Ref ID: 88401

Prediction of whirling disease in basin-fed streams in the Blackfoot Watershed, Montana

Ron Pierce
Montana Fish, Wildlife and Parks

Lisa Eby
University of Montana

Wease Bollman
Rhithron Associates

Dick Vincent
Montana, Fish Wildlife and Parks

Abstract

The parasite *Myxobolus cerebralis* has successfully invaded river systems throughout the interior West where it now poses a serious threat to wild salmonids. Given the variable effects and patchy distribution of whirling disease, it is important to identify the physical and biological characteristics of watersheds that influence and predict the disease. In the Blackfoot Basin of western Montana, we investigated relationships between a group of five landscape-and four reach-scale environmental conditions and the presence of whirling disease within basin-fed tributaries to the Blackfoot River in order to determine variables correlated with the disease. Our results show that, streams with higher gradients, lower levels of fines within the substrate and low summer temperatures supported little to no infection despite the near proximity of higher infection in adjacent waters. Infections were present in streams with summertime maximum water temperatures over 19°C; a logistic regression model including maximum water temperatures, fine sediment (<0.84mm) and channel gradient explained the presence of disease within our thirteen study streams. In our study area, infection was present and severity of whirling disease was high in meandering streams in broad valleys with gentle relief and warmer summer temperatures. We also examined the relationship between invertebrate taxa and the presence of whirling disease. Species richness of stenohaline species was the single best predictor of whirling disease; a logistic regression with stenohaline species, richness of Ephemeroptera, Plecoptera and Trichopera (EPT) species, and sediment sensitive species richness described was the best model for describing the variation in whirling disease in our dataset. In western Montana, infected environments described in this study are often prone to synergistic effects of habitat degradation often related to excessive grazing and other land use activities. Thus, changes in temperature or sediment regimes, whether natural or anthropogenic, likely influence infection and severity in wild trout populations.

Key words: Blackfoot River Basin, whirling disease, *Myxobolus cerebralis*, environmental characteristics, water temperature, macroinvertebrate assemblage, habitat

Introduction

Whirling disease is a chronic disease caused by the invasive myxosporean parasite *Myxobolus cerebralis* (Hoffman 1990) that was introduced into North America in the 1950s (Bartholomew and Reno 2002). *Myxobolus cerebralis* has a complex, two-host life cycle involving the aquatic oligochaete worm *Tubifex tubifex* and most members of the salmonidae family. Young fish (age-0 fry) are most susceptible to whirling disease (Ryce 2004); high mortality and recruitment collapse has occurred in certain infected rainbow trout (*Oncorhynchus mykiss*) populations in Montana (MacConnell and Vincent 2002) and Colorado (Nehring and Walker 1996). First detected in Montana in 1994 in the Madison River, this disease has spread to salmonid-dominated river systems throughout western Montana. The distribution and severity of disease is geographically highly variable among and within watersheds despite the ubiquitous presence of the salmonid host. This high variation in disease across regions, within drainages and within streams has been observed throughout the West including Colorado, Idaho, Utah, California and Montana (Nehring and Walker 1996, Modin 1998, Hiner and Moffitt 2001, Sandell et al. 2001, de la Hoz Franco and Budy 2004, Krueger et al. 2006).

The extent of contact between vulnerable fry and the release of the infective triactinomyxon (TAM) stage of the parasite determine the degree of exposure for young fish and, ultimately, the magnitude of population-level effects. Thus, watershed characteristics and aspects of tributaries that influence environmental conditions influencing *T. tubifex* availability and spore production can alter exposure rates. We can identify watershed characteristics governing whirling disease by examining distribution patterns in disease within the broader landscape.

Environmental factors play a critical role in determining the result of host and parasite interactions (MacConnell and Vincent 2002). Temperature influences the growth, reproduction and survival of *T. tubifex* (de la Hoz Franco and Budy 2004) as well as spore and infective TAM production (El-Matbouli et al. 1999). Water velocity may also influence TAM survival and concentrations (Kerans and Zale 2002, MacConnell and Vincent 2002). Substrate size and nutrients influence the distribution and abundance of *T. tubifex* (Sauter and Gude 1996, Arndt et al. 2002). Fewer studies examine how these factors interact in a field setting (but see Hiner and Moffitt 2002, de la Hoz Franco and Budy 2004, Krueger et al. 2006) to help predict the distribution and prevalence in the environment. Linking these potentially limiting factors to patterns on the landscape in a variety of different system types and regions is useful as we attempt to separate the effects of correlated variables in the field, understand different limiting factors across different types of systems, and provide generalizations of vulnerability to disease at both the landscape and reach scale. Response variables associated with infection (e.g., severity, spore production) increase with average water temperature (and variation in water temperature) in Idaho and Utah studies (Hiner and Moffitt 2002, de la Hoz Franco and Budy 2004), but decrease with water temperature in the Madison River MT (Krueger et al. 2006). Given that the temperature range observed in the Madison River study is similar to the other studies (range in study 10.1-13.6°C), this temperature could be due to the inverse correlation among fine sediment and temperature in the dataset. Beyond temperature, the other variables that have a positive relationship of disease severity include water velocity (de la Hoz Franco and Budy 2004), fine sediments (Krueger et al. 2006), and density of oligochaetes and chironomids (Hiner and Moffitt 2002).

The distribution of whirling disease in basin-fed environments seems to adhere to a fairly predictable geographic pattern of increasing infection (and severity) in the downstream direction (Smith 1998, Sandell et al. 2001, Hubert et al. 2002). Although the longitudinal relationship of whirling disease has been described in some areas (Sandell et al. 2001, de la Hoz Franco and Budy 2004), the variables used to predict disease vary by geographic region and the specific physical characteristics influencing infection have not been quantitatively evaluated in western Montana tributaries. Improving our understanding to better predict disease in the field will require expanding our understanding of the environmental mechanisms that result in spatial overlap of fry with high concentrations of triactinomyxon (Downing et al. 2002, Kerans and Zale 2002). This would allow fisheries managers to predict the species and streams in which high or low severity might be expected. If fisheries managers could predict disease potential on the landscape (based on geomorphic and physiochemical predictors), they could forecast which species, life histories or stream-types are most vulnerable to whirling disease and better determine broad-scale (versus localized) population effects. In addition, depending on the factors determining exposure potential, managers may be able to offset disease effects in a particular tributary through habitat restoration or other management techniques.

