Characteristics of streams infected by whirling disease (*Myxobolus cerebralis*) on the Flathead Indian Reservation of northeastern Montana

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Abstract

Whirling disease, caused by the parasite *Myxobolus cerebralis,* is sweeping through the streams of the intermountain west. Native and endangered species of trout are dying from this debilitating disease, damaging stream ecosystems and the recreation and fisheries they support. This study examines the relationship of stream characteristics to the abundance of *Tubifex tubifex*, an oligochaete host of the parasite. As *T. tubifex* were unable to be located, presence and abundance of other types of oligochaetes were used as a proxy to determine which streams may support the most tubifex worms. Streams with low velocity and temperature and high levels of cobble substrate seem to support the most oligochaetes. Also, stream characteristics between infected and uninfected streams were compared to determine which streams are most at risk of infection. Infected streams tend to have low cobble, and high dissolved oxygen and conductivity. These results can help to determine vulnerable streams that should be monitored and protected.

Introduction

Whirling disease has become a major problem in trout populations of the western United States. After its introduction from Europe in 1958, it has spread from fish farms to wild populations in streams throughout the intermountain west (Hedrick et al. 1998). The disease is caused by a myxozoan parasite, *Myxobolus cerebralis*. The parasite has a complex life cycle involving two hosts: it first resides in the digestive tract of *Tubifex tubifex*, an oligochaete worm, and once expelled it infects a salmonid fish (Wolf and Markiw 1984). *Myxobolus cerebralis* enters the fish through the epidermis, then travels through the fish's nervous system to the spine and cranium where it consumes cartilage (El-Matbouli and Hoffman 1998). This results in spinal deformities and blackening of the fish's tail, and can cause the fish to swim in circles, or "whirl." Affected fish are often unable to feed or avoid predation. The severity of these symptoms appears to depend on the amount of *M. cerebralis* in the fish's system and on the age of the fish, with fry being most susceptible (Hedrick et. al. 1999). Rainbow trout (*Oncorhynchus mykiss*) are most often infected, but other species such as the endangered cutthroat trout (*Oncorhynchus clarki*) and bull trout (*Salvelinus confluentus*) are also at risk (Gilbert and Granath 2003). As the threat of whirling disease spreads across the United States decimating trout populations, the need for tools to predict, detect, and prevent infection intensifies.

Since *M. cerebralis* must be hosted by *T. tubifex* to complete its life cycle, studies on these worms could create tools to locate vulnerable streams. Studies have shown that certain populations of *T. tubifex* are genetically predisposed to infection (Beauchamp et al. 2002). Isaak and Hubert looked at stream characteristics such as temperature and substrate composition to try to determine where infected worms are most prevalent, and found the main factor is how far the worms are from a heavily infected stream (1999). Another study found that streams with fine, silty sediment and cold water had the most infected worms (Krueger et al. 2006).

For all the research done on determining which worms are most likely to become infected, there does not appear to be a study testing whether there is a threshold density of *T. tubifex* that is necessary for the stream to support an *M. cerebralis* infection. Since *T. tubifex* is necessary for the parasite to live, I hypothesize that a certain threshold density of these worms must be reached in order for the disease to persist. Additionally, I plan to test whether stream characteristics, especially those related to water quality and land use, affect *T. tubifex* and overall annelid abundance and whether these characteristics differ between infected and uninfected streams. Tubificid worms have been proposed as a water quality indicator, actually preferring eutrophic conditions (Gilbert and Granath 2003). Therefore, I predict that streams polluted with silty run-off from agricultural fields will support more *T. tubifex*, possibly putting those streams at greater risk of whirling disease. Accordingly, these same types of streams should have higher levels of other annelids, and also higher incidence of whirling disease.

