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ARTICLE

Spatial and Temporal Variation of Whirling Disease Risk in Montana Spring Creeks and Rivers

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Abstract
Spring creeks are important spawning and rearing areas for wild trout, but the stable flows, cool temperatures, and high nutrient levels that characterize these unique habitats may also make them highly susceptible to establishment and proliferation of the whirling disease pathogen *Myxobolus cerebralis*. We evaluated the spatial and temporal dynamics in whirling disease risk by using sentinel rainbow trout *Oncorhynchus mykiss* fry in nine different spring creeks and their conjoining rivers or reservoirs in Montana over a 20-month period. Whirling disease risk was high in five of the seven pathogen-positive spring creek study sites; at these sites, prevalence levels exceeded 90% and over 50% of sentinel fry had moderate to high infection severity scores. Spring creeks generally had higher disease prevalence and severity than paired river or reservoir sites. Fine sediment levels varied widely among springs creeks with high and low whirling disease risk, and we found no significant association between fine sediment level and infection severity. The low risk measured for some spring creeks was likely attributable to the pathogen invasion being in its early stages rather than to environmental characteristics limiting the severity of infection. High whirling disease risk occurred over a wide range of temperatures at spring creek sites (4.5–13°C) and river sites (1.7–12.5°C). There was an unusual seasonal cycle of infection in spring creeks, with peak infection levels occurring from late fall to early spring and declining to near zero in late spring to early fall. The low infection risk during spring suggests that spring-spawning trout would be at a low risk of infection, even in spring creeks with otherwise high disease severity. In contrast, fry of fall-spawning trout may be much more susceptible to infection in spring creek environments.

Groundwater-fed streams, or "spring creeks," are important for wild trout populations as the consistent cool temperatures, high nutrient levels, and stable discharge provide near-optimum conditions for spawning, growth, and rearing. In particular, spring creeks provide critical spawning areas, drawing large numbers of spawners from long distances, thereby contributing to recruitment over a large geographic area (Kiefling 1978; Decker-Hess 1987; Clancy 1988).

The same characteristics that make spring creeks unique and valuable as trout habitat may also make them highly susceptible to establishment and proliferation of *Myxobolus cerebralis*, the nonindigenous myxosporean parasite that causes whirling disease in salmonids. Whirling disease has spread rapidly throughout the USA over the past 20 years (Bartholomew and Reno 2002) and has been associated with significant declines in some highly valued fisheries for wild rainbow trout *Oncorhynchus mykiss* in the western USA (Vincent 1996; Nehring 2006; McMahon et al. 2010). *Myxobolus cerebralis* has a complex, two-host life cycle involving salmonids and the aquatic oligochaete *Tubifex tubifex* (Wolf and Markiw 1984; Hedrick

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et al. 1998; Kerans and Zale 2002). Trout that are younger than 9 weeks of age are the most susceptible to disease from infection by the free-floating triactinomyxon stage (Ryce et al. 2004) that is released from the oligochaete host (Wolf and Markiw 1984). *Myxobolus cerebralis* attacks cartilage in the cranial and skeletal regions of trout and, at high infection rates, produces the eponymous "whirling" behavior, cranial and skeletal deformities, blackened tails, and high mortality (Hedrick et al. 1998; Baldwin et al. 2000; MacConnell and Vincent 2002; Ryce et al. 2004), ultimately leading to sharp declines in survival and recruitment of age-0 fish (Vincent 1996; Nehring 2006; McMahon et al. 2010). Disease severity in young trout depends on pathogen abundance (Markiw 1992; Vincent 2002; Ryce et al. 2004) and species-specific differences in susceptibility (Hedrick et al. 1999; Thompson et al. 1999; Vincent 2002). Infection also requires spatiotemporal overlap between triactinomyxon release and the narrow window of high infection sensitivity in young trout (Downing et al. 2002; MacConnell and Vincent 2002; Pierce et al. 2009). Factors that have been associated with a high risk of whirling disease infection include many common features of spring creek environments (Burckhardt and Hubert 2005), such as an abundance of fine sediments, which are favored as habitat by the *T. tubifex* host (Hiner and Moffitt 2001; Sandell et al. 2001; Burckhardt and Hubert 2005; Krueger et al. 2006); water temperatures of 10–15°C (El-Matbouli et al. 1999; Baldwin et al. 2000); low flows (MacConnell and Vincent 2002; Hallett and Bartholomew 2008); low gradient (Anlauf and Moffitt 2008); anthropogenic disturbances that increase sedimentation and organic inputs, thereby increasing *T. tubifex* density (Zendt and Bergersen 2000; Kaeser et al. 2006; Granath et al. 2007); and the presence of disease-resistant brown trout *Salmo trutta*, which serve as an infection reservoir for sustained parasite production (Nehring 2006).

Whirling disease was first confirmed in Montana in 1994 after sharp declines in Madison River rainbow trout (Vincent 1996). The discovery prompted initiation of a statewide program to monitor parasite distribution and infection risk by using caged sentinel fish (Baldwin et al. 1998). *Myxobolus cerebralis* is now widespread in many western Montana drainages (Vincent 2000; McGinnis 2007); however, little is known about the role spring creeks have in the spread of whirling disease. Over 100 spring creeks occur in western Montana, where they typically originate in the agricultural lands of river valley bottoms; many spring creeks are important spawning and rearing areas for wild trout (Decker-Hess 1987). Few spring creeks in Montana have been tested for *M. cerebralis* presence, but the potential for spring creeks to be foci of whirling disease infection appears to be high. In a survey of *M. cerebralis* in trout of the Salt River drainage, Wyoming, spring creeks had the highest incidence of infection among all stream types examined (Isaak and Hubert 1999).

The purpose of our study was to investigate the occurrence of *M. cerebralis* in Montana's spring creeks and to determine factors that may affect infection severity. We also measured seasonal dynamics of whirling disease risk in relation to timing of the vulnerable early fry rearing period because wide temporal variation in infectivity has been observed, even in rivers with very high infection risk (Sandell et al. 2001; Downing et al. 2002; MacConnell and Vincent 2002). We hypothesized that whirling disease infection risk would be of higher magnitude and of longer duration in spring creeks than in adjoining river systems due to habitat and thermal characteristics that support high pathogen production.

METHODS

Study sites.—Whirling disease risk was estimated for nine spring creeks within eight different drainages; each spring creek was paired with its confluent river or reservoir (Figure 1). Sites were chosen to represent a diversity of spring creeks across southwestern Montana. Site selection criteria included (1) previous detection of the pathogen in the spring creek or nearby river and (2) known importance of the spring creek as a spawning tributary. Sampling was conducted over a 20-month period from January 2000 to August 2001. Spring creeks ranged from 0.5 to 9.7 km in length, with discharge ranging from 0.1 to 1.4 m^3/s (Table 1). At all sites, sentinel rainbow trout were exposed

TABLE 1. List and characteristics of spring creek sites and their conjoining river or reservoir sites in Montana. Discharge data are from Decker-Hess (1989).

