EVIDENCE OF RELATIONSHIPS BETWEEN TUBIFEX HABITAT AND *MYXOBOLUS CEREBRALIS* ACROSS A MOUNTAIN WATERSHED

Jason C. Burckhardt[1] , U.S. Geological Survey, Wyoming Cooperative Fish and Wildlife Research Unit, University of Wyoming, Department 3166, 1000 East University Avenue, Laramie, WY 82071
Wayne A. Hubert, U.S. Geological Survey, Wyoming Cooperative Fish and Wildlife Research Unit, University of Wyoming, Department 3166, 1000 East University Avenue, Laramie, WY 82071

ABSTRACT

We tested the hypothesis that the proportions of age-0 salmonids infected by *Myxobolus cerebralis*, the causative agent of whirling disease, and histologic evidence of whirling disease in stream reaches across a watershed are related to measures of suitable habitat for Tubifex tubifex, the oligochaete host for M. cerebralis. We assumed habitat quality for *T. tubifex* increased with the amount of fine sediment, aquatic macrophytes, or low-gradient mesohabitat, i.e., pools and glides, and decrease with increasing channel slope in stream reaches. The highest rates of infection and tissue damage were observed among age-0 salmonids sampled from a cluster of spring streams in the upstream portion of the valley with low channel slopes or modifications to enhance deep-pool cover for adult salmonids, side channels of the main stem river with large amounts of sediment deposition, and mountain streams downstream from beaver (*Castor canadensis*) ponds with accumulations of fine sediment.

Key words: *Myxobolus cerebralis*, Salmonids, trout, *Tubifex tubifex*, stream, watershed

INTRODUCTION

Myxobolus cerebralis, the myxozoan parasite that causes whirling disease, is recognized as a threat to wild salmonid populations (Vincent 1996, Nehring and Walker 1996). The life cycle of *M. cerebralis* is complex because it has two alternating spore-forming phases. One phase involves formation of single-celled spores in salmonids that are released from the fish and consumed by the oligochaete, *Tubifex tubifex* (Rognlie and Knapp 1988). While in the gut of *T. tubifex*, *M. cerebralis* produces a multicellular spore known as a triactinomyxon (TAM, Wolf et al. 1986). Triactinomyxons are released into the water column and infect fish through epithelial contact. The parasite migrates to the cartilage of the skull and vertebral column where damage to the upper spinal cord and compression of the brain stem causes behavioral changes and clinical signs of disease (Schisler et al. 1997, Rose et al. 2000).

Physical characteristics of streams conducive to *T. tubifex* may lead to high infection rates in fish and manifest as whirling disease (Hiner and Moffit 2001, de la Hoz Franco and Budy 2004). *Tubifex tubifex* flourishes in areas with accumulations of fine sediment rich in organic matter (Lazim and Learner 1987, Sauter and Gude 1996, Lampert and Sommer 1997, Zendt and Bergersen 2000, Arndt et al. 2002). Stream substrate composition is largely a function of channel slope in mountain streams with low-gradient reaches typically having finer particles rich in organic matter, ideal habitat for *T. tubifex*.

Our goal was to evaluate the relationships of age-0 salmonids infected with *M. cerebralis*, occurrence of histologic evidence of tissue damage, and physical habitat features conducive to *T. tubifex*. We chose age-0 salmonids to minimize probable bias due to mortality associated with *M. cerebralis* infection in older salmonids. We tested the hypothesis that proportions of age-0 salmonids infected by *M. cerebralis* or with histologic evidence of whirling disease

---

[1] Current address: Wyoming Game and Fish Department, 2820 State Highway 120, Cody, WY 82414