Materials and Methods

Using data from a study performed by Montana Fish, Wildlife, and Parks, six streams on the Flathead Indian Reservation were selected as study sites (Vincent 2005). Three of these streams showed no signs of whirling disease as of 2005: Crow Creek upstream of its dam, Spring Creek, and Finley Creek. The other three have shown strong evidence of whirling disease infection: Mission Creek, Valley Creek, and the Jocko River. These streams pass through a variety of land use types including wildlife preserves, cattle pastures, irrigated and fertilized fields, towns, residential areas, and highways, and originate from both springs and montane snow melt. Crow Creek passes through agricultural fields and a wildlife area. Spring Creek is fed from a cold spring in a residential and agricultural area before running through the town of Ronan. Finley Creek passes through a largely residential and forested area, with a few cattle ranches. Mission Creek crosses the National Bison Range and agricultural fields after originating in the Mission Mountains. Valley Creek runs from mountains through cattle pasture and meadows. The Jocko River flows along highways near the base of mountain ranges, and through the town of Arlee. The diversity of their locations should provide the necessary variety to test my hypotheses on stream characteristics.

Ten sites in each stream were sampled at a random distance from an access point (Crow Creek: N 47.49729 W 114.14779, Spring Creek: N 47.54217 W 114.08888, Finley Creek: N 47.10183 W 114.05486, Mission Creek: N 47.36114 W 114.17760, Valley Creek: N 47.21714 W 114.23554, Jocko River: N 47.24717 W 114.17081). A random number generator was used to determine whether to sample upstream or downstream of the access point, and to determine the distances of each sampling site from the access point. Sampling alternated between infected and uninfected streams to minimize time- and weather-dependent effects. Water temperature, dissolved oxygen content and conductivity were measured at each site using a YSI meter. Water velocity was determined using the orange method. Stream bottom was visually characterized into percentages of cobble, silt, sand, vegetation, algae, and large woody debris (LWD). Water samples were collected and analyzed in the lab using Hach kits to test nitrate and phosphate levels. A 5.1 cm diameter sediment core was taken in a silty or sandy location at each site, and returned to lab to determine *T. tubifex* abundance. Tubificid worms typically burrow in the top 6 to 10 cm of sediment, so coring was performed to between a depth of 10 and 15 cm to ensure collection of all worms (Karlckhoff and Morris 1985). Cores were stored in Kahle's solution for one week before being rinsed through a 500 µm sieve (see Krueger et al. 2006). The remaining fraction was stored in ethanol and the *T. tubifex* worms, other annelids, and other invertebrates were counted.

Data was analyzed using Systat. After arcsine and log transformations failed to normalize the data, nonparametric statistics were used. Mann-Whitney U-tests were performed to determine whether stream characteristics differ between streams with and without whirling disease.

Logistic regression was used to determine which stream characteristics affect worm presence. A linear regression was run between worm abundance and stream characteristics.

Results

No *T. tubifex* were found in the 60 sediment cores taken. Statistics were therefore run to determine differences in stream characteristics between streams with and without whirling disease, and to determine which variables predict abundance of other annelids. After arcsine transforming the percentages of the stream bottom types and log transforming the other stream characteristics, most variables were still non-normal (Shapiro-Wilk p-value ≤ 0.05). Therefore, non-parametric statistics and robust regression models were used.

Stream characteristics in streams with and without whirling disease

The mean values of all stream chemistry characteristics, stream bottom characteristics, and the numbers of annelids and other invertebrates were calculated for each stream (Figs. 1, 2, 3). Mann-Whitney U-tests were performed on these mean values to determine whether differences exist between streams with and without whirling disease (Table 1). Only the number of non-annelid invertebrates had a significant difference, with higher numbers in streams without whirling disease (p=0.0495).