Spring creek	River or reservoir	Length (km)	Mean width (m)	Discharge (m^3/s)	Surface fines (%)
Anceny Spring Creek	Gallatin River	0.5	8.8	–	39.3
Ben Hart Spring Creek	East Gallatin River	4.8	10.5	0.7–1.0	48.5
Blaine Spring Creek	Madison River	8.3	11.1	0.9–1.0	40.3
Clark Canyon Spring Creek	Clark Canyon Reservoir	0.8	4.5	0.1–0.2	42.0
Kleinschmidt Creek	Blackfoot River	4.8	4.1	0.4–0.5	30.0
Mitchell Slough	Bitterroot River	9.7	14.6	0.9	33.0
Nelson Spring Creek	Yellowstone River	3.2	19.6	1.1–1.4	52.0
Rock Creek	Blackfoot River	4.8	5.2	0.6	13.3
Willow Springs Creek	Jefferson River	1.6	8.5	0.4	57.0

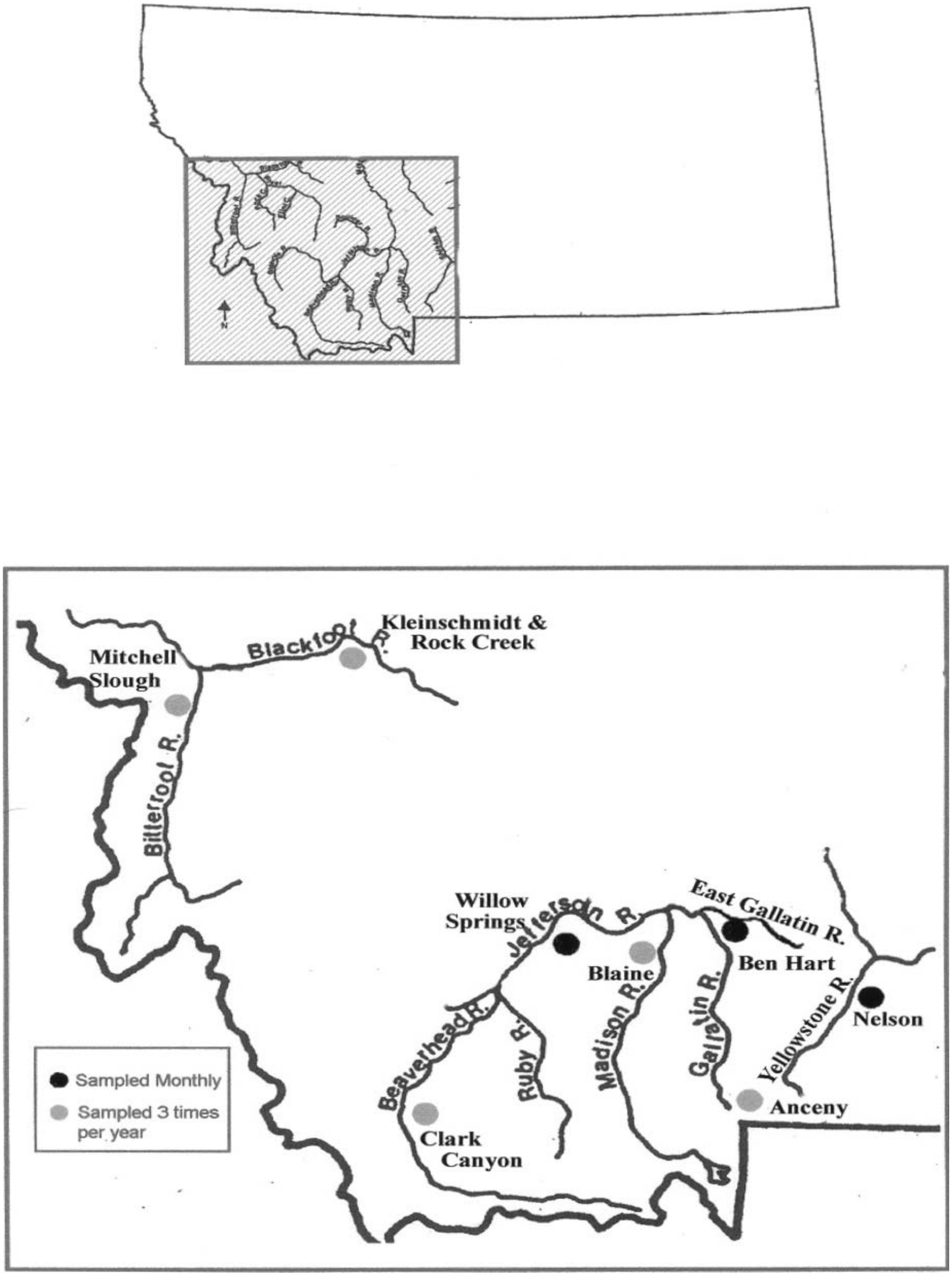

FIGURE 1. Locations of spring creek study sites and associated river drainages in southwestern Montana, USA.

twice in the spring (April and May) of 2000 and 2001 and once in the fall (October) of 2000 to assess the spatial distribution of whirling disease risk over a wide geographic area. Spring and fall sampling periods were chosen because they coincided with the periods of peak infectivity identified in previous studies of seasonal infection cycles in Montana and Wyoming streams (Downing et al. 2002; Murcia et al. 2006). Three spring creeks (Ben Hart Spring Creek, Nelson Spring Creek, and Willow Springs Creek) and their conjoining rivers (East Gallatin, Yellowstone, and Jefferson rivers, respectively) were sampled monthly to assess the seasonal dynamics of whirling disease risk.