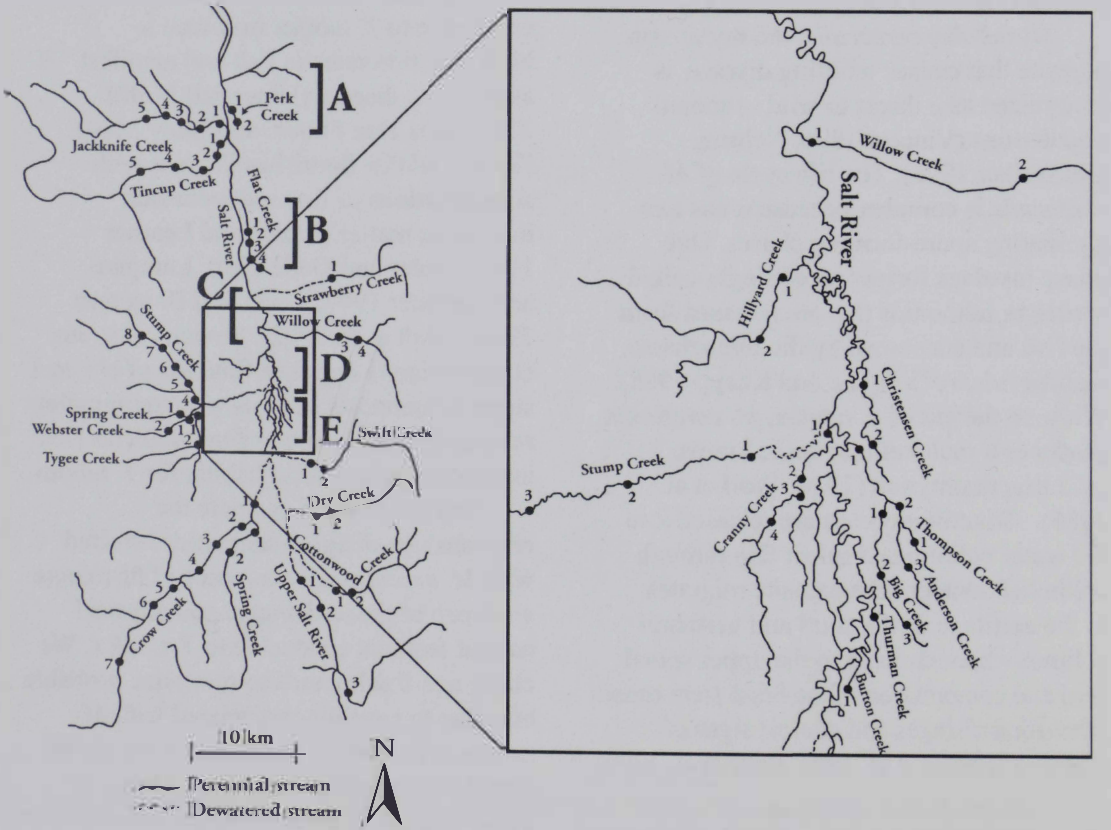
in reaches across a watershed are correlated with measures of suitable habitat for *T. tubifex*. We also hypothesized that infection rates and histologic evidence of disease would be positively related to the amount of fine sediments, aquatic macrophytes, and low-gradient mesohabitat, i.e., pools and glides, but negatively related to channel slope among reaches.

STUDY AREA

The Salt River is a fifth-order (after Strahler 1957) stream with a 2150-km^2 watershed near the Wyoming-Idaho border that has a mean annual discharge of 22.5 m^3/s into the Snake River. The Salt River Range to the east, Gannet Hills to the south, and Caribou and Webster ranges to the west border the watershed. Mountain tributaries and the headwaters of the Salt River have steep channels with the highest slopes among tributaries in the Salt River Range. Numerous streams formed by springs occur on the valley floor and flow short distances to the Salt River (Kennington and Hamblin 1989, Isaak 2001). The mainstem of the Salt River flows through a broad alluvial valley for 45 km with several high-gradient, highly braided reaches.

Native salmonids in the Salt River watershed included the fine-spotted form of Yellowstone cutthroat trout (*Oncorhynchus clarki bouvieri*) and mountain whitefish (*Prosopium williamsoni*). Rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), and brook trout (*Salvelinus fontinalis*) have been introduced and become naturalized in the watershed. *Myxobolus cerebralis* was first detected in the Salt River in 1995 (Money and Wanner 1997) and has spread within the watershed (Gelwicks et al. 2000, Hubert et al. 2002).

Figure 1. Location of reaches sampled in mountain and spring streams that are tributaries to the Salt River and identification of the four segments of the Salt River where reaches in side channels were sampled.



METHODS

We selected sample sites of 100-m reaches to represent the array of stream habitats across the watershed. From one to eight reaches were systematically selected in each of nine mountain tributaries and the Salt River headwater. Mountain tributaries were sampled with an upstream progression that included one reach above the most upstream barrier to fish passage for streams in which barriers occurred (Fig. 1). One to four reaches were systematically selected in each of 11 spring streams progressing from the mouth of the spring stream to the headwater spring(s). We sampled from four to 17 reaches in side channels among four segments of the Salt River. Side channels were sampled because they had potential spawning and nursery habitat for salmonids.

Four features were measured over each 100-m reach during summer and fall 2000: (1) fine sediment, (2) aquatic macrophytes, (3) channel slope, and (4) low-gradient mesohabitat. Patches of fine sediment (<1 mm diameter), > 2 cm deep, were measured and recorded as the percentage of the water surface area of non-riffle mesohabitats covered. Similarly, we measured aquatic macrophytes as the percentage of the water surface area with aquatic vascular plants covering the streambed. Measurement of channel slope within a reach followed Isaak et al. (1999) using a surveyor's rod and Abney level. Pools were scoured or dammed areas with water surface slopes near zero. Glides consisted of areas with generally uniform depth and no surface turbulence with water surface slopes ≤ 1 percent. Our measure of low-gradient mesohabitat included the percentage of water surface area of a reach comprised of pools and glides.