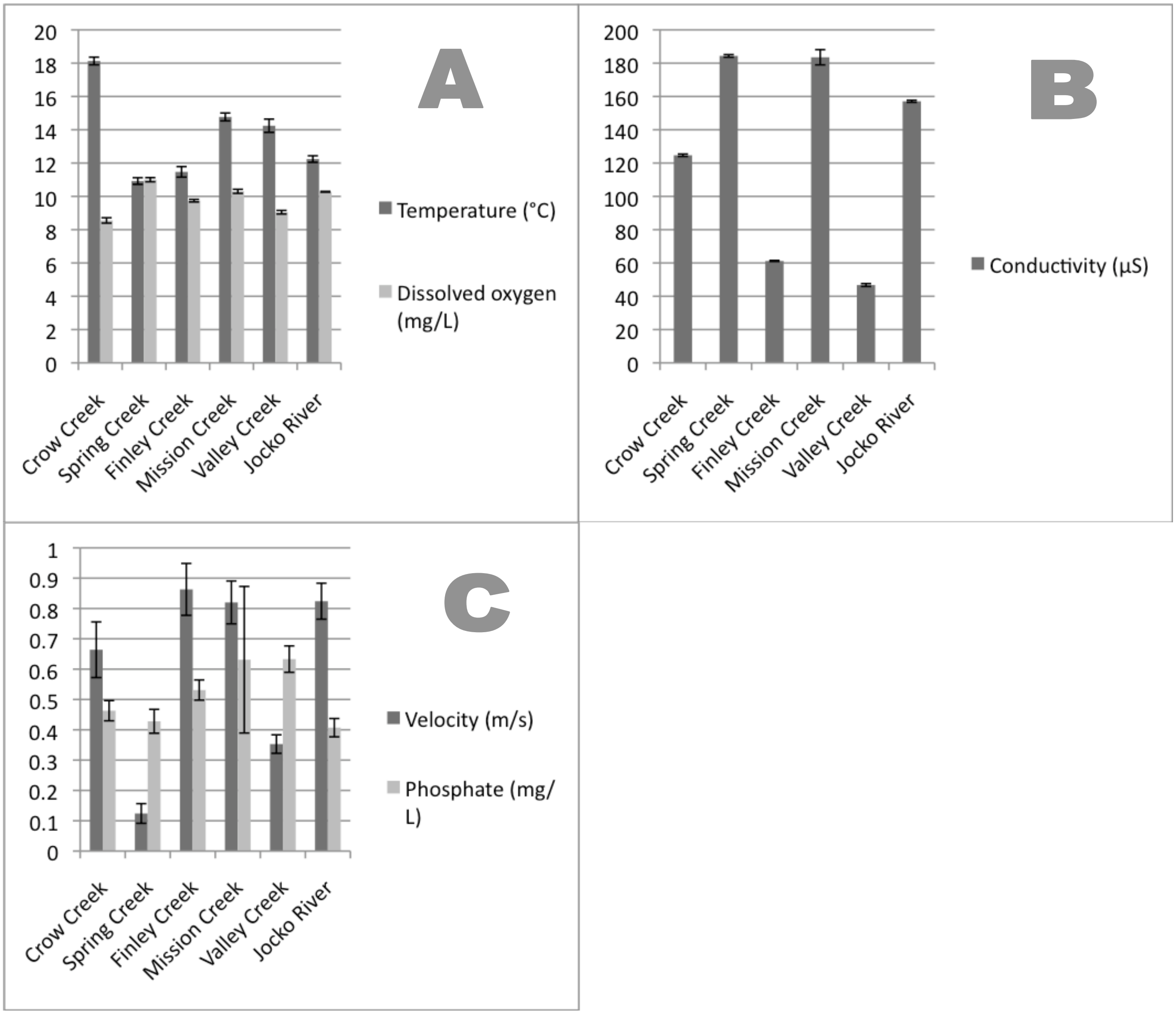

Figure 1. Average stream chemistry characteristics by stream. "A" shows mean dissolved oxygen and temperature, "B" shows mean conductivity, and "C" shows mean water velocity and phosphate levels. Crow Creek, Spring Creek, and Finley Creek do not have whirling disease; and Mission Creek, Valley Creek, and the Jocko River are infected.