Whirling disease risk.—Sentinel fish exposures were used to measure relative abundance of the infective, free-floating *M. cerebralis* triactinomyxon parasite and the resultant effect on whirling disease severity in salmonids. Use of uninfected rainbow trout fry as a biological filter to determine prevalence (percent infected) and severity of infection has been a standard test for measuring whirling disease risk (Baldwin et al. 2000; Sandell et al. 2001; Downing et al. 2002; Krueger et al. 2006). Sentinel fish were held in cages consisting of a wire-mesh cylinder (0.5-m diameter; 0.6 m deep). Sixty juvenile rainbow trout, which were obtained from certified whirling-disease-free hatcheries, were measured (mm TL) and placed into each cage. In total, 167 sentinel cage exposures were conducted during the study. Six strains of rainbow trout (Arlee, Eagle Lake, Madison, Erwin, Shasta, and Fish Lake) were used for sentinel exposures; each of these strains has high to very high susceptibility to whirling disease (Vincent 2002; Wagner et al. 2006). Sentinel fish were similar in strain, age, and size within each sampling period, but these factors differed among sampling periods due to limitations in year-round availability of a particular strain during the study. Sentinel fish ranged from 29 to 53 mm TL and from 24 to 119 d posthatch. Ryce et al. (2004, 2005) showed that sentinel rainbow trout older than 63 d and larger than 40 mm had a diminished infection severity and fewer clinical signs of disease than younger, smaller individuals when exposed to similar numbers of *M. cerebralis* in the laboratory. To evaluate the possible confounding effect of sentinel fish size and age differences on infection severity, we examined infection severity (percentage of sentinel fish with histology scores ≥ 3; see below) in relation to the size and age of fish from the two spring creek study sites with the highest infection severity and longest time series of sentinel cage data (Willow Springs Creek and Ben Hart Spring Creek). We hypothesized that there would be a negative association between disease severity and size or age if either of these factors had a significant effect on infection severity among test fish.

Sentinel fish were held in cages for 10 d and then were transferred to laboratory aquaria at either the Whirling Disease Laboratory in Pony, Montana (Vincent 2002), or the Wild Trout Research Laboratory at Montana State University, Bozeman (Ryce et al. 2004); the choice of laboratory site depended on tank availability. Fish were reared in the tanks for 80–89 d at 10–13°C to allow for full development of *M. cerebralis* spores (Baldwin et al. 1998). Test fish were fed a standard commercial trout diet twice daily to satiation. At the end of the rearing period, the rainbow trout were sacrificed with an overdose of tricaine methanesulfonate (MS-222). Heads were removed and fixed in a 10% solution of buffered formalin for 72 h prior to transfer into individually marked, sterile plastic bags containing 70% alcohol. At the Washington Animal Disease Diagnostic Laboratory (Pullman, Washington), the heads of the fish were sectioned, and infection severity was scored by using the MacConnell–Baldwin histology rating system (Baldwin et al. 2000; Vincent 2002) based on a lesion severity scale of 0 (no infection) to 5 (severe infection). For the first 6 months of the study (January–June 2000; 80 of the total 294 exposure groups), 50 fish from each 60-fish exposure group were randomly selected and scored histologically. For the final 14 months of the study, the sample size was decreased to 30 fish/exposure group in order to accelerate the time-consuming and costly scoring process. To assess the effect of the reduced sample size, 32 exposure groups were randomly selected and mean disease severity scores were compared between sample sizes of 30 and 50 randomly selected heads. The mean difference in histology scores between sample sizes was low (0.04), and we did not detect significant differences between the paired sample scores (Wilcoxon's signed rank test: $P = 0.32$); therefore, subsequent analyses were performed on the reduced data set for the remainder of the study.

The study design involved deploying sentinel cages in each spring creek and the conjoining river or reservoir into which it flowed. Generally, at least two cages were placed in each spring creek (the exception was that Willow Springs Creek had only one cage from January to June 2000) along protected stream margins, the typical habitat of age-0 trout fry (Downing et al. 2002). At least one cage was simultaneously placed in each conjoining river or reservoir upstream of the spring creek's mouth to avoid the influence of spring creek water. The number of months sampled was not equal for all sites due to cage loss from high or variable flows during cage deployment in rivers. Generally, only one sentinel cage was placed within a particular location due to the significant time and cost associated with deploying replicate cages in close proximity. To assess the degree of precision in estimation of disease severity with only one sentinel cage at a site, we compared mean histology scores from replicate sentinel cages that were placed in close proximity (1–7 m apart) at four different study sites during 20 sampling periods. Mean histology scores differed by 0.40 or less between replicate cages, and we did not detect significant differences in disease severity among paired exposure groups (Wilcoxon paired-sample test: $P = 0.29$).

Spawning and emergence timing.—Redd counts were conducted within the three intensively sampled spring creeks one to two times per month from February 2000 to September 2001 to compare the timing of spawning and fry emergence in relation to whirling disease risk. For each spring creek, counts were conducted in sections with a high percentage of spawning gravels by walking along the banks while wearing polarized glasses. Redds were identified based on a characteristic lighter color and the presence of an upstream depression or pit with several larger rocks in its center and a downstream tailspill of disturbed smaller gravel (Thurow and King 1994). Each new redd was identified with a colored rock to avoid counting a redd more than once. Rainbow trout and brown trout redds were distinguished by identification of individual fish near a redd and by spawn timing (fall and early winter: brown trout; late winter and spring: rainbow trout).

Timing of fry emergence from redds was estimated by using the estimated date of redd construction in combination with published values for the accumulated number of thermal units (degree-days, °C) required for emergence: 589 thermal units for rainbow trout emergence (Downing 2000) and 727 thermal units for brown trout emergence (Crisp 1988). The number of degree-days was calculated by summing mean daily water temperatures (°C) obtained from thermographs. The "disease susceptiblity window" (Downing et al. 2002; MacConnell and Vincent 2002) was determined as the 9-week interval after the estimated peak in fry emergence, coinciding with the period of highest susceptibility of young trout to *M. cerebralis* infection (Ryce et al. 2004).

Temperature.—Water temperature was measured at 30-min intervals during each sentinel exposure period by using electronic thermographs. Thermographs were also used to record hourly temperature in the three intensively studied spring creeks (Ben Hart Spring Creek, Nelson Spring Creek, and Willow Springs Creek).

Habitat characteristics.—The length of each habitat type (pool, riffle, and glide) was measured along the entire length of each spring creek by following the procedures of Overton et al. (1997). Channel width was measured at transects (n = 6–10) positioned at systematic intervals of 50–500 m, depending on the overall length of the spring creek. Percent aquatic vegetation was visually estimated within a 10-m band upstream and downstream of each transect. Percent surface fines (<2 mm), the preferred habitat of *T. tubifex* (Krueger et al. 2006), was measured at each transect by using a Wolman pebble count together with a surface fines grid (Overton et al. 1997). Pebble counts were performed by walking heel to toe across the stream transect until 100 samples were obtained. A surface fines grid was randomly tossed once upstream and once downstream at each transect. Mean surface fines calculated from each method were highly correlated (Pearson's product-moment correlation coefficient $r = 0.86$, $P = 0.003$), so only the Wolman surface fines results are reported herein.