Whirling disease was identified in the Blackfoot Basin in 1995. The disease is currently present throughout the mainstem Blackfoot River and lower reaches of many tributaries. Although not quantified, there tends to be a general inverse relationship between channel elevation, channel slope and disease severity, which appears to result from a lack of suitable habitat (slow water and fine substrate) to support *T. tubifex* (Smith 1998). Biotic predictors (e.g., sentinel exposures, *T. tubifex* or TAMs) are time consuming, expensive, and require specialized identification training and equipment. The ability to predict high disease risk areas potentially before severe infection levels occur and based on abiotic attributes at a watershed scale is critical for practitioners to focus research and management effort.

We investigated the relationships of whirling disease presence with abiotic attributes of streams within a heterogeneous area of the Blackfoot Basin prone to variable infection. We hypothesized that whirling disease will be limited to specific streams based on physical features of the tributaries, such as temperature, sediment characteristics, and channel morphology. In addition, we examined whether the riffle invertebrate assemblage was correlated with the presence of whirling disease, as these data are routinely collected in environmental or water quality assessments. We quantified differences in physical attributes and invertebrate taxa among 13 basin-fed spawning streams, and related these to whirling disease using exposure of sentinel fish at both the landscape and reach level. Our goals are both to develop a model that will help identify abiotic environmental

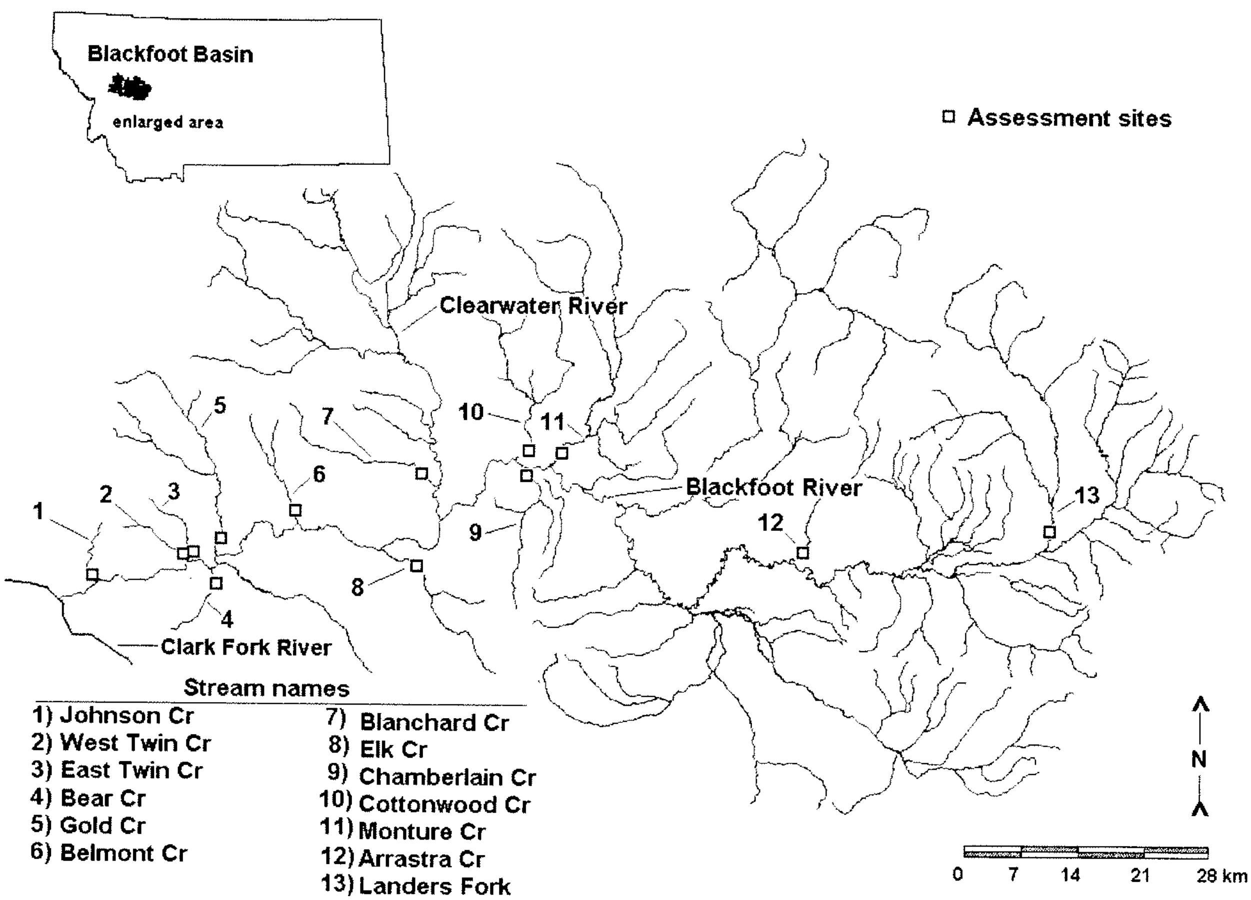


Figure 1. Study area: Blackfoot Basin including the names of study streams and locations of physical assessments and sentinel exposures. Whirling disease is present from the confluence of the Blackfoot and Clark Fork Rivers to upstream of Landers Fork.

conditions that predict the occurrence of whirling disease, and to examine the relationship of the occurrence of the disease with the invertebrate assemblage to determine whether they may be a good indicator of vulnerability to disease for a tributary.

Methods

Study area - The Blackfoot River, a 5th order tributary (Strahler 1957) of the upper Columbia River, lies in west-central Montana and flows west 211 km from the Continental Divide to its confluence with the Clark Fork River in Bonner, Montana. The River drains a 3,728 km^2 watershed through 3,040 km of perennial streams and generates a mean annual discharge of 45.2 m^3/s (United States Geological Survey 2006). The geography of the watershed is a physically diverse, geo-structurally controlled glacial landscape with alpine and subalpine mountains at the upper elevations, montane forests at the mid-elevations and semi-arid glacial pothole and outwash topography on the valley floor. Many tributaries of the Blackfoot River begin in high cirque basins, flow through alluvial valleys with meandering streams in broad valleys with gentle relief, while others flow through confined steeper channels of non-glacial origin before entering the Blackfoot River. Lands in the upper Blackfoot Basin are mostly public (65%) headwater areas with about 35 percent privately held lands consisting primarily of timbered foothills and agricultural bottomlands. Degradation of riparian areas primarily on private lands is common throughout the low-elevations of the Blackfoot Basin and has resulted in fisheries impairments on a majority of streams (Pierce et al. 2005).

