18.18	2.5
	7/16/1999	0	0	
	8/7/1999	0	0	
	9/14/1999	1.47	1.6	
	10/21/1999	2.58	2.82	
	3/24/2000	0.51	0.56	
	4/16/2000	0	0	
	5/18/2000	0	0	
	6/14/2000	3.33	3.64	
	6/22/2000	4.76	5.19	
	Mean	**2.93 ± 1.61**	**3.20 ± 1.76**	
Lyons Bridge	6/24/1999	0.5	0.55	48.87
	7/16/1999	0.51	0.56	
	8/7/1999	1.5	1.65	
	9/14/1999	1.47	1.61	
	10/21/1999	0.99	1.01	
	3/24/2000	.	.	
	4/16/2000	1.04	1.14	
	5/18/2000	1.49	1.63	
	6/14/2000	2.01	2.21	
	6/22/2000	4	4.39	
	Mean	**1.55 ± 0.32**	**1.70 ± 0.35**	
Sun Ranch	6/24/1999	0	0	1.2
	7/16/1999	0	0	
	8/7/1999	3.14	3.35	
	9/14/1999	.	.	
	10/21/1999	2.2	2.34	
	3/24/2000	0	0	
	4/16/2000	0	0	
	5/18/2000	1.59	1.69	
	6/14/2000	.	.	
	6/22/2000	.	.	
	Mean	**0.99 ± 0.50**	**1.06 ± 0.53**	

APPENDIX D

DIFFERENTIAL INFECTION IN SENTINEL RAINBOW TROUT

The mean grade of *Myxobolus cerebralis* infection and the proportion of rainbow trout infected in upstream and downstream sentinel cages in eight side channels in the Madison River, Montana, USA in 1999. The amount of *M. cerebralis* produced within a side channel was assessed for each side channel as the mean severity of infection in rainbow trout in sentinel cages placed at the downstream end of each side channel minus the mean severity of rainbow trout infection in sentinel cages placed at the upstream end of each side channel. Infection grade was based on the modified MacConnell-Baldwin scale (0-5) (Hedrick et al. 1999a). The cage means, proportions, and standard errors presented in columns 2 through 5 are the means across dates of each upstream and downstream cage, whereas the means in columns 6 and 7 are the means of the differences between cages across dates.

Side channel	Downstream cage		Upstream cage		Difference in mean RBT infection grades (lower cage – upper cage)	Difference in proportion of RBT infected (lower cage – upper cage)
	mean grade (range)	Proportion infected	mean grade (range)	Proportion infected		
NS	1.05 ± 0.15 (0-4)	0.37 ± 0.31	0.10 ± 0.03 (0-1)	0.1 ± 0.05	0.78 ± 0.74 n=2	0.27 ± 0.26
SS	2.31 ± 0.17 (0-5)	0.83	0.0 ± 0.0 (0-0)	0	2.54 n=1	0.83
GR	4.13 ± 0.12 (0-5)	0.97 ± 0.01	4.20 ± 0.12 (0-5)	0.97 ± 0.01	-0.11 ± 0.19 n=2	0.01 ± 0.01
PB	1.15 ± 0.12 (0-4)	0.62 ± 0.06	1.03 ± 0.12 (0-4)	0.56 ± 0.05	0.12 ± 0.08 n=2	0.05 ± 0.01
KB	2.01 ± 0.14 (0-5)	0.87 ± 0.1	2.89 ± 0.17 (0-5)	0.9 ± 0.05	-0.59 ± 0.16 n=2	-0.02 ± 0.05
LB	3.84 ± 0.14 (0-5)	0.95 ± 0.03	1.70 ± 0.16 (0-5)	0.73 ± 0.18	2.10 ± 0.24 n=2	0.22 ± 0.16
SR	2.92 ± 0.12 (0-5)	0.97 ± 0.01	3.15 ± 0.15 (0-5)	0.94 ±0.02	-0.20 ± 0.45 n=2	0.03 ± 0.03
PL	2.98 ± 0.15 (0-5)	0.92 ± 0.02	2.56 ± 0.15 (0-5)	0.89 ± 0.01	0.36 ± 0.16 n=2	0.04 ± 0.01

MONTANA STATE UNIVERSITY - BOZEMAN
3 1762 10357986 6