Statistical analysis.—Metrics that were used to summarize disease severity for each exposure group included (1) prevalence, or the total percentage of sentinel fish showing signs of infection; (2) mean histology score; and (3) the percentage of fish with histology scores of 3 or greater. The percentage of histology scores greater than or equal to 3 was considered a measure of high disease severity potential because fish with these infection severity scores exhibit significant cartilage damage, numerous lesions and granulomas, a higher incidence of clinical signs of disease, and reduced performance and survival (Baldwin et al. 1998; Ryce et al. 2004, 2005). Declines in wild rainbow trout populations have been observed when 50% or more of the sentinel fish demonstrate infection severity scores of 3 or higher (Vincent 2000; McMahon et al. 2010).

Disease severity differences in paired spring creek and river or reservoir sites were analyzed by comparing infection prevalence, mean histology scores, and the proportion of fish with high disease severity by using the Wilcoxon paired-sample test (Daniel 1990). When multiple cages were sampled at a site, we used disease severity data from the cage with the highest lesion score as a measure of infection risk for use in paired comparisons. Simple linear regression was used to evaluate the association between percent surface fines and infection severity. Linear and nonlinear relationships between temperature and disease severity were evaluated using the curve-fitting program in SigmaPlot (SigmaPlot 2008), with the highest r^2 values used to select the best-fitting models. Pearson's product-moment correlation was used to test for a possible negative correlation between disease severity and the size and age of sentinel fish. All tests were performed at an α level of 0.05.

RESULTS

Spatial and Temporal Variation in Whirling Disease Risk

Infection severity in sentinel fish varied widely among the 17 study sites (Table 2). *Myxobolus cerebralis* was not detected at three of the study sites (Anceny Spring Creek, Clark Canyon Spring Creek, and the Bitterroot River) and was detected at very low levels (infection prevalence ≤12%) at four other sites (Mitchell Slough, Nelson Spring Creek, Gallatin River, and Yellowstone River). In contrast, seven study sites had a high whirling disease risk (Rock Creek, Ben Hart Spring Creek, Blaine Spring Creek, Kleinschmidt Creek, Willow Springs Creek, Madison River, and East Gallatin River), as evidenced by 50% or more of the sentinel fish having histology severity scores of 3 or higher (i.e., indicating moderate to severe infection risk). Sentinel fish that were exposed in the Blackfoot River, Jefferson River, and Clark Canyon Reservoir exhibited intermediate levels of infection risk.

Disease prevalence and severity were generally higher in spring creeks than in conjoining rivers or reservoirs (Table 2). Three spring creeks had significantly higher mean histology scores than their paired river sites, and four spring creeks had a significantly greater proportion of high disease severity than their paired river sites. Disease severity in three spring creeks (Kleinschmidt Creek, Rock Creek, and Willow Springs Creek) reached very high levels (77–97% of sentinel fish had histology scores ≥3), whereas only one river site (Madison River) had a disease severity of this magnitude.

Monthly exposure sampling of several study sites revealed a distinct seasonal pattern in infectivity. In both Willow Springs Creek and Ben Hart Spring Creek, infection severity was highest

TABLE 2. Paired comparisons of sentinel rainbow trout infection ratings for spring creeks and their conjoining river or reservoir sites during April 2000–August 2001. For a given pair of sites, asterisks indicate the significantly higher values of percent infected, mean histology score, or mean percentage of sentinel fish with histology scores of 3 or greater (Wilcoxon paired-sample test: $P \leq 0.05$).

Spring creek and paired river or reservoir	Mean (range) percentage infected	Mean (range) histology score	Mean (range) percentage with histology score ≥ 3	Number of months sampled (total number of cage exposures)
Anceny Spring Creek	0	0	0	5 (10)
Gallatin River	0.6 (0–3)	0.02 (0–0.1)	0.6 (0–3)	5 (8)
Ben Hart Spring Creek	62.2 (6–100)	1.5 (0.1–2.8)	25.9 (0–54)*	20 (62)
East Gallatin River	52.9 (0–96)	1.23 (0–2.8)	19.2 (0–60)	18 (29)
Blaine Spring Creek	51.0 (4–93)	1.2 (0–2.6)	19.1 (0–50)	5 (9)
Madison River	87.6 (83–100)	2.6 (1.8–3.8)*	53.1 (28–93)*	5 (8)
Clark Canyon Spring Creek	0	0	0	5 (10)
Clark Canyon Reservoir	53.3 (26–68)*	0.9 (0.4–1.6)*	11.6 (7–25)*	3 (5)
Kleinschmidt Creek	89.5 (76–100)*	3.4 (2.3–4.0)*	74.1 (53–86)*	5 (9)
Rock Creek	81.0 (76–90)*	2.7 (2.0–3.9)*	53.6 (38–77)*	5 (8)
Blackfoot River	31.0 (2–76)	0.8 (0–2.3)	10.8 (0–43)	4 (6)
Mitchell Slough	0.3 (0–2)	0.04 (0–0.02)	0	5 (8)
Bitterroot River	0	0	0	3 (4)
Nelson Spring Creek	1.5 (0–6)	0.02 (0–0.1)	0	19 (38)
Yellowstone River	2.0 (0–12)	0.03 (0–0.2)	0	12 (19)
Willow Springs Creek	64.7 (0–100)*	2.2 (0–4.1)*	42.2 (0–97)*	20 (35)
Jefferson River	9.1 (0–45)	0.1 (0–0.9)	1.6 (0–10)	19 (25)

from November to May, declined to very low or undetectable levels during June–September, and increased sharply again in October (Figure 2); this pattern occurred during both years of the study. In conjoined river sites (Jefferson and East Gallatin rivers), peak infectivity occurred during October–November and May–June, whereas infectivity was very low during other months of the year. The East Gallatin River showed substantial year-to-year variation in infectivity, with low severity during the first year of the study and high severity during the second year; in the second year, infectivity followed a seasonal pattern similar to that observed in the spring creeks, with high infectivity during November–June and very low infectivity during July–September.

Relationship to Spawning and Emergence Timing

Overall, 314 redds were observed in Willow Springs Creek, 163 were observed in Ben Hart Spring Creek, and 327 were observed in Nelson Spring Creek during the 20-month survey period. Spawning generally occurred over extended periods at all sites (Figure 3). Rainbow trout spawning periods ranged from as early as January to as late as July at some sites, with peak spawning occurring from about early March to early May. Brown trout spawning began as early as September, peaked in mid-November, and extended through January. Infection risk was low during the postemergence disease susceptibility window for spring-spawning rainbow trout (1 June–15 August) and was very high during the susceptibility window for fall-spawning brown trout (1 March–15 May; Figure 3).