The Blackfoot River is a renowned trout river in Montana and contains diverse self-sustaining wild trout populations. Fish species in the Blackfoot watershed that are vulnerable to whirling disease include brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*), bull trout (*S. confluentus),* mountain whitefish (*Prosopium williamsoni*), rainbow trout (*Oncorhynchus mykiss)*, and westslope cutthroat trout (*O. clarkii lewisi*), many of which possess some level of whirling disease susceptibility (MacConnell and Vincent 2002).

Tributary Selection - Whirling disease exposures and physical characteristics were examined among a group of 13 basin-fed tributaries to the Blackfoot River (Figure 1). For this study, we emphasized basin-fed rainbow trout spawning tributaries and avoided spring creeks due to more continuously high infections present within groundwater environments (Anderson 2004, Pierce and Podner 2006). Study streams included those streams where the disease was known to be present within the tributary (Belmont, Elk, Cottonwood, Chamberlain, Monture and Arrastra Creeks), as well as those where the disease was absent (Johnson, East Twin, West Twin, Bear, Gold and Blanchard Creeks and Landers Fork). In all cases, assessment sites were located in tributaries downstream of known infected waters of the Blackfoot River, thus within this study all tributaries had the potential for direct exposure at the confluence with the Blackfoot River main channel (Pierce and Podner 2006). Site selection criteria also included the presence of the whirling disease transmission vector between the tributary assessment site and Blackfoot River (i.e. identified migration and spawning for salmonids from the Blackfoot River) and were identified as morphologically stable (Rosgen 1996).

Physical channel assessments – Physical assessments included geomorphic characterization using the methods described by Rosgen (1996). These surveys included measurements of bankfull width, bankfull depth, bankfull area, width/depth ratios, percent channel slope, entrenchment ratios, channel and valley type and a modified Wolman 100 particle substrate samples at riffle cross-sections (Wolman 1954).

We further examined channel substrate by extracting McNeil core samples from riffles using modified methods first describe by McNeil and Arhnel (1964). For this assessment, a hollow core sampler was pushed 10 cm into the streambed (six samples per stream) and the substrate extracted. The turbid water within the cone was sampled for sediment content utilizing an Imhoff cone as described in Shepard and Graham (1982) and Shepard et al (1984). The water in the core sampler was measured to the nearest half inch to calculate the intracore water volume and to assist in the conversion of the volume of the sediment captured in the cone to dry weight. The substrata was removed from the core area and placed in bags for transport to a USFS lab. Streambed samples were oven dried and shaken through sieve series containing 76.2, 50.8, 25.4, 12.7, 6.3, 4.76, 2.38, 0.85, 0.074 mm mesh screens. The material retained within each sieve and the pan was weighed to the nearest hundredth of a gram. The estimated dry weight of the sediment within the Imhoff cone was added to the weight of material <0.84mm. We calculated Fredle index, geometric mean and the weight of particle size classes of <0.84, 0.84-4.6 and <6.35mm. Substrate composition was reported as the percent of each size class by weight.

The remaining parameters included basic water chemistry (pH, conductivity, Total Dissolved Solids) and water temperatures logged at 48-minute intervals using Onset™ thermographs; both water chemistry and temperature were collected during summer base flow periods (July and August).

Invertebrate assessment – Benthic invertebrates were sampled on July 26-27, 2006 from single riffles in each of the tributaries. A D-frame net with 1,000-micron mesh was used. The substrate was disturbed by kicking along transects for an average of 6 minutes and 36 seconds and 10.58m. Samples were preserved in 95% ethanol at streamside and delivered to Rhithron Associates in Missoula for sorting and identification of organisms. In the laboratory the samples were sorted under dissecting stereoscopes, using 10x-30x magnification. Ephemeroptera, Trichoptera, and Plecoptera (EPT) were picked from the entire sample and identified to the lowest taxonomic level possible considering the maturity of the animals and the availability of appropriate keys. Generally, the lowest taxonomic level was at least genus, but in many cases species could be identified. Several common water and habitat quality indicators including, cold stenotherm richness, burrower richness, clinger richness, sediment sensitive richness, sediment tolerant richness, filterer richness, predator richness, Plecoptera richness, and EPT richness were calculated.

Whirling disease exposures – Sentinel cage exposures of 50 hatchery (age-0) rainbow trout cohorts at 13 streams were used to identify disease occurrence in individual streams and the spatial variation of *M. cerebralis* among tributaries (Figure 1). Exposures were completed in July within 9 weeks post-hatch to coincide with fry emergence in Blackfoot tributaries (Pierce et al. In review) and the known seasonal peak of TAM production within western Montana Rivers (Vincent 2000). The exposure period for each live cage was standardized at 10 days. At the end of that time, trout were transferred to Pony, MT, where they were held for an additional 80 days at a constant 10°C to ensure the whirling disease infection if present would reach maximum intensity (Vincent 2000). At the end of the holding period, all surviving fish were sacrificed and sent to the Washington State University Animal Disease Diagnostic Laboratory at Pullman, WA. At the lab, the heads were histologically examined using the MacConnell-Baldwin histological grading scale, which ranks whirling disease from 0 (absent) to 5 (severe) (Baldwin et al. 2000). Severity was considered high if a majority (%) of exposed RBT had histological (lesion) scores of $\geq$3 on the MacConnell-Baldwin scale. As an index to severity, mean lesion scores of >2.75 have been associated with significant levels of mortality in wild rainbow trout populations (Vincent 2002).

Analyses - Scatterplots of the response variables (index of mean infection or % of fish with infection grade $\geq$3) indicted non-linear relationships with many of our predictor variables (e.g., temperature). Typically streams either had no infection or a relatively high (majority $\geq$ grade 3) severity, with very few intermediate streams. In addition, streams with no or very low (<0.05 mean score) or relatively high exposure ($\geq$3 severity) have remained relatively consistent from year to year. Given these considerations we decided to use whirling disease presence and non-detect in our analyses. We used two approaches to predict whirling disease. First, we used classification and regression trees (CART, Venables and Ripley 1997) to examine whether the groups (stream with versus without disease) reflect differences in any of the environmental predictor variables. Classification and regression trees attempt to partition a data set by recursively explaining subsets of the data using either continuous or categorical variables (Breiman et al. 1984). Because of the small data set (and only 5 streams with whirling disease present), in Splus we set the minimum node size to be 3 but reduced the number of potential splits of the dataset ("pruned the tree") to prevent overfitting the data. In addition to CART analyses, we used logistic regression to identify the environmental variables that best explained the variation in disease presence/nondetect data. Logistic regressions were run with a backward elimination method (likelihood ratio) and performed in SPSS version 11.

