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Linking Environmental Heterogeneity to the Distribution and Prevalence of *Myxobolus cerebralis*: A Comparison across Sites in a Northern Utah Watershed

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Abstract.—Given the variable effects of *Myxobolus cerebralis* (the parasite that causes whirling disease) on trout populations in different streams across the intermountain West, it is important to understand the role of environmental variation in determining the distribution and prevalence (percent infected) of *M. cerebralis* in newly infected watersheds. We investigated the relationship between a selected group of environmental factors and the distribution and prevalence of *M. cerebralis* in wild salmonid populations in the Logan River, Utah. We also compared the results of polymerase chain reaction analyses of wild (free-ranging) fish and fish reared in sentinel cages. These results indicated that despite its recent widespread distribution, the prevalence of the parasite varied greatly across sites. The lowest prevalence among cutthroat *Oncorhynchus clarki* was found at the headwaters, where the average summer temperature was below 9.5°C, whereas high prevalence was associated with temperatures above 12°C. Furthermore, prevalence in brown trout *Salmo trutta* and cutthroat trout increased with discharge, reaching its highest levels at sites where the average base flow ranged between 0.7 and 1.1 m^3/s. Despite hypothesized mechanistic links to one or more stages or hosts in the *M. cerebralis* life cycle, we observed no relationship between *M. cerebralis* prevalence and substrate composition, nutrients (total nitrogen and total phosphorus), periphyton, and oligochaetes. However, multiple linear regression models that included average temperature and discharge explained more than 70% of the variability in prevalence across sites for both species. The diagnosis of the parasite also revealed inconsistencies among wild and sentinel fish, suggesting that fish movement and life history may be key components leading to the spread and effects of the parasite along the drainage. These results indicate that changes in stream temperature or discharge, either natural or anthropogenic, could reduce or increase the prevalence and ultimate effect of *M. cerebralis* on wild trout populations.

The response of trout populations susceptible to *Myxobolus cerebralis* (Myxozoa: Myxosporea), the parasite that causes whirling disease, has varied widely across and within different geographic areas. This parasite has been associated with declines of wild rainbow trout *Oncorhynchus mykiss* populations in Montana and Colorado (Nehring and Walker 1996; Vincent 1996), but population-level responses have not been consistently observed in areas of other states where the parasite is also present (e.g., California; Modin 1998). Further, Hiner and Moffitt (2001) found the effects of *M. cerebralis* varied within drainages and even within streams. The reported inconsistency in responses of wild rainbow trout populations to *M. cerebralis*, suggests that environmental factors may influence the variability of the responses in infected environments (Schisler et al. 2000). Environmental factors and anthropogenic stressors can affect parasite–host interactions by influencing the physiological condition, reproduction, and survival of both groups (e.g., Lenihan et al. 1999).

Understanding the interactions among environmental factors, hosts, and pathogens is critical for assessing the potential effects of *M. cerebralis* and developing management strategies to minimize the effect of the parasite in wild trout populations. However, identifying the environmental factors that influence *M. cerebralis* is complicated by the complexity of its life cycle, which involves two obligate hosts (fish and the oligochaete worm-*Tubifex tubifex*) and two spore stages (myxospore and triactinomyxon [TAM]; Wolf and Markiw 1984). Environmental factors such as water temperature, substrate composition, water velocity, and discharge may influence the life cycle of *M. cerebralis*.

Among these factors, temperature directly influences the parasite (spores and the infective TAM), the tubificid secondary host, and fish. Experiments have shown that 12–15°C is the optimal temper-

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ature range for TAM production in infected *T. tubifex*. Lower temperatures may retard the development and maturation of the spores and extend the period of spore production (El-Matbouli et al. 1999), whereas higher temperatures may decrease TAM persistence (Markiw 1992). In contrast, somewhat lower temperatures, between 10°C and 13°C, have been associated with the optimal range for *T. tubifex* growth (Reynoldson 1987). Furthermore, temperature has been directly related to infectivity, lesion severity, and prevalence of *M. cerebralis* on wild and naturally exposed rainbow trout (Nehring and Thompson 2001; Hiner and Moffitt 2002).

In addition to temperature effects, the distribution of tubificid worms can be influenced by their preference for silt (<125 μm) and fine (<10 mm) sediments in general (Sauter and Gude 1996; Arndt et al. 2002). Experiments have shown that *T. tubifex* prefer fine substrates where the associated microflora may offer concentrated bacterial food (Lazim and Learner 1987). Despite their preference for fine substrates, the abundance of heterotrophic aerobic bacteria may be more important in controlling substrate selection (McMurtry et al. 1983). In addition to a preference for fine substrate, high abundances of *T. tubifex* have been associated with increasing concentrations of nitrogen and phosphorous compounds (Lestochova 1994).

Water velocity may also determine, in part, the prevalence of *M. cerebralis* through its effects on sedimentation rates, TAM destruction, and dilution effects. Low, water-velocity areas in streams, where silty organic material may be more abundant (e.g., backwaters, pools), has been related to higher abundances of tubificid worms (Lazim and Learner 1987). Kerans and Zale (2002) suggest that myxospores may not be very abundant in natural environments and point out the possibility of their passive dispersal to areas of low water velocity and fine sediments, where tubificids may be more prolific. Conversely, high water velocity may reduce the rate of *M. cerebralis* infection by destroying TAMs, and high discharge may result in a reduction of their concentration (Kerans and Zale 2002; MacConnell and Vincent 2002).

Numerous studies have focused on the biology of the *M. cerebralis* (Halliday 1976), *T. tubifex* (Hedrick and El-Matbouli 2002) and on the effects of the parasite on fish (MacConnell and Vincent 2002). Fewer studies have been designed to identify and enhance the understanding of the environmental factors that may be associated with the distribution and prevalence of the parasite (Hiner and Moffitt 2002). We investigated the relationship between environmental factors (i.e., water temperature, discharge, substrate size, nutrient concentration, primary productivity, and relative abundance of *T. tubifex*) and the distribution and prevalence (percent infected) of *M. cerebralis* in wild salmonid populations and sentinel fish in the main stem of the Logan River, Utah, and two of its tributaries. These factors were chosen a priori, based on suggested relationships and hypothesized mechanistic links as discussed above.