Relationship between Infection Risk and Habitat Features

Temperature.—Temperature ranged from about 7°C to 12°C in spring creeks and from 1°C to 19°C in river sites during exposure sampling (Figure 2). Infection risk varied widely with temperature at spring creek sites and river sites (Figure 4). Among spring creeks that tested positive for *M. cerebralis*, there was a significant curvilinear relationship between temperature and infection risk, with peak infection severity occurring at 6–8°C. However, high infection risk occurred at temperatures as low as 4.5°C and as high as 13°C. There was a significant negative linear relationship between infection severity and temperature at Willow Springs Creek, the spring creek with the highest infection severity, with severity peaking at about 7°C and declining sharply to low levels at temperatures above 10°C. For river sites, there was no significant relationship ($P > 0.22$) between temperature and infection severity. Infection was detected at a wide range of temperatures (0.1–17°C), with high severity occurring mostly in the temperature range of 7.5–12°C but also at temperatures as low as 1.7°C (Figure 4).

Habitat characteristics.–Spring creek study sites comprised a high percentage of glide habitat (mean = 71%), fine sediment (mean = 39%), and abundant rooted aquatic vegetation (mean = 59%). There was no significant association between disease severity and percent fine sediment for all nine spring creeks combined ($r^2 = 0.02$, $P = 0.73$; Table 1) or for the subset

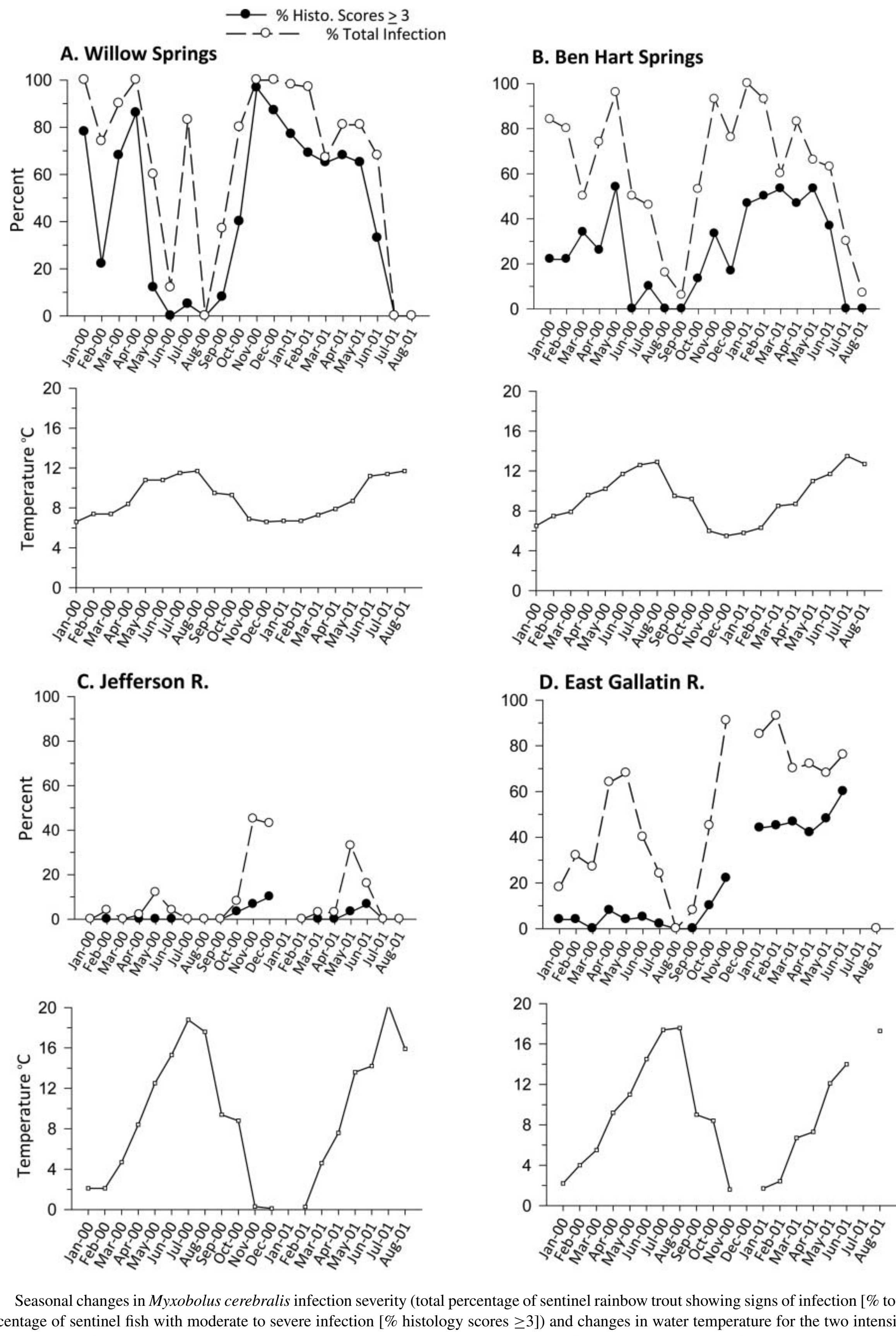

FIGURE 2. Seasonal changes in *Myxobolus cerebralis* infection severity (total percentage of sentinel rainbow trout showing signs of infection [% total infection]; and the percentage of sentinel fish with moderate to severe infection [% histology scores ≥3]) and changes in water temperature for the two intensively sampled spring creeks (Willow Springs Creek and Ben Hart Spring Creek) and their conjoining river sites (Jefferson and East Gallatin rivers, respectively) over the 20-month study period (January 2000–August 2001). Data for Nelson Spring Creek and the Yellowstone River are not shown because infection was very low (total infection <1.2%).