Age-0 salmonids were sampled by electrofishing during habitat sampling in the summer and fall of 2000. We sampled up to 200 m of stream encompassing each 100-m reach in an effort to obtain up to 25 age-0 fish of each species present at a site. Fish were identified to species, with the exception that *Oncorhynchus* were pooled due to difficulty in discerning age-0 rainbow trout, cutthroat trout, and rainbow trout x cutthroat trout hybrids in the field. Each fish was placed in an individual plastic bag and stored on dry ice. In the laboratory their heads were removed and laterally dissected along the dorsal midline with half preserved in ethanol for DNA extraction and half preserved in 10-percent buffered formalin for histologic assessment. The occurrence of *M. cerebralis* was determined by nested polymerase chain reaction (PCR) following Andree et al. (1998). Histologic assessment was conducted on those fish that tested positive for *M. cerebralis* to determine the extent of tissue damage. Individual fish were graded 0 (no abnormalities noted) to 4 (severely affected) following the grading protocol of Baldwin et al. (2000). The Washington Animal Disease Diagnostic Laboratory, Pullman, conducted all histologic assessments.

Infection rates and histologic grades of different salmonid species were assessed using a paired-*t* test to detect evidence of differences between species when two species occurred in the same reach. We used linear regression analysis to assess relationships between the extent of infection (% fish in samples with *M. cerebralis*), mean histologic scores of tissue damage, and habitat features among reaches. Dependent variable proportions were transformed with an arcsine square-root transformation before analysis to improve homoscedascity. All trout species were pooled for regression analysis. Regressions were computed for all reaches combined and for reaches in each of three stream types: (1) mountain tributaries and headwater of the Salt River, (2) spring streams, and (3) side channels along the mainstem of the Salt River.

Regression analyses were also conducted to account for spatial autocorrelation, i.e., the spatial independence of sample sites. We used a geographic information system (GIS) to map the locations of sampled sites and measure distances between them. The residuals derived from the conducted regressions were tested for spatial autocorrelation using Moran's I (Moran 1948) and a row-standardized spatial weights matrix based on linear distances between sample locations obtained from the GIS. A correlogram was used to assess the influence of the distance between sampled reaches on the regression analysis.

We used one-way analysis of variance to assess differences in physical habitat features, infection rates, and histologic scores of tissue damage among the three stream types and among reaches of the Salt River. Significance was determined at $P < 0.05$ for all tests.

RESULTS

In 2000, we sampled 46 reaches on nine mountain tributaries and the headwater of the Salt River, 24 reaches on 11 spring streams, and 37 reaches in side channels over four segments of the Salt River (Fig. 1, Appendix A). Measured habitat features differed among the three types of streams (Table 1). Reaches in mountain tributaries tended to have less fine sediment and higher channel slopes than reaches in spring streams or side channels of the Salt River. Spring streams had the lowest channel slopes, were dominated by low-gradient mesohabitats, i.e., pools and glides, had substantial aquatic vegetation, and contained high amounts of fine sediment.

Age-0 salmonids were found in 99 of 107 reaches sampled in the Salt River watershed (Appendix A). *Myxobolus cerebralis* was detected in age-0 salmonids at all but four reaches where they were collected. Reaches where age-0 salmonids occurred but did not test positive for *M. cerebralis* included the headwater areas of Cottonwood (2 sites) and Dry (1 site) creeks upstream from barriers to fish passage, and the most upstream reach on Willow Creek. The reach on Willow Creek was upstream of a long series of cascades that may inhibit upstream movement of fish. We found fish with *M. cerebralis* upstream of major barriers to upstream movement by fish on Swift Creek (see Reach 2, Appendix A).

We compared the percentages of *Oncorhynchus* spp. and brown trout from which we detected *M. cerebralis* at 14 reaches where at least five individuals of each species were collected and observed no difference ($P = 0.16$) between species. Similarly, when mean histologic grades were compared at the 14 reaches, we detected no difference ($P = 0.99$) between species. Five or more brook trout and brown trout were collected in only three reaches, five or more *Oncorhynchus* spp. and brook trout were collected in only two reaches, so we made no statistical comparisons of these species.

The proportions of fish with *M. cerebralis* detected in their heads varied widely among sampled reaches. The highest rates of infection by *M. cerebralis* were observed among fish from side channels of the Salt River followed by fish from spring streams (Table 1). Mean histologic scores followed a similar pattern (Table 1). Infection rate was related ($r^2 = 0.29$, $P = 0.006$) to mean histologic grade:

$$HG = -0.194 + 0.521\ IR$$

where HG is the mean histologic score among all fish in a reach and IR is the arcsine square-root transformation of the percentage of fish in a reach with *M. cerebralis* detected in their head among the 99 reaches where age-0 salmonids were found. In many instances the highly sensitive PCR technique detected the presence of the parasite in fish with no histologic evidence of infection.

We rarely encountered clinical signs of whirling disease, i.e., black tail, shortened opercula, and spinal or cranial deformities. Clinical signs of whirling disease were most common in reaches with high infection rates. High histologic grades of tissue damage occurred in a cluster of spring streams in the upper valley, in side channels of the Salt River adjacent to and immediately downstream of the cluster of springs in the upper valley, and in mountain tributaries on the west side of the valley downstream from beaver ponds.

