Occurrence and seasonal dynamics of the whirling disease parasite, Myxobolus cerebralis, in Montana spring creeks
by Ryen Aasheim Anderson

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Fish and Wildlife Management
Montana State University

Abstract:
Spring creeks provide a unique ecosystem for wild trout populations, characterized by high nutrient levels, chemical stability, steady flow regimes, and near optimum temperatures for spawning and rearing. However, several of these factors may accentuate whirling disease in spring creeks by providing favorable Tubifex tubifex habitat, the alternate host for the whirling disease parasite, and promoting the release of the infectious triactinomyxin (TAM) stage over prolonged periods. This study evaluated the prevalence and severity of whirling disease in nine different spring creeks and adjoining rivers or reservoirs in southwestern Montana. I investigated the influence of temperature, T tubifex abundance, water quality, and habitat characteristics on infection severity. Infection severity was measured with sentinel fish exposures in the spring and fall at all sites to evaluate the extent of infection. Seasonal dynamics of infection severity was assessed through monthly sampling in three spring creeks over a 20-month period. I found that infection severity was common (7 of 9 sites) but that severity among infected sites varied widely. Only five of nine sites had high infection levels (> 50% of sentinel fish exhibiting moderate to severe lesion scores ≥ grade 3) where population declines would be expected. The seasonal cycle of infection in spring creeks was much different than observed in non-spring fed streams as peak infection occurred during winter months, declined in late spring, and remained at low levels until fall when infection began to rise again. No significant relationships were found between infection severity and habitat features, T. tubifex abundance, and water quality, with the exception of infection severity and phosphorus in sites with moderate to high infection levels. Peak infections in spring creeks occurred at temperatures ranging from 6 to 12 °C. Redd counts and estimated emergence periods, correlated with sentinel fish exposure results indicated that fry emerging and rearing during late spring and summer are likely to avoid high infection, even in highly infected spring creeks. In contrast, given the high infection in spring creeks in late fall and winter, fry of fall spawning trout may be much more susceptible to infection in these systems.

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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ABSTRACT

Spring creeks provide a unique ecosystem for wild trout populations, characterized by high nutrient levels, chemical stability, steady flow regimes, and near optimum temperatures for spawning and rearing. However, several of these factors may accentuate whirling disease in spring creeks by providing favorable *Tubifex tubifex* habitat, the alternate host for the whirling disease parasite, and promoting the release of the infectious triactinomyxin (TAM) stage over prolonged periods. This study evaluated the prevalence and severity of whirling disease in nine different spring creeks and adjoining rivers or reservoirs in southwestern Montana. I investigated the influence of temperature, *T. tubifex* abundance, water quality, and habitat characteristics on infection severity. Infection severity was measured with sentinel fish exposures in the spring and fall at all sites to evaluate the extent of infection. Seasonal dynamics of infection severity was assessed through monthly sampling in three spring creeks over a 20-month period. I found that infection severity was common (7 of 9 sites) but that severity among infected sites varied widely. Only five of nine sites had high infection levels (> 50% of sentinel fish exhibiting moderate to severe lesion scores ≥ grade 3) where population declines would be expected. The seasonal cycle of infection in spring creeks was much different than observed in non-spring fed streams as peak infection occurred during winter months, declined in late spring, and remained at low levels until fall when infection began to rise again. No significant relationships were found between infection severity and habitat features, *T. tubifex* abundance, and water quality, with the exception of infection severity and phosphorus in sites with moderate to high infection levels. Peak infections in spring creeks occurred at temperatures ranging from 6 to 12 °C. Redd counts and estimated emergence periods, correlated with sentinel fish exposure results indicated that fry emerging and rearing during late spring and summer are likely to avoid high infection, even in highly infected spring creeks. In contrast, given the high infection in spring creeks in late fall and winter, fry of fall spawning trout may be much more susceptible to infection in these systems.

INTRODUCTION

Groundwater-fed streams or spring creeks are important to wild trout populations as they provide a unique ecosystem characterized by consistent cool temperatures (Hopkins 1971), high nutrient levels, chemical stability, and stable discharge (Stern and Stern 1969; Hynes 1970; Williams 1991; Hubbs 1995). As a result, trout production is supported throughout the year by an abundance of food and cover and a thermal regime near the optimum for year-round spawning and growth. Thus, total biomass and growth rates of trout inhabiting spring creeks may far exceed more environmentally variable surface water-fed streams (Decker-Hess 1989).

Spring creeks can provide excellent trout spawning habitat offering increased permeability of the gravel substratum, decreased sedimentation due to upwelling, stable flows, and favorable temperatures for developing embryos (Hansen 1975). As a result, trout spawning may occur earlier (Hubert et al. 2002) and over a longer duration in spring creeks than in seasonally variable rivers and streams (Roberts 1988). In addition, the favorable spawning conditions may draw spawners from long distances, and spring creeks may thereby contribute to recruitment over a large geographic area (Clancy 1988; Poff and Ward 1989).

The same physical characteristics that make spring creeks unique and valuable trout habitat may also make them highly susceptible to whirling disease. Whirling disease, caused by the myxosporean parasite *Myxobolus cerebralis*, has been associated with significant declines in some wild rainbow trout populations in the western United

States (Nehring and Walker 1996; Vincent 1996). *Myxobolus cerebralis* has a complex, two-host life cycle involving salmonids and the aquatic oligochaete, *Tubifex tubifex.* Young trout less than 9 weeks of age are the most susceptible to the disease, with the development and severity of the disease dependent on fish age, size, species, and parasite dose at time of exposure (Markiw 1991; Markiw 1992a; Hedrick et al. 1998; Hedrick et al. 1999; Thompson et al. 1999; MacConnell and Vincent 2002; Vincent 2002; Ryce 2003). Clinical signs of whirling disease include "whirling" behavior, blacktail, and skeletal deformities (Hedrick et al. 1998; Rose 2000; MacConnell and Vincent 2002), which may lead to high mortality and recruitment collapse at the population level when disease is severe (Nehring and Walker 1996; Vincent 1996; MacConnell and Vincent 2002).

Whirling disease was first detected in Montana in 1994 in the Madison River and resulted in a severe population decline of rainbow trout (Vincent 1996). Whirling disease has been considered to be the single greatest threat to wild trout populations (MWDTF 1996). Since 1994, Montana Fish, Wildlife and Parks has extensively monitored the progression of the disease and mapped its distribution. Whirling disease has now been confirmed in twelve different drainages and 118 different waters in the state (Jim Peterson, Montana Fish, Wildlife and Parks, personal communication).

Little is known about the role spring creeks have in the spread of whirling disease. Over 100 spring creeks occur in Montana, particularly in southwest Montana (Decker-Hess 1987), where they typically originate in the agricultural lands of river valley bottoms (Wells 1981). Many are important wild trout spawning and rearing areas and support recreationally important wild trout fisheries (MTDFG 1975; MTFWP 1987;

Clancy 1988; Shepard 1992). Few spring creeks in Montana have been tested for whirling disease infection, and though the data are limited, the potential for spring creeks to be reservoirs for the spread of whirling disease appears high (Vincent 2000).

Several ecological risk factors have been hypothesized to influence whirling disease severity. These factors include: high productivity, lack of flushing flows, low gradient, human altered or enriched habitats that amplify the density of *T. tubifex*, and the presence of brown trout (*Salmo trutta*) that can act as a reservoir for the disease (Modin 1998; McWilliams 1999; Zendt and Bergersen 2000). A recent study on the Madison River, MT, revealed that *M. cerebralis* infections occurred in slack water habitats with a high percentage of fine sediments (Krueger 2002). Such habitats may retain *M. cerebralis* spores better than substrates dominated by coarse particles such as gravel or cobble (Lemmon and Kerans 2001). Brown trout exposed in the Ruby River and Poindexter Slough, Montana, were found to be infected with *M. cerebralis*, and though infection was light in most cases and clinical signs were not abundant, this study suggests these fish could be carriers (Opitz 1999). Most of these factors, such as fine sediments, the presence of brown trout, high productivity, low gradient, and non-flushing flows, are common in spring creeks in Montana. Spring creeks could, therefore, be a significant source of "hot spots" of whirling disease infection that could affect trout populations over a wide area.

Spring creeks typically support abundant populations of *T. tubifex*, a critical host in the whirling disease life cycle. In the Salt River watershed, Wyoming, spring creeks had the highest incidence of whirling disease among streams sampled (Isaak and Hubert 1999). *Tubifex tubifex* thrives in environments with increased amounts of siltation and nutrient enrichment (Casellata and Caneva 1994; Finogenova 1996; Zendt and Bergersen 2000; Sandell et al. 2001). Spring creeks may provide prime habitat for *T. tubifex* populations as many are affected by siltation caused by agricultural practices and grazing (Decker-Hess 1989).

The moderate and consistent temperatures of spring creeks may also promote severe *M. cerebralis* infections. Temperature has been shown to affect whirling disease in several ways with respect to the development (Halliday 1976; El-Matbouli et al. 1999; Kerans and Zale 2002), viability, and infectivity of triactinomyxons (TAMs), the waterborne stage of the parasite that is infective to fish (Markiw 1992c; Markiw 1992b; El-Matbouli et al. 1999; MacConnell and Vincent 2002; Smith et al. 2002). Temperature also affects the intensity of infection from *M. cerebralis* (Hedrick et al. 1998). Highest infection severity of rainbow trout in sentinel cage studies in Montana occurred at water temperatures ranging from 12 °C to 16 °C, with infection severity decreasing markedly at lower and at higher temperatures (Vincent 2002). Other field studies reported the highest prevalence of infection occurred at temperatures between 10 °C to 12 °C (Baldwin et al. 2000; Downing et al. 2002).

The moderate and consistent temperatures of spring creeks may also affect the duration over which *M. cerebralis* infection can occur. In whirling disease-infected streams, infection patterns have followed a seasonal cycle of peaks in the spring and fall during moderate temperatures, and declines in the summer and winter at warmer and colder temperatures (Vincent 1999). Temperatures in spring creeks, however, often do not fluctuate more than a few degrees seasonally, and typically average near the apparent optimal infection range of 10 °C to 12 °C (e.g., Pierce et al. 2002). Thus, it is possible that spring creeks could exhibit a prolonged infection cycle. Constant water temperatures have encouraged a longer duration of triactinomyxon release in previous laboratory controlled studies (El-Matbouli et al. 1999; Krueger 2002). Alternatively, relatively constant temperatures lacking in seasonal fluctuations and thermal peaks may depress high infection levels. Thermal cues appear to be necessary for the timing of numerous life history events of aquatic insects (Glazier 1991; Ward and Dufford 1979). Seasonal shedding of triactinomyxons from worms may occur when water temperatures cycle seasonally (Hedrick et al. 1998). In addition, a seasonal periodicity of triactinomyxon release by *T. tubifex* may be cued by water temperature fluctuations (Granath and Gilbert 2002). The absence of such cues in the stable thermal environment of spring creeks therefore could decrease disease severity.

The purpose of this study was to investigate how widespread *M. cerebralis* is in Montana's spring creeks and to determine factors that may affect its infection severity. I assessed seasonal changes in *M. cerebralis* infection in relation to temperature and trout spawning and rearing in nine different spring creeks in western Montana. *Tubifex tubifex* abundance, water quality, and habitat were also investigated to determine if such factors were related to the occurrence or severity of *M. cerebralis* infection. My specific objectives were to: 1) examine the extent of *M. cerebralis* infection in a subset of spring creeks across southwestern Montana; 2) assess seasonal incidence and severity of *M. cerebralis* infection in spring creeks and in comparison to adjoining river systems; 3) examine how water temperature, *T. tubifex* abundance, and habitat characteristics relate to disease severity; and 4) assess degree of overlap between cycles of infection and the period of emergence and early fry rearing when fry are most susceptible to infection. I hypothesized that whirling disease severity will be of higher magnitude and longer duration in spring creeks as compared to adjoining river systems due to habitat and thermal characteristics that support abundant *T. tubifex* populations and high *M. cerebralis* production.

STUDY AREAS

To assess the occurrence of *M. cerebralis* among spring creeks in western Montana, I sampled nine spring creeks within eight different drainages in southwestern Montana over a 20-month period from January 2000 through August 2001 (Figure 1). Individual spring creeks ranged from 0.5 to 9.7 km in length and from 0.4 to 1.4 cms in discharge (Table 1). Spring creeks where whirling disease had previously been detected in the spring creek or adjacent river, or sites that were known to be important spawning tributaries for trout, were selected. All nine spring creeks were sampled for *M. cerebralis* infection twice in the spring (April and May) in 2000 and 2001, and once in the fall (October) in 2000 to assess the extent of infection over a wide geographic area. Spring and fall sampling was chosen as these were the time periods of peak infection in previous studies of seasonal infection cycles in streams (Vincent 1999). Three intensive sites (Ben Hart, Nelson and Willow Springs) also were sampled monthly throughout the study period to assess seasonal dynamics of *M. cerebralis* infection in relation to temperature, degree of overlap with period of high susceptibility to infection for trout, and *T. tubifex* abundance.

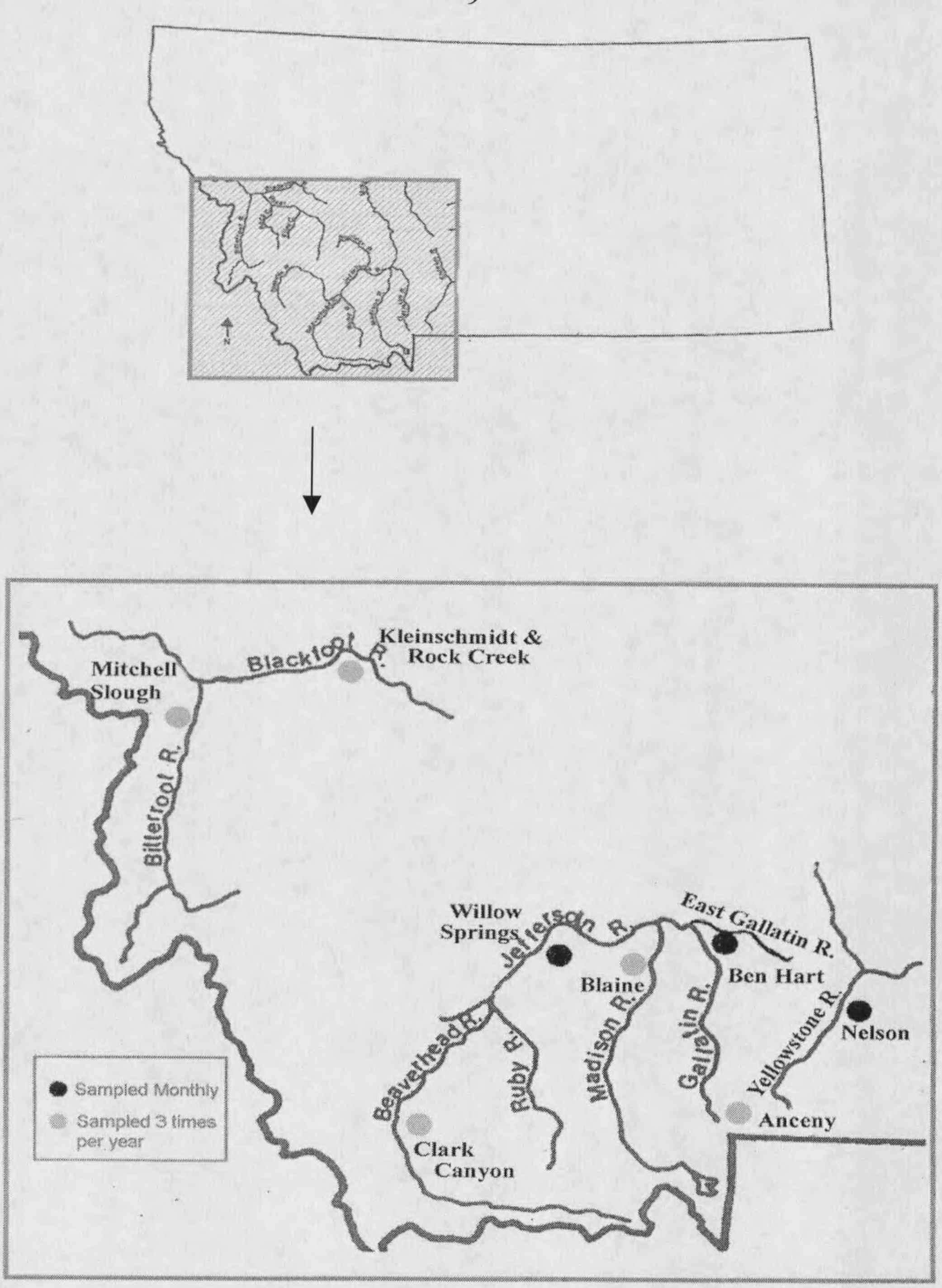


Figure 1. Study spring creeks and associated river drainages.

Spring Creek	Drainage	Length (km)	Discharge (cms)	Trout Species present	Spawning Tributary	*M. cerebralis* confirmed
Anceny	Gallatin	0.5	NA	NA	Y	NS
Ben Hart	East Gallatin	4.8	0.7-1.0	RBW, BN, EB	Y	Y
Blaine	Madison	8.3	0.9-1.0	RBW, BN	Y	Y
Clark Canyon	Beaverhead	0.8	0.1-0.2	RBW, BN	Y	NS
Kleinschmidt	Clark Fork	4.8	0.4-0.5	RBW, BN, BT, EB, WCT	Y	Y
Mitchell Slough	Clark Fork	9.7	0.9	RBW, BN, EB, WCT	Y	NS
Nelson	Yellowstone	3.2	1.1-1.4	RBW, BN, EB, YCT	Y	NS
Rock Creek	Clark Fork	4.8	0.6	RBW, BN, EB	Y	NS
Willow Springs	Jefferson	1.6	0.4	RBW, BN, EB	Y	Y

Table 1. Drainage, stream length, discharge (Decker-Hess 1989), trout species (RBW = rainbow, BN = brown trout, EB = brook trout, BT = bull trout, WCT =Westslope cutthroat, YCT = Yellowstone cutthroat), spawning use, and *M. cerebralis* confirmation data for the nine spring creek study sites.

Anceny

Anceny Spring Creek flows for 0.5 km in a northeasterly direction, paralleling Highway 93, before entering the Gallatin River at river kilometer (rkm) 111. Prior to this study, the lower Gallatin River had tested positive for *M. cerebralis;* however, the infection status of Anceny Spring Creek had not been examined. It was included because it is an important spawning tributary for the Gallatin River drainage.

Blaine

Blaine Spring Creek flows for 8 km and enters the Madison River at rkm 89. Blaine Spring Creek was chosen as one of our study sites because *M. cerebralis* had previously been detected.

Kleinschmidt / Rock Creek

Kleinschmidt and Rock Creek spring creeks flow into the North Fork of the Blackfoot River between rkm 16 and 18 near the town of Ovando, Montana. They converge approximately 250 m above their confluence with the river. We included these two spring creeks in our study because Kleinschmidt had tested positive for *M. cerebralis* earlier and Rock Creek was considered to be at high risk for *M. cerebralis* infection due to its proximity to Kleinschmidt. Both are important spawning tributaries for brown and brook trout, and Rock Creek also supports a run of spawning rainbow trout.

Clark Canyon

Clark Canyon Spring Creek flows in a northerly direction and enters Clark Canyon Reservoir near the mouth of Red Rock River. Clark Canyon Spring Creek had not been tested for *M. cerebralis* but was included because it is an important site for spawning rainbow trout migrating from Clark Canyon Reservoir (Dick Oswald, MFWP, personal communication.)

Mitchell Slough

Mitchell Slough consists of a series of old river channels supplemented by both groundwater springs and water from the Bitterroot River. It joins the Bitterroot River at rkm 56 (Decker-Hess 1989). Mitchell-Slough was included because it was known to support populations of spawning brown trout and rainbow trout from the Bitterroot River (Chris Clancy, personal communication). It had not been tested for whirling disease prior to this study, but *M. cerebralis* had been detected at low levels in the Bitterroot River.

Ben Hart

Ben Hart Spring Creek flows for 5 km in a northerly direction before entering the East Gallatin River at rkm 23 near Belgrade, MT (Decker-Hess 1989). Inclusion in this study was based on Ben Hart testing positive for whirling disease in 1999 and supporting populations of spawning trout.

Nelson

Nelson Spring Creek originates from a series of coldwater springs and parallels the Yellowstone River in a northerly direction before entering the river south of Livingston, MT at rkm 813 (Roberts 1988; Decker-Hess 1989). Nelson Spring Creek was included in this study because it supports high populations of spawning trout and whirling disease had previously been detected in the nearby Armstrong Spring Creek. It was unknown if Nelson Spring Creek was positive for whirling disease, but because whirling disease occurs in the Yellowstone, it was considered to be at high risk for infection.

Willow Springs

Willow Springs Spring Creek originates from a low area in a hayfield and then flows in an easterly direction where it enters the Jefferson River at rkm 88 near Waterloo, Montana. Willow Springs Spring Creek was chosen as an intensive site because it had previously tested positive for whirling disease and it is one of the few spawning tributaries to the upper Jefferson River (Ron Spoon, Montana Department of Fish, Wildlife and Parks, personal communication).