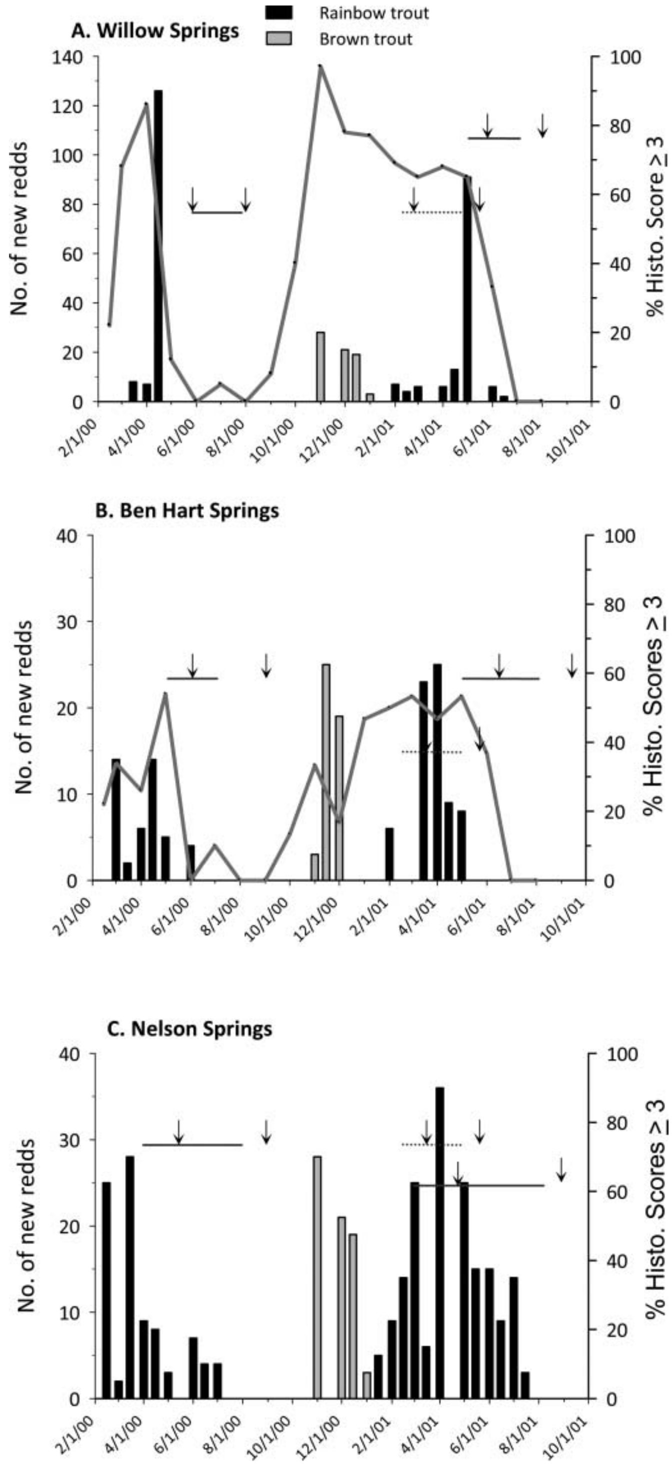

FIGURE 3. Numbers of newly observed spawning redds of rainbow trout (black bars) or brown trout (gray bars) during each survey period in Ben Hart Spring Creek, Nelson Spring Creek, and Willow Springs Creek, 2000 and 2001. Solid line refers to the percentage of sentinel rainbow trout with moderate to severe infection (histological [histo] scores ≥3; note that moderate to severe infections were absent among sentinel fish in Nelson Spring Creek). Horizontal lines indicate periods of peak fry emergence from redds (solid lines = rainbow trout; dashed lines = brown trout); vertical arrows frame the disease susceptibility window, the 9-weeks-posthatch period when young trout are most susceptible to *Myxobolus cerebralis* infection and disease.

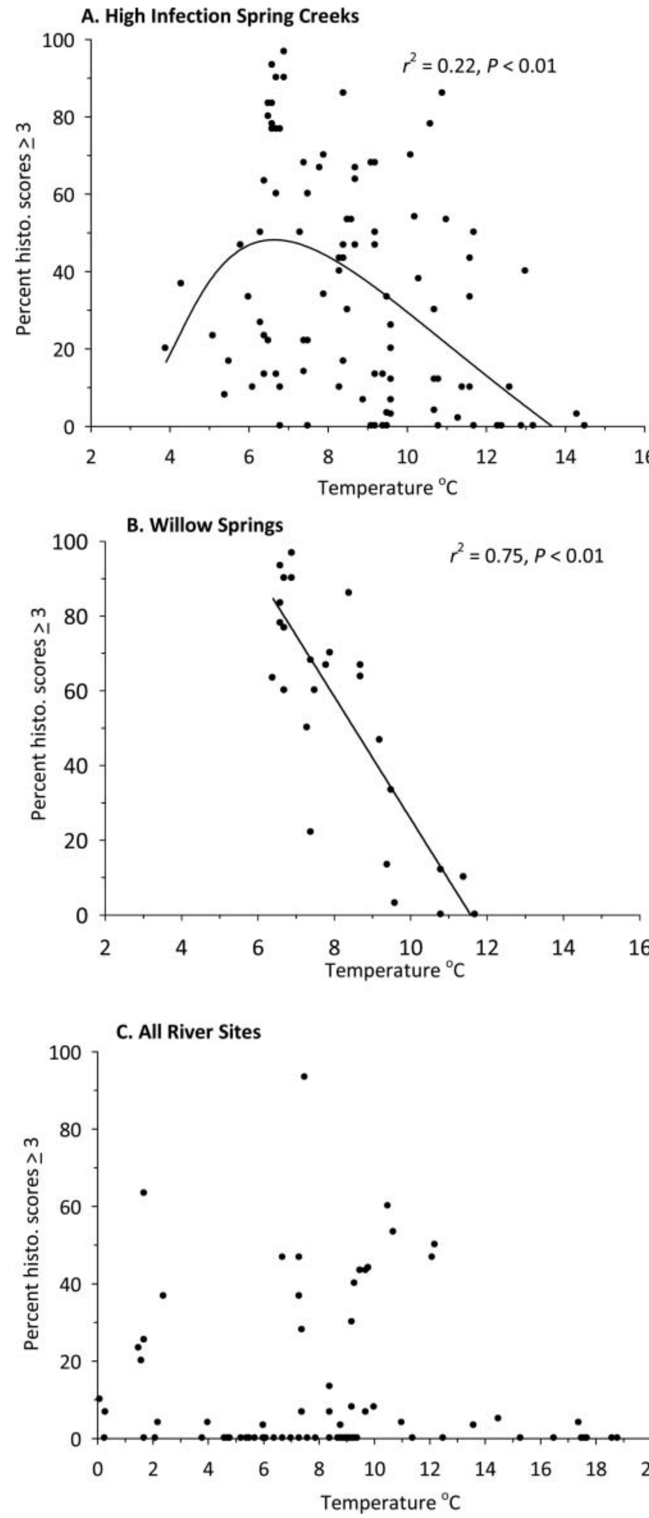

FIGURE 4. Percentage of sentinel rainbow trout with moderate to severe *Myxobolus cerebralis* infection (histology [histo] scores ≥3) in relation to average water temperature during the exposure period for **(A)** the five high-infection spring creeks (Ben Hart Spring Creek, Blaine Spring Creek, Kleinschmidt Creek, Rock Creek, and Willow Springs Creek; $n = 103$ exposure groups); **(B)** Willow Springs Creek, the site with the highest infection severity ($n = 26$ exposure groups); and **(C)** the river or reservoir exposure sites ($n = 90$ exposure groups). Lines represent the best fit of the data (highest r^2 value); no curve had a significant r^2 value for panel C (all $P > 0.19$).

of five spring creeks with high infection risk (Ben Hart Spring Creek, Blaine Spring Creek, Kleinschmidt Creek, Rock Creek, and Willow Springs Creek; $r^2 = 0.00$, $P = 0.98$; Table 2). Percent surface fines ranged from 13% to 57% among the five high-risk sites and from 33% to 52% among the four low-risk sites.