Comparisons of habitat features, rates of infection by *M. cerebralis*, and histologic grades of tissue damage among the four segments of the Salt River indicated no significant differences in habitat features or mean histologic grades (Table 2). However, a highly significant ($P < 0.001$) difference

Table 1. Means and standard errors (in parentheses) of measured habitat features, infections rates by *M. cerebralis*, and histologic scores of tissue damage among the three stream types in the Salt River watershed measured during 2000. *P*-values are from one-way analysis of variance comparing the three stream types. Included in the analysis were 99 reaches where both age-0 salmonids and infection by *M. cerebralis* were observed.

	Stream type						
Variable	**Mountain streams (41 reaches)**		**Spring streams (22 reaches)**		**Salt River side channels (36 reaches)**		***P***
Fine sediment (%)	30.0[bc]	(4.1)	70.0[a]	(5.3)	66.4[a]	(5.0)	<0.001
Aquatic macrophytes (%)	4.4[b]	(1.3)	37.3[ac]	(5.5)	5.3[b]	(2.1)	<0.001
Channel slope (%)	1.02[b]	(0.12)	0.32[a]	(0.09)	0.62	(0.09)	<0.001
Low-velocity mesohabitat (%)	47.2[b]	(4.2)	73.8[a]	(5.4)	63.2	(5.5)	0.002
Infection rate (%)	39.0[bc]	(4.7)	63.0[a]	(5.4)	77.5[a]	(3.7)	<0.001
Mean histologic score	0.08[c]	(0.03)	0.25	(0.07)	0.33[a]	(0.06)	0.001

[a] significantly different from mountain streams
[b] significantly different from spring streams
[c] significantly different from Salt River side channels

in rates of infection was observed among segments. A mean infection rate of 92 percent was observed in the two most upstream segments (Segments C and D), whereas mean infection rates of 54 percent (Segment B) and 62 percent (Segment A) were observed in the two most downstream segments.

The four habitat features were significantly correlated among the 99 reaches where age-0 salmonids were collected (Table 3). Abundance of low-velocity mesohabitat was positively correlated with the abundance of fine sediment and aquatic macrophytes, and negatively correlated with channel slope.

Linear regression identified several significant relationships between infection rate and physical habitat features (Table 4). Among all of the sampled reaches, infection rate was positively related to abundance of fine sediment, aquatic macrophytes, and low-velocity mesohabitat, but negatively related to channel slope. Infection rate was positively related to abundance of fine sediment and low-velocity mesohabitat but negatively related to channel slope among reaches in mountain streams. Infection rate was positively related to the abundance of fine sediment among reaches in spring streams. We observed no significant relationships among reaches in the Salt River.

Mean histologic grade of tissue damage was positively correlated to abundance of fine sediment, aquatic macrophytes, and low-velocity mesohabitat among all sampled reaches where age-0 salmonids were found (Table 4). Mean histologic grade was positively related to the abundance of fine sediment among reaches in mountain streams and the abundance of aquatic macrophyptes among reaches in spring streams. We again observed no significant relationships among reaches in the Salt River.

Spatial autocorrelation was evident in all of the significant linear regressions. The range of spatial correlation, i.e., the inter-plot distance beyond which the data from two sample sites were essentially uncorrelated, varied from 9 to 14 km. Many sampled reaches were within 9-14 km of each other suggesting our sampling reaches were not spatially independent (see Fig. 1). Adjusting regression models for spatial autocorrelation resulted in no statistically significant relationships between physical habitat features and infection rates or histologic grades of tissue damage.

DISCUSSION

Results from this study partially supported our hypothesis, i.e., the proportions of age-0 salmonids infected by *M. cerebralis* and with histologic evidence

Table 2. Means and standard errors (in parentheses) of measured habitat features, infections rates by *M. cerebralis*, and histologic scores of tissue damage among the four segments of the Salt River watershed measured during 2000. *P*-values are from one-way analysis of variance comparing the four segments. Included in the analysis were 36 reaches where both age-0 salmonids and infection by *M. cerebralis* were observed in side channels.

	Reach				
Variable	**A (6 reaches)**	**B (9 reaches)**	**C (17 reaches)**	**D (4 reaches)**	***P***
Fine sediment (%)	70 (14)	68 (8)	60 (8)	84 (10)	0.546
Aquatic macrophytes (%)	1.2 (1.2)	0	8.5 (3.7)	10.0 (10.0)	0.280
Channel slope (%)	0.89 (0.12)	0.31 (0.14)	0.68 (0.16)	0.63 (0.13)	0.179
Low-velocity mesohabitat (%)	62 (13)	60 (7)	58 (9)	93 (7)	0.283
Infection rate (%)	62[cd] (8)	54[cd] (7)	92[ab] (2)	92[ab] (3)	< 0.001
Mean histologic score	0.22 (0.12)	0.22 (0.07)	0.37 (0.08)	0.57 (0.28)	0.286

[a] significantly different from A Reaches.
[b] significantly different from B Reaches.
[c] significantly different from C Reaches.
[d] significantly different from D Reaches.