We also explored the use of these environmental factors to build a predictive model for potential increases in prevalence of *M. cerebralis*, because the effects of *M. cerebralis* on salmonid populations along the Logan River are uncertain. The parasite poses a threat to salmonid populations, particularly to the endemic Bonneville cutthroat trout *O. clarki utah* population, which may be one of the largest metapopulations spread over its historical range (Thompson et al. 2000). The recent detection of the parasite in this system (Wilson 1999), the higher resistance to infection of cutthroat trout and brown trout *Salmo trutta* in relation to rainbow trout, and heterogeneity in environmental characteristics in the Logan River, make this area an ideal study site to explore processes of invasion, persistence, and the role of environmental factors in determining the parasite's distribution and prevalence.

Methods

Eight index sites within the Logan River drainage in northern Utah were selected to represent a wide range of environmental conditions that exist within this watershed. Sites included reaches at headwaters, tributaries, and lower stream sections developed for water management and influenced by artificial impoundments (Figure 1; Table 1). The river is free-flowing from Franklin Basin through Twin Bridges and considered to be in relatively pristine condition. There are three dams on the lower river. The Third Dam index site was in the free-flowing river section between Second and Third dams; significant water withdrawals (about 1.4 m^3/s during base flows) occur between this index site and the next one upstream (Twin Bridges). Below Third dam, more water is withdrawn and the river is affected by urban influences (e.g., golf course) and agricultural uses. The tributary index sites were primarily on U.S. Forest Service land, Cache National Forest; we believed these sites to be in relatively good physical condition,

TABLE 1.—Number of wild cutthroat trout and brown trout infected with *Myxobolus cerebralis* and environmental variables measured in the Logan River, Utah, in 2001 and 2002. Sample size (*n*) is also given.

		Trout species					
		Cutthroat		Brown		Average temperature (°C)	
Site	Elevation (m)	No. infected	*n*	No. infected	*n*	Summer	Diel
			2001				
Franklin Basin	2,023	1	20	0	0	9.2	7.3
Red Banks	1,923	9	14	0	0	11.0	7.7
Forestry Camp	1,855	11	17	0	0	12.1	8.8
Twin Bridges	1,691	10	19	0	0	11.8	6.2
Third Dam	1,509	8	8	0	0	12.1	6.0
Lower Logan	1,352	0	0	6	10	16.0	2.5
Temple Fork	1,745	4	13	0	5	10.6	8.5
Right Hand Fork	1,588	0	0	0	10	10.8	1.8
			2002				
Franklin Basin	2,023	3	18	0	0	8.8	6.7
Red Banks	1,923	15	20	0	0	10.6	7.3
Forestry Camp	1,855	15	20	0	0	11.6	8.5
Twin Bridges	1,691	15	19	7	20	11.7	5.5
Third Dam	1,509	12	14	13	20	12.3	4.1
Lower Logan	1,352	0	0	15	19	15.7	3.2
Temple Fork	1,745	9	19	1	11	10.6	8.1
Right Hand Fork	1,588	0	0	0	10	10.7	1.7

[a] 10-m fixed-time collection.
[b] Of 415 mature tubificids collected at this site, 50 were identified.

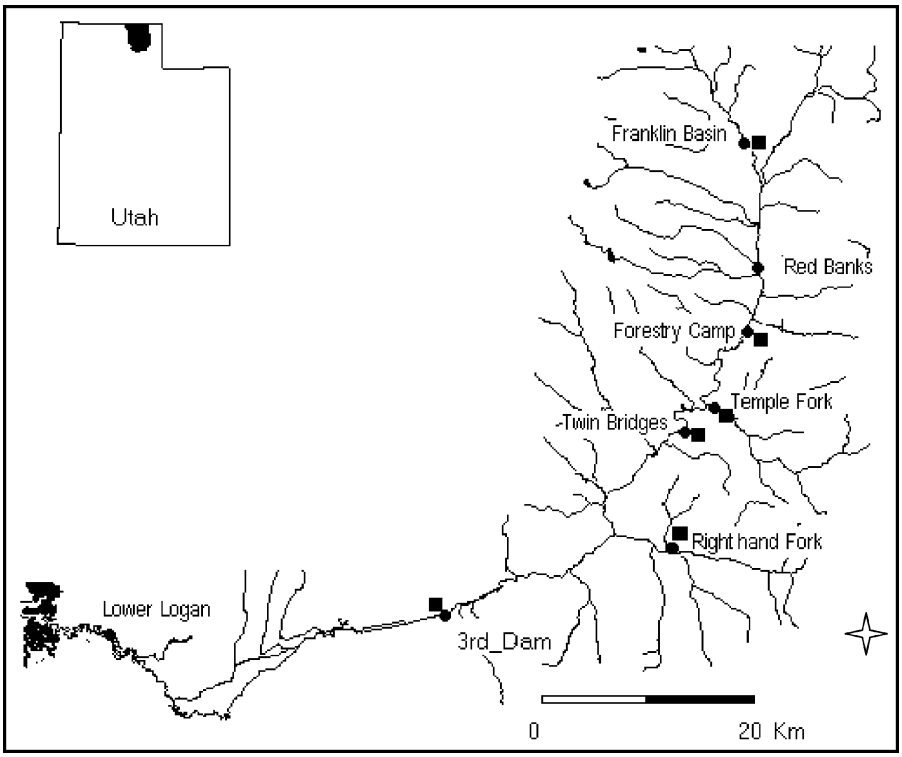

FIGURE 1.—Map depicting the locations of *M. cerebralis* study sites within the Logan River basin, Utah (see Table 1 for elevations). Circles represent sites where wild trout where collected and environmental factors measured. Squares indicate sites where sentinel fish (i.e., fish previously unexposed to *M. cerebralis*) where exposed.

TABLE 1.—Extended.