Our first set of analyses examined which landscape-scale parameters best explained the presence of whirling disease in tributaries. We included percent valley slope, percent forest cover, sinuosity, channel type, and stream order. Valley type and valley slope were not both used in this analysis because of the inherent relatedness of the factors (Table 1).

Next we used the same analytical techniques to examine which tributary characteristics best explained whether streams were vulnerable to whirling disease. To remove correlated predictive variables, we first examined the relationships among the sediment measures. There were significant correlations among all of the McNeil coring measures, including the Fredle and geometric mean measures, as well as many of the Wolman pebble count measure (e.g., D50). To reduce correlated predictor variables and given the known relationship of *T. tubifex* with fine sediment, we used the percentage of substrate less than 0.84 mm to describe substrate composition in our analyses. This measure of substrate composition significantly correlated with several stream measures, including entrenchment and sinuosity. Conductivity was significantly correlated with pH, Total Dissolved Solids, entrenchment, and reach slope. In addition, average temperature, maximum temperature, and reach slope were significantly correlated. Given the known importance of temperature to the biology of *T. tubifex* and spore production, we included temperature (versus slope) in the analyses. To examine what stream reach characteristics predicted the presence of whirling disease, we excluded correlated predictor variables and included the following variables: (1) maximum summertime temperature, (2) substrate less than 0.84mm, (3) width/depth ratio, and (4) conductivity (Table 2).

Finally, we used similar analytical techniques to examine how much variation in whirling disease presence could be described by the invertebrate community indices. There are many potential indices to describe the invertebrate community; we focused on those that were linked to ecological mechanisms and we excluded highly correlated indices. We included cold stenotherm richness, EPT richness, sediment sensitive richness, and sediment tolerant richness in analyses to investigate how well invertebrate community indices describe variation in whirling disease.

Results

When we examined the landscape predictors of whirling disease presence (Table 1), the classification and regression tree analysis predicted whirling disease presence based on percent valley slope (if valley slope is <0.8 present, Figure 2a). In this Blackfoot River Basin dataset, if the valley slope is less that 0.8 then it is also a Valley type 8 (Rosgen classification). These valley types are characterized by wide, gentle valley slopes with well-developed floodplain adjacent to river or glacial terraces typically containing alluvial valley fills (Rosgen 2006). This analysis misclassified two tributaries, where there was a predicted absence when in fact the disease was present. The misclassified tributaries were Chamberlain Creek (valley type 6, valley slope 2.1) and Belmont Creek (valley type 5, valley slope 1.3); although these two streams had higher slopes they did have warm summertime temperatures (max temp > 18°C). In this data set, valley type is significantly correlated with valley slope, channel slope, temperature, and percent of substrate less than 0.84mm, pH, conductivity and Total Dissolved Solids. Thus, whirling disease is present in wide, gradually sloped valleys with warm temperatures and fine substrate. Results from the logistic regression were consistent with the CART analysis that valley slope was the only parameter not excluded from the final model (valley slope p=0.022, 67% misclassification, Figure 2b).

In our analyses examining which reach level variables predicted whirling disease presence, the CART model predicted disease presence in streams with maximum summertime temperatures above 19.02°C and the only misclassified tributary was Belmont Creek (maximum temp <19.02°C but whirling disease present). Two different logistic regression models correctly classified all of the streams with two predictor variables: maximum temperature and conductivity or maximum temperature and width/depth ratio. The best individual predictor of disease presence was maximum summertime temperature (Figure 3b). Not surprisingly, there was a significant correlation between our landscape level (valley slope) and several reach level variables (maximum temperature Pearson Correlation R=-0.74, p=0.006; fine sediment <0.8mm R=0.585, p=0.046; and Total Dissolved Solids R=-0.806, p=0.002).

Sixty-seven EPT taxa were identified in the samples and a total of 10,644 EPT individuals were present. Several taxa were collected in more than one stream and exclusively in streams with no incidence of whirling disease, including *Drunella spinifera* (2 sites), *Caudatella edmundsi* (2 sites), *Epeorus longimanus* (5 sites), *Epeorus deceptivus* (2 sites), *Rhithrogena sp.* (4 sites), *Ironodes sp.* (2 sites) *Baetis alius* (2 sites), *Baetis flavistriga* (2 sites), *Visoka cataractae* (2 sites), *Calineuria californica* (2), *Megarcys signata* (4 sites), *Parapsyche elsis* (4 sites), *Agraylea sp.* (2 sites), *Lepidostoma unicolor* (4 sites), *Rhyacophila alberta* (3 sites). The CART model predicted disease presence in streams with cold stenotherm richness of less than 6 species (Figure 4a). This model misclassified 3 of the 13 streams, with Belmont incorrectly classified as a whirling disease absent stream and Gold and Blanchard incorrectly classified as streams with disease presence. The logistic regression model indicated that we could correctly classify 12 of our 13 streams with two predictor variables: EPT species richness and cold stenotherm species richness. In this analysis, Belmont Creek was the only stream incorrectly classified (Figure 4b).

Stream	mean infection severity	% ≥ grade 3 infected	Detect / Nondetect	Sinuosity	Channel type	% watershed forested	Valley Slope	Stream order	Valley type
Johnson	0	0	0	1.13	B4	75.8	8.6	2	II
E.Twin	0	0	0	1.11	C4	73.4	7.7	2	II
W.Twin	0	0	0	1.28	C4	92.6	5	3	II
Bear	0	0	0	1.57	C4b	86.8	5.7	2	VI
Gold	0	0	0	1.35	C3	90	1.4	3	V
Belmont	2.48	48.5	1	1.05	B4	95.1	1.3	3	V
Elk	4.82	95.3	1	1.77	E4	83.1	0.4	3	VIII
Blanchard	0	0	0	1.31	C4	88.9	3	2	VI
Cottonwood	3.78	100	1	1.11	C3	77.4	0.7	3	VIII
Monture	4.81	96.9	1	1.46	C3	74.6	0.7	4	VIII
Arrastra	0.02	0	0	1.3	C4	86.4	1.2	2	V
Chamberlain	3.78	78	1	1.14	C4	95	2.1	2	VI
Landers Fork	0	0	0	1.2	C4	87.3	0.9	4	V

Table 1. Variables used in the landscape scale analyses. Valley slope and valley type are correlated and were not included in the same analysis.