Size and Age Effects

Larger size or greater age of sentinel rainbow trout was not associated with a significant decline in infection severity (Figure 5). The percentage of sentinel rainbow trout with moderate to severe infection was equal between the largest (53 mm) or oldest (108 d posthatch) sentinel fish (>85% with histology scores ≥3) and smaller or younger sentinal fish, indicating that the size and age differences among sentinel fish did not have a strong influence on the observed patterns of infection severity.

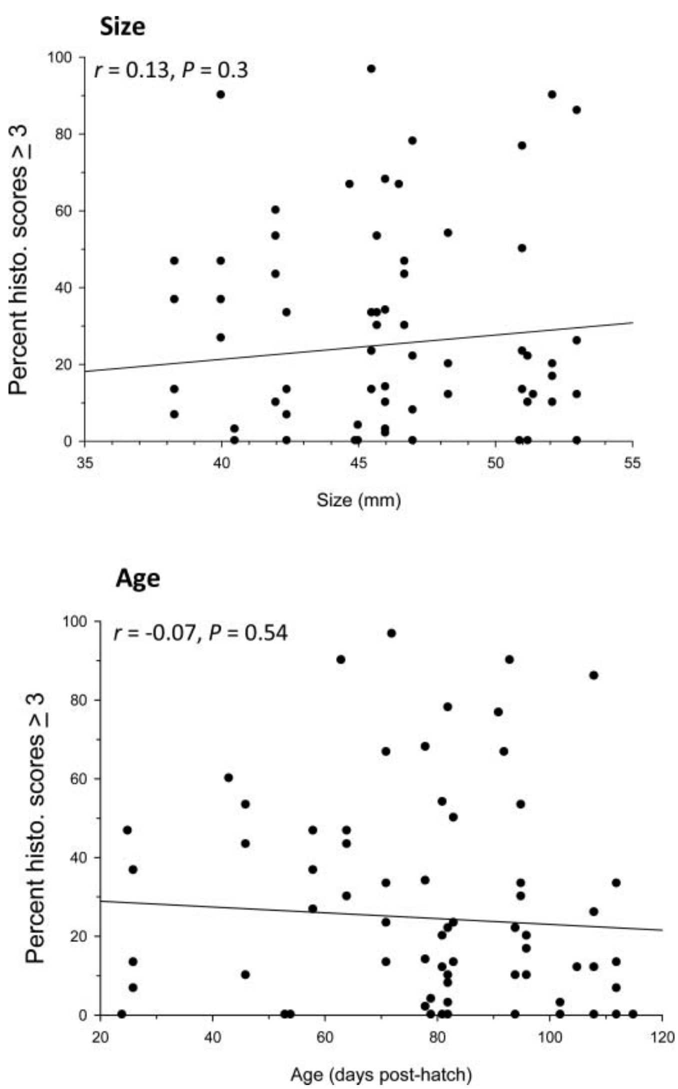

FIGURE 5. Results of Pearson's product-moment correlation comparisons between *Myxobolus cerebralis* infection severity (percentage of sentinel fish with moderate to severe infection [histology scores ≥3]) and the **(A)** size (TL, mm) or **(B)** age (d posthatch) of sentinel rainbow trout from the two most infected and intensively sampled spring creek sites (Willow Springs Creek and Ben Hart Spring Creek).

DISCUSSION

Examination of whirling disease risk by using sentinel rainbow trout fry confirmed that *M. cerebralis* was common across a subset of Montana spring creeks but indicated that infection severity varied substantially among sites. Overall, *M. cerebralis* was detected in seven of nine spring creek study sites, and five of the seven pathogen-positive spring creeks exhibited infection severity levels that have been correlated with significant declines in recruitment of age-0 rainbow trout (i.e., >50% of sentinel fry with moderate to high infection severity scores; Vincent 2000; McMahon et al. 2010). Spring creeks generally had higher disease prevalence and severity than their paired river or reservoir sites. Seven of the eight river or reservoir sites tested positive for the pathogen, but only two sites had infection risk levels that approached the peak infectivity observed during disease exposures in the spring creeks.

High spatial variation in *M. cerebralis* prevalence and abundance, both among and within drainages, has been noted for many other systems (e.g., Baldwin et al. 1998; Hiner and Moffitt 2001; Sandell et al. 2001; Downing et al. 2002; Thompson et al. 2002; Granath et al. 2007; Pierce et al. 2009). Such variation has been hypothesized to be a function of several factors, including how long the parasite has been in a drainage and the degree of spore production by infected fish (Kerans and Zale 2002; Krueger et al. 2006); the amount of fine sediment habitat available for *T. tubifex* (Zendt and Bergersen 2000; Hiner and Moffitt 2001; Krueger et al. 2006); and differences in the strain of *T. tubifex*, as the strains vary markedly in their production of infective triactinomyxons (Kerans et al. 2004; Beauchamp et al. 2005; Lodh et al. 2011). We did not find a strong association between infection severity and fine sediment level. We suspect that the very low infectivity levels in Anceny Spring Creek, Clark Canyon Spring Creek, Mitchell Slough, and Nelson Spring Creek were attributable to *M. cerebralis* having not yet invaded those systems or the invasion being in its early stages; we do not believe that environmental characteristics were responsible for preventing the spread of *M. cerebralis* in those creeks. At all four sites, fine sediment levels were high and similar to those at the five highly infected spring creeks. Moreover, Anderson (2004) found that *T. tubifex* densities in Nelson Spring Creek, our very-low-infection study site, were two to four times higher than those in highly infected Ben Hart Spring Creek and Willow Springs Creek, which indicates that *T. tubifex* density was not limiting the development of high infectivity in our low-severity spring creeks (see also Alexander et al. 2011). We know of no systematic evaluation of *T. tubifex* lineages within spring creeks, but the *T. tubifex* lineages that are known to be highly susceptible to *M. cerebralis* infection (lineages I and III) are common in shallow depositional areas with abundant fine sediments (Dubey and Caldwell 2004; Beauchamp et al. 2005;

Lodh et al. 2011), and such habitat conditions are common in spring creeks.