Site	Substrate		Average nutrients		Chlorophyll *a* (mg/m^2)	Oligochaetes	
	Average (mm)	% Fines	Total N (mg/L)	Total P (μg/L)		All	*T. tubifex*
				2001			
Franklin Basin	209	1	0.07	17.7	47		
Red Banks	287	0	0.08	21.9	44		
Forestry Camp	283	10	0.07	21.8	120		
Twin Bridges	143	6	0.03	21.9	64		
Third Dam	38	6	0.09	15.2	182		
Lower Logan	137	1	0.21	22.5	95		
Temple Fork	23	24	0.12	21.3	183		
Right Hand Fork	46	13	0.16	24.2	12		
				2002			
Franklin Basin	173	14	0.14	21.0	74	62	6
Red Banks	252	14	0.07	19.2	80	12	7
Forestry Camp	258	14	0.05	19.4	86	857	49[b]
Twin Bridges	236	4	0.06	15.5	78	16	4
Third Dam	205	1	0.10	11.7	75	92	
Lower Logan	40	5	0.27	28.6	91	6	
Temple Fork	123	30	0.13	18.3	75	39	2
Right Hand Fork	105	11	0.22	21.7	96	126	11

except for varying degrees of grazing pressure (P. Chase, U.S. Forest Service, Logan Ranger District, personal communication). Overall the Logan River watershed is characterized as having a high base flow, low flood magnitude, and habitat predominated by runs and riffles in the main stem.

Field surveys of fish populations and habitat characteristics were conducted at all sites during the summers of 2001 and 2002. Sentinel fish were exposed to natural stream conditions at six of these sites. Bonneville cutthroat trout and brown trout dominate the fish community throughout this watershed, brown trout occupying the lower-elevation reaches and cutthroat trout occupying the higher-elevation reaches; overall, there is a distinct pattern of allopatry (de la Hoz Franco and Budy, in press). Cutthroat trout in this system typically spawn in tributary streams in early spring. Their fry emerge in late spring and early summer, and the juveniles rear for varied periods in the tributaries before migrating out into the main stem (Behnke 1992). Brown trout typically spawn in the main stem in fall. Their fry emerge in early spring, and the juveniles rear in the main stem.

Fish Sampling and Myxobolus cerebralis Diagnosis

Wild salmonid populations were sampled at all sites using three-pass electrofishing depletion techniques during low-flow conditions in August of 2001 and 2002. All fish captured were identified to genera and evaluated for clinical signs of whirling disease (e.g., black tail, whirling behavior); 100 of each species were additionally weighed and measured. Where possible 10 juveniles (subadults), and 10 adults from each species (Bonneville cutthroat trout and brown trout) were sacrificed and further analyzed for *M. cerebralis*, the parasite that causes whirling disease. The head, including all gill arches and anterior spinal cord, was removed from the sacrificed fish and frozen for PCR (polymerase chain reaction) analyses, as required to officially confirm *M. cerebralis* (per American Fisheries Society Blue Book; MacConnell 2003). For PCR analyses, samples were heated at 95°C for either 15 min (tubes) or 30 min (large head in sample cups; J. Wood, Pisces Molecular, LLC, personal communication). Each head was placed on a clean polypropylene cutting mat and defleshed with scalpels. The fragments of skull and gill arch bone and cartilage were collected and placed in a microcentrifuge tube. Total DNA was extracted from all samples using a spin-column DNA purification procedure. All sample DNA preparations were assayed for *M. cerebralis* via presence of the *Hsp70* gene segment by single-round PCR amplification and four controls for each PCR run (positive DNA, negative DNA, no DNA in reagents, no DNA carryover). Results were scored over a range of positive signals versus no signal or below the limit of detection. For ecological analyses, only positive and negative categories were used; prevalence was quantified as the per-

centage of samples that tested positive as a function of the total number tested.

Sentinel Fish Exposures

Sentinel fish experiments were conducted at a subset of index sites to evaluate the prevalence of whirling disease (caused by the *M. cerebralis* parasite) in fish held at and thus exposed only to the sites of interest. Cutthroat trout alevins (<5 weeks posthatch) were obtained from a rearing facility free of *M. cerebralis*. Alevins were transported to a fish holding facility and maintained in pathogen-free water at 10°C until natural exposures were conducted. Natural field exposures were completed at three sites during summer of 2001, and at three additional sites during summer of 2002 (Table 1). In 2001, three sentinel cages, each holding 30 fish (<9 weeks posthatch), were deployed at each site. In 2002, we used the same number of cages per site but 14 fish (<9 weeks post hatch) per cage. Fish were exposed 21 d at each site. We did not add additional feed to the cages. After the exposure, survivors were returned to the laboratory and maintained in pathogen-free well-water at 10°C. Fish from different sentinel cages were held in separate aquaria. Daily observations were made to detect clinical signs of whirling disease and to remove dead fish. At 90 d postexposure, fish were euthanatized, and the heads, including all gill arches and anterior spinal cord, were removed and frozen. Fish heads were tested for the presence of *M. cerebralis* using the same PCR method for the *Hsp70* gene segment described above for wild fish. The PCR data from sentinel cage fish were compared with data from wild fish to determine the potential effect of movement and life history on prevalence.

Environmental Variables

Temperature.—Water temperature was recorded from July to September at all sites at 2-h intervals by means of temperature loggers. Minimums, maximums, averages, and fluctuations (daily maximum − daily minimum) were determined for each day; monthly and summer averages were calculated from these daily indices. Temperature was also recorded from October 2001 to June 2002 at five selected sites. In addition, thermographs were placed at the stream sites along with sentinel cages during the field fish exposures.

Discharge.—Biweekly measurements were conducted during summer 2001 and 2002 at each sampling location. Discharge was estimated from cross-sectional measurements of water velocity at 10–20 equally spaced sites using an electromagnetic flowmeter (Bain and Stevenson 1999). Measurements were also conducted bi-weekly during the field exposures at each site.

Substrate.—Substrate composition was determined for each site during low flow conditions in summer of 2001 and 2002. Substrate particles were collected randomly at riffle zones from four transects within each reach that were evenly spaced and perpendicular to stream flow (Wolman 1954). At least 100 particles were collected at each site (reach). The middle width (*B*-axis) of each particle was measured to determine average substrate size and the percentage of fines (<10 mm in diameter; Kondolf 2000). Substrate was classified according to the Wentworth Scale (Allan 1995); substrate measurements were meant to provide a relative (across site) and quantitative index of general bed substrate (Kondolf 1997).