METHODS

Myxobolus cerebralis infection

Sentinel fish were used to determine the presence and seasonal dynamics of *M. cerebralis*. Sentinel fish have commonly been used to test for the prevalence of *M. cerebralis* (Thompson 1999; Baldwin 2000; Densmore et al. 2001; Hiner and Moffitt et al. 2001; Sandell et al. 2001; Downing et al. 2002), and provide an indirect measure of *M. cerebralis* triactinomyxon abundance (Kerans and Zale 2002). Sentinel cages consisted of a 0.5 m diameter by 0.6 m deep cylinder covered with a fine-mesh wire screen. Sixty juvenile rainbow trout obtained from certified whirling disease-free hatcheries were placed into each cage. Six strains of rainbow trout with relatively equal whirling disease susceptibility (Arlee, Eagle Lake, Madison, Erwin, Shasta, and Fish Lake) were used for sentinel exposures (Vincent 2002; Jerri Bartholomew, Oregon State University, personal communication). Within an exposure group, all fish were similar in strain, age, and size, but varied between exposure groups based on fish availability during the 20-month study period (January 2000-August 2001). Fish ranged from 29 to 53 mm in size and 24 to 119 days post-hatch in age. Descriptions of fish strain, size, and age used for each exposure group are listed in Appendix A.

Sentinel fish were held in cages for 10 d, then transferred to the Pony Trout Lab, Pony, MT, or the Wild Trout Research Lab (WTRL) Montana State University, Bozeman, MT, (depending on tank availability) where they were held for 80 to 89 days to allow for full development of *M. cerebralis* spores (Baldwin et al. 1998; Vincent 2000).

Upon arrival at the laboratory, each exposure group was treated in either a salt (Pony) or formalin (WTRL) bath to eliminate fungus and parasites (Piper et al. 1982). Exposure groups were reared separately in split 114 L aquaria supplied with flow-through well water (10.4 °C to 13 °C) at the Pony lab and in 38 L aquaria supplied with recycled, filtered and oxygenated water (13 °C) at the WTRL. All fish were fed a standard commercial trout feed twice daily. At the end of the holding period, trout were sacrificed with an overdose of tricaine methane sulfonate (MS-222), measured (mm), and the heads removed. Individual heads were fixed in 10% buffered formalin for 72 h prior to transfer into sterile "whirlpak" bags containing 70% alcohol. Each bag had an internal and external tag for identification. Heads were sectioned and disease severity scored using the MacConnell-Baldwin rating system, based on a scale of 0 (none) to 5 (severe) (Table 2; Baldwin et al. 2000; Vincent 2002), at the Washington Animal Disease Diagnostic Laboratory (WADDL; Pullman, Washington). Metrics used to summarize disease severity for each exposure group included proportion by each lesion score category; proportion with lesion scores ≥ 3; and mean lesion score. The proportion of lesion scores ≥ 3 was considered a measure of high disease severity because the moderate grade (grade 3) is the initial stage when severe cartilage damage and disperse inflammatory response occurs in an infected fish (Baldwin et al. 1998). A fish graded as moderate or higher will have multiple foci of cartilage degeneration/necrosis indicated by the large granulomatous lesion and is likely to suffer the greatest from the parasite. These fish will also generally have a higher level of disease severity than those of lower grades, and may exhibit decreased stamina, increased spore burdens and a higher incidence of clinical signs (E. Ryce, Montana State University, personal communication). Therefore, fish with grade 3 or higher lesion scores will indicate how many fish from an exposure group will have the highest potential of becoming severely impacted by the disease.

From January through June 2000, 50 randomly selected fish from each exposure group were scored histologically. However, in order to increase the efficiency of the histological processing, the number of heads examined was decreased to 30 per exposure group beginning in July 2000. To test the precision of the reduced sample size, I randomly selected thirty-two exposure groups and compared mean lesion scores between 30 and 50 randomly selected heads. There were no significant differences in the disease severity scores between the two paired sample sizes and the mean difference was 0.04 (Wilcoxon Signed Rank test, P = 0.32). For subsequent samples, 30 of 60 fish from each exposure group were randomly selected for disease severity scoring. In addition, negative control samples (two groups of 50 fish that had not been exposed to *M. cerebralis)* were sent blind to WADDL to test accuracy of histology evaluation. All negative control fish were scored as negative for *M. cerebralis* infection.

My study design involved pairing sentinel cages in spring creek and adjoining river or reservoir sites at each study location. Generally, at least two cages were placed in each spring creek (exception: Willow Springs had only one cage from January to June 2000) along protected stream margins once per month. At least one cage was simultaneously placed in each adjoining river or reservoir upstream of the mouth of the spring creek so as to avoid the influence of spring creek water.

The number of sentinel cages deployed was constrained by significant time lags in obtaining data (approximately 9 months from sampling to lesion scoring) and need for extended rearing facilities in the lab for each exposure. Therefore, to assess the degree of precision in estimation of infection severity with only one exposure group in an area, I compared mean lesion scores from sentinel cages in close proximity (1 to 7 m apart) at

Table 2. Grading scheme used to quantify *M. cerebralis* lesion severity (Baldwin et al. 2000)

Grade	Description
0	No abnormalities noted.
1	Discrete, rare (usually single), small foci of cartilage degenerative with intralesional *M. cerebralis* myxospores/generative stages present. Any associated leukocyte infiltrates are small.
2	Single, locally extensive focus or several smaller foci (usually 2) of cartilage degeneration/necrosis with intralesional *M. cerebralis* myxospores/generative stages are seen. Lytic foci typically surrounded and/or infiltrated by few to moderate number of leukocytes.
3	Multiple foci of cartilage degeneration/necrosis (usually 3 to 4) with intralesional *M. cerebralis* myxospores/generative stages are present. Moderate numbers of leukocytes are typically associated with lytic cartilage.
4	Multifocal (usually 4 or more sites) to coalescing, often locally extensive areas of cartilage degeneration/necrosis with intralesional *M. cerebralis* myxospores/generative stages are present. Moderate to large numbers of leukocytes typically border and/or infiltrate degenerate cartilage.
5	Multifocal (usually 6 or more sites) to coalescing areas of cartilage necrosis, with locally extensive destruction in at least one focus and preferably more are present, and have intralesional *M. cerebralis* myxospores/generative stages. Moderate to large numbers of leukocytes typically border and/or infiltrate necrotic cartilage. This classification is reserved for the most severely affected fish, and when in doubt, classify as a grade 4.

four different study sites during 20 sampling periods. There were no significant differences in mean infection severity among paired exposure groups and all paired lesion scores differed by ≤ 0.40 (Wilcoxon Signed Rank Test; P = 0.29).

To test for possible spatial differences of *M. cerebralis* infection within a spring creek, I placed three cages approximately equidistant along the length of Ben Hart Spring Creek. I tested for seasonal differences in disease severity by comparing dynamics of *M. cerebralis* infection between two sites with moderate-to-high disease severity (Ben Hart and Willow Springs Spring Creeks). The twelve months in 2000 were subjectively assigned to one of four seasons: winter (November, December, January and February); spring (March, April and May); summer (June, July and August); and autumn (September and October).

Spawning and Emergence Timing

Redd counts were conducted within the three intensively sampled spring creeks one to two times monthly from February 2000 to September 2001 to compare timing of spawning and fry emergence in relation to *M. cerebralis* infection levels. Counts were conducted in sections of each spring creek with a high percentage of spawning gravels (Kondolf 2000). Study reaches ranged in length from 400 to 1500 m and the percentage of stream surveyed was 15% at Nelson, 20% at Ben Hart and 100% at Willow Springs. Redd counts were conducted by hiking along the creek banks or wading the spring creek while wearing polarized glasses. Redds were recognized by a characteristic upstream depression or pit with several larger rocks in its center and a downstream tailspill of disturbed smaller gravel (Chapman 1988; DeVries 1997). Redds were also identified by their lighter color as compared to neighboring, undisturbed substrate (Thurow and King 1994). Each new redd was identified with a colored rock to avoid counting a redd more than once. To gauge surveyor accuracy and the incidence of false redds, approximately 10 % of the redds were randomly selected and carefully checked for eggs or fry (Witzel and MacCrimmon 1983; Knapp and Vredenburg 1996). Rainbow trout and brown trout redds were distinguished by identification of individual fish near a redd and spawning timing. Spawning timing and duration was assessed by comparing the total number of new redds observed during each count. Peak spawning dates were indicated by the largest number of new redds counted relative to the previous survey date.

Timing of emergence of fry from redds was estimated using temperature units in relation to the observed date of redd construction. A mean spawning date was estimated for each redd count by using the mean date between consecutive redd counts. Date of predicted emergence for each redd was then determined by using 589 thermal units (degree-days, ^{o}C) 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 (^{o}C) obtained from thermographs. The period of susceptibility for young trout to the *M. cerebralis* parasite was determined as the time period from emergence and the 9-weeks following.

I attempted to verify predicted emergence dates by directly measuring timing of emergence using redd caps on brown trout redds (n = 9) and rainbow trout redds (n = 3). A redd cap consisted of a 1 m by 2 m net tapering towards a perforated PVC pipe trap. Each cap was secured over the redd with pins and the perimeter was lined with cobble to prevent fry escape. Water current through the netting and across the redd transferred newly hatched fry into the detachable PVC pipe. At each site, redds containing ova or sac fry with known spawning date were capped. Each cap was then checked for fry and cleaned at 3 to 7 day intervals. Emergent fry were identified to species (Weisel 1966) and released.

Habitat

Temperature

I measured water temperature at each sentinel cage at 30-minute intervals during each exposure period using Optic StowAway thermographs. Also, temperatures were recorded hourly with continuous recording thermographs deployed from January 2000 to June 2001 in the intensively studied spring creeks (Ben Hart, Nelson, Willow Springs), and from April to September 2001 in the other study sites.

Water Quality

To assess if water quality influenced disease severity, water samples were collected from spring creeks and the adjacent river or reservoir from July 24 to July 27, 2000, using sterile, 250-mL plastic bottles. Individual samples were then transferred to a cooler. Samples were analyzed for nitrate, calcium carbonate, phosphorus, sulfur and ammonia at the MSU Soils, Plant and Water Analytical Lab. Dissolved oxygen and pH were measured using a YSI Model 57 Dissolved Oxygen Meter and Hach Kit.

Physical Habitat

Habitat evaluations were conducted at all spring creek sites during summer 2001 to quantify individual stream channel characteristics, specifically in relation to abundance of potential *T. tubifex* habitat. Starting at the mouth, habitat type (glide, riffle, run, etc) was sequentially described (Overton et al.1997) and measured (m) while wading upstream. The length of unstable banks characterized by slumping, tension fractures, bare soil, and/or vertical and eroding soil were also measured (Overton et al. 1997). Systematic transects perpendicular to stream flow were sampled at predetermined intervals (50 to 500 m) dependent on overall spring creek channel length. The number of transects performed on each spring creek ranged from 6 to 10.

At each transect, channel width and depth were measured, percent aquatic vegetation estimated, and substrate composition analyzed using a Wolman pebble count and a surface fines grid. Percent aquatic vegetation was assessed by visually estimating the percent of substrate covered by vegetation in an area 20 m upstream and downstream from each transect. Pebble counts were performed walking heel to toe across the stream transect (with each "toe" delineating the next observation site) until 100 samples were obtained (Overton et al. 1997). A surface fines grid (Overton et al. 1997) was also used to estimate the percentage of fines (substrate < 2 mm). The grid consisted of a 32 x 32 cm clear Plexiglas square on which a 7 by 7 grid was drawn using 2 mm wide lines spaced 40 mm apart. This resulted in 49 grid intersection points with each point corresponding to the upper size limit of a surface fine (i.e. 2 mm). The Plexiglas square was bordered by a 3.8 cm-wide metal bar to provide the grid with negative buoyancy.

Two random tosses (one upstream and downstream) of the grid were made at each transect. At each toss location the 49 grid intersection points were examined, and every point that had individual substrate particles visible under each two millimeter point (> 2 mm) was counted. The proportion of fines was then calculated by dividing the number of counts > 2 mm by 49, and then subtracting this number from 1 to obtain percentage of fines < 2 mm.

Tubifex tubifex abundance

To assess the potential relation between *T. tubifex* abundance and *M. cerebralis* infection, oligochaetes were collected using a timed kick net sample (200 µm mesh size; one minute) within the three intensively studied spring creeks during September and October in 2000. One sample was collected every 500 m beginning at the mouth along a transect perpendicular to stream flow. Samples were filtered in the field with a 200-micron sieve to remove fines and the remaining sample was then transferred into a 500-ml plastic bottle and preserved with Kahles solution and 70%-recycled ethanol. The number of samples obtained at each spring creek ranged from 4 to 10.

In the laboratory, samples were filtered using a 500 µm sieve to remove sediments and then transferred into 70% ethanol until split and sorted. A plankton splitter was used to split the sample into eighths. Depending on "split" sample size, a subsample or the entire remaining sample was sorted (keeping the number of slides generated at each sample site < 15) and all oligochaetes were subsequently slide-mounted with CMCP-10 (mounting and clearing medium, Polysciences, Incorporated, Warrington, Pennsylvania) and identified using a Nikon Optiphot-2 compound microscope with phase contrast. Identification was completed to family level for all oligochaetes. Tubificidae were separated into immature or mature categories, and further delineated based on the presence of hair and pectinate chaetae (Kathman and Brinkhurst 1998). Mature worms were identified to species. Identification could not be completed to the species level on immature worms, as this is dependent upon reproductive parts. However, it was important to differentiate immature worms as having either hair and pectinate chaetae or bifid chaetae, as immature worms with hair and pectinate chaetae could be *T. tubifex* (Kathman and Brinkhurst 1998). Immature worms with hair and pectinate chaetae were assigned to the *T. tubifex* or *Rhyacodrilus* species category based on the relative abundances of mature *T. tubifex* or *Rhyacodrilus* species identified in each spring creek. Oligochaetes at each sample site were assigned to one of five categories: 1) *T. tubifex* 2) *Rhyacodrilis* species, 3) Naididae 4) *Limnodrilis* species + immature oligochaetes with bifid chaetae, and 5) Lumbriculidae + any remaining oligochaetes.

Statistical Analysis:

Index of *M. cerebralis* infection severity of exposure groups was expressed as the proportion of fish with moderate or more severe lesions, corresponding to lesion scores of ≥ 3 on the MacConnell-Baldwin histological scale. Peak infection severity was defined as the highest proportion of fish graded ≥ 3 at each spring creek or river site during the 20-month sampling period. Infection severity differences in paired spring creek and river sites were compared using the Mann-Whitney Rank Sum test. To test for longitudinal infection differences within a spring creek, the proportion of exposure groups graded into each of the five histological categories at each cage location were compared using the chi-square test of homogeneity (Daniel 1990). Differences in annual and seasonal variation of lesion scores between and within spring creeks were also assessed using the chi-square test of homogeneity. Relationships between water quality, physical habitat, and peak infection were evaluated using simple linear regression. Simple linear regression was also used to compare the average number of oligochaetes and peak infection severity in three spring creek sites. All tests were performed at the alpha 0.05 level of significance.

RESULTS

Myxobolus cerebralis infection

Peak infection severity varied widely among the nine spring creek study sites (Figure 2). In four sites, infection was absent (Anceny, Clark Canyon) or at very low levels (Mitchell Slough, Nelson), whereas three sites (Rock Creek, Kleinschmidt, Willow Springs) had a high proportion (> 77%) of sentinel fish exhibiting moderate to severe lesion scores (≥ grade 3). Ben Hart and Blaine Spring Creeks had moderate levels of infection present. Infection patterns throughout the 20-month sampling period in whirling disease positive sites are displayed in Figure 3. There was no apparent relationship between disease severity and geographic area as spring creeks in both western and eastern portions of the sampling area varied in severity from high to low. The number of exposure groups and dates of sampling for each site are listed in Appendix A.

Spring creeks generally had much higher infection severity than adjacent rivers (Figure 4). In study sites with moderate to severe disease severity (> 50% of lesion scores ≥ 3), peak severity was at least two times greater in the spring creek in three of the five paired spring creek and river sites. Statistically significant differences were detected in paired spring creek and river sites when comparing peak infections (Mann-Whitney Rank Sum test, $P = 0.006$). The average overall proportion value for the spring creek sites during the 17-month study period was 24.9 % compared to 10.3 % for the river sites. Moderately significant differences in three sites were also found when comparing the

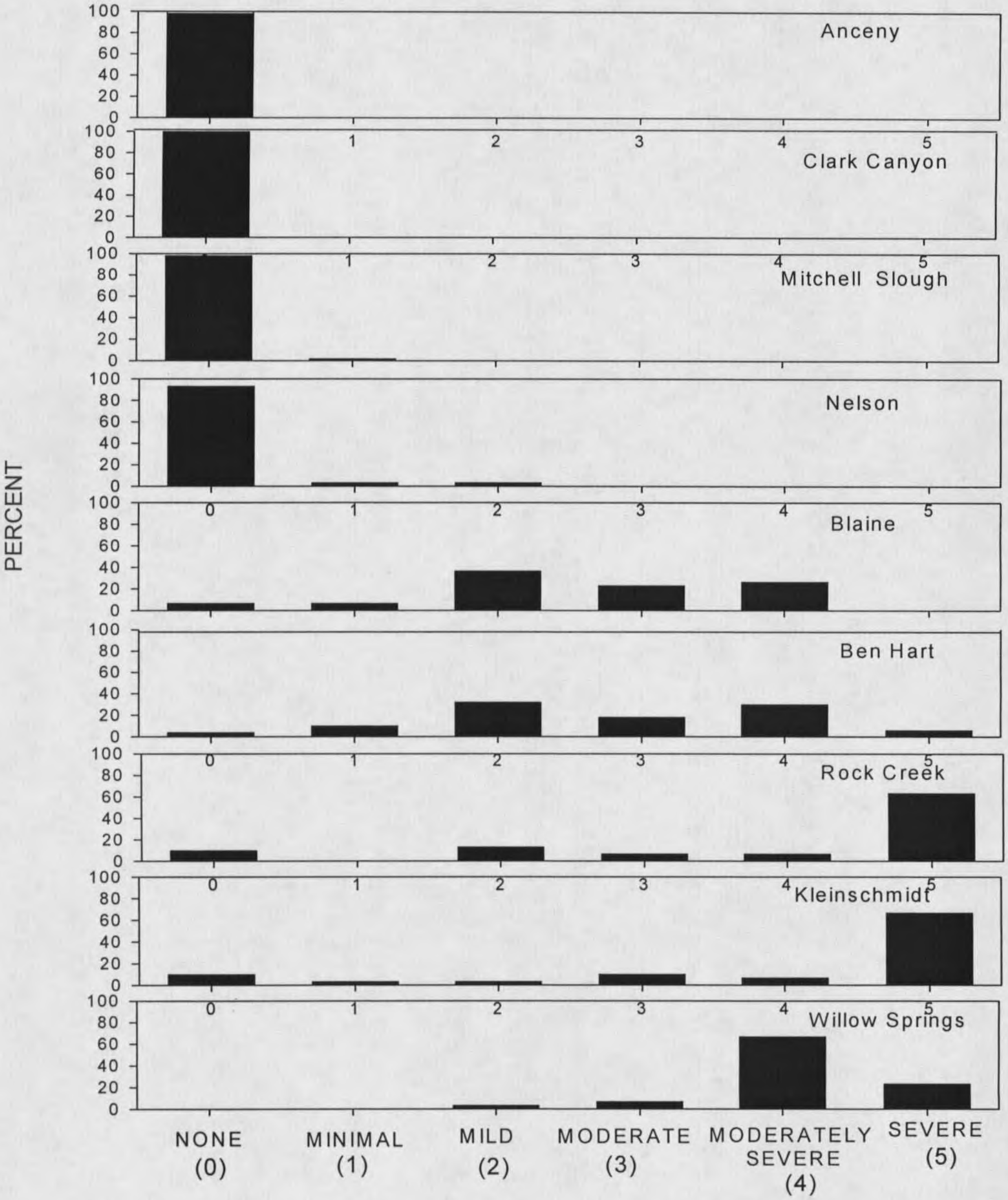


Figure 2. Proportion of sentinel fish by lesion score category. For each site, results represent the highest infection levels recorded in each spring creek during sampling from January 2000 to June 2001.