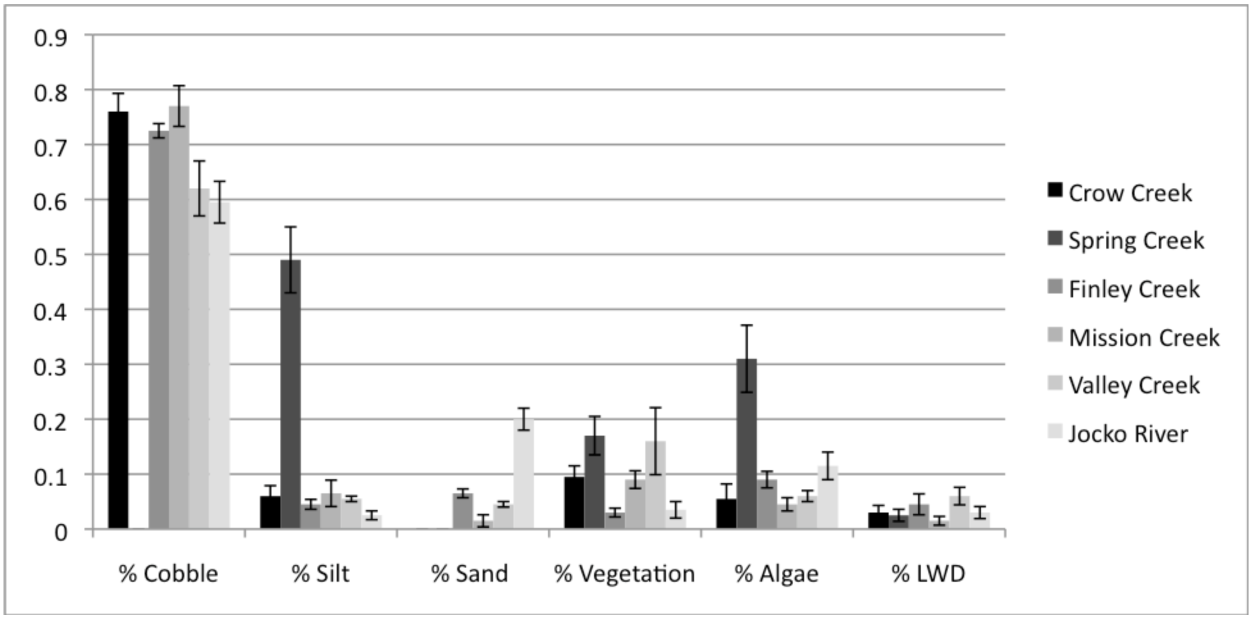

Figure 2. Stream bottom characterization of each stream. Mean percent cover by each substrate type in each stream. Crow, Spring, and Finley Creeks are not infected by whirling disease. Mission and Valley Creeks and the Jocko River are infected by whirling disease. Note that Spring Creek is often an outlier.

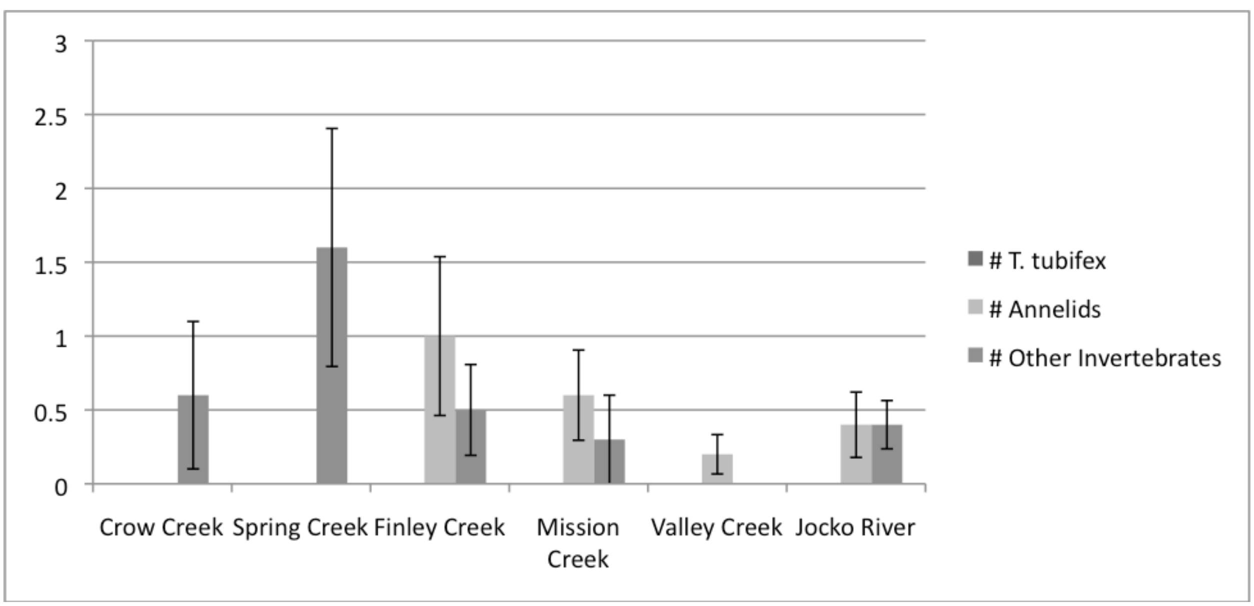

Figure 3. Mean number of invertebrates per sediment core by stream. Each cylindrical core was at least 10 cm long, and 5.1 cm in diameter. No *T. tubifex* were found, but numbers of other types of annelids and non-annelid invertebrates are shown here. Crow Creek, Spring Creek, and