Nutrient analyses.—Water samples were collected for nutrient analyses on 1 d during late spring and 1 d during summer at each sampling site in 2001 and 2002. Bottles were prewashed with 1N HCl and rinsed with stream water before sample collection. Samples were kept on ice in the field and frozen until total nitrogen (TN) and total phosphorous (TP) analyses were conducted. Total nitrogen was determined by high-temperature catalytic oxidation with chemiluminescent nitrogen detection (Merriam et al. 1996). The ascorbic acid method was used for total phosphorous analysis (APHA et al. 1992).

Periphyton.—Chlorophyll *a* extracted from periphyton was used as an index of primary productivity (Wetzel and Likens 1991). In 2001, rocks were randomly collected in riffles at each site by walking three transects perpendicular to the stream flow. Ten rocks from each transect were collected, placed in plastic bags, and frozen. Methanol extraction of chlorophyll *a* was conducted for 24 h at room temperature and in the dark. From the extract, three 6-mL aliquots were analyzed fluorometrically (Welschmeyer 1994). The surface area of each rock was estimated by measuring three axes, length, width, and depth, and it was assumed that the area covered by periphyton was 60% of this estimated surface (Biggs and Close 1989). In summer 2002, three to five unpolished tiles (30 × 30 cm) were individually deployed across a riffle at each sampling site. Tiles where retrieved after 36 d, placed in plastic bags, stored in a cooler in the field, and frozen. Chlorophyll *a* was extracted and measured following the same procedures used for periphyton on rocks.

Oligochaetes and Tubifex tubifex.—Oligochaetes were collected during a 10-min (fixed time) collection at each site. Oligochaetes were collected during spring 2002 from habitats with soft, fine sediments using a 500-μm kick net. Samples were washed, sorted, and preserved in 70% methanol. Subsequently, oligochaetes were sorted and counted. All mature tubificids (bifid chaetae and hair with pectinate chaetae) were mounted on microscope slides and identified. If more than 100 tubificids were collected, 50 were randomly selected and mounted for identification.

Statistical Analyses and Modeling

We used a two-way analysis of variance (ANOVA) to examine variability in the prevalence of *M. cerebralis* in wild cutthroat trout and brown trout and to evaluate differences in explanatory variables (e.g., average temperature, discharge) among all sites and between years. Subsequently, data for each variable were pooled across the 2 years because the variables of interest did not differ significantly between years. Sample sizes varied because both cutthroat and brown trout were not present at all eight sites; in both years cutthroat trout were present at six sites and brown trout at four sites.

Regression analysis was used to identity the environmental variables that best explained the variation in *M. cerebralis* prevalence in cutthroat trout and brown trout across sites. Scatterplots of the response versus each explanatory variable were examined for preliminary assessment of potential relationships and to evaluate the form of relationship if present (i.e., linear, nonlinear). Scatterplots were also used to assess relationships among explanatory variables; apparent associations among these variables provided information about collinearity and were used to select a subset of variables for multiple regression model selection. The selection of variables for model development was based on a best-fit model regression; analysis of covariance (ANCOVA) among variables selected by best-fit indicated that these variables were independent. An analysis of residuals was included to assess assumptions of normality, homogeneity of variance, and linearity. Prevalence data were transformed with an arcsine square-root function to meet assumptions of normality for statistical analyses.

Results

Diagnosis of Myxobolus cerebralis

Over 4,200 fish representing five salmonid species (cutthroat trout, brown trout, brook trout *Salvelinus fontinalis*, rainbow trout, and mountain whitefish *Prosopium williamsoni*) where collected during 2001 and 2002. Clinical signs that could be attributed to whirling disease (e.g., black tail, cranial or spinal deformities) were observed on less than 1% of the trout captured. However, *M. cerebralis* was detected with PCR assays at seven of eight sampling sites, demonstrating that the parasite was widespread in the basin. The overall prevalence of *M. cerebralis* (percentage of fish testing positive) across the basin in 2001 was 47% for cutthroat trout ($N = 91$), 24% for brown trout ($N = 25$), 0% for mountain whitefish ($N = 10$), 75% for rainbow trout ($N = 4$), and 0% for brook trout ($N = 4$). In 2001 and 2002, respective prevalence among cutthroat trout across sites ranged from 5% and 17% at the uppermost site (Franklin Basin) to 100% and 84% at a low-elevation site on the main stem (Third Dam; Figure 2).

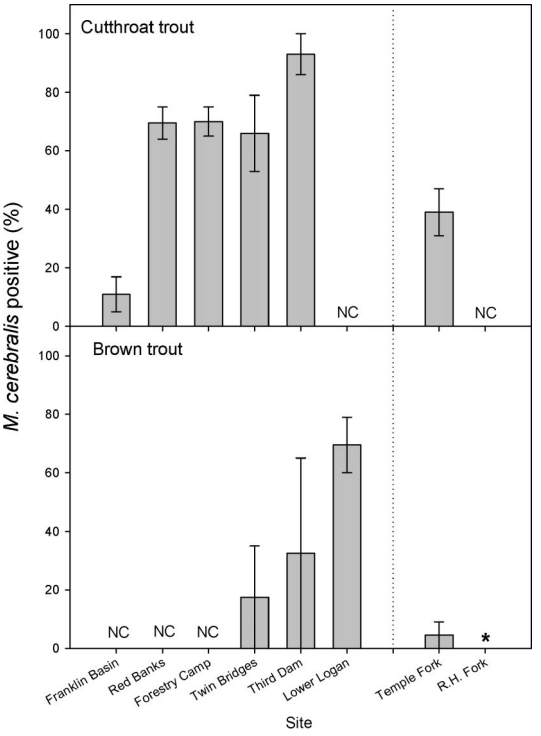

FIGURE 2.—Average prevalence of infection (percent testing positive for *M. cerebralis*) in wild cutthroat trout (upper panel) and brown trout (lower panel) at sample sites along the Logan River, Utah, in 2001 and 2002. Vertical bars represent 2-year ranges. Sites to the left of the dashed line are main-stem sites, whereas sites to the right are tributaries. The acronym ''NC'' indicates sites where fish were not captured. Asterisks show the sites where the parasite was not detected.