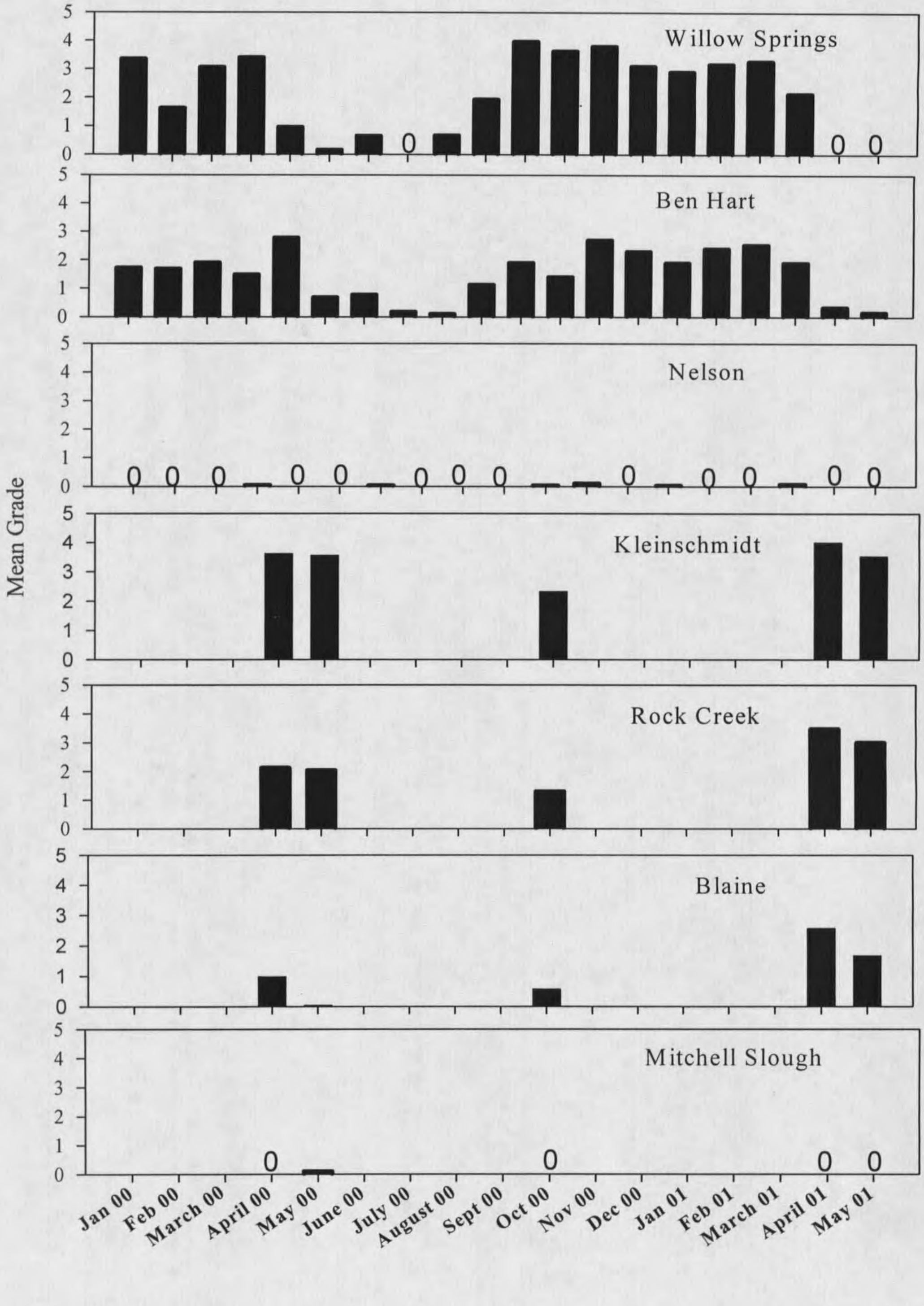


Figure 3. Infection pattern in seven *M. cerebralis*- positive spring creeks during 20-month study period.

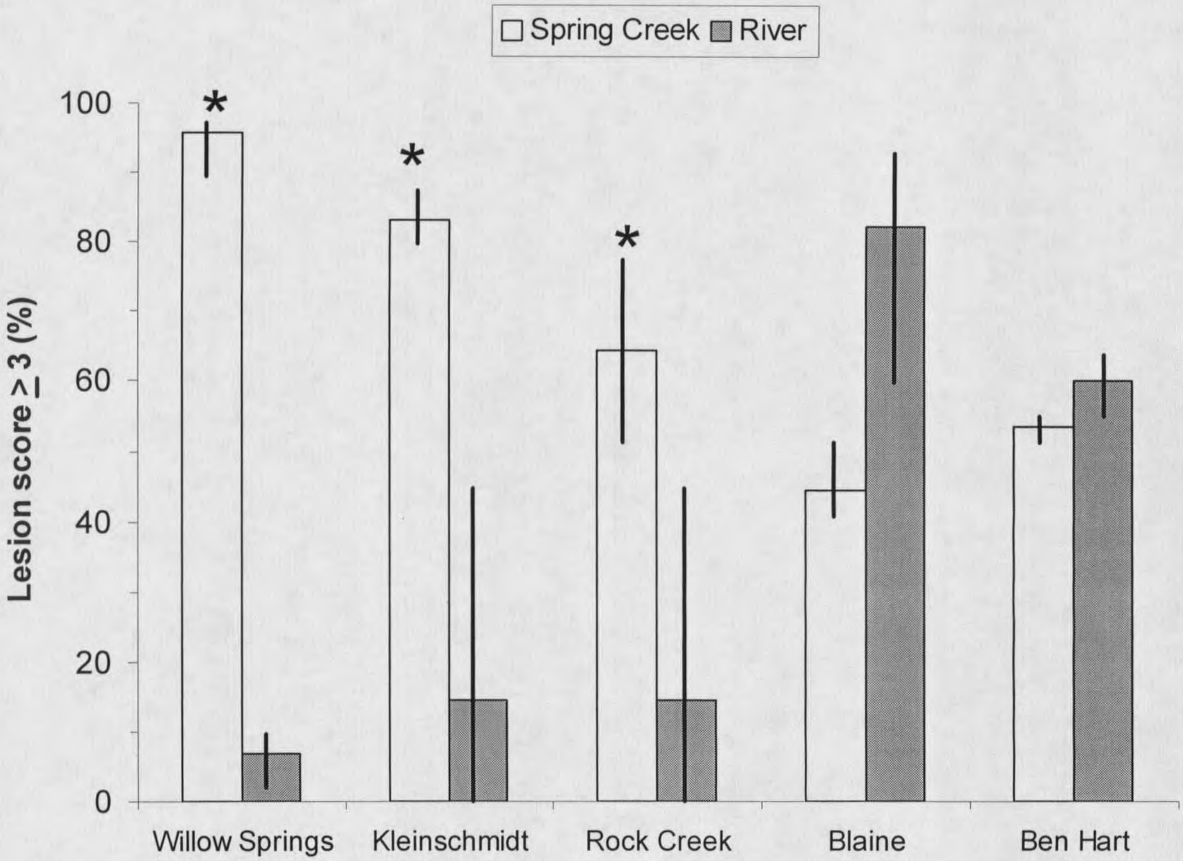
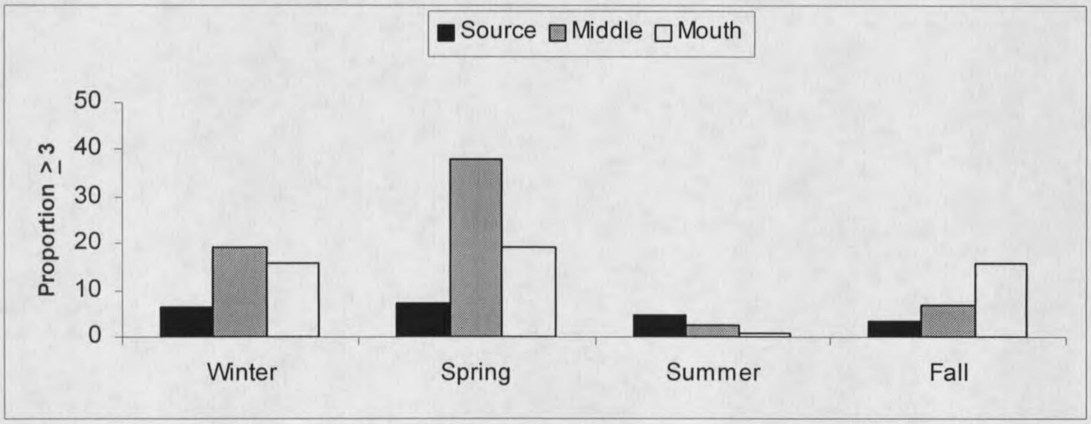


Figure 4. Average infection severity and range of the three highest infections (measured as proportion of fish graded with lesion score ≥ 3) observed in sentinel trout exposed in paired spring creek and river sites during the 17-month sampling period. * Indicates sites where significant differences (P ≤ 0.10) were detected.

three highest infection values between paired spring creek and river sites (Mann-Whitney Rank Sum test; Willow Springs and Jefferson, $P = 0.1$; Rock Creek and North Fork Blackfoot, $P = 0.1$; Kleinschmidt and North Fork Blackfoot, $P = 0.1$; Blaine and Madison, $P = 0.2$; Ben Hart and East Gallatin, $P = 0.4$) (Figure 4).

Myxobolus cerebralis infection of sentinel fish also varied longitudinally within a spring creek. Frequency distributions of lesion scores were significantly different during all four seasons among three different cages in Ben Hart spring creek [(Chi-square test of homogeneity, winter: $P < 0.001$; spring: $P < 0.001$; summer: $P = 0.018$; autumn: $P = 0.002$)]. Infection severity was typically highest in the middle and lower cage sites, and lowest near the spring source (Figure 5).

Figure 5. Infection severity differences among three cage locations in Ben Hart Spring Creek during winter, spring, summer and fall seasons.



Among infected sites, sharp seasonal and annual differences in infection severity were evident. Infection severity of sentinel fish in April and May 2000 was significantly higher than October at all sites except for Blaine Spring Creek and Ben Hart source (Chi-square test of homogeneity, $P < 0.0001$- 0.02; Figure 6). Infection severity during these time periods ranged from 0 % to 86 % (Table 3). There was also a significant difference in infection severity between years for all sites, excluding Nelson and Mitchell-Slough where only one fish was positive (Chi-square test of homogeneity, $P < 0.0001$- 0.006). Disease severity was lower in April and May 2000 compared to April and May 2001. For example, proportion of fish graded ≥ 3 in Blaine Spring Creek in April and May 2000 averaged 1.0 %, compared to 43.4 % in April and May of 2001 (Table 3).

Two intensive study sites with moderate to severe infections showed a distinct seasonal pattern in infection severity. Frequency distributions of pathology categories were significantly different between winter, spring, summer and autumn months for both Willow Springs (Figure 7) and Ben Hart (Figure 8) Spring Creeks (Chi-square test of homogeneity, $P < 0.0001$-0.012). In both spring creeks, infection severity was highest in the winter and early spring, declined to very low or undetectable levels throughout the summer and began to increase again in the fall (Figure 9). For example, the peak infection in Willow Springs occurred in November (2000) (96.7 %) and in January (2001) (90 %). In Ben Hart, infection in 2000 peaked during May (54 %) and in March and May of 2001 (53.3 %).

Although infection in the adjacent river sites did not typically escalate to levels as high as those found in spring creeks, infections in the East Gallatin River peaked in November 2000 (23.3 %) and January of 2001 (63.3 %). Infection levels in the Jefferson River were at their maximum in December 2000 (10 %) and in May 2001 (3.3 %) (Figure 10). No sentinel fish in the Yellowstone River had scored ≥ 3.

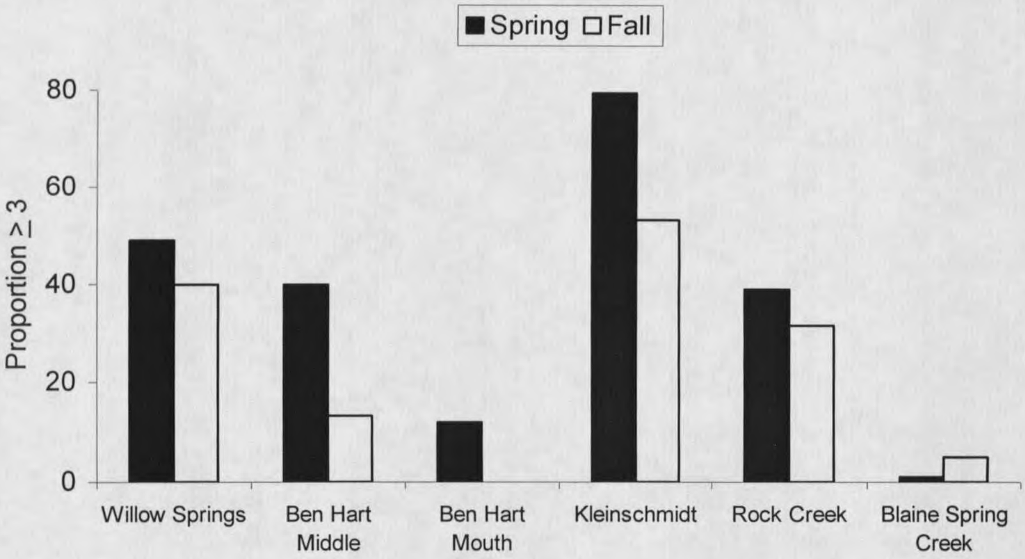


Figure 6. *Myxobolus cerebralis* infection (measured as the average proportion of fish graded ≥ 3) in six cage locations comparing spring (April and May) with fall (October), 2000. Infection severity was significantly higher during spring months in all sites compared with the fall, except for Blaine Spring Creek where infection was significantly higher in the fall.

Site	2000 April	2000 May	2000 October	2001 April	2001 May
Ben Hart	12.7	28.7	6.7	40	37.8
East Gallatin	8	4	10	41.7	48.4
Nelson	0	0	0	0	0
Yellowstone	0	0	0	0	0
Willow Springs	86	12	40	68.4	65.2
Jefferson	0	0	1.7	0	3.3
Anceny	0	0	0	0	0
Gallatin	0	0	0	0	0
Blaine	2	0	5	46.7	40
Madison	28	44	93.3	35	56.7
Clark Canyon Spring	0	0	0	0	0
Clark Canyon Reservoir	8	–	–	6.7	15.9
Mitchell Slough	0	0	0	0	0
Bitterroot	0	–	0	0	–
Kleinschmidt	68	82	53.3	81.7	75
Rock Creek	40	38	31.7	76.7	58.4
N.Fork Blackfoot	–	0	43.3	0	0

Table 3. Proportion of sentinel fish graded with a lesion score ≥ 3 in paired spring creek and river sites in spring 2000 and 2001, and fall 2000.

Spawning and Emergence Timing

A total of 163 redds were observed in Ben Hart, 327 in Nelson, and 314 in Willow Springs Spring Creeks throughout the 20-month redd survey period. Spawning occurred over extended periods in both 2000 and 2001 (Figure 11). Rainbow trout spawning periods ranged from as early as January to as late in July in some sites, but peak spawning occurred from mid-March to mid-April in 2000, and from early March to early May in 2001 in all three intensively-studied spring creeks. Brown trout spawning commenced during September to late November, and extended through December or January. Peak spawning occurred during mid-November. In 2001, spawning surveys ended in September so brown trout redds were not observed.

Overall, water clarity conditions were ideal for spawning surveys in the three spring creeks. Redd surveys in 2000 may be an underestimated as redd identification was challenging due to much of the substrate having a "clean appearance" which took some practice to differentiate from substrate "cleaned" from redd excavation.

Estimated rainbow trout emergence occurred as early as April and extended as late as August (Nelson and Willow Springs) (Figure 12). Peak emergence in all sites was similar between both years and occurred from late May to early-to-late June. Brown trout fry were estimated to emerge from late December (Willow Springs) to mid-April (Ben Hart) in 2000. Peak emergence occurred from early February (Nelson) to early April (Ben Hart) (Figure 12).

Only two of 12 redd caps were successful, one each in Ben Hart and Nelson. Brown trout fry were caught in the trap in Ben Hart on March 16, 2001. This redd was

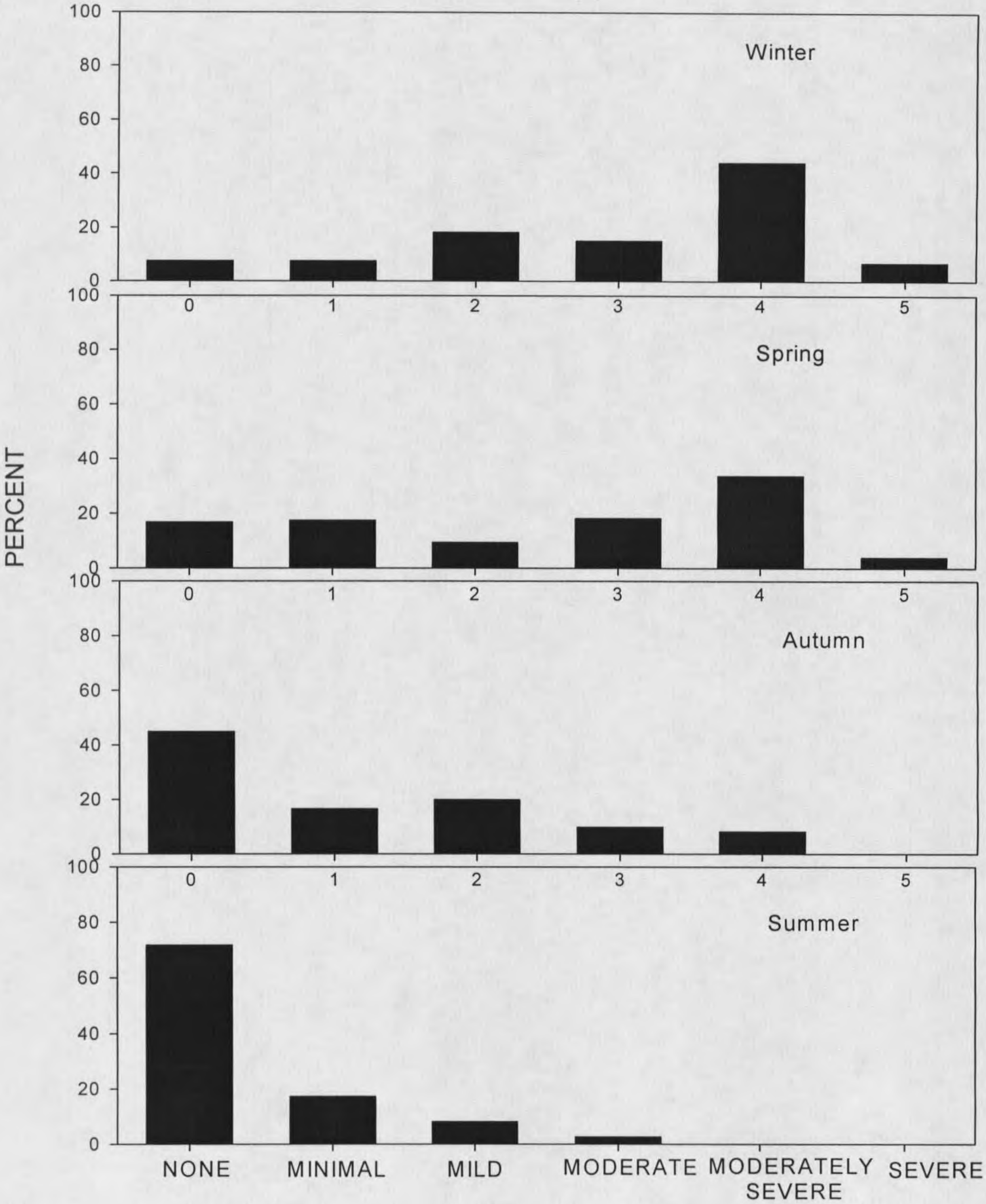


Figure 7. Frequency distribution of microscopic pathology category for sentinel rainbow trout exposed in Willow Springs Spring Creek during the winter (November, December, January and February), spring (March, April and May), autumn (September and October), and summer (June, July and August) 2000.