of whirling disease in reaches across a watershed are related to measures of suitable habitat for *T. tubifex*. We found that infection rates and histologic scores of tissue damage tended to be positively correlated to the amount of fine sediments, aquatic macrophytes, and low-gradient mesohabitat, i.e., pools and glides, but negatively correlated to channel slope among reaches throughout the Salt River watershed when spatial autocorrelation was not considered. However, these physical habitat features accounted for relatively small amounts of variation, and statistically significant relationships were not observed when we accounted for spatial autocorrelation. Nonetheless, our data provide insight into locations with high rates of infection or tissue damage and the habitat features at those locations. Sampled reaches with high infection rates and high histologic grades of tissue damage occurred in a cluster of spring streams in the upper valley, in side channels of the Salt River adjacent to and immediately downstream of the cluster of springs in the upper valley, and in mountain tributaries on the west side of the valley downstream from beaver ponds.

Our findings suggest linkages among land use, high sediment deposition, occurrence of *M. cerebralis* infection, and tissue damage in age-0 salmonids. We found high rates of infection in most of the spring streams sampled in the upstream portion of the valley. The sampled reaches in the spring streams with high rates of infection typically had high (> 75%) amounts of fine sediments and low (< 0.2%) channel slopes. Livestock grazing had deteriorated riparian vegetation and accentuated bank erosion in many of the reaches on spring streams, thereby increasing fine sediment accumulation. Sediment loading can lead to stream segments with high *T. tubifex* densities and TAM production (Lampert and Sommer 1997, Zendt and Bergersen 2000).

A few of the spring streams in the upstream portion of the valley had modifications to enhance deep-pool habitat for adult salmonids that created silt-laden pools with abundant aquatic macrophytes. Additionally, spawning habitat had been enhanced by the construction of riffles between pools. The highest rates of infection (> 97%) and highest mean histologic scores of tissue damage (> 0.9) were observed in one of these spring streams with extensive habitat modification. High densities of adult brown trout and cutthroat trout, substantial spawning, and abundant age-0 fish have

Table 3. Pearson correlation coefficients among the four physical habitat variables measured at 99 reaches where age-0 salminids infected by *M. cerebralis* were found in the Salt River watershed during 2000. Correlations coefficients greater than 0.195 are significant at $\alpha = 0.05$.

Variable	Fine sediment	Aquatic macrophytes	Channel slope
Aquatic macrophytes	0.3689		
Channel slope	- 0.4181	- 0.3258	
Low-velocity mesohabitat	0.5967	0.4450	- 0.6170

been observed in modified spring streams compared to unmodified spring streams in the Salt River watershed (Joyce and Hubert 2004). The combination of good habitat for both *T. tubifex* and salmonids possibly makes these reaches in spring streams point sources of TAMs that infect age-0 fish in the spring streams and downstream in the mainstem of the Salt River.

Dams constructed by beaver (*Castor canadensis*) were common among mountain tributaries that flowed from the Caribou and Webster ranges on the west side of the Salt River valley. Several reaches immediately downstream from beaver dams on Jackknife (Reach 2), Tincup (Reach 1), Webster (Reaches 1 and 2), and Crow (Reaches 2, 3, and 6) creeks had higher infection rates than reaches with similar physical habitat characteristics elsewhere in the watershed (Appendix A). A common feature among these reaches was the presence of beaver ponds with substantial fine-sediment deposition in the ponds a short (< 1 km) distance upstream. We did not include the presence of upstream beaver dams as an independent variable in our analysis because we did not search the entire length of all tributary streams for presence of beaver dams. However, beaver dams trap sediment and organic material (Naiman et al. 1988), and the activity of bacteria on organic matter in beaver ponds can consume oxygen allowing *T. tubifex* to flourish due to lack of competition from other invertebrate species (Hynes 1966, Hart and Fuller 1974). Hiner and Moffitt (2002) found that salmonids downstream from beaver dams with high accumulations of organic matter had higher histologic grades of tissue damage than fish in other locations.

The poor predictive abilities of our models were likely due to complex interactions of pathogen, hosts, and environmental features. Numerous factors were not accounted for in this study, including fish density, contact rate with the pathogen, immunity, natural survival rates of both fish and *T. tubifex* populations, and parasite development within *T. tubifex* (Reno 1998). *M. cerebralis* colonization throughout the Salt River watershed also may not have been complete so that maximum *M. cerebralis* density had occurred throughout the watershed (Kennedy 1976).

Spatial autocorrelation of the sampled reaches indicated that the dependent variables, e.g., infection rates and histologic scores, were similar among reaches in close proximity to each other. Additionally, measuring habitat features at the reach scale did not reflect habitat conditions upstream of the sampled reaches. These insights contribute to interpretation of the data. For example, high rates of infection were observed in side-channel reaches of the Salt River with the highest of these rates immediately downstream from the cluster of spring streams in the upper valley. High rates of *M. cerebralis* infection in the side-channel reaches likely were due to high densities of TAMs drifting from the upstream cluster of spring streams (Hubert et al. 2002).