TABLE 2.—Survival, confidence intervals (CIs), and prevalence of infection (percent testing positive for *Myxobolus cerebralis*) in field-exposed sentinel trout (previously not exposed) at sample sites on the Logan River, Utah, in 2001 and 2002. Sample size (*n*)—the number of survivors tested—is also given.

Year	Sample site	Survival	95% CI	Percent positive	*n*
2001	Franklin Basin	0.18	0.10–0.26	0	16
	Temple Fork	0.26	0.16–0.36	0	23
	Twin Bridges	0.04	0.00–0.08	50	4
2002	Forestry Camp	0.57	0.40–0.73	56	24
	Third Dam	0.60	0.54–0.76	12	25
	Right Hand Fork	0.50	0.44–0.66	0	21

Differences in prevalence across sites were significant (df = 7, $P < 0.01$), but differences between years were not (df = 1, $P = 0.1$). Similarly, the highest prevalence of the parasite in brown trout was observed at the lowest site in 2001 and 2002 (Lower Logan; Figure 2). Prevalence at brown trout sites ranged from 0% to 60% in 2001 and from 0% to 79% in 2002. Despite the high percentage of fish that were identified to be infected with *M. cerebralis*, through 2003, we observed no population-level declines in cutthroat or brown trout (de la Hoz Franco 2003). However, it may be too early to detect a response at the population level given the recent date of first detection of the parasite (1999) and the age-structure of the fish populations (Budy et al. 2003).

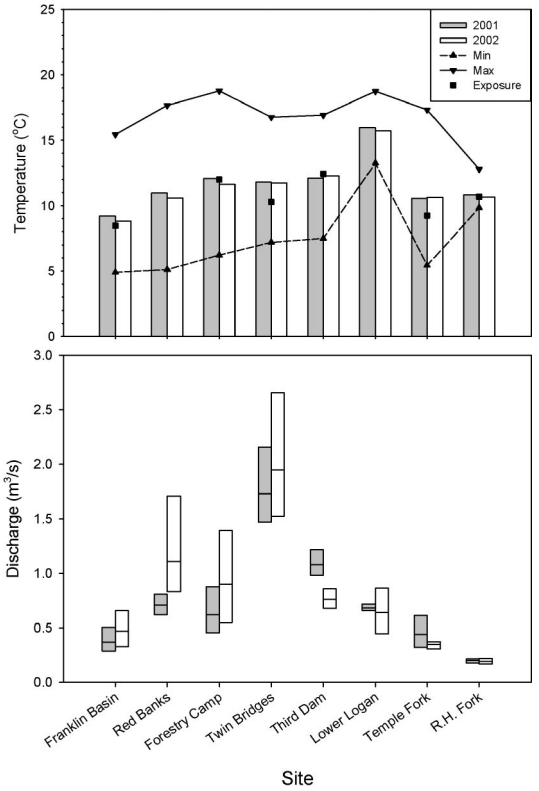

FIGURE 3.—The top panel depicts the average temperature at sample sites on the Logan River, Utah, 2001. Minimum and maximum average summer temperatures are shown, along with average temperatures during sentinel fish exposures (squares). The bottom panel depicts discharge at main-stem sites and tributaries. The boxes indicate the minimum and maximum discharges during summer 2001 (shaded) and 2002 (unshaded); the horizontal lines in the boxes indicate averages. Significant water withdrawals occur between the Twin Bridges and Third Dam sites.

Sentinel Fish Exposures

Field survival of fish in sentinel cages ranged from 18% to 60% (Table 2). Sentinel fish exposed to natural stream conditions did not develop clinical signs of whirling disease during the 90 d postexposure period. The complete lack of clinical signs observed during the postexposure period suggests that sentinel fish that died during the field exposure period did not die as a result of severe infection. The PCR analyses, however, indicated that up to 56% of the sentinel fish became infected with *M. cerebralis* during the 21 d field exposure (Table 2). Prevalence of *M. cerebralis* ranged from undetected at the uppermost site and the two tributaries to 56% at an upper-elevation site on the main stem (Forestry Camp; Table 2). Results from PCR analyses for sentinel fish contradicted those for wild fish for the headwater site and one tributary site. In these cases, sentinel fish in tributaries tested negative for *M. cerebralis* whereas wild fish tested positive.

Environmental Variables

Temperature.—Summer temperatures along the stream increased considerably at sites from high to low elevations. Average summer daily temperatures (July–September) ranged from 9.2°C to 15.9°C in 2001 and from 8.8°C to 15.7°C in 2002 (Figure 3). Daily average temperatures differed

significantly among sites (df = 7, $P < 0.01$) but not between years (df = 1, $P = 0.06$). The lowest daily average temperature during the 21-d sentinel fish exposure was recorded at the uppermost site (Franklin Basin; 8.5°C); the highest was recorded at a low-elevation site (Third Dam, 12.4°C; Figure 3). Average summer temperature fluctuation (diel = daily maxmum—daily minmum) in 2001 ranged from 1.8°C at a tributary site to 8.8°C at a main-stem site; a similar pattern was observed in 2002 (Table 1). The daily maximum temperatures recorded from September to June at selected sites reached 8°C during fall, 4°C during winter, and 10°C during spring.

Discharge.—The highest summer discharge was recorded at the Twin Bridges site on the middle main stem in 2001 (1.73 m^3/s) and 2002 (1.95 m^3/s). The lowest average discharge was observed in 2001 at one of the tributaries (Right Hand Fork; 0.19 m^3/s; Figure 3). In general, estimates of discharge were lower at sites at high and low elevations sites and at tributary sites; the highest estimates occurred at main-stem sites. Significant differences in discharge among years were not detected across all sites (df = 1, $P = 0.7$).

Substrate.—Small boulders and large cobbles were predominant in headwaters and main-stem sites. Coarse gravel was the most common substrate at the lowest site (Lower Logan). Substrate at tributary sites (Temple Fork, Right Hand Fork) was predominantly small cobbles. The highest percentage of fine substrates (≤10 mm) occurred in one of the tributaries (Temple Fork; 27%); lower percentages were estimated at low-elevation sites (Lower Logan, 3%; Third Dam, 3.5%; Table 1).