Finley Creek do not have whirling disease; and Mission Creek, Valley Creek, and the Jocko River are infected.

Table 1. Mann-Whitney U-test results of mean stream characteristics in streams with and without whirling disease. Mean values of each characteristic were calculated for 3 streams without whirling disease (Crow Creek, Spring Creek, Finley Creek) and 3 streams with whirling disease (Mission Creek, Valley Creek, Jocko River) and used in this test.

Statistic	Temp. (°C)	D.O. (mg/L)	Cond. (μS)	V (m/s)	PO3 (mg/L)	% Cobble	% Silt
U	6	5	4	5	6	5	3
df	1	1	1	1	1	1	1
p	0.5127	0.8273	0.8273	0.8273	0.5127	0.8273	0.5127
Statistic	**% Sand**	**% Veg.**	**% Algae**	**% LWD**	**# Annelids**	**# Other Inverts**	
U	7	4	3	4.5	6	0	
df	1	1	1	1	1	1	
p	0.2683	0.8273	0.5127	1	0.5066	0.0495	

*D.O.=dissolved oxygen, Cond.=conductivity, V=velocity, PO3=phosphate, Veg.=vegetation

Examination of the mean levels of each stream bottom type revealed that Spring Creek may be an outlier (Fig.2). It appears to have higher levels of silt and algae, and much lower levels of cobble than the rest of the streams. The Mann-Whitney U-tests on mean stream characteristics between streams with and without whirling disease were re-run without Spring Creek to see if results differed without this outlier (Table 2). Results showed no significant differences between infected and uninfected streams ($p > 0.05$).

Table 2. Mann-Whitney U-test results of mean stream characteristics in streams with and without whirling disease, excluding Spring Creek. Mean values of each characteristic were calculated for 2 streams without whirling disease (Crow Creek, Finley Creek) and 3 streams with whirling disease (Mission Creek, Valley Creek, Jocko River) and used in this test.

Statistic	Temp. (°C)	D.O. (mg/L)	Cond. (μS)	V (m/s)	PO3 (mg/L)	% Cobble	% Silt
U	3	5	4	2	4	2	3
df	1	1	1	1	1	1	1
p	1	0.2482	0.5637	0.5637	0.5637	0.5637	1
Statistic	**% Sand**	**% Veg.**	**% Algae**	**% LWD**	**# Annelids**	**# Other Inverts**	
U	4	4	3	2.5	3	0	
df	1	1	1	1	1	1	
p	0.5637	0.5637	1	0.7671	1	0.0833	

*D.O.=dissolved oxygen, Cond.=conductivity, V=velocity, PO3=phosphate, Veg.=vegetation

The limited replication and loss of data inherent in using means prompted me to also analyze the full data set of 60 sites (10 per stream). Although this is pseudoreplication, it may reveal information lost in averaging the data. Mann-Whitney U-tests were again run for the stream characteristics of all the sites sampled grouped by whether the stream was infected or uninfected (Table 3). The percent of silt in the stream bottom was the only significant difference between stream types, with higher silt in streams without whirling disease ($p=0.0093$). Again, Spring Creek was removed as it is a high-silt, low-cobble outlier and the Mann-Whitney U-tests were re-run (Table 4). Dissolved oxygen and conductivity were significantly higher in streams with whirling disease ($p=0.0008$, 0.0477). Cobble was significantly higher in streams without whirling disease ($p=0.046$).

Table 3. Mann-Whitney U-test results of stream characteristics in streams with and without whirling disease at each sampling site. Ten sites per stream were used in the analysis. Three streams without whirling disease (Crow Creek, Spring Creek, Finley Creek) and 3 streams with whirling disease (Mission Creek, Valley Creek, Jocko River) were sampled.