Management implications may be drawn from our observations of physical habitat features, *M.cerebralis* infection rates, and histologic evidence of disease. We found the highest rates of infection and tissue damage in reaches with substantial accumulation of fine sediment in the reach due to habitat degradation or efforts to

Table 4. Significant ($P < 0.05$) linear regressions accounting for variation in infection rates (IR) by *M. cerebralis* and histologic scores (HS) of tissues in age-0 salmonids from the Salt River watershed during 2000. Infection rates were arcsine square-root transformed. Independent variables were expressed as percentages.

Dependent variable	Independent variable	Slope	Intercept	Adjusted r^2	*P*
		All sites (99 reaches)			
IR	Fine sediment	0.0044	0.547	0.201	<0.001
	Aquatic macrophytes	0.0032	0.740	0.031	0.045
	Channel slope	- 0.1838	0.909	0.144	<0.001
	Low-velocity mesohabitat	0.0033	0.584	0.091	0.001
HS	Fine sediment	0.0029	0.061	0.084	0.002
	Aquatic macrophytes	0.0034	0.170	0.041	0.025
	Low-velocity mesohabitat	0.0020	0.094	0.029	0.049
		Mountain streams (41 reaches)			
IR	Fine sediment	0.425	26.3	0.118	0.016
	Channel slope	-17.7	57.0	0.209	0.003
	Low-velocity mesohabitat	0.390	20.6	0.104	0.023
HS	Fine sediment	0.0026	0.0023	0.097	0.027
		Spring streams (22 reaches)			
IR	Fine sediment	0.0040	0.546	0.167	0.034
HS	Aquatic macrophytes	0.0065	0.005	0.215	0.017

improve habitat for adult salmonids, or upstream from the reach due to the presence of beaver ponds with substantial amounts of fine-sediment deposited in them. Zendt and Bergersen (2000) proposed that habitats rich in organic matter may serve as point sources of TAM production, and that management of riparian habitats could reduce *T. tubifex* and effects of *M. cerebralis* on salmonids. Our study suggested that not only the management of riparian habitats, but also the management of beaver in mountain streams and habitats modified for adult salmonids in spring streams, may be important in the control of *T. tubifex*, *M. cerebralis*, and the impacts of whirling disease on salmonids across a watershed.

ACKNOWLEDGEMENTS

We thank R. Gipson, D. Zafft, K. Gelwicks, D. Hawk, and D. Money with the Wyoming Game and Fish Department for their extensive help in coordinating and conducting the research; D. Miller, E. Williams, and T. Wesche for assistance in planning and critical review; L. Leslie and M. Stuart for assistance with laboratory analysis; S. Merritt, B. Smith, and T. Burckhardt for assistance in the field; and the many landowners in the Salt River valley who allowed access to their land. The research was supported by the Wyoming Game and Fish Department and the Whirling Disease Initiative.

LITERATURE CITED

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

Arndt, R. E., E. J. Wagner, Q. Cannon, and M. Smith. 2002. Triactinomyxon production as related to rearing substrate and diel light cycle. Pp. 87-91 *in* J. L. Bartholomew and J. C. Wilson, editors. Whirling disease: review and current topics. American Fisheries Society, Symposium 29, Bethesda, MD. 262 pp.

Baldwin, T. J., E. R. Vincent, R. M. Silflow, and 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 Investigation 12:312-321 de la Franco, E., and P. Budy. 2004. Linking environmental heterogeneity to the distribution and prevalence of *Myxobolus cerebralis*: a comparison across sites in a northern Utah watershed. Transactions of the American Fisheries Society 113:1176-1189.

Gelwicks, K., D. Zafft, and R. Gipson. 2000. Effects of *Myxobolus cerebralis* on salmonids in the mainstem Salt River, Wyoming. Pp. 95-97 *in* Proceedings of the Whirling Disease Symposium "Solutions to whirling disease: putting the pieces together." Coer d'Alene, Idaho, February 3 and 4, 2000. Whirling Disease Foundation, Bozeman, MT.

Hart, C. W. Jr., and S. L. H. Fuller, editors. 1974. Pollution ecology of freshwater invertebrates. Academic Press, New York, NY. 389 pp.

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

Hiner, M., and C. M. Moffitt. 2002. Modeling *Myxobolus cerebralis* infections in trout: associations with habitat variables. Pp. 167-179 *in* J. Bartholomew and C. Wilson, editors. Whirling disease: reviews and current topics. American Fisheries Society Symposium 26, Bethesda, MD. 262 pp.

Hubert, W. A., M. P. Joyce, R. Gipson, D. Zafft, D. Money, D. Hawk, and B. Taro. 2002. Whirling disease among Snake River cutthroat trout in two spring streams in Wyoming. Pp. 181-193 *in* J. Bartholomew and C. Willson, editors. Whirling disease: reviews and current topics. American Fisheries Society Symposium 26, Bethesda, MD. 262 pp.

Hynes, H. B. N. 1966. The biology of polluted waters. Liverpool University Press, Liverpool, England. 555 pp.