Stream	Detect / Nondetect	Width / Depth ratio	% substrate < 0.084mm	Conductivity	Mean Temp °C (July/Aug)	Max Temp °C (July/Aug)
Johnson	0	9.5	7.9	39	10.33	13.72
E. Twin	0	11.5	5.9	18	11.83	14.83
W. Twin	0	10.8	5.3	9	11.78	15.61
Bear	0	14.4	5.9	82	11.22	17.50
Gold	0	20.3	11	162	13.00	18.67
Belmont	1	34.2	9.2	258	13.22	18.28
Elk	1	11.2	29.1	230	13.94	20.56
Blanchard	0	33.3	6.3	95	14.89	18.28
Cottonwood	1	17.6	11.3	220	11.83	20.94
Monture	1	48.0	12.1	135	14.67	20.56
Arrastra	0	34.1	9.3	159	10.11	14.44
Chamberlain	1	19.2	6.6	67	13.94	19.39
Landers Fork	0	27.3	6.5	204	11.50	17.11

Table 2. Variables used in the reach scale analyses included detect / Nondetect, width/depth ratio, % substrate < 0.84mm, conductivity, and temperature. Channel slope, entrenchment, other substrate composition measures, pH, and total dissolved solids were significantly correlated with these variables

Tributary	Cold Stenotherm Richness	Sediment Sensitive Richness	EPT Richness	Sediment Tolerant Richness
Arrasta	7	3	30	0
Bear	7	2	29	0
Belmont	7	4	30	0
Blanchard	5	4	39	2
Chamberlain	5	3	26	1
Cottonwood	1	4	21	0
East Twin	12	4	34	0
Elk	1	2	26	2
Gold	2	1	33	1
Johnson	8	3	29	0
Landers Fork	7	2	33	2
Monture	2	3	32	2
West Twin	10	4	29	0

Table 3. Indices developed from the invertebrate data and used in analyses.

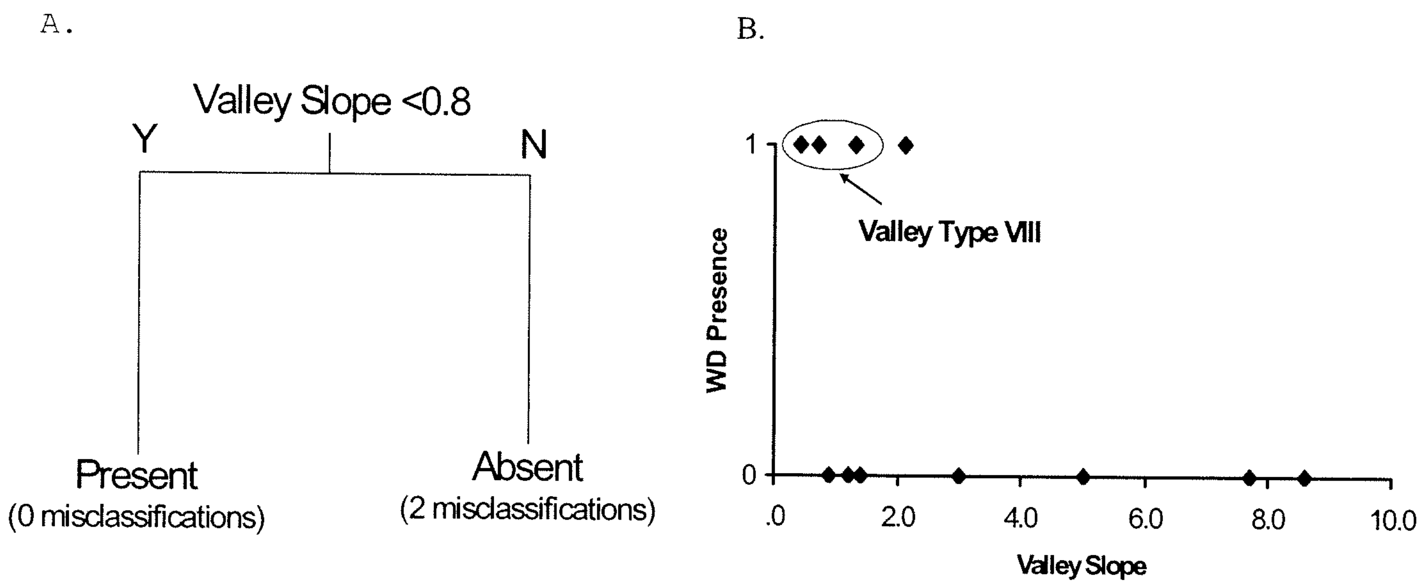
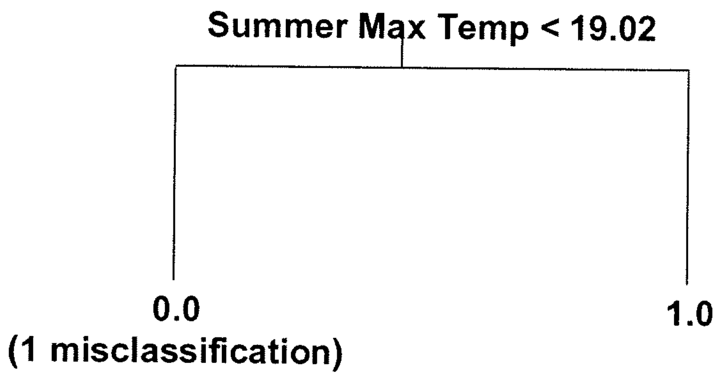


Figure 2. A) Results from the CART analysis of the landscape variables. The 2 misclassifications are Belmont and Chamberlain Creeks which we predicted whirling disease to be absent but it was present. B) Plot of the whirling disease detection associated with valley slope and valley type.

Figure 3. A) CART results for the reach scale variables. Maximum summertime temperature was the only significant predictor of disease presence. Belmont Creek was predicted to have no whirling disease, while in fact whirling disease was present. B) Scatterplots of the variables that were significant predictors of whirling disease presence at the reach scale from the logistic regression analysis. A whirling disease score of 1.0 indicates detection and 0 indicates the disease was not detected in the stream.

A.



B.