Statistic	Temp. (°C)	D.O. (mg/L)	Cond. (μS)	V (m/s)	PO3 (mg/L)	% Cobble	% Silt
U	334.5	416.5	483.5	351	422	399.5	616.5
df	1	1	1	1	1	1	1
p	0.0876	0.6204	0.6204	0.1433	0.6787	0.4509	0.0093
Statistic	% Sand	% Veg.	% Algae	% LWD	# Annelids	# Other Inverts	
U	500.5	500.5	561	431	381.5	519.5	
df	1	1	1	1	1	1	
p	0.44	0.44	0.0928	0.7569	0.1586	0.1647	

*D.O.=dissolved oxygen, Cond.=conductivity, V=velocity, PO3=phosphate, Veg.=vegetation

Table 4. Mann-Whitney U-test results of stream characteristics in streams with and without whirling disease at each sampling site, excluding Spring Creek. Ten sites per stream were used in the analysis. Two streams without whirling disease (Crow Creek, Finley Creek) and 3 streams with whirling disease (Mission Creek, Valley Creek, Jocko River) were sampled.

Statistic	Temp. (°C)	D.O. (mg/L)	Cond. (μS)	V (m/s)	PO3 (mg/L)	% Cobble	% Silt
U	329	131	200	345	308	399.5	317.5
df	1	1	1	1	1	1	1
p	0.5657	0.0008	0.0477	0.3728	0.8741	0.046	0.7018
Statistic	% Sand	% Veg.	% Algae	% LWD	# Annelids	# Other Inverts	
U	277.5	277.5	288.5	299	276.5	328	
df	1	1	1	1	1	1	

p	0.6413	0.6413	0.8133	9827	0.5443	0.4261	

*D.O.=dissolved oxygen, Cond.=conductivity, V=velocity, PO3=phosphate, Veg.=vegetation

Predicting annelid presence and abundance

No *T. tubifex* were found, but other species of annelids were occasionally present in the sediment cores (Fig.3). A stepwise binary logistic regression was run to determine which variables can predict annelid presence or absence at a cored site. All 60 sampling sites were used in the analysis. The only significant variable was velocity (coefficient=-2.436, Z=-2.088, p=0.037) when LWD is also used as a nonsignificant variable (coefficient=4.127, Z=1.520, p=0.128). The equation's constant was 2.511 (Z=2.716, p=.007). The model had significant goodness-of-fit (χ^2= 7.587, df=2, p=0.023, R^2=0.183).

A backwards-stepping linear regression was also run between the stream characteristics and the number of annelids found per core. All 60 sampling sites were again used. The amount of cobble and water temperature were significant (coefficients=1.044, -0.128; t=3.099, -2.601; p=0.003, 0.012) when the number of non-annelid invertebrates is also used as a non-significant variable (coefficient=0.125, t=1.512, p=0.136). The equation's constant was 1.181 (t=1.967, p=0.054). The regression was significant (F=3.911, df=3, p=0.013, R^2=0.129).

Discussion

The complete lack of *T. tubifex* worms in all 60 sediment cores is a surprising result. It is widely assumed that *T. tubifex* is an obligate host for *M. cerebralis* (Stevens et al. 2001, Gilbert and Granath 2003, Kerans et al. 2004). Interestingly, Mission Creek, Valley Creek, and the Jocko River have documented cases of whirling disease and have turned up no tubifex worms (Vincent 2005). This suggests that an alternative host exists for *M. cerebralis*. Without an oligochaete host, the pathogen cannot mature and therefore cannot infect salmonid fish (El-Matbouli and Hoffmann 1998). If no tubifex worms are present in these streams, the pathogen must be maturing in another type of worm. In future studies, a wide variety of potential oligochaete hosts, especially other tubificids, should be tested to determine whether other species are possible hosts for whirling disease. Alternatively, tubifex worms may not have been located due to problems locating silty substrate in many streams. Also, many of the worms collected lacked chetae and reproductive structures, limiting my ability to identify them. However, based on gross morphological characteristics, I believe that my assessment was accurate that none of the worms in the sediment cores were *T. tubifex.*

Although *T. tubifex* was not found and therefore cannot be used to determine a threshold density required for infection, other stream characteristics could be used to determine which streams are most susceptible to infection. The Mann-Whitney U-test of the means for each stream showed that there were higher numbers of non-annelid invertebrates in streams without whirling disease (Table 1). This makes sense, as *T. tubifex* flourishes in polluted streams, and some other species of invertebrates such as caddis flies are sensitive to low water quality (Resh and Unzicker 1975). Therefore, since streams without whirling disease should be more pristine, they should also have higher levels of non-annelid invertebrates.