Isaak, D. J., W. A. Hubert, and K. L. Krueger. 1999. Accuracy and precision of stream reach water surface slopes estimated in the field and from maps. North American Journal of Fisheries Management 19:141-148.

Isaak, D. J. 2001. A landscape ecological view of trout populations across a Rocky Mountain watershed. Doctoral dissertation. University of Wyoming, Laramie. 148 pp.

Joyce, M. P., and W. A. Hubert. 2004. Spawning ecology of finespotted Snake River cutthroat trout in spring streams of the Salt River Valley, Wyoming. Western North American Naturalist 64:78-85.

Kennedy, C. R., editor. 1976. Ecological aspects of parasitology. North Holland Publishing Company, Amsterdam, Holland. 474 pp.

Kennington, F. W., and K. K. Hamblin. 1989. A history of the Star Valley 1800-1900. Valley Graphics, Salt Lake City, UT. 289 pp.

Lampert, W., and U. Sommer. 1997. Limnoecology: the ecology of lakes and streams. Oxford University Press, New York, NY. 382 pp.

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.

Money, D., and S. Wanner. 1997. New findings and current status of whirling disease in Wyoming. Pp. 35-38 *in* Whirling disease symposium, expanding the database: 1996 research progress reports, Logan, Utah, March 6-8, 1997. Whirling Disease Foundation, Bozeman, MT.

Moran, P. 1948. The interpretation of statistical maps. Journal of the Royal Statistical Society B 10:243-251.

Naiman, R., C. Johnston, and J. Kelley. 1988. Alteration of North American streams by beaver. BioScience 38:753-761.

Nehring, B. R., and P. G. Walker. 1996. Whirling disease in the wild: the new reality in the Intermountain West. Fisheries 21(6):29-30.

Reno, P. W. 1998. Factors involved in the dissemination of disease in fish populations. Journal of Aquatic Animal Health 10:160-171.

Rognlie, 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.

Rose, J. D., G. S. Marrs, C. Lewis, and G. Schisler. 2000. Whirling disease behavior and its relation to pathology of brain stem and spinal cord in rainbow trout. Journal of Aquatic Animal Health 12:107-118.

Sauter, G., and H. Gude. 1996. Influence of grain size on the distribution of tubificid oligochaete species. Hydrobiologia 333:97-101.

Schilser, G. L., R. B. Nehring, P. G. Walker, K. G. Thompson, and E. P. Bergersen. 1997. Assessment of the distribution of clinical signs of whirling disease among rainbow and brown trout fingerlings *in* the Upper Colorado River. Pp. 103-106 *in* Whirling disease symposium. Expanding the database: 1996 research progress reports. Whirling Disease Foundation, Bozeman, MT.

Strahler, A. N. 1957. Quantitative analysis of watershed geomorphology. Transactions of American Geophysical Union 38:913-920.Vincent, E. R. 1996. Whirling disease and wild trout: the Montana experience. Fisheries 21(6):32-33.

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

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

Received 1 April 2005
Accepted 15 September 2005

Appendix A. Measurements of habitat features, number of age-0 salmonids collected, rate of infection by *Myxobolus cerebralis*, and mean histologic scores of tissue damage for 107 reaches sampled across the Salt River watershed during 2000.

Stream	Reach	Fine sediment (%)	Aquatic macrophytes (%)	Channel slope (%)	Low-velocity mesohabitat (%)	Fish collected	Infection rate (%)	Histologic score
				MOUNTAIN TRIBUTARIES				
Cottonwood Creek	1	1	0	2.78	18	6	0	0.00
	2	0	0	1.29	0	30	0	0.00
Crow Creek	1	0	0	0.38	0	6	50	0.00
	2	40	24	0.44	52	3	67	0.00
	3	23	7	0.28	50	25	60	0.16
	4	40	8	0.12	70	16	38	0.00
	5	55	19	0.28	43	9	44	0.00
	6	27	12	0.63	46	27	63	0.04
	7	46	7	1.34	32	36	3	0.00
Spring Creek (tributary to Crow Creek)								
	1	88	6	0.68	52	26	8	0.00
	2	55	3	0.40	65	36	25	0.03
Dry Creek	1	0	0	2.53	26	5	20	0.00
	2	0	0	.50	11	8	0	0.00
Jackknife Creek	1	63	90	0.38	72	0		
	2	63	10	0.79	78	4	75	0.25
	3	33	11	0.94	68	13	8	0.23
	4	38	0	0.82	57	18	33	0.00
	5	55	0	1.02	76	26	4	0.00
Upper Salt River	1	19	0	0.96	52	14	29	0.00
	2	12	0	1.37	34	14	50	0.07
	3	6	0	1.57	28	25	8	0.00
Strawberry Creek	1	0	0	1.80	5	0		
Stump Creek	1	14	0	0.01	100	3	67	0.33
	2	15	0	0.55	53	2	100	0.00
	3	8	3	1.08	23	20	50	0.00
	4	54	13	0.16	81	25	60	0.04
	5	38	2	0.42	40	27	48	0.00
	6	32	4	0.34	77	16	25	0.00
	7	22	2	0.62	46	28	25	0.04
	8	6	0	1.71	19	18	11	0.06
Spring Creek (tributary to Tygee Creek)								
	1	26	73	0.63	94	0		
Tygee Creek	1	68	41	0.26	71	4	50	0.25
	2	63	8	1.08	61	7	71	0.00
Webster Creek	1	0	0	2.12	0	25	80	0.40
	2	85	0	1.51	46	25	100	1.08
Swift Creek	1	0	0	2.82	24	0		
	2	60	1	0.01	100	25	92	0.00

Appendix A (cont.)