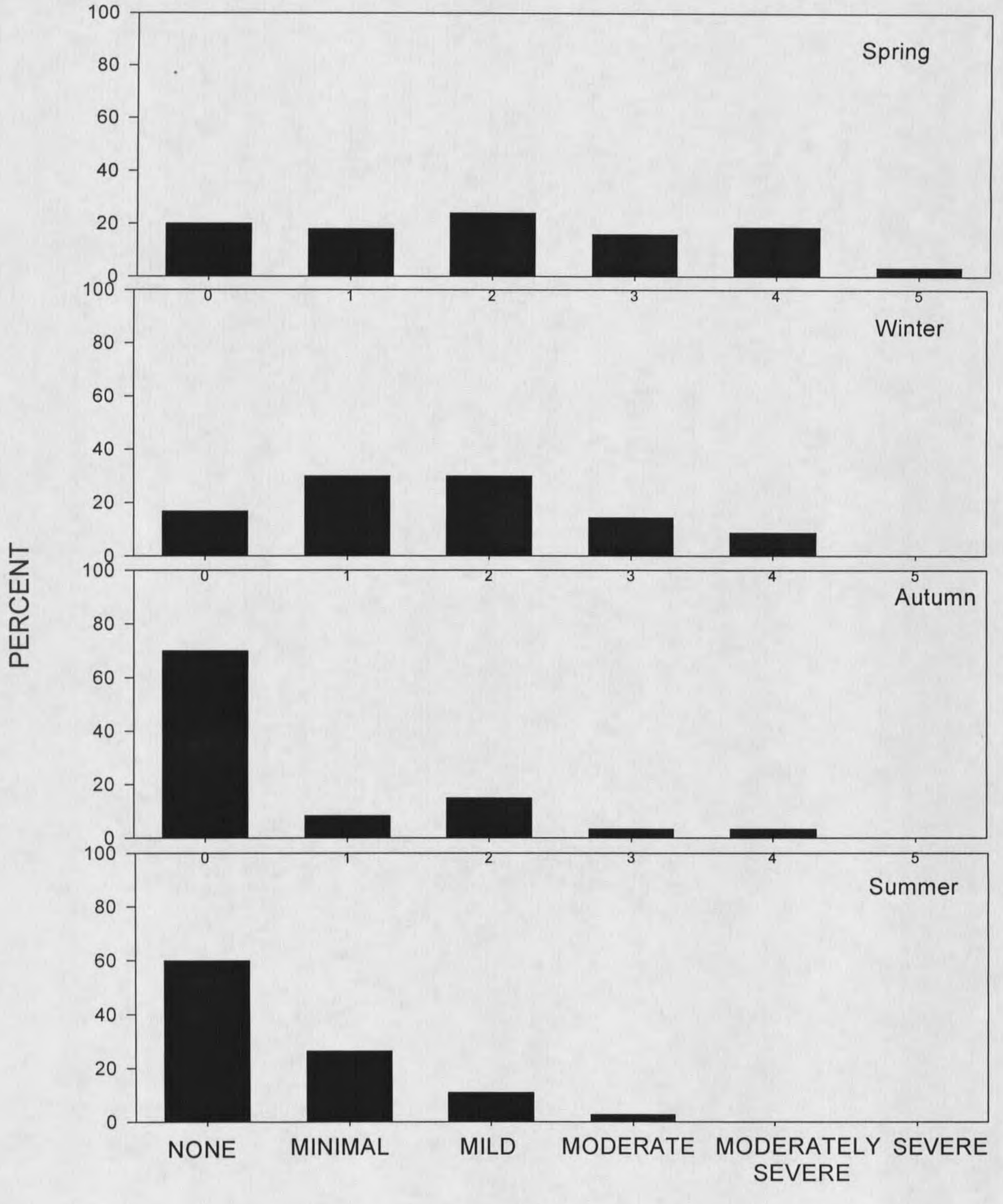


Figure 8. Frequency distribution of microscopic pathology for sentinel rainbow trout exposed in Ben Hart Spring Creek (middle cage) during the spring (March, April and May), winter (November, December, January, and February), autumn (September and October) and summer (June, July and August) 2000.

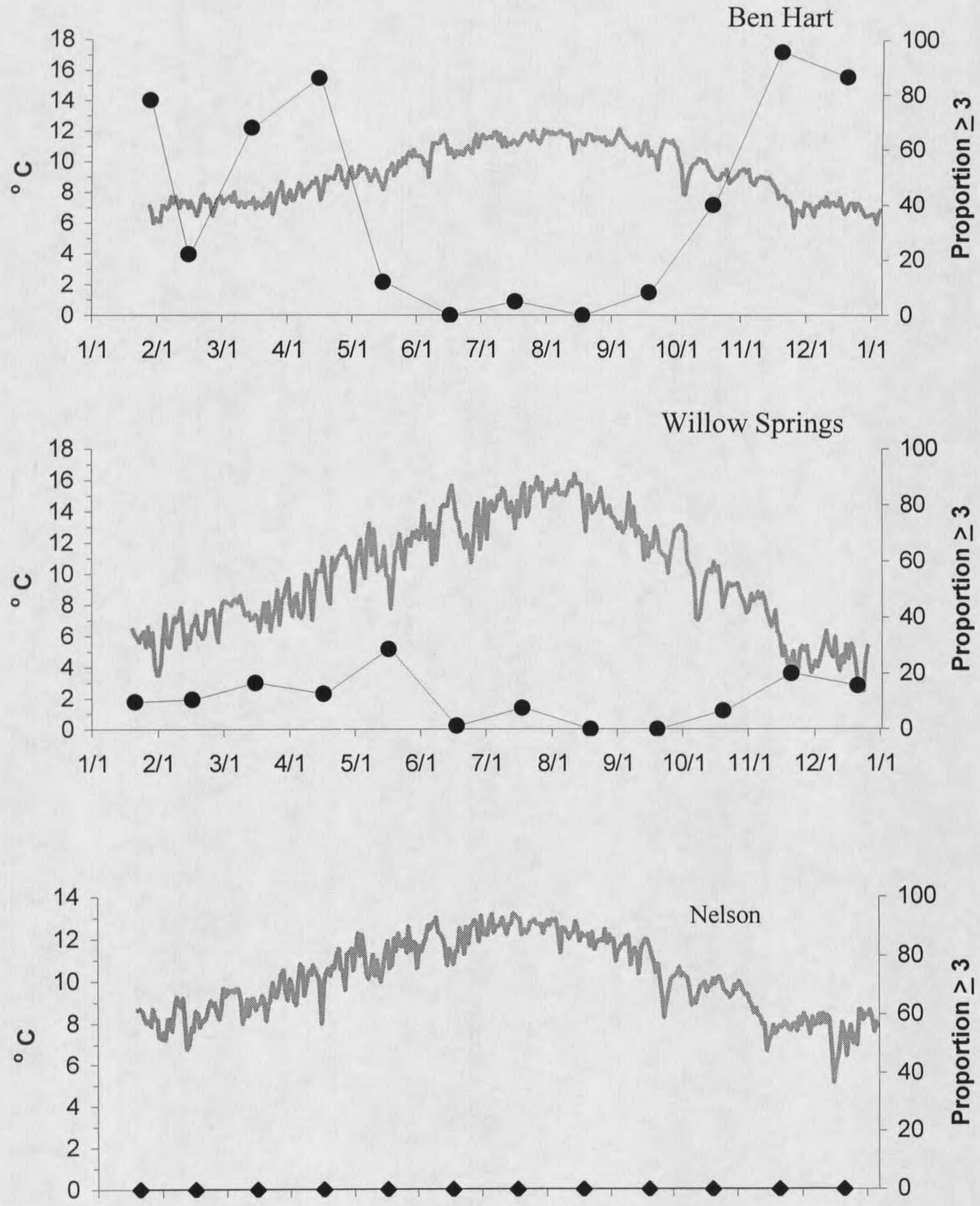


Figure 9. Average proportion of fish graded with a lesion score ≥ 3 in all cages during January through December 2000 and average daily temperatures during 2000 in Ben Hart, Willow Springs and Nelson Spring Creeks.

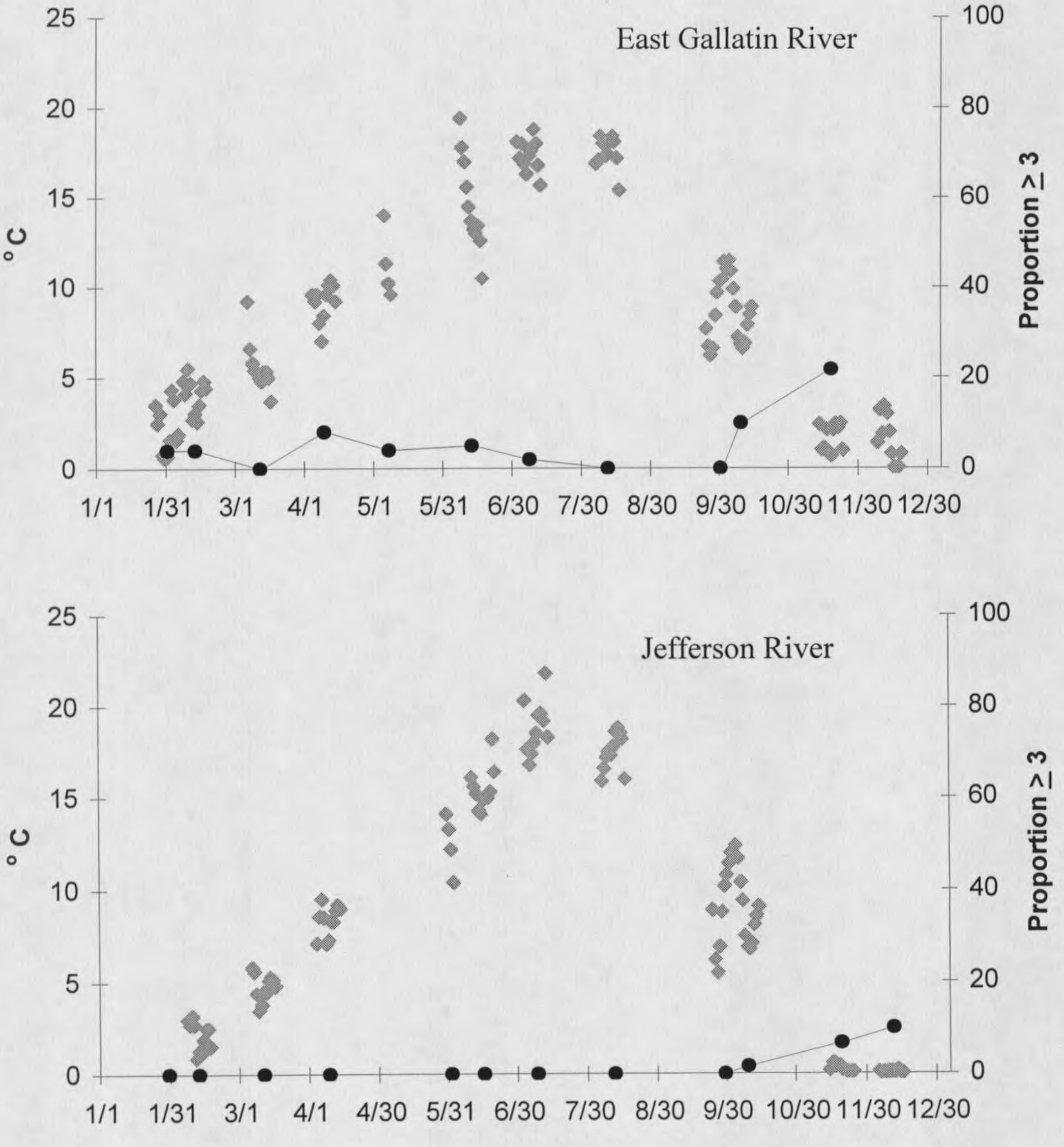


Figure 10. Comparison of average daily temperature and average proportion of fish graded with a lesion score ≥ 3 in the East Gallatin and Jefferson Rivers January-December 2000.

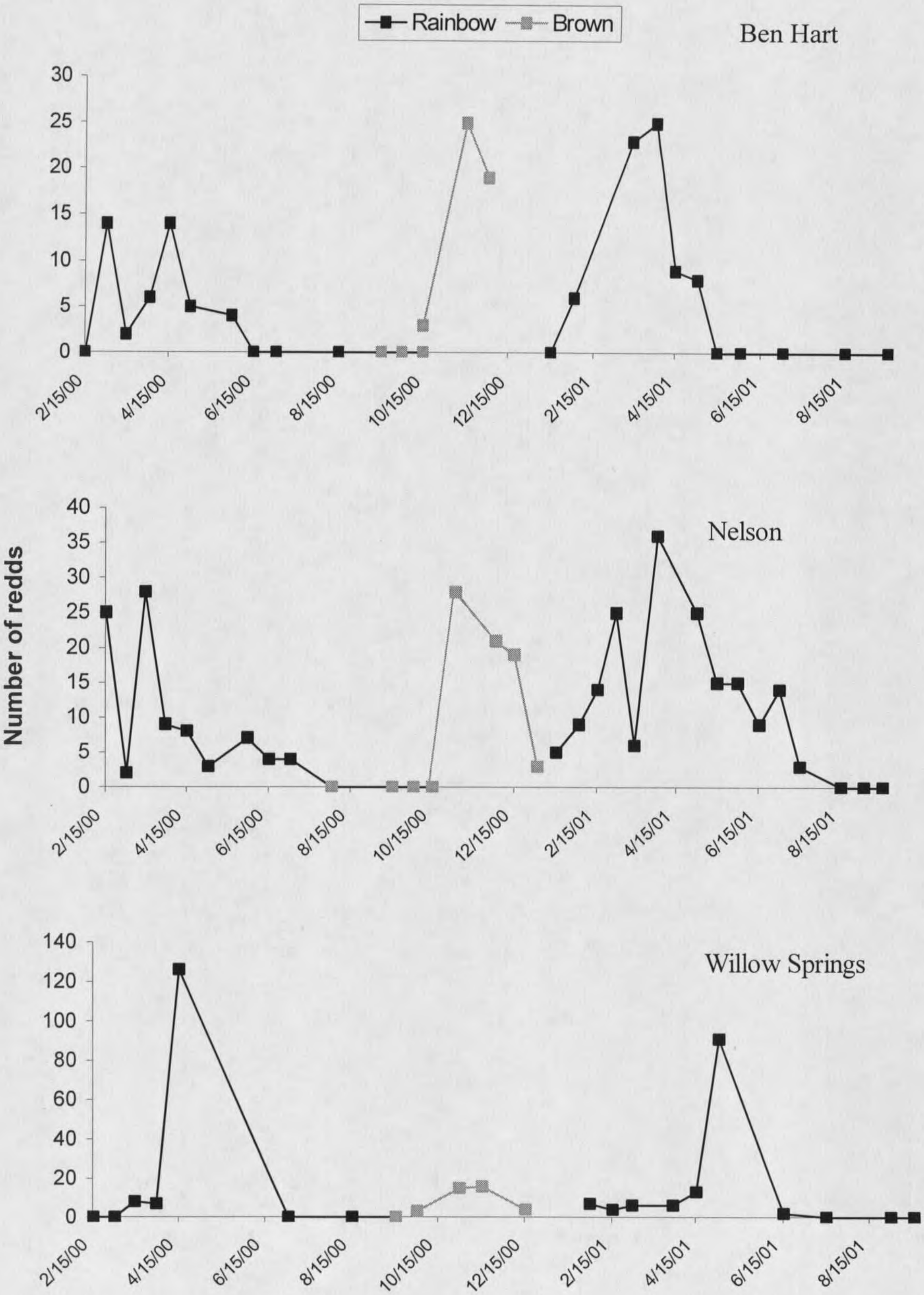


Figure 11. Number of new redds observed during each survey period in Ben Hart, Nelson and Willow Springs Spring Creeks, 2000 and 2001.

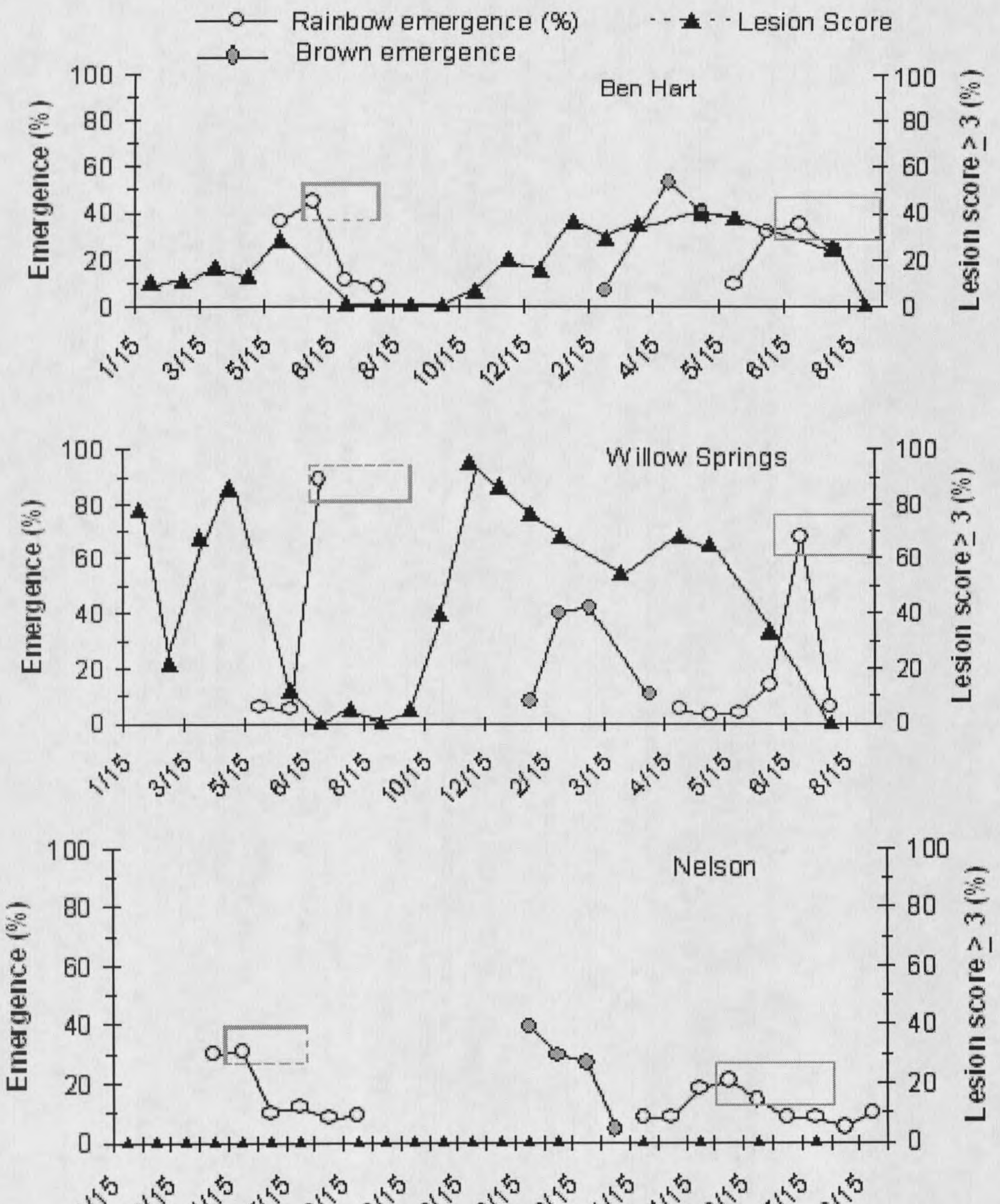


Figure 12. Infection severity (proportion ≥ lesion score 3) in relation to timing of rainbow (open symbols) and brown trout (gray symbols) emergence during 2000 and 2001 in Ben Hart, Willow Springs and Nelson Spring Creeks, Montana. Boxes indicate 9-week period of highest vulnerability to infection of rainbow trout fry.

formed approximately November 17, and accumulated 560 thermal units. In Nelson, brown trout fry from a redd formed December 5 were caught February 27 after 639 thermal units had accumulated. The low efficiencies of the redd capps could be attributed to the large amount of sediment that accumulated very rapidly under the caps, possibly suffocating the developing eggs. Based on this, redd caps do not appear to be an effective monitoring tool in spring creeks or those sites with non-flushing flows and high sediment buildup.

Habitat

Temperature

Average water temperatures during each 10 d exposure at spring creek and river sites are summarized in Appendix A. For intensively-studied spring creeks, temperatures ranged from 3.8 to 14.3 in Ben Hart, 5.1 to 14.0 in Nelson, and 5.2 to 13.2 in Willow Springs, spring creeks (Figure 9). Average temperature over the 17-month study period was 9.9 for Ben Hart, 10.3 for Nelson and 9.1 for Willow Springs. Temperatures in spring creeks were similar among sites, but had higher seasonal variation than expected. However, spring creek temperatures were considerably more stable than adjoining river sites. The majority of spring creek sites had warmer temperatures in the winter, spring and fall months. River sites had warmer temperatures in summer months and cooler temperatures in winter months as compared to adjoining spring creek sites (Figure 10).

Temperature appeared to influence infection severity. Although infection in spring creeks occurred over a wide range of water temperatures (4 °C to 14 °C) (Figure

13, (A)), highest infection occurred at temperatures ranging from 6 °C to 12 °C (Figure 13, (B)). Highest infection in Willow Springs Spring Creek (study site where infection severity was highest) occurred within a narrower temperature range (6 °C to 9 °C) (Figure 13 (C)).

Sentinel fish positive for *M. cerebralis* exposed in river sites were detected at temperatures ranging from 0.1 °C to 17 °C, temperatures considerably higher and lower than spring creeks (Figure 14). Cages where ≥ 50 % of fish had lesion scores ≥ 3 were found at temperatures ranging from 7.5 °C to 12 °C, but also as low as 1.7 °C.

Water Quality

Water quality data from each spring creek and river site are summarized in Table 4. There was no significant relationship between any water quality parameter and peak infection detected (simple linear regression; Figure 15). Significant differences in water quality were also not detected among the five spring creek sites with moderate to high levels of infection, except for a significant positive association between infection severity and phosphorus (simple linear regression, $r^2 = 0.81$; $P = 0.038$). At all sites, phosphorus, nitrate, and ammonia values were within acceptable levels according to Montana Numeric Water Quality Standards (Montana DEQ 2001). A value was not listed for sulfur.