Study results did not support our hypothesis that fish in spring creeks are exposed to *M. cerebralis* infection for a longer portion of the year than fish in rivers. In both environments, the duration of infectivity was about 9 months on an annual basis. However, the timing of disease risk in spring creeks was generally different from that observed in rivers. In both of the highly infected spring creeks that were sampled monthly (Willow Springs Creek and Ben Hart Spring Creek), whirling disease risk was highest in the late fall through early spring. For example, in Willow Springs Creek, very high disease risk (>80% of sentinel fish with moderate to severe lesions) was observed during November–February, but the risk then dropped to zero during July and August. The timing of infectivity was surprising, as most previous studies of rivers have shown that whirling disease risk is very low to zero during winter and early spring (Thompson and Nehring 2000) and that peak infection risk occurs during relatively brief time windows in late spring and early fall (Downing et al. 2002; Murcia et al. 2006) or during summer (Thompson and Nehring 2000; Sandell et al. 2001). Seasonal infection risk in our river sites also generally followed this latter pattern, but we did observe an unexpectedly high infection risk in the East Gallatin River during winter and spring months (January–June).

High seasonal variation in whirling disease infection risk has been largely attributed to seasonal temperature changes. Temperature affects development rate and spore production of *M. cerebralis* in both hosts (El-Matbouli et al. 1999; Blazer et al. 2003; Kerans et al. 2005). El-Matbouli et al. (1999) found that peak triactinomyxon release from *T. tubifex* under controlled laboratory conditions occurred at 10–15°C. Evaluation of sentinel fish to determine infection risk in rivers has also generally shown that infectivity peaks during the spring and fall within this same temperature range (Baldwin et al. 2000; Downing et al. 2002; MacConnell and Vincent 2002). However, in the present study, high infection risk occurred over a much broader range of temperatures in both spring creek sites and river or reservoir sites. High infection risk in spring creeks (>40% of sentinel fish with moderate to severe lesion scores) occurred at temperatures as low as 4.5°C and as high as 13°C, and peak infection occurred at 6–8°C. In river study sites, we observed high infection risk over a wide range of temperatures (1.7–12.5°C). Moreover, we detected moderate levels of infection (43% of sentinel fish infected, with 10% showing moderate to severe infection) even at very cold temperatures (0.1°C in the Jefferson River, December 2000). Although winter sampling for infection risk has been limited, observations from other studies concur with our findings of the potential for high infectivity during winter and early spring at low temperatures (Munson and Johnson 2001; Nehring et al. 2003; Lukins 2004).

We hypothesize that the unusual timing of peak winter infection risk we observed in spring creeks and the more common spring pulse of infection risk in rivers were a result of different thermal accumulation rates. Triactinomyxons are produced by *T. tubifex* approximately 1,350 degree-days after ingestion of myxospores (range = 1,300–1,456 degree-days; Markiw 1986; Kerans et al. 2005). Interestingly, the estimate of 1,350 degree-days equates to a back-calculated myxospore input timing of mid- to late August in the spring creek and river study sites, corresponding to the period in which heavily infected age-0 rainbow trout would likely begin dying and releasing myxospores (Kerans and Zale 2002). For spring creeks, warmer winter temperatures accelerate spore development within the *T. tubifex* host, resulting in a midwinter peak in triactinomyxon release, whereas in non-spring-fed rivers the depressed winter temperatures would prolong spore development and delay triactinomyxon release until the rapid rise in spring temperature. Wide variation in infectivity responses among different systems therefore suggests that the timing of infection risk is a site-specific response based on differences in thermal accumulation and myxospore input (Kerans and Zale 2002; Kerans et al. 2005) and differences in discharge (Hallett and Bartholomew 2008).

The lack of overlap between high infection risk and the disease susceptibility window for rainbow trout fry suggests that spring-spawning trout would be at a low risk of infection, even in spring creeks with high infection risk during other time periods. During the period of highest infection susceptibility for rainbow trout fry in Ben Hart Spring Creek and Willow Springs Creek (1 June–15 August), the incidence of moderate to severe lesions in sentinel fish was 0–10% compared with 40–97% during winter to early spring months. This contrasts markedly with other studies of surface-water-fed streams, which demonstrated a strong temporal overlap between high infectivity and high vulnerability for rainbow trout and other spring-spawning trout (Downing et al. 2002; MacConnell and Vincent 2002; Pierce et al. 2009). In contrast, our results showed that in spring creeks, fall-spawning brown trout had a much higher risk of whirling disease infection than did spring-spawning rainbow trout. Although brown trout are highly resistant to whirling disease, they can still become infected and produce myxospores (Hedrick et al. 1999; Baldwin et al. 2000), thus serving as a source of spores for infection of more susceptible species. Two fall-spawning species that may be susceptible to whirling disease infection in spring creeks are the brook trout *Salvelinus fontinalis* and kokanee *O. nerka*. Both species use spring-fed sites for spawning (Curry et al. 1995; Garrett et al. 1998), and both are also susceptible to whirling disease (Vincent 2002). Brook trout numbers have declined markedly in recent years in Kleinschmidt Creek (Ron Pierce, Montana Fish, Wildlife, and Parks, personal communication), a spring creek that produced one of the highest infectivity levels among sentinel rainbow trout in our study (see also Kaeser et al. 2006).

Our findings have several important implications for the monitoring and management of whirling disease. The discovery of high infectivity over a wide range of months and temperatures indicates that effective monitoring of infection risk will require careful tailoring of infection severity measurement in relation to the timing of disease susceptibility windows on a site-by-site basis. From a risk assessment perspective (Bartholomew et al. 2005), the very high infectivity we observed at low water temperatures does not support the inference that colder water associated with winter, higher elevations, or higher latitudes imparts protection from severe impacts of whirling disease (Schisler and Bergersen 2002; Arsan et al. 2007).

The high spawning and rearing usage of spring creeks, in combination with their high susceptibility for severe whirling disease infection, indicates that these sites are important "hot spots" for the proliferation and intensification of the disease. We only sampled a small subset of the numerous spring creeks in Montana, and more extensive evaluation of infection risk is warranted. Detailed examination of *T. tubifex* lineages in spring creeks would be a useful component of such infection risk assessments. Anthropogenic disturbances that increase sedimentation and organic enrichment are common in spring creeks (Burckhardt and Hubert 2005; our personal observations) and would likely serve to intensify whirling disease impacts (Zendt and Bergersen 2000; McGinnis 2007). Research is needed to test the idea that reducing sedimentation by use of habitat restoration practices can decrease *T. tubifex* habitat and reduce whirling disease infectivity in these key trout spawning and rearing areas (e.g., Thompson 2011).

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