Stream	Reach	Fine sediment (%)	Aquatic macrophytes (%)	Channel slope (%)	Low-velocity mesohabitat (%)	Fish collected	Infection rate (%)	Histologic score
Tincup Creek	1	70	2	0.14	87	24	75	0.33
	2	57	1	0.87	86	6	50	0.00
	3	95	0	0.23	100	0		
	4	9	0	.60	37	8	50	0.00
	5	10	0	1.08	0	26	35	0.00
Willow Creek	1	7	0	1.33	40	26	19	0.00
	2	13	0	1.08	58	0	3	0.00
	3	1	0	2.57	32	17	6	0.00
	4	0	0	3.01	23	25	0	0.00
				SPRING STREAMS				
Anderson Creek	1	90	60	0.09	100	51	76	0.24
	2	95	1	0.13	76	26	85	0.23
	3	61	23	0.33	32	25	36	0.00
Big Creek	1	46	24	0.02	60	29	79	0.39
	2	80	76	0.13	100	21	57	0.43
	3	67	20	0.21	67	28	64	0.04
Burton Creek	1	89	31	0.14	100	30	73	0.03
Christensen Creek	1	95	70	0.14	88	30	97	0.93
	2	79	66	0.39	90	51	98	1.22
Cranny Creek	1	67	24	0.01	58	25	88	0.44
	2	100	90	0.01	100	0		
	3	100	75	0.14	97	4	75	0.25
	4	37	11	0.14	80	0		
Dave Creek	1	86	4	0.16	64	16	38	0.06
Flat Creek	1	19	3	0.12	81	35	11	0.00
	2	10	0	0.17	7	51	29	0.00
	3	75	47	0.37	92	25	32	0.00
	4	67	1	1.13	33	25	80	0.00
Hillyard Creek	1	64	40	0.25	65	14	93	0.07
	2	67	45	0.05	64	26	62	0.85
Perk Creek	1	69	36	1.94	73	36	33	0.00
	2	90	53	0.17	96	35	37	0.06
Thompson Creek	1	93	58	0.50	80	37	76	0.08
Thurman Creek	1	29	61	0.36	100	26	65	0.23
				SALT RIVER SIDE CHANNELS				
Reach A	1	83	0	0.88	90	16	56	0.06
	2	90	0	1.34	18	23	87	0.13
	3	95	7	0.64	69	21	48	0.38

Appendix A (cont.)

Stream	Reach	Fine sediment (%)	Aquatic macrophytes (%)	Channel slope (%)	Low-velocity mesohabitat (%)	Fish collected	Infection rate (%)	Histologic score
Reach A (cont.)								
	5	10	0	1.00	28	4	75	0.75
	6	51	0	1.00	66	8	75	0.00
Reach B								
	1	49	0	0.09	65	22	82	0.27
	2	85	0	0.57	57	29	38	0.14
	3	68	0	0.22	37	29	38	0.07
	4	60	0	0.05	79	13	23	0.00
	5	64	0	0.17	69	27	63	0.30
	6	94	0	0.01	93	11	64	0.45
	7	75	0	0.21	68	28	82	0.57
	8	22	0	0.18	29	34	38	0.16
	9	90	0	1.30	46	22	59	0.00
Reach C								
	1	65	0	1.11	70	14	86	0.36
	2	90	60	0.01	100	6	83	0.00
	3	6	0	0.99	31	0		
	4	96	11	0.63	55	5	100	0.00
	5	99	0	0.01	100	2	100	1.50
	6	36	11	0.01	35	29	90	0.31
	7	98	5	0.01	100	26	96	0.20
	8	97	25	0.08	92	25	96	0.20
	9	39	0	0.03	95	26	100	0.42
	10	60	0	1.49	2	33	88	0.70
	11	60	0	1.50	11	26	96	0.38
	12	92	14	0.39	72	27	85	0.24
	13	70	0	0.52	85	26	92	0.12
	14	20	0	1.50	46	7	100	0.29
	15	74	0	0.34	100	19	84	0.37
	16	0	5	1.00	0	28	75	0.21
	17	0	0	1.50	0	16	100	0.56
	18	30	13	1.50	25	24	92	0.38
Reach D								
	1	95	0	0.50	100	15	87	0.20
	2	95	40	0.50	100	8	100	1.38
	3	55	0	0.50	74	25	88	0.20
	4	92	0	1.00	100	23	91	0.48