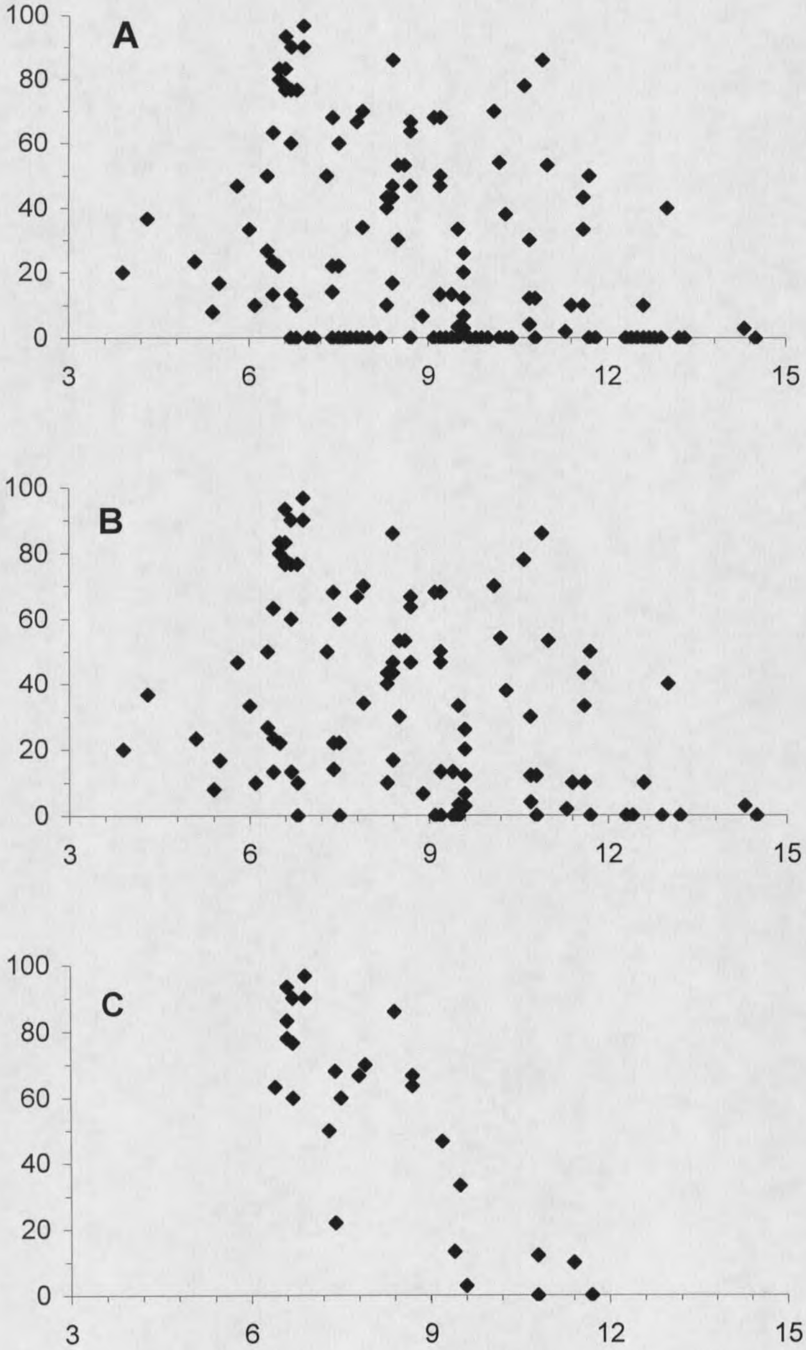


Figure 13. Proportion of fish graded ≥3 and average temperature (^oC) during each exposure period. (A) represents temperature and infection data from all spring creeks; (B) represents exposure groups where ≥ 50 % of fish had lesion scores ≥ 3; (C) represents temperature and infection data from a spring creek site (Willow Springs) where infection severity was highest.

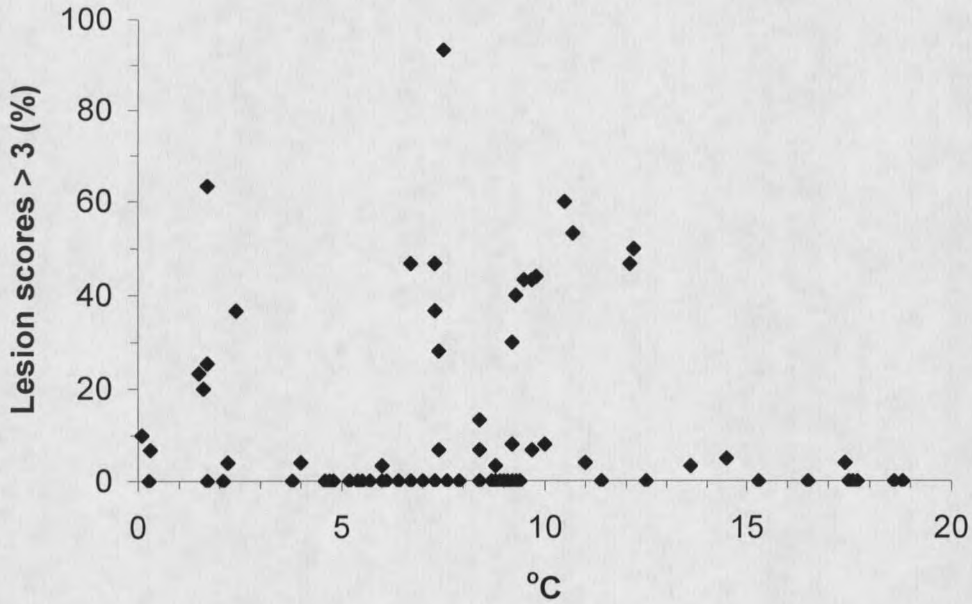


Figure 14. Proportion of fish graded with a lesion score ≥ 3 and average water temperature during each 10-d exposure in river study sites.

Physical Habitat

Habitat and substrate data for each spring creek study site are summarized in Tables 5 and 6. The majority of sites were comprised of high percentages of "glide" habitat type, high percentage of fines, and abundant aquatic vegetation. There was no significant relationship between habitat factor and peak infection in any of the spring creeks, including percentage of fines (simple linear regression, $r^2 = 0.00\text{-}0.27$; P = 0.19-0.98; Figure 16). Significant differences in physical habitat features and disease severity were also not detected when only the five sites with moderate to high levels of infection were included ($r^2 = 0.00\text{-}0.57$; P = 0.14-0.98).

Tubifex tubifex abundance

The total and average number of oligochaetes for each spring creek sample site is summarized in Appendix C. Mean density of oligochaetes for each spring creek is shown in Figure 17. Ben Hart had the highest number of oligochaetes, while the highest proportion of *T. tubifex* was found in Nelson Spring Creek, a site with very low infection levels (Figure 2). The number of *T. tubifex* and other oligochaetes was highly variable resulting in large variability (Figure 17). There was no significant associations between infection severity and mean density of *T. tubifex*, *Rhyacodrilis,* Naididae, *Limnodrilis* species + immature oligochaetes with bifid chaetae, or Lumbriculidae + remaining oligochaetes (simple linear regression, $r^2 = 0.05\text{-}0.63$; P = 0.42-0.89).

Spring Creek / River Sampled	pH	DO	NO3 -N mg/L	NH4 -N mg/L	P mg/L	S mg/L	Alkalinity mg CaCO3 /L
Nelson SC	8.5	10.4	0.3	< 0.1	0.23	6.8	129
Yellowstone R	8.3	8.4	< 0.1	< 0.1	0.11	5.3	54
Ben Hart SC	7.8	7	0.8	< 0.1	0.4	7	224
E. Gallatin R	8.7	6.1	0.8	< 0.1	0.41	6.7	202
Willow Springs SC	7.9	8.3	0.8	< 0.1	0.26	9.1	161
Jefferson R	8.6	9.1	< 0.1	< 0.1	0.32	20	183
Anceny SC	7.8	7.2	< 0.1	< 0.1	0.26	47.1	142
Gallatin R	8.4	7.7	< 0.1	< 0.1	0.22	19.2	122
Blaine SC	8.4	10.2	0.1	< 0.1	0.4	18.9	175
Madison R	8.5	9	< 0.1	< 0.1	0.15	3.3	80
Clark Canyon Spring	7.4	5.1	0.5	< 0.1	0.34	35.7	218
Clark Canyon Reservoir	8.9	8.2	< 0.1	< 0.1	0.35	33.5	209
Mitchell-Slough	8.8	13.2	0.1	< 0.1	0.25	1.8	147
Bitterroot R	7.5	7.6	< 0.1	< 0.1	0.1	0.6	45
Kleinschmidt SC	8.6	9	0.1	< 0.1	0.24	1.3	147
Rock Creek SC	8.4	9	0.1	< 0.1	0.24	1.4	139
N. Fork Blackfoot R	7.8	8.2	0.2	< 0.1	0.23	1.4	138

Table 4. -Summary of water quality characteristics analyzed at nine study spring creeks(SC) and river sites (R), July 24-27, 2000.

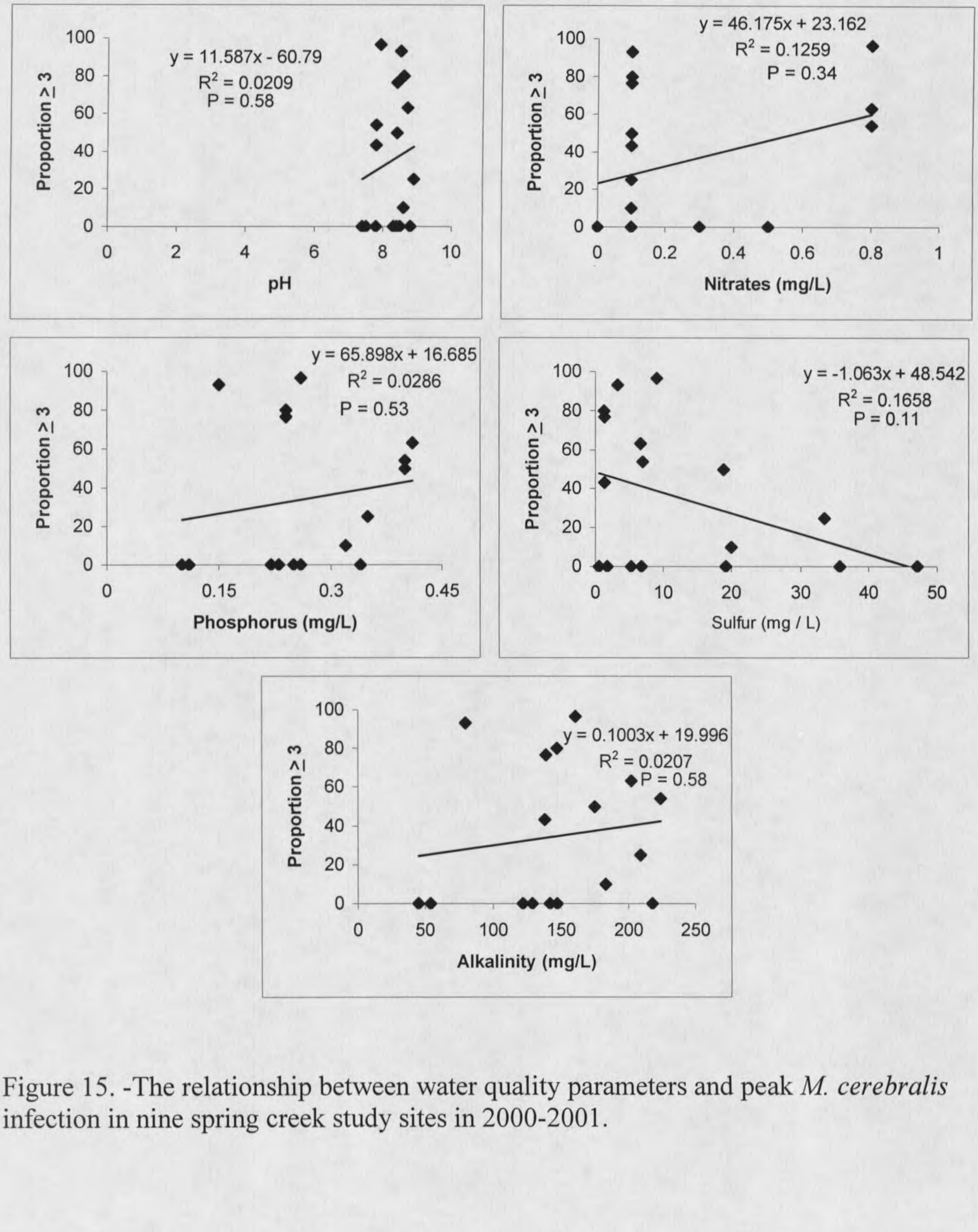


Figure 15. -The relationship between water quality parameters and peak *M. cerebralis* infection in nine spring creek study sites in 2000-2001.

Spring Creek Inventoried	Habitat Type (%) Glide(%) (m)	Riffle(%) (m)	Pool(%) (m)	Backwater(%) (m)	Width (m)	Depth (m)	Aquatic Vegetation(%)	Unstable Bank (m)
Anceny	75.1	24.9	0	0.9	8.8	0.17	67	0
Ben Hart	80.5	14.3	5.2	9.4	10.46	0.27	79	2
Blaine	85.8	14.2	0	12.2	11.1	0.31	74	0
Clark Canyon	51.7	41.7	6.6	4.9	4.5	0.29	43	0
Kleindschmidt	28.7	45.3	26	2.8	4.11	0.34	36	0
Mitchell Slough	100	0	0	0	14.6	0.45	89	0
Nelson	77.4	22.6	0	11.2	19.6	0.28	68	0
Rock Creek	56	42	2	0.88	5.23	0.26	19	0
Willow Springs	81.8	18.2	0	5.1	8.5	0.25	55	0
Average	70.8	24.8	4.4	5.3	9.7	0.3	58.9	0.2

Table 5.-Habitat type (%), average width, average depth, aquatic vegetation (%), and unstable banks (%) measured at nine spring creek study sites in 2001.

Spring Creek Inventoried	Proportion of Fines (SFG)	Wolman Pebble Count: Fines < 2mm	Small Gravel 2-8 mm	Gravel 8-64 mm	Small Cobble 64-128 mm	Cobble 128-256 mm	Small Boulder 256-512 mm	Boulder >512 mm	Bedrock
Anceny	0.48	39.3	15.8	25	10.5	6.7	1	1.2	0.5
Ben Hart	0.49	48.5	13.1	27.1	10.5	0.8	0	0	0
Blaine	0.43	40.3	2.7	15.6	18.7	18.3	4.3	0.1	0
Clark Canyon	0.43	42	17.5	29.1	9.8	1.5	0.1	0	0
Kleindscmidt	0.42	30	14.7	18.6	19.9	15.1	1.5	0	0
Mitchel-Slough	0.49	33	15.2	44.5	7	0.2	0.2	0	0
Nelson	0.46	52	5	22.8	7	11.5	2	0	0
Rock Creek	0.04	13.3	21.6	33.7	23	7.1	1.1	0.1	0
Willow Springs	0.62	57	9.4	23.4	6.9	3.4	0	0	0

Table 6. Substrate compositions of nine spring creek study sites inventoried during 2001. (SFG = Surface fines grid).

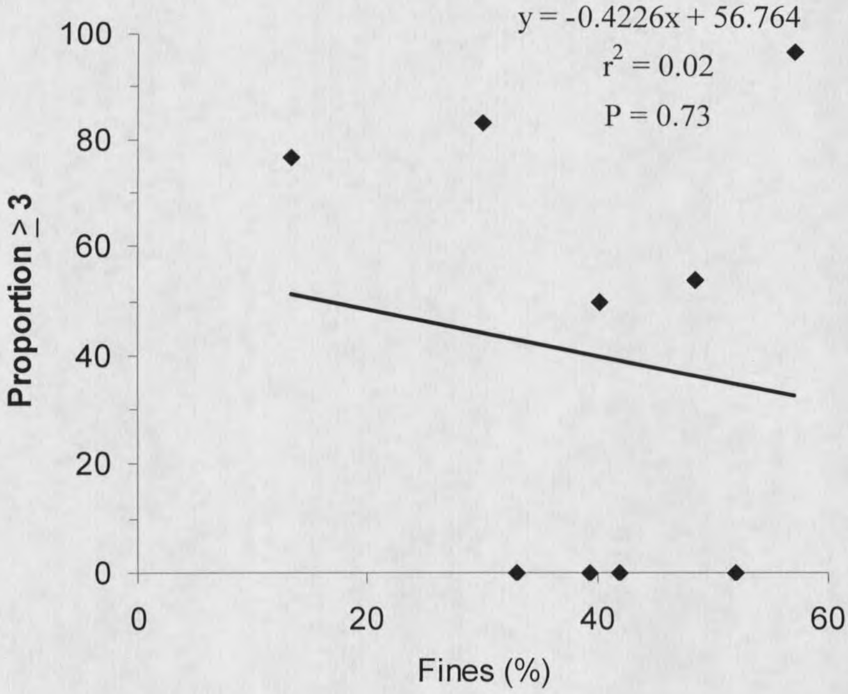


Figure 16. The relationship between fines (%) (derived from Wolman pebble count) and peak *M. cerebralis* infection observed in nine spring creek sites in 2000-2001.

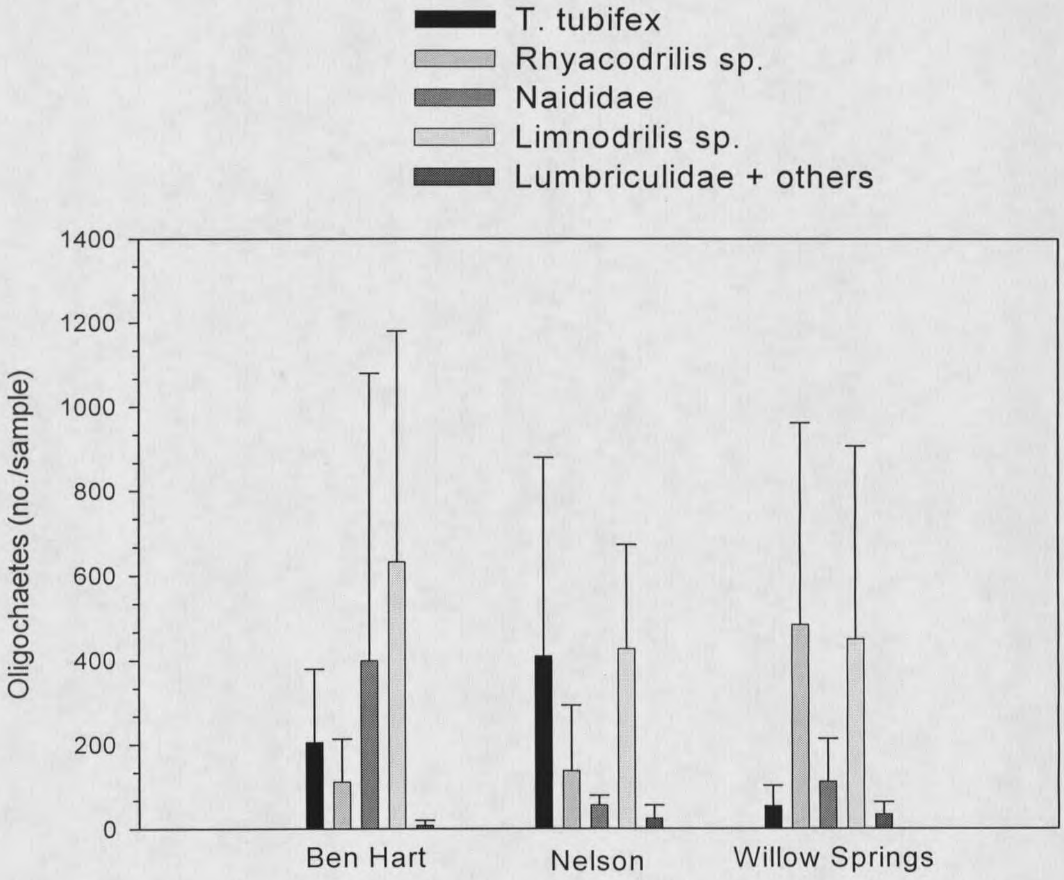


Figure 17. Average number (± standard error) of oligochaetes per sample in Ben Hart Spring Creek (n = 10), Willow Springs Spring Creek (n = 4), and Nelson Spring Creek (n = 5), Montana.

DISCUSSION

The purpose of this study was to determine the occurrence and seasonal dynamics of *M. cerebralis* in Montana spring creeks and to assess what factors influence infection severity. I found *M. cerebralis* infection was common (7 of 9 sites) across a subset of spring creeks but that infection severity varied substantially among sites. Only four of nine sites (44 %) had moderate to high infection severity at levels where population declines would be expected (MacConnell and Vincent 2002). Prevalence and severity of whirling disease among drainages sampled for wild trout have previously been shown to vary in Montana (Baldwin et al. 1998; Vincent 2000). This high variation in whirling disease occurrence and severity has been hypothesized to be a function of several factors, including how long the parasite has been in a drainage; lack of suitable habitat supporting the life cycle of the *T. tubifex* host (Kerans and Zale 2002); differences in *M. cerebralis* spore loading; or abundance of *T. tubifex* (Hedrick et al. 1999; Zendt and Bergersen 2000).