Nutrient analyses.—Higher nitrogen concentrations were detected at sites at high and low elevations and in the tributaries compared with sites in middle sections of the main stem. Differences between years were not significant (df = 1, $P = 0.06$; Table 1) but sites were significantly different (df = 7, $P < 0.01$). Total nitrogen concentrations ranged from 0.07 to 0.21 mg N/L, corresponding to the uppermost (Franklin Basin) and lowest (Lower Logan) sites. No significant differences were detected in total phosphorous concentrations among sites (df = 7, $P = 0.09$) or between years (df = 1, $P = 0.4$; Table 1).

Periphyton.—Extracts of chlorophyll *a* from rocks in 2001 and from artificial substrates in 2002 did not reveal a consistent pattern in primary productivity along the stream. Chlorophyll-*a* concentrations were 12–183 mg/m^2 for rocks and 74–96 mg/m^2 for tiles (Table 1). There were no significant differences between sites (df = 7, $P = 0.56$) or years (df = 1, $P = 0.64$).

Oligochaetes and Tubifex tubifex.—A total of 1,210 oligochaetes were collected at the eight sites: four species of Tubificidae (*Tubifex tubifex*, *Rhyacodrilus coccineus*, *Limnodrilus hoffmeisteri*, and *Telmatodrilus vejdovskyi*) and five other families (Naididae, Enchytraeidae, Lumbriculidae, Lumbricidae, and Sparganophilidae; *Eiseniella tetraedra* represented the only mature lumbricid). The largest numbers of oligochaetes were collected at an upper-elevation site on the main stem (Forestry Camp; Table 1). Tubificids with hair and pectinate chaetae predominated at this site (854 out of 857 worms), and of the 50 we mounted and identified, 49 were *T. tubifex*. The estimated proportion of the total number of oligochaetes identified as tubificids varied across sites: Franklin Basin = 15%, Red Banks = 58%, Forestry Camp = 92%, Twin Bridges = 31%, Third Dam = 0%, Lower Logan = 0%, Temple Fork = 5%, and Right-Hand Fork = 11%. Samples from headwaters and high-elevation main-steam sites contained mostly tubificids, whereas tributaries and low-elevation sites contained more lumbriculids or lumbricids.

Relationships among Environmental Factors and Prevalence

The prevalence of *M. cerebralis* was similar for cutthroat trout and brown trout. In cutthroat trout, *M. cerebralis* prevalence showed a nonlinear relationship with discharge (Figure 4); the highest prevalence was at a low-elevation main-stem site (Third Dam) associated with discharge estimates of 0.6–1 m^3/s. Prevalence of *M. cerebralis* in cutthroat trout also appeared to increase with water temperature (Figure 5); the highest prevalence was detected in water temperatures around 12°C. A similar pattern was observed for prevalence in brown trout in relation to temperature and discharge.

There was no apparent relationship between *M. cerebralis* prevalence in wild trout and periphyton (chlorophyll *a*), nutrient concentration (TN, TP), substrate size, percent fines (<10 mm), or relative density of oligochaetes. Furthermore, scatterplots did not reveal any apparent associations between oligochaete density and productivity (chlorophyll *a*), nutrient concentration (TN, TP), or substrate composition (Figure 5).

Scatter plots and Pearson's correlation coefficient indicated that sediment size was correlated with diel temperature, *T. tubifex* abundance was

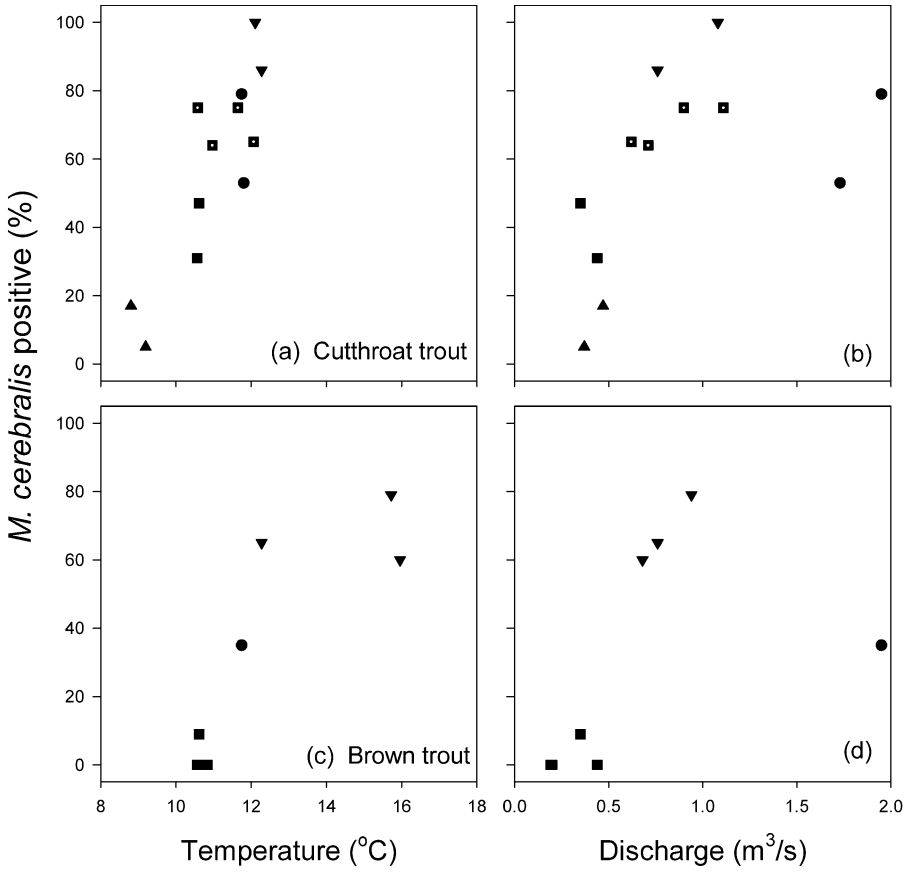

FIGURE 4.—Prevalence of infection (percent positive for *M. cerebralis*) in **(a)**—**(b)** wild cutthroat trout and **(c)**—**(d)** brown trout as a function of average summer temperature and discharge in the Logan River, Utah, in 2001 and 2002. Symbols depict sampling sites as follows: upward-pointing triangles = headwaters; solid squares = tributaries; open squares = reaches at high elevations; circles = reaches at middle elevations; and downward-pointing triangles = reaches at low elevations.