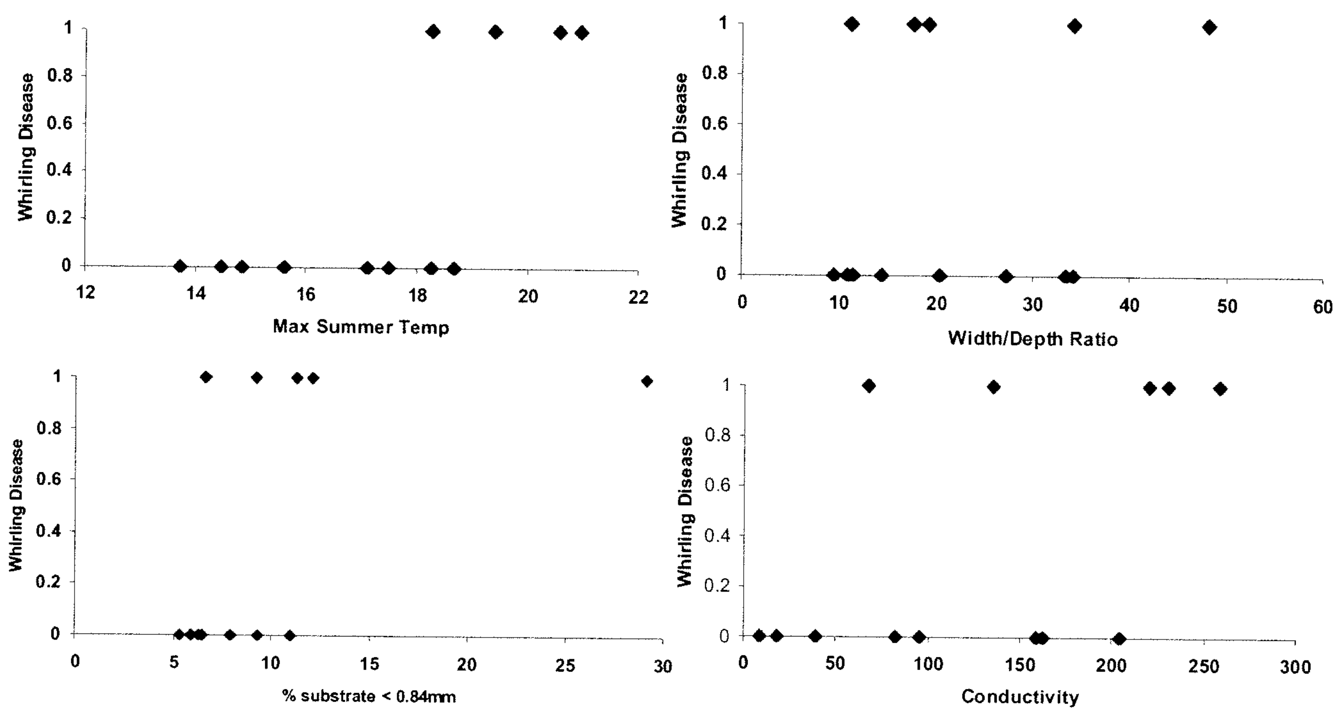
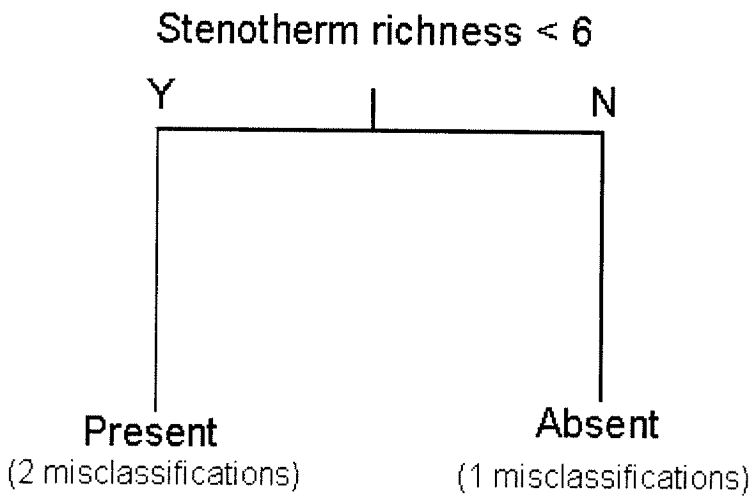
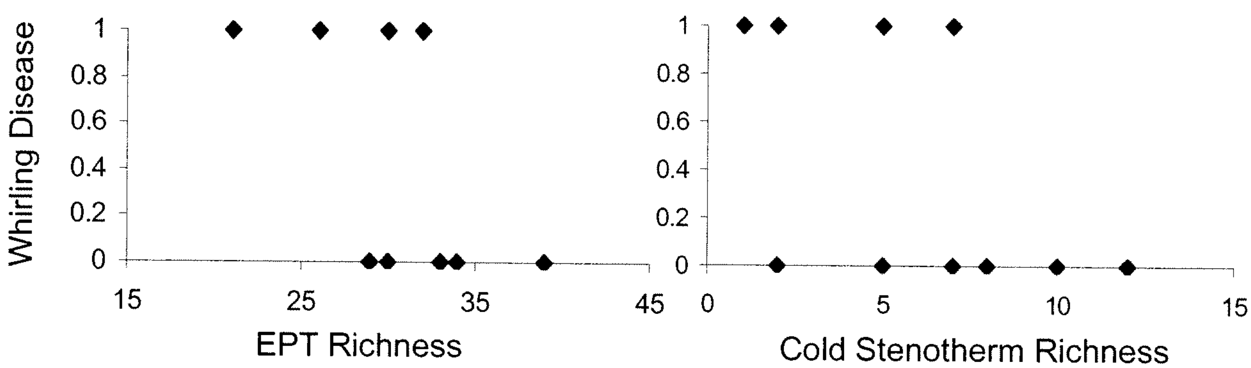


Figure 4. A) CART results examining how well the invertebrate taxa predict whirling disease presence. B) Scatterplots of the invertebrate variables that were significant predictors of whirling disease presence from the logistic regression analysis. A whirling disease score of 1.0 indicates detection and 0 indicates the disease was not detected or has a severity score <0.5.

A.



B.