My original hypothesis was that that *M. cerebralis* infection would be of higher magnitude and longer duration in spring creeks, due to habitat and thermal characteristics that support abundant *T. tubifex* populations and high triactinomyxon production. *Myxobolus cerebralis* severity was generally shown to be of higher magnitude in spring creek sites compared to adjacent river sites. My data were inconclusive as for the hypothesis of a longer duration in sentinel fry exposed in spring creeks versus rivers.

However, as discussed below, the timing of infection in spring creeks was very different from that previously reported for infection cycles in rivers.

Spatial variation of whirling disease also existed within a spring creek site (Ben Hart) with infection severity increasing downstream. A similar pattern was reported from studies in Idaho (Hiner and Moffitt 2001), Oregon (Sandell et al. 2001), and Wyoming (Hubert et al. 2002). This occurrence could be explained in part by a lack of spawning sites in upstream portions of study areas (Hubert et al. 2002), and the coinciding lack of migratory fish as a possible source of spores. However, this intra-site variation contrasts with the similarity of infection severity along a 30 km reach of Little Prickly Peak Creek, Montana, where mean lesion scores averaged more than 3.4 at all eight sites (R. Vincent, Montana Fish, Wildlife and Parks, Bozeman, MT, unpublished data).

Although infection was shown to vary longitudinally in a spring creek, no significant differences in paired sentinel cages were found between paired sentinel cages (less than seven meters apart). The similarity of scores between cages of close proximity suggests that a single cage is representative of the immediate site; however, how far the representative area reaches is unclear. Disease severity was shown to vary widely over distances as small as 100 m in the Madison River (Downing et al. 2002).

Seasonal differences in infection severity were found within spring creek sites when comparing spring and fall seasons. Among sites where *M. cerebralis* was present, infection severity was higher during spring months in most sites. Annual differences in disease severity were also found in infected sites, with infection being significantly higher in April and May 2001, compared to April and May 2000. This is similar to a study in the Madison River, Montana, where large annual differences in infection were observed (Downing et al. 2002). This annual variation in infection severity has been hypothesized to be related to yearly differences in flow, water temperature, or recruitment within *T. tubifex* populations (Zendt and Bergersen 2000). The stability of spring creek environments doesn't support this idea as flow and water temperature regimes were similar within and between the two sampling years in my study. This annual variation in infection severity within spring creeks could possibly be related to differences in *T. tubifex* populations between years, or a function of increasing numbers of the parasite as time progresses.

Timing of infection in spring creeks was very different from that previously reported in river or non-spring creek tributaries. The seasonal cycle of *M. cerebralis* detected in sentinel fish exposed in intensively sampled spring creeks during 2000 followed a pattern where infection was highest in the winter and early spring, and decreased to low levels throughout the summer, increasing again in the late fall. For example, peak infection in Willow Springs Spring Creek was seen in November (96.7 %) of 2000, and in January of 2001 (90 %). This contrasts with late May and late June peaks observed in a recent study in the Madison River (Downing et al. 2002) and with a study in the Colorado River where triactinomyxons were shown to be at their highest density during June through September (Thompson and Nehring 2000).

Similar to infection patterns observed in sentinel fish exposed in spring creeks, *M. cerebralis* infection in river sites was not limited to spring and fall months. I expected *M. cerebralis* infection to peak in river sites during spring and fall months based on previous studies (Vincent 1999). Infection was detected in spring and fall months, but was seldom at its peak and infection was not limited to these time periods. I detected infection during all four seasons, and infection in one river site (East Gallatin River) followed a pattern similar to that detected in the intensive spring creek sites with high infection levels in the winter. However, two out of three intensive river sites did not show infection beyond low levels, so a pattern of annual variation of infection could not be extrapolated to all river sites.

The lack of overlap between infection timing and emergence and early rearing of rainbow trout fry (Figure 12) suggested spring-spawning trout would be at low risk of infection, even in spring creeks with high infection severity. Sentinel fish exposure studies indicated the risk of infection for rainbow trout fry during peak emergence and the nine weeks following, (the time period when rainbow are most susceptible to whirling disease; Markiw 1991; Ryce 2003), in Ben Hart and Willow Springs ranged was low during this time period. For example, the highest average number of fish scored ≥ 3 during emergence and the 9-weeks following in Ben Hart was 29 %, and 12 % in Willow Springs. Based on my estimation of emergence timing, the majority of fry (84 %) emerged and reared during periods of low infection risk, even in heavily infected spring creeks. This contrasts with other studies from surface-fed streams where there was a high overlap between the "vulnerable period" and high infection levels in the spring (Sandell et al. 2001; Downing et al. 2002).

In contrast to the lack of overlap between emergence and infection risk for spring spawners, fall spawning trout were shown to have a much higher risk of infection during hatching, emergence, and early rearing. Brown trout fry emergence occurred from December through early April, which coincided with very high infection levels in my two intensively sampled spring creeks. Although brown trout have been reported to be much more resistant to *M. cerebralis* infection as compared to rainbow and cutthroat trout (Hedrick et al. 1999; Baldwin et al. 2000), brown trout can still be infected and could be a source of future spores, thus supporting the continuation of the life cycle. Other fall spawning species, such as brook trout and kokanee, have been shown to be susceptible to *M. cerebralis* (Vincent 2002) and emerging fry are likely to be infected in spring creeks. For example, brook trout numbers have dramatically declined in recent years in Kleinschmidt Spring Creek, a system where infection levels are very high (Ron Pierce, Montana Department of Fish, Wildlife and Parks, personal communication).

Although fall spawning trout are likely to be exposed to high infection levels, stable habitat characteristics in spring creeks may provide a better opportunity for infected fish to survive. Salmonids endure a critical period after emergence (Knight et al. 1999), and rearing in areas with high infection is likely to hinder recruitment. Additional stressors on infected fish have been documented to further hinder survival (e.g., Snieszko 1974). Decreased swimming performance was found with increasing parasite dose level (Ryce 2001) and mortality increased with elevated water temperatures for rainbow trout fry exposed to *M. cerebralis* (Schisler et al. 2000). The lack of high flow events, moderate temperatures and abundant food may positively influence the survival of young trout, including infected fry, by providing a refuge during development. Spring emerging fry that do remain in spring creeks are subject to reduced exposure to *M. cerebralis* during their vulnerable period as infections decline in the spring and summer months throughout this period. Although I didn't examine rearing life history tendencies in this study, addressing if age-0 fish stay in spring creeks would be beneficial. Many studies have documented trout remain in close proximity to their natal areas (Magee et al. 1996; Downing et al. 2002), while others have documented long distance dispersal after emergence (Knight et al. 1999).

Temperature appears to have an important role in determining when infections will occur. The developmental rate of the *M. cerebralis* parasite in both the worm and fish hosts is contingent on temperature (Halliday 1973; El-Matbouli et al. 1999). Previous studies have shown an optimal temperature range for the parasite to flourish (MacConnell and Vincent 2002). Peak infection was shown to occur at temperatures ranging near 10-13 °C (Baldwin et al. 2000; Downing et al. 2002; and Krueger 2002). However, studies in Idaho (Hiner and Moffit 2001) and Oregon (Sandell et al. 2001) did not find a relationship between infection and mean water temperatures. The range of temperatures at which *M. cerebralis* was detected in my study included a broad range of temperatures. Infection in fish exposed in spring creeks was detected at temperatures ranging from 4 °C to 14 °C and in rivers from 0.1 °C to 17.5 °C. Peak infections in spring creeks were found at temperatures ranging from 6 to 12 °C, and from 7.5 °C to 12 °C in river sites. These temperature ranges differ from previous work. MacConnell and Vincent (2002) found *M. cerebralis* infection declined rapidly at temperatures above 17 °C and below 5 °C and found infection to be most severe at temperatures ranging from 12-15 °C. My findings are similar to results from a study in Willow Creek, Montana, where triactinomyxons were recovered at temperature ranging from 1 to 19 °C, with peak numbers found from 4 °C to 12 °C (Hally Lukins, Montana State University, personal communication). According to studies previously mentioned, the temperatures found in spring creeks were favorable for prolonged triactinomyxon release and high infections almost year round; yet this was not detected. Seasonal changes in infection have been previously related to changing water temperature, however, the infection timing in spring creeks is likely not linked directly with this occurrence, as temperature did not vary drastically during the decline of infection. This suggests other factors influence the effects of mean temperature on infection.

There was no clear relationship between disease severity and either habitat features or water quality. Previous studies reported that triactinomyxon viability was reduced as pH decreased or increased from 7 (Smith 2002). Conductivity was positively correlated with *M. cerebralis* infection of rainbow trout in the Lostine River, Oregon (Sandell et al. 2001). However, alkalinity, which is similar to conductivity in being a measure of the productivity of the water, was not correlated with infection levels in spring creek or river sites in my study. However, a significant relationship was detected between the five sites with moderate to high levels of infection and phosphorus. Phosphorus is an important element for plant growth and essential in the metabolic reactions of plants and animals (Mitchell and Stapp 1997). The importance of phosphorus in relation to infection severity needs to be examined more closely as its function was unclear in this study. Several habitat conditions speculated to increase *M. cerebralis* infection (e.g., elevated percentages of fine sediments, presence of brown trout, and non-flushing flows) were also observed in the spring creek study sites. For example, eight of nine spring creeks had high percentages of fines present. However, a statistical relationship was not found between any of the habitat conditions and substrate compositions measured and peak infection detected in the spring creek sites.

Although not statistically supported, other studies have observed high infection to coincide with degradation of habitat. Sentinel fish exposed in the South Fork of Boise River developed severe infections at a site containing high amounts of fines immediately downstream of a beaver dam (Hiner and Moffit 2001). Sentinel fry tested in the Lostine River, Oregon had a higher prevalence of infection with *M. cerebralis* exposed at a site with large accumulations of fines and a low river gradient (Sandell et al. 2001). When tested in a laboratory, *T. tubifex* produced significantly more triactinomyxons when reared in silt substrate as compared with those reared in sand or no substrate (Arndt et al. 2002). However, studies conducted on Rock Creek, Montana found no apparent correlation between *T. tubifex* and the habitat condition of the stream (Granath and Gilbert 2002). *Myxobolus cerebralis* infected *T. tubifex* were collected in pristine areas with little sedimentation and also from degraded sections containing high amounts of fine sediments. There appears to be consensus that eutrophic conditions foster the development of abundant *T. tubifex* populations, but due to the variety of conditions that *T. tubifex* inhabit, habitat does not appear to be the limiting factor.

I found no clear association between disease severity and *T. tubifex* abundance. Infection levels were highest in Willow Springs Spring Creek, the same spring creek where I found the lowest abundance of *T. tubifex*. These finding were unexpected;

however, this could be explained by a recent study on the Madison River that found low densities of *T. tubifex* can still produce sufficient triactinomyxons to be highly infective to trout (Krueger 2002). In the upper Colorado River basin, *T. tubifex* was found to be widespread, but fluctuated in abundance throughout sites, suggesting there are point sources of *M. cerebralis* infection (Zendt and Bergersen 2000). As suggested, although these individual sources may not be significant, the collective effect of these "hot spots" could lead to high levels of infection in a stream. I collected oligochaete samples every 500 m, so *T. tubifex* samples could have been inadvertently overlooked if a "point source" was missed. Nelson Spring Creek had the highest abundance of *T. tubifex*, but infection was consistently found at low to non-detectable levels. Minimal infection could be attributed to the parasite's lack of time in the area and low densities of myxospores in the environment. *Tubifex tubifex* populations have also been shown to fluctuate in their capability of triactinomyxon production. Production effectiveness has been shown to vary by geographic region (Stevens et al. 2001), and preliminary results reveal at least three different genotypes among *T. tubifex*, and only *T. tubifex* with the dominant genotype have been infected with *M. cerebralis* (Granath and Gilbert 2002). The presence and density of *T. tubifex* was not the most important indicator of infection severity in this study and perhaps a combination of many variables including tubificid maturity, suitable habitat to support *T. tubifex,* and an input of viable *M. cerebralis* spores (Markiw 1986) is a better predictor of infection severity.

Timing of peak infection in this study was not limited to the range of temperatures previously reported. Temperature correlated with infection in previous studies, with peak infection shown to occur within a specific range, during two distinct seasons. A seasonal periodicity in triactinomyxon release was proposed to explain previous infection patterns of spring and fall peaks shown in Montana rivers. *Tubifex tubifex* are capable of living for several seasons and can remain infected for the duration of their lives, with the potential of shedding triactinomyxons during various and separate time periods (Granath and Gilbert 2002). In this study, infection was not limited to a specific range of temperatures, and fluctuations in infection did not appear to be linked with water temperature. Other factors not examined in this study such as shifting diel light cycles, discharge, or infection timing in *T. tubifex* have also been suggested to be connected to a seasonality of triactinomyxon production (Arndt et al. 2002).

One possible explanation for unusual infection cycle observed in spring creeks could be related to the temperature-related effects on development rate of *M. cerebralis* (E. MacConnell, U.S. Fish and Wildlife Service, personal communication). Approximately 2250 temperature units (TU) are required for the parasite to complete its entire life cycle (950 TU in trout, 1300 TU in *T. tubifex* (Markiw 1992c)). An important difference between spring creek and river environments is that the accumulation of temperature units in a spring creek is relatively consistent year round, even during winter months when temperatures average near 6-8 °C. Considering the spring and fall infection peaks detected in river sites and the required temperature units for the parasite to complete its life cycle, this suggests parasite development slows during winter months in the river. An input of spores occurs presumably when an infected trout moves into an area to spawn and then either dies or expels spores through excrement in the spring and fall months, or upon death of diseased young trout (Kerans and Zale 2002). The spore is then ingested by a *T. tubifex*, and in 1300 degree-days, triactinomyxons will start to be released. If viable *M. cerebralis* spores are consumed by *T. tubifex* in the spring, triactinomyxons could be released in a river during early fall depending on water temperatures. Alternatively, if the worm ingests a spore in the fall, when temperatures start to decrease, degree-day accumulation will be delayed during winter months, presumably hindering parasite development until the next spring.

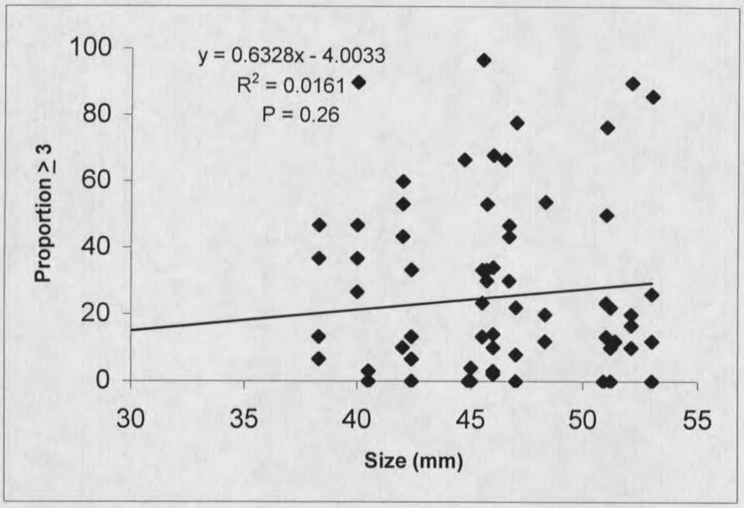
In contrast, degree-day accumulation is not depressed during winter months in spring creeks. Timing of peak infection during winter and early spring and the decrease in summer months, could be explained in part by the timing of spore input and the influence of temperature on development. Instead of an "optimal range" of temperatures for triactinomyxons to be successfully released, therefore, a necessary accumulation of temperature units might instead help to better explain the relationship between water temperature and *M. cerebralis* infection, in both spring creek and river environments. Therefore, an input of viable myxospores, in association with water temperature, may be the factor controlling the timing of peak infection severity.

Conclusions

My findings have important implications with respect to monitoring and managing whirling disease in spring creeks. These results suggest that the appropriate time to monitor peak infection in spring creeks is during winter months, rather than spring and fall months, the common period for monitoring non spring-fed streams. As infection was also detected in river sites during winter months, data collection should also not be limited to spring and fall months in river sites as well. Rivers and creeks with known populations of spawning salmonids should also be monitored at times and locations that are biologically significant, such as near known spawning reaches during emergence periods of susceptible trout.

Sentinel cages provide an indirect measure of triactinomyxon abundance in a tested water body, but are time consuming, expensive, and result in data delays. At the time of this study, they were the most reliable method to test for *M. cerebralis* in rivers and streams. Recently a new drum filtration system has been developed to collect and count triactinomyxons in the field (Hally Lukins, personal communication). This new system could dramatically improve efficiencies in determining triactinomyxon densities. Timely results could be available to inform biologists of current patterns of *M. cerebralis* escalation in their areas, which could aid in determining management plans for dealing with whirling disease.

A potential limitation of my study was the variation in size, age, and strain of rainbow trout fry used in sentinel fish exposures. Recent research has suggested that rainbow trout fry reared for longer than 756 degree-days of development, or at sizes greater than 40 mm have less susceptibility to whirling disease (Ryce 2003). Due to logistical constraints on the availability of rainbow trout fry, the majority of our sentinel rainbow fry were older or larger than this recommended limit. However, in our study, age or size differences did not appear to greatly influence infection severity. Plots of infection severity in relation to age and size (Figure 18) of sentinel fish in exposure



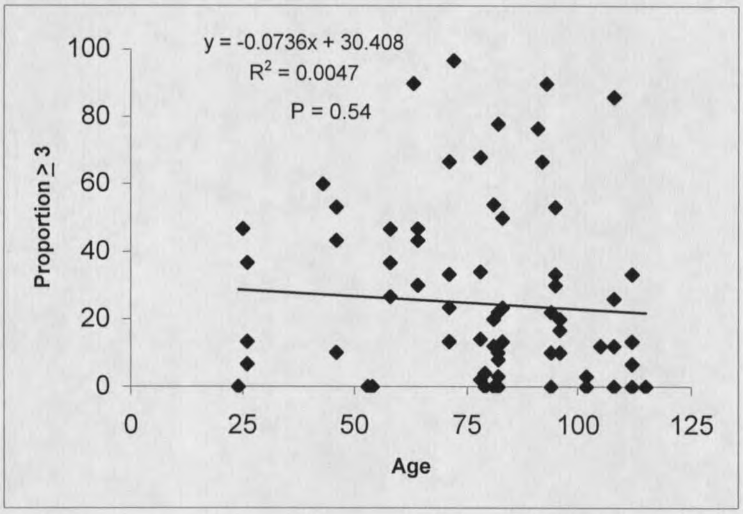


Figure 18. Size and age correlated with proportion of fish graded ≥ 3 in Willow Springs and Ben Hart Spring Creeks, during the 20-month sampling period.

groups showed no apparent relationship, and neither age (r = 0.01, P = 0.54) nor size (r = 0.02, P = 0.26) was correlated with infection severity (Pearson correlation) in the two highly infected spring creeks (Ben Hart and Willow Springs) throughout the 20- month sampling period. Six different rainbow trout strains were used in the study. While no strong evidence for susceptibility among these strains currently exists (Vincent 2002), it is possible that all rainbow trout strains may not be identical in susceptibility to the whirling disease parasite.

Myxobolus cerebralis infection was detected at very high levels in several spring creeks systems, often at a higher magnitude than in adjoining rivers. However, my results suggest that the timing of infection may limit exposure to *M. cerebralis* for spring spawning trout. In contrast, fall spawning trout are likely to be much more vulnerable to high infection risk. This pattern of infection timing was detected in two spring creeks sites and further research is needed to see if this pattern holds in other sites. Moreover, incorporating time sequence studies (e.g., Vincent 2000) to determine exactly when peak infection occurs, when it begins to decline, and when it starts to rise again would be very beneficial, to determine the degree of overlap with vulnerable salmonid fry to infection.

I sampled only a small portion of Montana's spring creeks (< 10%), and sampling of additional sites is warranted given their value to spawning and rearing trout. Determining if the rate of spread of *M. cerebralis* is likely to intensify over time will also be important. For example, Nelson Spring Creek was monitored for 20 continuous months and infection was only detected in a few fish. Other sites, such as Blaine Spring Creek, had higher infection in 2001 compared to 2000, therefore suggesting that infection may be at early stages in some sites. Tubificid populations should be further studied in spring creeks, perhaps by examining particular genotypes, life cycles and interactions within oligochaete assemblages. In addition, further testing of the degree-day hypothesis should be assessed. The affects of habitat improvement in minimizing whirling disease impacts should also be examined as this influence has been speculated, but is uncertain.

Spring creeks have been noted to provide essential spawning and rearing habitat for salmonids, and my results suggests they may become even more important sources for recruitment of rainbow trout and other spring spawners like native cutthroat trout, in whirling disease infected river systems. In contrast, fall spawning trout in these sites will have a higher risk of infection. The preservation of spring creeks should be prioritized so that they can continue to support the strong recruitment of young trout to Montana's rivers and streams.

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APPENDICES

APPENDIX A

SUMMARY OF SENTINEL EXPOSURE DESCRIPTIONS AND DATA.