associated with sediment size, and percent fines was associated with average discharge. Based on these initial analyses of association and collinearity, temperature and discharge were included in the regression analyses; these variables were independent (based on an ANCOVA), and the assumptions of normality and homogeneity of variance were met. Multiple linear regression models (Table 3) that included average water temperature and discharge were significant overall and explained a large portion of the variation in prevalence of *M. cerebralis*. For cutthroat trout, the model accounted for 74% of the variability in prevalence observed across sampling sites (df = 11, adjusted $R^2 = 0.74$, $P \leq 0.01$; Table 4). A similar model explained 83% of the variability in prevalence among brown trout (df = 7, adjusted $R^2 = 0.83$, $P = 0.018$).

Discussion

Since the time *M. cerebralis* was first detected in the Logan River in 1999, its range has broadened along the main stem and its tributaries. Suspected vectors of the parasite include, fish eating birds, anglers' equipment, and fish (Taylor and Lott 1978; Bergersen and Anderson 1997; Schisler and Bergersen 2002). The diagnosis of *M. cerebralis* revealed that at some sites, the parasite was not detected among caged sentinel trout but was detected among wild free-ranging trout. This difference suggests that fish movement may be one of the vectors leading to the spread of the parasite along the stream and its tributaries. Differences in prevalence among juvenile (not infected) and adult trout (infected) at some sites (Budy et al. 2003) also support this hypothesis and suggest that life

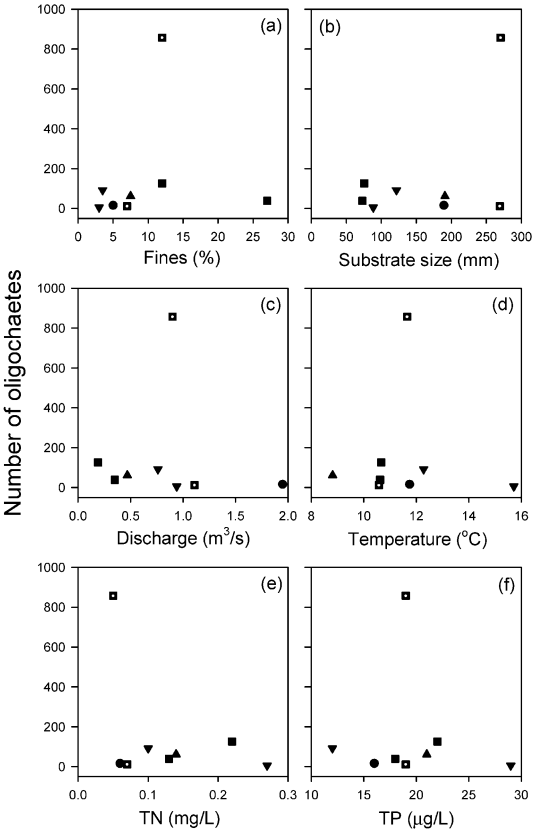

FIGURE 5.—Total number of oligochaetes collected at sample sites in the Logan River, Utah, in 2002 in relation to **(a)** percent fines, **(b)** average substrate size, **(c)** average summer discharge, **(d)** average summer temperature, **(e)** total nitrogen (TN), and **(f)** total phosphorous (TP). See the caption to Table 4 for an explanation of the symbols used.

history may be an important factor determining the level of infection in this system. Cutthroat trout in these systems are known to migrate to tributaries as adults to spawn; juveniles move back down into the main stem after rearing in these smaller tributaries (Behnke 1992). Infected fish may thus act as important vectors for the transport and spread of *M. cerebralis* spores, whereas at the same time, juveniles are temporarily protected from infection while rearing in tributaries. Based on tagging studies in the Logan River watershed, the majority of adult fish of both species demonstrate extremely high across-year site fidelity (percentage of tagged fish recaptured in the same reach; Budy, unpublished data). However, a small proportion of cutthroat trout display frequent and wide-ranging movements; the extent of movement depends on time, season, and life history stage (Hilderbrand 1998). Angler equipment and activity cannot be ruled out as an important vector. The sites where *M. cerebralis* was first detected in the Logan River and sites with the highest prevalence of *M. cerebralis* coincided with a high degree of angler activity (Budy et al. 2003). Our results demonstrate the importance of clearly delineating between infected fish versus infected sites.

Despite its widespread distribution, the prevalence of *M. cerebralis* along the Logan River varies greatly within the basin. This high variability in prevalence was not surprising; other studies have shown evidence of variability in prevalence and severity of infection across and within drainages (Baldwin et al. 1998; Hiner and Moffitt 2001). In our study, differences in average summer temperature and discharge along the river explained most of the variability (>70%) in prevalence observed across sites. Across sites where cutthroat trout were present, the lowest prevalence was observed in the headwaters, where the daily average water

TABLE 3.—Parameter estimates for the linear regression models for the prevalence of *M. cerebralis* in cutthroat and brown trout in the Logan River, Utah.

Dependent variable	df	Parameter estimate	Standard error	*t*	*P*
		Cutthroat trout			
Intercept	1	−1.7796	0.5880	−3.03	0.0164
Average temperature	1	0.1816	0.0646	2.81	0.0229
Average discharge	1	0.0405	0.0178	2.28	0.0524
(Average discharge)^2	1	−0.0004657	0.0002087	−2.23	0.0262
		Brown trout			
Intercept		1.1070	0.4540	−2.44	0.0713
Average temperature	1	0.0669	0.0522	1.28	0.2693
Average discharge	1	0.546	0.0231	2.37	0.0767
(Average discharge)^2	1	−0.0005913	0.0002862	−2.07	0.1077

TABLE 4.—Statistical summary of linear regression models for the prevalence of *M. cerebralis* in cutthroat and brown trout in the Logan River, Utah.