Discussion

Our findings are consistent with down-valley morphological transitions of stream valleys and channel-types (Rosgen 1996) and a central tenet of stream ecology that abiotic (conditions and biotic communities) change predictably along longitudinal (upstream-downstream) gradients (Vannote et al. 1980). In western Montana watersheds, channel features often transition in a predictable downstream path. However due to basin heterogeneity, stream environments conducive to infection such as those in the Blackfoot Basin also vary substantially at a reach and sub-basin scales. In contrast to the lower Blackfoot Basin, present in the middle Blackfoot Basin are tributary environments suitable to *T. tubifex,* high TAM exposure and high infection rates.

In our analysis of landscape variables, environments conducive to whirling disease were found in broad valleys with gentle, down-valley elevation relief (Valley type 8, Rosgen 1996). Streams within these valleys occupy C and E-type channels, which are slightly entrenched. In our study area, high severities were prevalent in meandering streams in broad valleys with gentle relief and warmer summer temperatures. Alluvial floodplains are the most predominant landforms, which can produce relatively high sediment supply. Soils are developed over alluvium; thus, streams in this valley are susceptible to accelerated bank erosion. These streams appear to be naturally predisposed to whirling disease infection, but also prone to anthropogenic activities (e.g. elevated sediment and water temperatures) that tend to favor the pathogen. By contrast, mountain streams of the lower Blackfoot Basin support lower temperatures, lower in-channel sediment levels within spawning areas, and low infection rates. These streams occupy steep forested valleys, narrow floodplains with moderate side-slopes formed primarily of colluvium.

At the reach scale, we had a strong positive correlation of whirling disease with water temperature, similar to other studies (Hiner and Moffitt 2002, de la Hoz Franco and Budy 2004). This was expected given the entire life cycle of the parasite in both the fish and worm host is temperature dependant and that natural outbreaks occur during temperatures optimal to the parasite (MacConnell and Vincent 2002). Summertime temperatures identify conditions favorably for spore TAM production and release, and thus the potential of a stream to support a higher severity of disease. Temperature also influence fish growth, development and immune response, all of which may influence the degree to which fish are susceptible to the development of the disease (MacConnell and Vincent 2002). Maximum annual water temperature also provides a simple and direct method of comparison to the other basins (de la Hoz Franco and Budy 2004).

Similar to other studies, we found a relationship with conductivity (in addition to temperature), the only water quality parameter correlated to disease severity in Oregon (Sandell et al. 2001). Although Sandell et al. (2001) provide a potential mechanistic explanation associated with conductivity influencing TAM recognition of living tissue, in our study conductivity is significantly correlated with Total Dissolved Solids, entrenchment, slope, and geometric mean sediment size. Therefore, high conductivity is indicative of stream reaches with low gradient channels, more fine sediments, greater entrenchment and higher Total Dissolved Solids. We did not find that stream size using bankfull area or stream-order as potential index to discharge explained a significant amount of the variation in the occurrence of the disease. But in a similar study, both water temperature and discharge were positively correlated with the prevalence of infection (de la Hoz Franco and Budy 2004). In the Blackfoot Basin, the variable geology produces large, cold-water bodies with very low incidence of disease. The results from analyses of our invertebrate indicators do corroborate results from the analyses from the abiotic factors with cold stenotherm species richness classifying whirling disease presence fairly well in the CART analysis and EPT richness and cold stenotherm species richness predicting whirling disease presence in the regression analyses.

Developments of high severity have been shown in areas of natural and man-made impoundments (Hiner and Moffit 2002), both of which are present in our study area and likely influence the prevalence of whirling disease. Impoundments can affect community structure of macroinvertebrate, likely increase organic and fine particulate matter. Impoundments serve as sediment traps creating optimal conditions for *T. tubifex*. In addition impoundments increase warming. These conditions can lead to the production and release of TAMs from infected worm populations in these ponds. Both Belmont and Chamberlain Creeks have ponds (either man-made or beaver) upstream of the sentinel cage sites. Throughout this study Belmont was an outlier, a cold stream with whirling disease. These outliers could be explained by the amount of high sediment levels generated from roads and beaver activity in the watershed creating a hot spot for *T. tubifex* and TAM production.

Management Implications - In watersheds like the Blackfoot, stream degradation and whirling disease may act synergistically having combined effects on fisheries. As we begin to understand the role of environmental factors at the landscape level, we can predict areas that we expect to be naturally prone to having severe impacts of the disease. Reach scale variables imply that within certain watersheds we might help manage the potential impact of the disease by protecting and restoring habitat in tributary spawning and rearing areas to minimize factors that favor habitat of *T. tubifex* (e.g. sediment) or otherwise increase whirling disease (e.g., temperature). In our study area, infections were identified in meandering streams in broad valleys with gentle relief and warmer summer temperatures. In western Montana, environments of this type often support prone to excessive grazing and other land-use activities that potentially elevate water temperatures and instream sediment levels. Zendt and Bergerson (2000) found the highest relative abundance of *T. tubifex* in areas where riparian zones were heavily disturbed.

Our study further suggests that anthropogenic warming or increases in sediment supply may increase infection (and severity) and shift disease distribution (and effects) in the upstream direction. For example, Monture Creek has been identified as the primary rainbow trout spawning tributary to the Blackfoot River. Water temperature monitoring beginning in 1993 has shown a 1°C increase in maximum annual temperatures. Relative influences of regional trends versus anthropogenic influences driving this temperature change are not clear, as habitat degradation of riparian areas is occurring within the watershed, which is likely influencing several environmental conditions conducive to the expansion of whirling disease. If the exposure potential shifts upstream, so will the likely impacts of the disease on various species that typically spawn higher in the watershed. While there is little we can do within the Blackfoot Basin to affect climate, land-management related water temperature changes may be able to reduce disease severity. If we can predict which streams are likely to be sites with high disease severity because of habitat degradation, we can develop and/or implement existing stream restoration techniques to correct human-related factors in areas that may contribute to severity. Examining the potential for restoration to prevent and/or reverse trends in disease severity is needed. In addition, *apriority* predictions may allow managers to change fishing regulations to decrease other sources of mortality that would allow these populations to better handle the stress of extra juvenile mortality resulting from whirling disease. Hopefully, this information will help us maintain self-sustaining wild salmonid populations in parasite positive streams.

Conclusions

Determining the physical variables correlate with whirling disease is critical to understanding the spatial characteristics of the disease and impacts on fish populations. This study improves our ability to better predict the aquatic environments prone whirling disease. Understanding the environmental and spatial relationships within the watershed allow more concise interpretation of the effects on susceptible species, when considered within a context of overlapping life-histories for vulnerable species (*see* Pierce et al. In review). The role of anthropogenic habitat degradation must also be considered in terms of combined effects of multiple threats including disease. Based on our study, increased water temperature appears to be coupled with infection. Habitat degradation often increases temperature, in which cases we can generally predict a higher incidence of disease.