Study Site	Exposure Period	Strain	Age at Exposure	Size (mm)	Rearing Period	Lab	Histological grade 0	1	2	3	4	5	Proportion ≥ 3	Mean Grade	% Infected	Average Water Temp °C
2000																
JANUARY																
Ben Hart Source	1/26-2/5	Arlee	82	47	81	Pony	49	1	0	0	0	0	0	0.02	2	6.8
Ben Hart Middle	1/26-2/5	Arlee	82	47	81	Pony	8	14	17	6	5	0	22	1.72	84	6.5
Ben Hart Mouth	1/26-2/5	Arlee	82	47	81	Pony	23	13	10	4	0	0	8	0.9	54	5.4
East Gallatin River	1/26-2/5	Arlee	82	47	81	Pony	41	6	1	2	0	0	4	0.28	18	2.2
Nelson Upper	1/26-2/5	Arlee	82	47	81	Pony	50	0	0	0	0	0	0	0	0	7.8
Nelson Lower	1/26-2/5	Arlee	82	47	81	Pony	50	0	0	0	0	0	0	0	0	7.7
Yellowstone River	1/26-2/5	Arlee	82	47	81	Pony	50	0	0	0	0	0	0	0	0	1.7
Willow Springs	1/26-2/5	Arlee	82	47	83	Pony	0	3	8	9	27	2	78	3.35	100	6.6
Jefferson River	1/26-2/5	Arlee	82	47	83	Pony	39	0	0	0	0	0	0	0	0	2.1
FEBRUARY																
Ben Hart Source	2/7-2/17	Arlee	94	51.2	82	Pony	50	0	0	0	0	0	0	0	0	7.5
Ben Hart Middle	2/7-2/17	Arlee	94	51.2	82	Pony	10	13	16	5	6	0	22	1.68	80	7.5
Ben Hart Mouth	2/7-2/17	Arlee	94	51.2	82	Pony	24	11	10	4	1	0	10	0.94	52	6.8
East Gallatin River	2/7-2/17	Arlee	94	51.2	82	Pony	34	9	5	1	1	0	4	0.52	32	4
Nelson Upper	2/7-2/17	Arlee	94	51.2	82	Pony	50	0	0	0	0	0	0	0	0	8
Nelson Lower	2/7-2/17	Arlee	94	51.2	82	Pony	48	0	0	0	0	0	0	0	0	7.9
Yellowstone River	2/7-2/17	Arlee	94	51.2	82	Pony	50	0	0	0	0	0	0	0	0	3.8
Willow Springs	2/7-2/17	Arlee	94	51.2	82	Pony	13	9	17	6	5	0	22	1.62	74	7.4
Jefferson River	2/7-2/17	Arlee	94	51.2	82	Pony	48	1	1	0	0	0	0	0.06	4	2.1
MARCH																
Ben Hart Source	3/6-3/16	Arlee	78	46	80	Pony	45	2	2	0	1	0	2	0.2	10	
Ben Hart Middle	3/6-3/16	Arlee	78	46	80	Pony	15	7	11	4	11	2	34	1.9	50	7.9
Ben Hart Mouth	3/6-3/16	Arlee	78	46	80	Pony	11	12	19	5	2	0	14	1.49	78	7.4
East Gallatin River	3/6-3/16	Arlee	78	46	80	Pony	36	11	2	0	0	0	0	0.31	27	5.5

Nelson Upper	3/6-3/16	Arlee	78	46	80	Pony	49	1	0	0	0	0	0	0.02	2	8.7
Nelson Lower	3/6-3/16	Arlee	78	46	80	Pony	50	0	0	0	0	0	0	0	0	8.7
Yellowstone River	3/6-3/16	Arlee	78	46	80	Pony	50	0	0	0	0	0	0	0	0	5.7
Willow Springs	3/6-3/16	Arlee	78	46	80	Pony	5	3	6	10	18	6	68	3.06	90	7.4
Jefferson River	3/6-3/16	Arlee	78	46	80	Pony	45	0	0	0	0	0	0	0	0	4.7
APRIL																
Ben Hart Source	4/3-4/13	Arlee	108	53	80	WTRL	37	8	5	0	0	0	0	0.36	26	9.1
Ben Hart Middle	4/3-4/13	Arlee	108	53	80	WTRL	13	15	9	11	2	0	26	1.48	74	9.6
Ben Hart Mouth	4/3-4/13	Arlee	108	53	80	WTRL	13	16	15	4	2	0	12	1.32	74	9.6
East Gallatin River	4/3-4/13	Arlee	108	53	80	WTRL	18	20	8	3	1	0	8	0.98	64	9.2
Nelson Upper	4/3-4/13	Arlee	108	53	80	WTRL	48	2	0	0	0	0	0	0.04	4	10.3
Nelson Lower	4/3-4/13	Arlee	108	53	80	WTRL	48	2	0	0	0	0	0	0.04	4	10.4
Yellowstone River	4/3-4/13	Arlee	108	53	80	WTRL	50	0	0	0	0	0	0	0	0	9
Willow Springs	4/3-4/13	Arlee	108	53	80	WTRL	0	5	2	11	32	0	86	3.4	100	8.4
Jefferson River	4/3-4/13	Arlee	108	53	80	WTRL	45	1	0	0	0	0	0	0.02	2	8.4
Anceny Upper	4/11-4/21	Arlee	115	53	80	WTRL	50	0	0	0	0	0	0	0	0	12.9
Anceny Lower	4/11-4/21	Arlee	115	53	80	WTRL	47	0	0	0	0	0	0	0	0	13.2
Gallatin River	4/11-4/21	Arlee	115	53	80	WTRL	50	0	0	0	0	0	0	0	0	6.7
Clark Canyon Spring Upper	4/11-4/21	Arlee	115	53	80	WTRL	50	0	0	0	0	0	0	0	0	9.7
Clark Canyon Spring Lower	4/11-4/21	Arlee	115	53	80	WTRL	23	0	0	0	0	0	0	0	0	9.7
Clark Canyon Reservoir	4/11-4/21	Arlee	115	53	80	WTRL	16	23	7	4	0	0	8	0.98	68	10
Blaine Upper	4/11-4/21	Arlee	115	53	80	WTRL	21	19	9	1	0	0	2	0.8	58	11.3
Blaine Lower	4/11-4/21	Arlee	115	53	80	WTRL	22	18	9	1	0	0	2	0.78	56	11.3
Madison River	4/11-4/21	Arlee	115	53	80	WTRL	8	10	18	10	4	0	28	1.84	84	7.4
Kleinschmidt Upper	4/14-4/24	Arlee	119	53	80	WTRL	0	5	2	7	29	7	68	3.62	100	9.1
Kleinschmidt Lower	4/14-4/24	Arlee	119	53	80	WTRL	3	9	4	4	26	4	68	3.06	94	9.2
Rock Creek	4/14-4/24	Arlee	119	53	80	WTRL	7	11	12	10	9	1	40	2.12	86	8.3
Mitchel-Slough	4/14-4/24	Arlee	119	53	80	WTRL	50	0	0	0	0	0	0	0	0	10.7
Bitterroot River	4/14-4/24	Arlee	119	53	80	WTRL	50	0	0	0	0	0	0	0	0	7.3

MAY																
Ben Hart Source	5/5-5/15	Eagle Lake	81	48.3	82	WTRL	18	7	15	7	3	0	20	1.4	64	9.6
Ben Hart Middle	5/5-5/15	Eagle Lake	81	48.3	82	WTRL	2	5	16	9	15	3	54	2.78	96	10.2
Ben Hart Mouth	5/5-5/15	Eagle Lake	81	48.3	82	WTRL	11	20	13	4	2	0	12	1.32	78	10.7
East Gallatin River	5/5-5/15	Eagle Lake	81	48.3	82	WTRL	16	20	12	2	0	0	4	1	68	11
Nelson Upper	5/5-5/15	Eagle Lake	81	48.3	82	WTRL	50	0	0	0	0	0	0	0	0	10.3
Nelson Lower	5/5-5/15	Eagle Lake	81	48.3	82	WTRL	49	1	0	0	0	0	0	0.02	2	10.3
Willow Springs	5/29-6/8	Eagle Lake	105	51.4	81	WTRL	20	18	6	6	0	0	12	0.96	60	10.8
Jefferson River	5/29-6/8	Eagle Lake	105	51.4	81	WTRL	44	5	1	0	0	0	0	0.14	12	12.5
Anceny Upper	5/5-5/15	Eagle Lake	81	48.3	82	WTRL	50	0	0	0	0	0	0	0	0	12.9
Anceny Lower	5/5-5/15	Eagle Lake	81	48.3	82	WTRL	50	0	0	0	0	0	0	0	0	12.6
Gallatin River	5/17-5/26	Eagle Lake	93	50.1	82	WTRL	50	0	0	0	0	0	0	0	0	4.8
Clark Canyon Spring Upper	5/29-6/8	Eagle Lake	105	51.4	81	WTRL	49	0	0	0	0	0	0	0	0	9.9
Clark Canyon Spring Lower	5/29-6/8	Eagle Lake	105	51.4	81	WTRL	50	0	0	0	0	0	0	0	0	10
Blaine Upper	5/29-6/8	Eagle Lake	105	51.4	81	WTRL	50	0	0	0	0	0	0	0	0	12.3
Blaine Lower	5/29-6/8	Eagle Lake	105	51.4	81	WTRL	47	2	0	0	0	0	0	0.04	4	12.4
Madison River	5/29-6/8	Eagle Lake	105	51.4	81	WTRL	6	11	11	14	8	0	44	2.14	88	9.8
Kleinschmidt Upper	5/22-5-31	Eagle Lake	98	50.1	81	WTRL	2	3	6	4	24	11	78	3.56	96	10.6
Kleinschmidt Lower	5/22-5/31	Eagle Lake	98	50.1	81	WTRL	2	1	4	10	28	5	86	3.25	96	10.9
Rock Creek	5/22-5/31	Eagle Lake	98	50.1	81	WTRL	10	7	14	9	10	0	38	2.04	80	10.3
N.Fork Blackfoot	5/22-5/31	Eagle Lake	98	50.1	81	WTRL	49	1	0	0	0	0	0	0.02	2	7.6
Mitchel-Slough	5/11-5/22	Eagle Lake	87	50.1	83	WTRL	49	1	0	0	0	0	0	0.02	2	10.5
JUNE																
Ben Hart Source	6/6-6/16	Eagle Lake	79	45	89	WTRL	28	18	2	2	0	0	4	0.56	44	10.7
Ben Hart Middle	6/6-6/16	Eagle Lake	79	45	89	WTRL	25	16	9	0	0	0	0	0.68	50	11.7
Ben Hart Mouth	6/6-6/16	Eagle Lake	79	45	89	WTRL	45	4	1	0	0	0	0	0.12	10	13.2
East Gallatin River	6/6-6/16	Eagle Lake	79	45	89	WTRL	24	7	7	2	0	0	5	0.68	40	14.5
Nelson Upper	6/6-6/16	Eagle Lake	79	45	89	WTRL	50	0	0	0	0	0	0	0	0	11.8
Nelson Lower	6/6-6/16	Eagle Lake	79	45	89	WTRL	50	0	0	0	0	0	0	0	0	11.7

Yellowstone River	6/6-6/16	Eagle Lake	79	45	89	WTRL	14	1	1	0	0	0	0	0.19	12	11.4
Willow Springs	6/8-6/18	Eagle Lake	81	45	87	WTRL	44	4	2	0	0	0	0	0.16	12	10.8
Jefferson River	6/8-6/18	Eagle Lake	81	45	87	WTRL	47	2	0	0	0	0	0	0.04	4	15.3
JULY																
Ben Hart Source	7/1-7/11	Eagle Lake	82	46	82	WTRL	18	8	1	3	0	0	10	0.63	40	11.6
Ben Hart Middle	7/1-7/11	Eagle Lake	82	46	85	WTRL	16	8	3	3	0	0	10	0.77	46	12.6
Ben Hart Mouth	7/1-7/11	Eagle Lake	82	46	85	WTRL	11	11	7	1	0	0	3	0.93	63	14.3
East Gallatin River Upper	7/1-7/11	Eagle Lake	82	46	82	WTRL	29	0	1	0	0	0	0	0.07	3	17.5
East Gallatin River Lower	7/1-7/11	Eagle Lake	82	46	82	WTRL	14	6	4	1	0	0	4	0.68	44	17.4
Nelson Upper	7/1-7/11	Eagle Lake	82	46	82	WTRL	30	0	0	0	0	0	0	0	14	12.7
Nelson Lower	7/1-7/11	Eagle Lake	82	46	82	WTRL	29	1	0	0	0	0	0	0.03	3	12.8
Yellowstone River Upper	7/1-7/11	Eagle Lake	82	46	82	WTRL	30	0	0	0	0	0	0	0	0	16.5
Yellowstone River Lower	7/1-7/11	Eagle Lake	82	46	82	WTRL	30	0	0	0	0	0	0	0	0	16.5
Willow Springs Upper	7/1-7/11	Eagle Lake	82	46	82	WTRL	29	1	0	0	0	0	0	0.03	3	11.7
Willow Springs Lower	7/1-7/11	Eagle Lake	82	46	82	WTRL	5	15	7	3	0	0	10	1.27	83	11.4
Jefferson River Upper	7/1-7/11	Eagle Lake	82	46	85	WTRL	30	0	0	0	0	0	0	0	0	18.8
Jefferson River Lower	7/1-7/11	Eagle Lake	82	46	82	WTRL	30	0	0	0	0	0	0	0	0	18.8
AUGUST																
Ben Hart Source	8/4-8/14	Eagle Lake	115	50.9	80	WTRL	29	1	0	0	0	0	0	0.03	3	11.7
Ben Hart Middle	8/4-8/14	Eagle Lake	115	50.9	80	WTRL	25	5	0	0	0	0	0	0.17	16	12.9
Ben Hart Mouth	8/4-8/14	Eagle Lake	115	50.9	80	WTRL	30	0	0	0	0	0	0	0	0	14.5
East Gallatin River Upper	8/4-8/14	Eagle Lake	115	50.9	80	WTRL	30	0	0	0	0	0	0	0	0	17.7
East Gallatin River Lower	8/4-8/14	Eagle Lake	115	50.9	80	WTRL	30	0	0	0	0	0	0	0	0	17.6
Nelson Upper	8/4-8/14	Eagle Lake	115	50.9	80	WTRL	30	0	0	0	0	0	0	0	0	12.3
Nelson Lower	8/4-8/14	Eagle Lake	115	50.9	80	WTRL	30	0	0	0	0	0	0	0	0	12.5
Yellowstone River Upper	8/4-8/14	Eagle Lake	115	50.9	80	WTRL	30	0	0	0	0	0	0	0	0	18.6
Yellowstone River Lower	8/4-8/14	Eagle Lake	115	50.9	80	WTRL	30	0	0	0	0	0	0	0	0	18.6
Willow Springs Upper	8/4-8/14	Eagle Lake	115	50.9	80	WTRL	30	0	0	0	0	0	0	0	0	11.7
Willow Springs Lower	8/4-8/14	Eagle Lake	115	50.9	80	WTRL	30	0	0	0	0	0	0	0	0	11.7

Jefferson River Lower	8/4-8/14	Eagle Lake	115	50.9	80	WTRL	30	0	0	0	0	0	0	0	0	17.6
SEPTEMBER																
Ben Hart Source	9/21-10/1	Madison	102	40.5	80	WTRL	27	3	0	0	0	0	0	0.1	10	9.2
Ben Hart Middle	9/21-10/1	Madison	102	40.5	80	WTRL	28	1	1	0	0	0	0	0.1	6	9.5
Ben Hart Mouth	9/21-10/1	Madison	102	40.5	80	WTRL	30	0	0	0	0	0	0	0	0	9.4
East Gallatin River Upper	9/21-10/1	Madison	102	40.5	80	WTRL	27	3	0	0	0	0	0	0.1	10	9.1
East Gallatin River Lower	9/21-10/1	Madison	102	40.5	80	WTRL	28	2	0	0	0	0	0	0.07	6	9
Nelson Upper	9/21-10/1	Madison	102	40.5	80	WTRL	30	0	0	0	0	0	0	0	0	9.9
Nelson Lower	9/21-10/1	Madison	102	40.5	80	WTRL	30	0	0	0	0	0	0	0	0	10
Yellowstone River Upper	9/21-10/1	Madison	102	40.5	80	WTRL	28	2	0	0	0	0	0	0.07	6	9.3
Yellowstone River Lower	9/21-10/1	Madison	102	40.5	80	WTRL	30	0	0	0	0	0	0	0	0	9.2
Willow Springs Upper	9/21-10/1	Madison	102	40.5	80	WTRL	17	5	4	3	1	0	13.3	0.86	43	9.4
Willow Springs Lower	9/21-10/1	Madison	102	40.5	80	WTRL	21	6	2	0	1	0	3	0.47	30	9.6
Jefferson River Upper	9/21/10/1	Madison	102	40.5	80	WTRL	30	0	0	0	0	0	0	0	0	9.3
Jefferson River Lower	9/21-10/1	Madison	102	40.5	80	WTRL	30	0	0	0	0	0	0	0	0	9.4
OCTOBER																
Ben Hart Source	10/1-10/11	Madison	112	42.4	81	Pony	16	5	7	1	1	0	6.7	0.87	46	8.9
Ben Hart Middle	10/1-10/11	Madison	112	42.4	81	Pony	14	4	8	2	2	0	13.3	1.13	53	9.2
Ben Hart Mouth	10/1-10/11	Madison	112	42.4	81	Pony	26	3	1	0	0	0	0	0.17	13	9.1
East Gallatin River Upper	10/1-10/11	Madison	112	42.4	81	Pony	12	10	6	2	0	0	6.7	0.93	60	8.4
East Gallatin River Lower	10/1-10/11	Madison	112	42.4	81	Pony	21	3	2	3	1	0	13.3	0.67	30	8.4
Nelson Upper	10/1-10/11	Madison	112	42.4	81	Pony	30	0	0	0	0	0	0	0	0	9.8
Nelson Lower	10/1-10/11	Madison	112	42.4	81	Pony	30	0	0	0	0	0	0	0	0	9.8
Yellowstone River Upper	10/1-10/11	Madison	112	42.4	81	Pony	29	0	1	0	0	0	0	0.07	3	8.8
Yellowstone River Lower	10/1-10/11	Madison	112	42.4	81	Pony	30	0	0	0	0	0	0	0	0	8.8
Willow Spring Upper	10/1-10/11	Madison	112	42.4	81	Pony	6	4	6	9	4	1	46.7	2.13	80	9.2
Willow Spring Lower	10/1-10/11	Madison	112	42.4	81	Pony	6	4	10	6	4	0	33.3	1.93	80	9.5
Jefferson River Upper	10/1-10/11	Madison	112	42.4	81	Pony	27	2	1	0	0	0	0	0.13	10	8.7
Jefferson River Lower	10/1-10/11	Madison	112	42.4	81	Pony	28	0	1	1	0	0	3.3	0.17	6	8.8

Anceny Upper	10/24-11/3	Erwin	55	50.8	80	Pony	30	0	0	0	0	0	0	0	0	13.2
Anceny Lower	10/24-11/3	Erwin	55	50.8	80	Pony	30	0	0	0	0	0	0	0	0	12.8
Gallatin River Upper	10/24-11/3	Erwin	55	50.8	80	Pony	30	0	0	0	0	0	0	0	0	6
Gallatin River Lower	10/24-11/3	Erwin	55	50.8	80	Pony	29	0	0	1	0	0	3.3	0.1	3	6
Clark Canyon Spring Upper	10/17-10/27	Erwin	48	50.8	80	Pony	30	0	0	0	0	0	0	0	0	10.2
Clark Canyon Spring Lower	10/17-10/27	Erwin	48	50.8	80	Pony	30	0	0	0	0	0	0	0	0	10
Blaine Upper	10/18-10/28	Erwin	49	50.8	80	Pony	25	0	3	1	1	0	6.7	0.43	16	9.6
Blaine Lower	10/18-10/28	Erwin	49	50.8	80	Pony	21	2	6	1	0	0	3.3	0.57	30	9.5
Madison River Upper	10/18-10/28	Erwin	49	50.8	80	Pony	0	1	1	6	17	5	93.3	3.8	100	7.5
Madison River Lower	10/18-10/28	Erwin	49	50.8	80	Pony	0	1	1	4	20	4	93.3	3.8	100	7.5
Kleinschmidt Lower	10/4-10/14	Erwin	115	50.8	80	Pony	7	0	7	9	6	1	53.3	2.33	76	8.6
Rock Creek Upper	10/4-10/14	Erwin	115	50.8	80	Pony	10	9	6	2	3	0	16.7	1.3	66	8.4
Rock Creek Lower	10/4-10/14	Erwin	115	50.8	80	Pony	3	4	9	9	5	0	46.7	2.3	90	8.4
N.Fork Blackfoot Upper	10/4-10/14	Erwin	115	50.8	80	Pony	7	4	6	3	7	3	43.3	2.27	76	9.5
N.Fork Blackfoot Lower	10/4-10/14	Erwin	115	50.8	80	Pony	8	4	5	6	5	2	43.3	2.06	73	9.7
Mitchel-Slough Upper	10/19-10/29	Erwin	50	50.8	80	Pony	30	0	0	0	0	0	0	0	0	9.4
Mitchel-Slough Lower	10/19-10/29	Erwin	50	50.8	80	Pony	30	0	0	0	0	0	0	0	0	8.9
Bitterroot River Upper	10/19-10/29	Erwin	50	50.8	80	Pony	30	0	0	0	0	0	0	0	0	7.9
NOVEMBER																
Ben Hart Source	11/9-11/19	Erwin	71	45.5	80	Pony	5	8	10	7	0	0	23.3	1.63	83	6.4
Ben Hart Source*	11/9-11/19	Erwin	71	45.5	80	Pony	13	6	7	3	1	0	13.3	1.1	56	6.4
Ben Hart Middle	11/9-11/19	Erwin	71	45.5	80	Pony	2	10	8	9	1	0	33.3	1.9	93	6
Ben Hart Mouth	11/9-11/19	Erwin	71	45.5	80	Pony	14	8	3	5	0	0	16.7	0.97	53	4.9
Ben Hart Mouth*	11/9-11/19	Erwin	71	45.5	80	Pony	10	7	9	4	0	0	13.3	1.23	66	4.9
East Gallatin River Upper	11/9-11/19	Erwin	71	45.5	80	Pony	2	9	12	5	2	0	23.3	1.87	93	1.5
East Gallatin River Lower	11/9-11/19	Erwin	71	45.5	80	Pony	3	14	7	3	3	0	20	1.63	90	1.6
Nelson Upper	11/9-11/19	Erwin	71	45.5	80	Pony	29	1	0	0	0	0	0	0.03	3	7.6
Nelson Lower	11/9-11/19	Erwin	71	45.5	80	Pony	29	1	0	0	0	0	0	0.03	3	7.4
Willow Springs Upper	11/10-11/20	Erwin	72	45.5	81	Pony	0	0	2	7	19	2	93.3	3.7	100	6.6