Source of variation	df	Sum of squares	Mean square	*F*	*P*	Adjusted R^2
Cutthroat trout						
Model	3	1.1467	0.3822	11.28	0.0030	0.7371
Error	8	0.2710	0.0338			
Total	11	1.4178				
Brown trout						
Model	3	1.3492	0.4497	12.12	0.0178	0.8265
Error	4	0.1485	0.0371			
Total	7	1.4977				

temperature was 9.2°C, whereas the highest was observed at a low-elevation site, where the average temperature was the highest (>12°C). Likewise, the low prevalence of the parasite in brown trout in the tributaries and high prevalence at the lowest site is consistent with the lowest (10–11°C) and highest (16°C) average summer temperatures across stream reaches where this species is distributed. Water temperatures that are close to the ideal for TAM production (12°C; Markiw 1992) and persistence (15°C; El-Matbouli et al. 1999) could explain the high prevalence of the parasite in both cutthroat trout and brown trout in lower sections of the river. Similarly, lower temperatures may retard spore development (El-Matbouli et al. 1999) and lead to lower prevalences, as we observed in trout from headwaters and tributaries.

Our study also demonstrated that differences in base flow discharge along the river may influence the variability in prevalence. The prevalence of the parasite in cutthroat trout increased with increasing discharge. Lower prevalence rates were observed at headwaters and at one of the tributaries where discharge was low, whereas the highest prevalence was observed at a low-elevation site of the main stem, where discharge was higher. These results are consistent with the pattern observed in prevalence among brown trout. Other authors have suggested that high flows could destroy or dilute TAMs, thus reducing infection in susceptible fish (Kerans and Zale 2002; MacConnell and Vincent 2002). Conversely, this study shows a nonlinear relationship between the range of flows observed in the Logan River and the prevalence of the parasite. Prevalence in cutthroat trout and brown trout increased with discharge reaching its highest levels at sites where average base flow ranged between 0.7 and 1.1 m^3/s; prevalence then decreased at the site where the highest discharge was estimated.

The asymptotic relationship between discharge and prevalence suggests that lower discharge at headwaters and tributaries may decrease the probability of spores contacting and infecting fish. On the other hand, higher discharge probably disturbs areas where spores may be concentrated, thus maximizing the probability of infection. Above this threshold, higher discharges lower infections by reducing the concentration of TAMs in the water column. Further, the presence of artificial impoundments in lower sections of the Logan River may favor higher spore concentrations because spores may be passively transported to areas of low water velocity (Hiner and Moffitt 2002; Kerans and Zale 2002; Nehring et al. 2003) and thus produce the higher prevalences observed at low-elevation reaches.

The lack of clinical signs (e.g., deformities, black tail) in wild and sentinel fish suggest that the abundance of TAMs along the Logan River is low. Similarly, we have observed no population-level declines in the trout populations of the Logan River since 1999 (Budy et al. 2003). Spore concentration (dose) is directly related to the development of clinical signs of whirling disease and its severity (Markiw 1992). However, other factors such as fish age (Markiw 1991), size (Thompson et al. 1999), species (Hedrick et al. 1999; Sollid et al. 2002; Vincent 2002), and environmental factors at the time of the exposure may also influence the susceptibility of fish to the disease. Highly susceptible cutthroat trout fry could be exposed to low TAM concentrations during spring and early summer; low temperatures may also retard spore development and production, and flushing or diluting effects may result from high discharge during this season. The effects of these environmental variables may also explain the response in brown trout fry that emerge during early spring. Results from this study are consistent with the hypothesis formulated by Hubert et al. (2002) that life history patterns of cutthroat trout in spring streams of the

Salt River drainage may reduce their susceptibility to *M. cerebralis* in two ways: (1) when adult fish migrate from the main stem to smaller tributaries and headwaters to spawn and (2) because these streams, which serve as nursery habitat for the fry, have lower water temperatures.

Despite the potential influence of other factors (e.g., primary productivity, relative abundance of oligochaetes, substrate composition, and nutrient concentrations such as TN and TP) on the life cycle of *M. cerebralis*, these factors were not related to the prevalence and distribution of the parasite among trout in the Logan River. The lack of relationship for those factors does not mean that a link does not exist and may be an artifact of some combination of low interyear variability, high within site variability in some factors, or perhaps the scale of measurement. Other authors have revealed that *T. tubifex* is not ubiquitous, and where present, densities can vary greatly (i.e., from <100 to >1000 worms/m^2; Särkkä 1987 ; Zendt and Bergersen 2000), which reflects the large differences in oligochaete abundance we observed across sites in the Logan River. The lack of correlation between oligochaete density and productivity (chlorophyll *a*), nutrient concentration (TN, TP), or substrate composition may indicate that, in the Logan River, other biological factors (e.g., abundance of heterotrophic bacteria) may be more important in substrate selection than are physical or chemical factors (McMurtry et al. 1983).

The combination of low variability in environmental factors (i.e., temperature, flow) between 2001 and 2002 and our field assessment period (mainly during base flow conditions) potentially limit our results. Logistical limitations impeded the measurement of environmental factors yearround. In addition, there are other variables we did not measure (e.g., abundance of food source or predation on tubificids) that may partially determine the pattern of distribution and abundance of *M. cerebralis*. However, results from our study should serve as a solid starting point or reference for others investigating potential linkages between environmental factors (e.g., water quality, discharge, and distribution and abundance of *T. tubifex*) and *M. cerebralis* distribution and prevalence. In addition, our results suggest that changes to stream temperature or discharge, either natural or anthropogenic, could alter the spread and effect of *M. cerebralis* in mountain streams.

Many authors have addressed the need to investigate pathogen-host-environment interactions to fully understand and assess the potential effects of disease in fish populations (Hedrick 1998; Reno 1998). Similarly, understanding the role of fish stressors, and synergistic effects of stress or disease and the environment, is also key to evaluating and managing the health and status of fish populations (Budy et al. 2002). This study was conducted to provide baseline information for the distribution and prevalence of *M. cerebralis* along the Logan River and to assess potential relationships between environmental factors and the parasite. Understanding the environmental variables that influence the distribution and prevalence of diseases and the mechanisms for its dispersal could produce tools that fishery biologists and managers could use to limit the spread of parasites and to minimize negative effects on wild salmonid populations.

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