Acknowledgments: We would like to acknowledge the USFS for sifting samples, NRCS and Blackfoot Challenge for funding the McNeil core samples, Northwestern Energy for funding field technicians Ryen Aasheim Anderson, Mike, and Craig Podner, and the Whirling Disease Foundation for contributing funds to help complete this project. We would also like to thank Eileen Ryce, for a thoughtful review of this manuscript.

References

Anderson, R.A. 2004. Occurrence and seasonal dynamics of the whirling disease parasite, *Myxobolus cerebralis*, in Montana spring creeks. Masters Thesis Montana State University Bozeman, MT.

Arndt, R.E., E.J. Wagner, Q. Cannon, and M. Smith. 2002. Triactinomyxon production as related to rearing substrate and diel light cycle. Pages 87-91 *in* J.L. Bartholomew and J.C. Wilson, editors. Whirling disease: reviews and current topics. American Fisheries Society, Symposium 29, Bethesda, Maryland.

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

Bartholomew, J.L. and P.W. Reno. 2002. The history and dissemination of whirling disease. Pages 3-24. *in* J.L. Bartholomew and J.C. Wilson, editors. Whirling disease: reviews and current topics. American Fisheries Society, Symposium 29, Bethesda, Maryland.

Breiman, L., J.H. Friedman, R.A. Olshen, and C.J. Stone. 1984. Classification and regression trees. Chapman and Hall, New York.

De la Hoz, E, and P. Budy. 2004. Linking environmental heterogeneity to the distribution of prevalence of *Myxobolus cerebralis*: a comparison across sites in a northern Utah watershed. Transactions of the American Fisheries Society. 133:1176-1189.

Downing, D.C., T.E. McMahon, B.L. Kearns, and E.R. Vincent. 2002. Relation of spawning and rearing life history of rainbow trout and susceptibility to *Myxobolus cerebralis* infection in the Madison River, Montana. Journal of Aquatic Animal Health 14:191-203.

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

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

Hiner, M. and C.M. Moffitt. 2002. Modeling *Myxobolus cerebralis* infections in trout: associations with habitat variables. Pages 167-179. *in* J.L. Bartholomew and J.C. Wilson, editors. Whirling disease: reviews and current topics. American Fisheries Society, Symposium 29, Bethesda, Maryland.

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

Hubert, W. A. and six coauthors. 2002. Whirling disease among Snake River cutthroat trout in two spring streams in Wyoming. Whirling disease: reviews and current topics. American Fisheries Society Symposium 29:181-193.

Kearns, 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.

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

Landolt, P, and M, Sartori. 1997. Ephemeroptera and Plecoptera: biology-ecology-sytematics.

MacConnell, E. and E. R. Vincent 2002. Review: the effects of *Myxobolus cerebralis* on the salmonid host. Pages 95-108 *in* J. L. Bartholomew and J. C. Wilson, editors. Whirling disease: reviews and current topics. American Fisheries Society, Symposium 29, Bethesda Maryland.

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

McNeil, W.J. and W.H Ahnell. 1964. Success of pink salmon spawning relative to size of spawning bed material. U.S. Fish and Wildlife Service Special Scientific Report Fisheries 469.

MWDTF (Montana Whirling Disease Task Force). 1996. Final report and action recommendations. Montana Whirling Disease Task Force, Helena, MT.

Nehring, B., and P. G. Walker. 1996. Whirling disease in the wild: the new reality in the intermountain west. Fisheries 21: 28-30.

Pierce., and R. Aasheim, and C. Podner 2005. An integrated stream restoration and native fish conservation strategy for the Big Blackfoot River Basin, Montana Fish, Wildlife and Parks, Missoula, MT.

Pierce, R., and C. Podner. 2006. The Big Blackfoot River Restoration Report for 2004 and 2005. Montana Fish, Wildlife and Parks. Missoula, MT.

Pierce, R., C. Podner, M. Davidson, and R. Vincent. In Review. Relationships of migratory (hybrid) rainbow trout spawning life histories to risk of *Myxobolus cerebralis* infection in the Blackfoot River Basin, Montana. In submission to Transactions of the American Fisheries Society.

Rosgen, D. 1996. Applied Fluvial Geomorphology. Wildlands Hydrology, Pagosa Springs Colorado.

Rosgen ,. D. 2006.

Ryce, E. K. N, A. V. Zale and E. MacConnell. 2004. Effects of fish age and parasite dose on the development if whirling disease in rainbow trout. Diseases of Aquatic Organisms Vol. 59 (3):225-233.

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 333:97-101.

Shepard, B. B., and P. J. Graham. 1982. Monitoring spawning gravel bed material used by bull trout on the glacier view district Flathead National Forest. Montana Fish Wildlife and Parks Completion Report.

Shepard, B., S. Leathe, T. Weaver, and M. Enk. 1984. Monitoring levels of fine sediments within tributaries to Flathead Lake and impacts of fine sediment to bull trout recruitment. Wild Trout III.

Smith, L. 1998. Study on the distribution and abundance of *Tubifex tubifex* within Cottonwood Creek in the Blackfoot drainage. Masters Thesis, University of Montana, Missoula, Montana.

Strahler, A.N. 1957. Quantitative analysis of watershed geomorphology. Transactions of the American Geophysical Union 38:913-920.

USGS. 2006. Gauging station 1234000 provisional unpublished data.

Vannote, R. L., G.W. Minshall, K.W. Cummins, JH. R. Sedell, and C.E. Cushing. 1980. The river continuum concept. Canadian Journal of Fisheries and Aquatic Sciences.; 37: 130-137.

Venables, W.N. and B.D. Ripley. 1997. Modern applied statistics with S-PLUS (2nd edition). Springer, New York

Vincent, E. R. 1996. Whirling disease and wild trout: the Montana experience. Fisheries 21 (6):32-33.

Vincent, E.R. 2000. Whirling disease report 1997-98. Montana, Fish, Wildlife and Parks. Project 3860. Helena, Montana.

Vincent, E. R. 2002. Relative susceptibility of various salmonids to whirling disease with emphasis on rainbow and cutthroat trout. Whirling disease: reviews and current topics. American Fisheries Society Symposium 29:109-115.

Wolman, M. 1954. A method of sampling coarse river-bed material. Transactions of the 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.