After removing Spring Creek as an outlier and re-running the Mann-Whitney U-test, none of the stream characteristics varied significantly between streams with and without whirling disease (Table 2). Even with Spring Creek only one characteristic was significant, indicating that the streams sampled are similar. Perhaps other factors are more important in determining which streams become infected with whirling disease. Isaak and Hubert found that distance from an already infected stream is the most important factor (1999). The amount of recreation by fishermen and subsequent stocking of potentially-infected trout could also influence how susceptible a stream is to infection. Further study into a connection between recreational use of streams and prevalence of infection would be informative. These non-significant results could also be due to low sample size; only three streams of each type were sampled.

Due to the low sample size, I also ran Mann-Whitney U-tests on all the data points collected as individual samples. This is pseudoreplication but could reveal information lost by averaging the data. The result was that only the percentage of silt was significantly different between stream types, with more silt in uninfected streams (Table 3). This seems counterintuitive, as *T.tubifex* prefers fine, silty sediments and infected worms release more *M. cerebralis* triactinomyxons in silt (Arndt et al. 2002). Looking at the stream bottom characteristics, it seems apparent that this result is likely due to the strong presence of silt in Spring Creek, one of the uninfected streams (Figure 2). Therefore, this stream was removed and the tests were re-run. The results then showed higher dissolved oxygen and conductivity and lower percentage of cobble in infected streams (Table 4). The higher levels of dissolved oxygen in infected streams is unusual since *T. tubifex* prefers polluted environments, which would have lower levels of dissolved oxygen. It is better able to compete in low-oxygen environments as it is capable of anaerobic respiration (Gilbert and Granath 2003). However, the higher conductivity levels are logical as higher conductivity is associated with clay soils and high levels of salt or runoff from mines. Higher conductivity is therefore associated with soft sediments and pollution, as are tubifex worms, and subsequently whirling disease. Also, the low level of cobble in infected streams is expected, since cobble is not preferred by *T. tubifex*. Of all the results obtained from the Mann-Whitney U-tests, these are the most logical, perhaps indicating that more replication testing a wider variety of streams would reveal additional information on which characteristics influence stream infection.

Since *T. tubifex* often lives nearby other oligochaetes due to beneficial feeding relationships (Brinkhurst et al. 1972), characteristics that affect annelid presence and abundance could be used as a proxy for characteristics that affect tubifex worms. The logistic regression showed that only velocity was a significant factor, with a negative relationship to worm presence. This is logical, as lower water velocity would allow silty sediments to accumulate, creating ideal worm habitat. LWD had a non-significant positive relationship to worm presence, which could indicate that decaying wood and plant matter contributes to the silty, nutrient-rich environment favored by annelids.

The linear regression showed that the amount of cobble and worm abundance are positively correlated, while temperature and worm abundance are negatively correlated. It seems odd that more cobble would correspond with more worms, they generally prefer to live in silty substrate. This also directly contradicts what was found in the Mann-Whitney U-test of all sampling sites excluding those at Spring Creek. However, a study by Smith et al. showed that tubifex worms are more abundant in lower elevation, cobble-substrate streams. Perhaps the sites with the most cobble were able to accumulate silt in pools sheltered behind large boulders, and therefore they yielded the most worms. However, I remain skeptical of this result, as more cobble seemed to correspond with less silt, and therefore less worm habitat. It follows that lower temperature would correlate with more worms, as tubificids prefer temperatures near the lower end of the range sampled, about 10-13 °C (Kerans et al. 2005). Additionally, *T. tubifex* infected by *M. cerebralis* are most prevalent at this temperature range (Krueger et al. 2006).

If the abundance of these other types of oligochaetes corresponds to the abundance of tubifex worms, and assuming that more tubifex worms translates to a greater risk of infection, this information could be useful for predicting which streams are most vulnerable to whirling disease. Low-velocity streams with low temperatures would be at risk, and should therefore be protected. Various conclusions can be drawn from the many permutations of Mann-Whitney U-tests performed in this study. The most promising results seem to indicate that streams with low cobble, high dissolved oxygen, and high conductivity are most at risk. This type of stream is often associated with silty, polluted agricultural runoff. These streams should not be stocked with salmonids, and signs should be posted advising anglers of measures they can take to prevent the spread of whirling disease. Future studies of a wider variety of streams should be performed to avoid complications in data analysis due to pseudoreplication. These studies would truly show which characteristics are associated with infected streams. Also, a modified coring or dredging technique should be devised to ensure capture of *Tubifex tubifex* to determine whether a threshold density of worms for infection exists. As whirling disease continues to threaten wild populations of trout throughout the American west, research of this nature gains importance and immediacy.

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Works Cited

Arndt, R.E., E. J. Wagner, Q. Cannon and M. Smith. 2002. Triactinomyxon production as related to rearing substrate and diel cycle. American Fisheries Society Symposium **29**: 87-91.

Beauchamp, K.A., M. Gay, G. O. Kelley, M. El-Matbouli, R. D. Kathman, R. B. Nehring and R. P. Hedrick. 2002. Prevalence and susceptibility of infection to *Myxobolus cerebralis*, and genetic differences among populations of *Tubifex tubifex.* Diseases of Aquatic Organisms **51**: 113-121.

Brinkhurst, R.O., K.E. Chua and N.K. Kaushik. 1972. Interspecific interactions and selective feeding by tubificid oligochaetes. Limnology and Oceanography **17**(1): 122-133.

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

Gilbert, M.A. and W.O. Granath, Jr. 2003. Whirling disease of salmonid fish: life cycle, biology and disease. The Journal of Parasitology **89**(4): 658-667.

Hedrick, R.P., M. El-Matbouli, M.A. Adkison,and 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. Giorgiadis and E. MacConnell. 1999. 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.

Isaak, D. J. and W. A. Hubert. 1999. Predicting the effects of *Myxobolus cerebralis* across a fifth-order Rocky Mountain watershed. 1999 Whirling Disease Symposium Proceedings: 146-151.

Karlckhoff, S.W. and K.R. Morris. 1985. Impact of tubificid oligochaetes on pollutant transport in bottom sediments. Environmental Science and Technology **19**: 51-56.

Kerans, B.L., C. Rasmussen, R. Stevens, A.E.L. Colwell and J.R. Winton. 2004. Differential propagation of the metazoan parasite *Myxobolus cerebralis* by *Limnodrilus hoffmeisteri*, *Ilyodrilus templetoni*, and genetically distinct strains of *Tubifex tubifex*. The Journal of Parasitology **90**(6): 1366-1373.

Kerans, B.L., R.I. Stevens and J.C. Lemmon. 2005. Water temperature affects a host-parasite interaction: *Tubifex tubifex* and *Myxobolus cerebralis*. Journal of Aquatic Animal Health **17**: 216-221.

Krueger, R.C., B.L. Kerans, E.R. Vincent and C. Rasmussen. 2006. Risk of *Myxobolus cerebralis* infection to rainbow trout in the Madison River, Montana, USA. Ecological Applications **16**(2): 770-783.

Resh, V.H. and J.D. Unzicker. 1975. Water quality monitoring and aquatic organisms: The importance of species identification. Journal of the Water Pollution Control Federation **47**(1): 9-19.

Smith, L.S., V. Watson, W. Granath and A. Sheldon. Distribution and abundance of *Tubifex tubifex* within Cottonwood Creek in the Blackfoot River drainage. University of Montana publication.

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. The Journal of Parsitology **87**(2): 315-321.

Vincent, E.R. 2005. Whirling disease infections in Salish-Kootenai streams. Montana Fish, Wildlife, and Parks. Unpublished manuscript.

Wolf, K. and M.E. Markiw. 1984. Biology contravenes taxonomy in the myxozoa: New discoveries show alternation of invertebrate and vertebrate hosts. Science **225**: 1449-1452.