Willow Springs Lower	11/10-11/20	Erwin	72	45.5	81	Pony	0	0	1	2	20	7	96.7	4.1	100	6.9
Willow Springs Lower *	11/10-11/20	Erwin	72	45.5	81	Pony	0	0	1	8	12	9	96.7	3.97	100	6.9
Jefferson River Lower	11/10-11/20	Erwin	72	45.5	81	Pony	12	4	4	1	0	1	6.7	0.91	45	0.3
DECEMBER																
Ben Hart Source	12/4-12/14	Erwin	96	52.1	82	Pony	17	6	4	3	0	0	10	0.76	43	6.1
Ben Hart Middle	12/4-12/14	Erwin	96	52.1	82	Pony	7	11	7	3	2	0	16.7	1.4	76	5.5
Ben Hart Mouth	12/4-12/14	Erwin	96	52.1	82	Pony	11	4	9	5	1	0	20	1.37	63	3.9
Nelson Upper	12/5-12/16	Erwin	97	52.1	80	Pony	28	1	1	0	0	0	0	0.1	6	7
Nelson Lower	12/5-12/16	Erwin	97	52.1	80	Pony	28	1	1	0	0	0	0	0.1	6	6.7
Willow Springs Upper	12/1-12/11	Erwin	93	52.1	81	Pony	0	1	4	7	15	3	83.3	3.5	100	6.6
Willow Springs Lower	12/1-12/11	Erwin	93	52.1	81	Pony	0	0	3	7	18	2	90	3.63	100	6.9
Jefferson River Lower	12/1-12/11	Erwin	93	52.1	81	Pony	17	7	3	3	0	0	10	0.73	43	0.1
2001																
JANUARY																
Ben Hart Source	1/18-1/28	Arlee	58	40	80	Pony	12	3	7	5	3	0	26.7	1.47	60	6.3
Ben Hart Middle	1/18-1/28	Arlee	58	40	80	Pony	0	3	13	4	10	0	46.7	2.7	100	5.8
Ben Hart Mouth	1/18-1/28	Arlee	58	40	80	Pony	8	1	10	7	4	0	36.7	1.93	73	4.3
East Gallatin River Upper	1/18-1/28	Arlee	58	40	80	Pony	1	5	5	8	9	2	63.3	2.83	96	1.7
East Gallatin River Lower	1/18-1/28	Arlee	58	40	80	Pony	8	6	5	6	5	0	25.4	1.8	73	1.7
Nelson Upper	1/18-1/28	Arlee	63	40	80	Pony	30	0	0	0	0	0	0	0	0	7.1
Nelson Lower	1/18-1/28	Arlee	63	40	80	Pony	30	0	0	0	0	0	0	0	0	7.6
Willow Springs Upper	1/23-2/2	Arlee	63	40	80	Pony	0	3	8	9	7	3	63.3	2.97	100	6.4
Willow Springs Lower	1/23-2/2	Arlee	63	40	80	Pony	1	1	1	3	18	6	90	3.8	96	6.7
FEBRUARY																
Ben Hart Source	2/12-2/22	Arlee	83	51	81	Pony	7	9	10	3	1	0	13.3	1.4	76	6.7
Ben Hart Middle	2/12-2/22	Arlee	83	51	81	Pony	2	7	6	10	4	1	50	2.3	93	6.3
Ben Hart Mouth	2/12-2/22	Arlee	83	51	81	Pony	3	8	12	3	4	0	23.3	1.9	90	5.1
East Gallatin River Upper	2/12-2/22	Arlee	83	51	81	Pony	1	3	10	12	4	0	53.3	2.5	96	
East Gallatin River Lower	2/12-2/22	Arlee	83	51	81	Pony	3	8	8	9	2	0	36.7	1.97	90	2.4

Nelson Upper	2/29-3/1	Arlee	90	51	81	Pony	29	1	0	0	0	0	0	0.03	3	7.9
Nelson Lower	2/29-3/1	Arlee	90	51	81	Pony	30	0	0	0	0	0	0	0	0	8.2
Willow Springs Upper	2/20-3/2	Arlee	91	51	80	Pony	2	3	8	11	5	2	60	2.65	93	6.7
Willow Springs Lower	2/20-3/2	Arlee	91	51	80	Pony	0	2	5	13	8	2	76.7	3.1	100	6.7
Jefferson Lower	2/20-3/2	Arlee	91	51	80	Pony	30	0	0	0	0	0	0	0	0	0.27
MARCH																
Ben Hart Source	3/16-3/26	Shasta	46	42	80	WTRL	10	6	11	2	1	0	10	1.27	66	8.3
Ben Hart Middle	3/16-3/26	Shasta	46	42	80	WTRL	12	0	2	11	5	0	53.3	1.9	60	8.5
Ben Hart Mouth	3/16-3/26	Shasta	46	42	80	WTRL	11	1	5	9	4	0	43.3	1.8	63	8.3
East Gallatin River Upper	3/16-3/26	Shasta	46	42	80	WTRL	10	3	3	7	4	3	46.7	2.03	66	6.7
East Gallatin River Lower	3/16-3/26	Shasta	46	42	80	WTRL	8	1	7	9	3	2	46.7	2.13	73	6.7
Nelson Upper	3/15-3/25	Shasta	45	42	80	WTRL	30	0	0	0	0	0	0	0	0	9.3
Nelson Lower	3/15-3/25	Shasta	45	42	80	WTRL	30	0	0	0	0	0	0	0	0	9.2
Yellowstone River Upper	3/15-3/25	Shasta	45	42	80	WTRL	30	0	0	0	0	0	0	0	0	6.4
Yellowstone River Lower	3/15-3/25	Shasta	45	42	80	WTRL	30	0	0	0	0	0	0	0	0	
Willow Springs Upper	3/13-3/23	Shasta	43	42	80	WTRL	12	1	2	4	7	4	50	2.17	60	7.3
Willow Springs Lower	3/13-3/23	Shasta	43	42	80	WTRL	8	1	3	2	6	10	60	2.9	73	7.5
Jefferson River Upper	3/13-3/23	Shasta	43	42	80	WTRL	29	0	1	0	0	0	0	0.07	3	4.7
Jefferson River Lower	3/13-3/23	Shasta	43	42	80	WTRL	29	1	0	0	0	0	0	0.03	3	4.6
APRIL																
Ben Hart Source	4/3-4/13	Shasta	64	46.7	80	WTRL	7	7	7	5	4	0	30	1.7	76	8.5
Ben Hart Middle	4/3-4/13	Shasta	64	46.7	80	WTRL	5	4	7	3	10	1	46.7	2.4	83	8.7
Ben Hart Mouth	4/3-4/13	Shasta	64	46.7	80	WTRL	8	3	6	9	4	0	43.3	1.93	73	8.4
East Gallatin River Upper	4/3-4/13	Shasta	64	46.7	80	WTRL	6	3	7	5	7	2	46.7	2.3	80	7.3
East Gallatin River Lower	4/3-4/13	Shasta	64	46.7	80	WTRL	11	4	4	5	6	0	36.7	1.7	63	7.3
Nelson Upper	4/3-4/13	Shasta	64	46.7	80	WTRL	30	0	0	0	0	0	0	0	0	9.2
Nelson Lower	4/3-4/13	Shasta	64	46.7	80	WTRL	30	0	0	0	0	0	0	0	0	9.4
Yellowstone River Upper	4/3-4/13	Shasta	64	46.7	80	WTRL	30	0	0	0	0	0	0	0	0	7
Yellowstone River Lower	4/3-4/13	Shasta	64	46.7	80	WTRL	29	1	0	0	0	0	0	0.03	3	7

Willow Springs Upper	4/10-4/20	Shasta	71	46.5	80	WTRL	4	2	3	1	9	11	70	3.4	86	7.9
Willow Springs Lower	4/10-4/20	Shasta	71	46.5	80	WTRL	7	0	3	1	9	10	66.7	3.17	76	7.8
Jefferson River Upper	4/10-4/20	Shasta	71	46.5	80	WTRL	29	0	1	0	0	0	0	0.07	3	7.6
Hells Canyon	4/10-4/20	Shasta	71	46.5	80	WTRL	19	1	8	2	0	0	6.7	0	36	
Anceny Upper	4/6-4/16	Shasta	67	46.7	80	WTRL	30	0	0	0	0	0	0	0	0	13.3
Anceny Lower	4/6-4/16	Shasta	67	46.7	80	WTRL	30	0	0	0	0	0	0	0	0	12.8
Gallatin River Upper	4/6-4/16	Shasta	67	46.7	80	WTRL	30	0	0	0	0	0	0	0	0	6
Gallatin River Lower	4/6-4/16	Shasta	67	46.7	80	WTRL	30	0	0	0	0	0	0	0	0	6.1
Clark Canyon Spring Upper	4/10-4/20	Shasta	71	46.5	80	WTRL	30	0	0	0	0	0	0	0	0	9.8
Clark Canyon Spring Lower	4/10-4/20	Shasta	71	46.5	80	WTRL	30	0	0	0	0	0	0	0	0	9.7
Clark Canyon Reservoir Upper	4/10-4/20	Shasta	71	46.5	80	WTRL	25	0	3	2	0	0	6.7	0.4	16	7.4
Clark Canyon Reservoir Lower	4/10-4/20	Shasta	71	46.5	80	WTRL	22	5	1	2	0	0	6.7	0.43	26	7.4
Blaine Upper	4/22-5/2	Shasta	83	47.7	84	WTRL	12	2	3	8	5	0	43.3	1.73	60	11.6
Blaine Lower	4/22-5/2	Shasta	83	47.7	84	WTRL	2	2	11	7	8	0	50	2.57	93	11.7
Madison River Upper	4/22-5/2	Shasta	83	47.7	84	WTRL	9	4	8	7	2	0	30	1.63	70	9.2
Madison River Lower	4/22-5/2	Shasta	83	47.7	84	WTRL	5	4	9	7	3	2	40	2.17	83	9.3
Kleindschmidt Upper	4/7-4/17	Shasta	68	46.5	80	WTRL	3	2	1	0	4	20	80	4	90	6.5
Kleinschmidt Lower	4/7-4/17	Shasta	68	46.5	80	WTRL	3	1	1	3	2	20	83.3	4	90	6.5
Rock Creek Upper	4/7-4/17	Shasta	68	46.5	80	WTRL	3	0	4	2	2	19	76.7	3.9	90	6.8
Rock Creek Lower	4/7-4/17	Shasta	68	46.5	80	WTRL	6	0	1	3	7	13	76.7	3.47	80	6.6
N.Fork Blackfoot Upper	4/7-4/17	Shasta	68	46.5	80	WTRL	30	0	0	0	0	0	0	0	0	6
N.Fork Blackfoot Lower	4/7-4/17	Shasta	68	46.5	80	WTRL	29	0	1	0	0	0	0	0.07	3	6
Mitchel-Slough Upper	4/19-4/29	Shasta	80	47.6	80	WTRL	30	0	0	0	0	0	0	0	0	10
Mitchel-Slough Lower	4/19-4/29	Shasta	80	47.6	80	WTRL	30	0	0	0	0	0	0	0	0	9.9
Bitterroot River Upper	4/19-4/29	Shasta	80	47.6	80	WTRL	30	0	0	0	0	0	0	0	0	8.8
Bitterroot River Lower	4/19-4/29	Shasta	80	47.6	80	WTRL	30	0	0	0	0	0	0	0	0	8.9
MAY																
Ben Hart Source	5/4-5/14	Shasta	95	45.7	80	WTRL	11	2	8	7	1	1	30	1.6	63	10.7

Ben Hart Middle	5/4-5/14	Shasta	95	45.7	80	WTRL	10	1	3	4	3	9	53.3	2.53	66	11
Ben Hart Mouth	5/4-5/14	Shasta	95	45.7	80	WTRL	10	2	8	7	3	0	33.3	1.7	66	11.6
East Gallatin River Upper	5/4-5/14	Shasta	95	45.7	80	WTRL	12	0	3	6	4	5	50	2.17	60	12.2
East Gallatin River Lower	5/4-5/14	Shasta	95	45.7	80	WTRL	7	5	4	4	5	5	46.7	2.33	76	12.1
Nelson Upper	5/4-5/14	Shasta	95	45.7	80	WTRL	28	2	0	0	0	0	0	0.07	6	9.2
Nelson Lower	5/4-5/14	Shasta	95	45.7	80	WTRL	30	0	0	0	0	0	0	0	0	9.4
Willow Springs Upper	5/1-5/11	Shasta	92	44.7	80	WTRL	4	3	4	2	8	9	63.7	3.13	86	8.7
Willow Springs Lower	5/1-5/11	Shasta	92	44.7	80	WTRL	7	1	2	1	5	14	66.7	3.27	76	8.7
Jefferson River Upper	5/1-5/11	Shasta	92	44.7	80	WTRL	20	8	1	1	0	0	3.3	0.43	33	13.6
Anceny Upper	4/30-5/10	Shasta	91	44.7	80	WTRL	30	0	0	0	0	0	0	0	0	12.8
Anceny Lower	4/30-5/10	Shasta	91	44.7	80	WTRL	30	0	0	0	0	0	0	0	0	12.9
Gallatin River Upper	4/30-5/10	Shasta	91	44.7	80	WTRL	30	0	0	0	0	0	0	0	0	5.2
Gallatin River Lower	4/30-5/10	Shasta	91	44.7	80	WTRL	30	0	0	0	0	0	0	0	0	5.4
Clark Canyon Spring Upper	5/1-5/11	Shasta	92	44.7	80	WTRL	30	0	0	0	0	0	0	0	0	9.9
Clark Canyon Spring Lower	5/1-5/11	Shasta	92	44.7	80	WTRL	30	0	0	0	0	0	0	0	0	9.9
Clark Canyon Reservoir Upper	5/1-5/11	Shasta	92	44.7	80	WTRL	8	2	8	5	0	1	25	1.58	66	
Clark Canyon Reservoir Lower	5/1-5/11	Shasta	92	44.7	80	WTRL	11	11	6	1	1	0	6.7	1	63	9.7
Blaine Upper	5/11-5/21	Shasta	102	46.4	80	WTRL	9	6	3	10	2	0	40	1.67	70	13
Madison River Upper	5/11-5/21	Shasta	102	46.4	80	WTRL	5	4	3	3	6	9	60	2.93	83	10.5
Madison River Lower	5/11-5/21	Shasta	102	46.4	80	WTRL	8	2	4	6	7	3	53.3	2.37	73	10.7
Kleinschmidt Upper	5/10-5/20	Shasta	101	47	80	WTRL	2	3	4	1	8	12	70	3.53	93	10.1
Kleinschmidt Lower	5/10-5/20	Shasta	101	47	80	WTRL	1	3	2	2	10	12	80	3.77	96	
Rock Creek Upper	5/10-5/20	Shasta	101	47	80	WTRL	6	3	6	3	8	4	50	2.53	80	9.2
Rock Creek Lower	5/10-5/20	Shasta	101	47	80	WTRL	7	1	2	3	9	8	66.7	3	76	
N.Fork Blackfoot Lower	5/10-5/20	Shasta	101	47	80	WTRL	17	7	6	0	0	0	0	0.63	43	
Mitchel-Slough Upper	5/12-5/22	Shasta	103	48.4	80	WTRL	30	0	0	0	0	0	0	0	0	10.9
Mitchel-Slough Lower	5/12-5/22	Shasta	103	48.4	80	WTRL	30	0	0	0	0	0	0	0	0	10.8

JUNE

Ben Hart Source	6/5-6/15	Fish Lake	26	38.3	80	WTRL	15	5	6	4	0	0	13.3	0.97	50	11.2
Ben Hart Middle	6/5-6/15	Fish Lake	26	38.3	80	WTRL	11	0	8	4	6	1	36.7	1.9	63	11.7
Ben Hart Mouth	6/5-6/15	Fish Lake	26	38.3	80	WTRL	21	5	2	2	0	0	6.7	0.5	30	12.7
East Gallatin River Upper	6/5-6/15	Fish Lake	26	38.3	80	WTRL	7	0	5	4	9	5	60	2.77	76	14
East Gallatin River Lower	6/5-6/15	Fish Lake	26	38.3	80	WTRL	30	0	0	0	0	0	0	0	0	
Nelson Upper	6/5-6/15	Fish Lake	26	38.3	80	WTRL	30	0	0	0	0	0	0	0	0	11.8
Nelson Lower	6/5-6/15	Fish Lake	26	38.3	80	WTRL	30	0	0	0	0	0	0	0	0	11.8
Willow Springs Upper	6/4-6/14	Fish Lake	25	38.3	80	WTRL	10	5	9	2	3	1	20	1.53	66	11.4
Willow Springs Lower	6/4-6/14	Fish Lake	25	38.3	80	WTRL	9	1	6	7	5	2	46.7	2.13	70	10.9
Jefferson River Upper	6/4-6/14	Fish Lake	25	38.3	80	WTRL	23	4	2	1	0	0	3.3	0.37	23	14.1
Jefferson River Lower	6/4-6/14	Fish Lake	25	38.3	80	WTRL	27	0	1	2	0	0	6.7	0.27	10	14.2
JULY																
Ben Hart Source	7/2-7/12	Fish Lake	53	44.9	80	WTRL	23	7	0	0	0	0	0	0.23	23	12.4
Ben Hart Middle	7/2-7/12	Fish Lake	53	44.9	80	WTRL	21	8	1	0	0	0	0	0.33	30	13.5
Ben Hart Mouth	7/2-7/12	Fish Lake	53	44.9	80	WTRL	26	3	1	0	0	0	0	0.17	13	15.1
Nelson Upper	7/2-7/12	Fish Lake	53	44.9	80	WTRL	30	0	0	0	0	0	0	0	0	12.6
Nelson Lower	7/2-7/12	Fish Lake	53	44.9	80	WTRL	30	0	0	0	0	0	0	0	0	13.2
Yellowstone River Upper	7/2-7/12	Fish Lake	53	44.9	80	WTRL	30	0	0	0	0	0	0	0	0	19.1
Yellowstone River Lower	7/2-7/12	Fish Lake	53	44.9	80	WTRL	30	0	0	0	0	0	0	0	0	19.2
Willow Springs Upper	7/3-7/13	Fish Lake	54	44.9	80	WTRL	30	0	0	0	0	0	0	0	0	11.6
Willow Springs Lower	7/3-7/13	Fish Lake	54	44.9	80	WTRL	30	0	0	0	0	0	0	0	0	11.3
Jefferson River Upper	7/3-7/13	Fish Lake	54	44.9	80	WTRL	30	0	0	0	0	0	0	0	0	20.3
AUGUST																
Ben Hart Source	8/25-9/4	Fish Lake	24	29.4	80	WTRL	30	0	0	0	0	0	0	0	0	11.9
Ben Hart Middle	8/25-9/4	Fish Lake	24	29.4	80	WTRL	13	0	1	0	0	0	.0	0.15	7	12.7
Ben Hart Mouth	8/25-9/4	Fish Lake	24	29.4	80	WTRL	30	0	0	0	0	0	0	0	0	14.2
East Gallatin River Lower	8/25-9/4	Fish Lake	24	29.4	80	WTRL	29	0	0	0	0	0	0	0	0	17.3

Willow Springs Upper	8/25-9/4	Fish Lake	24	29.4	80	WTRL	30	0	0	0	0	0	0	0	0	11.9
Willow Springs Lower	8/25-9/4	Fish Lake	24	29.4	80	WTRL	30	0	0	0	0	0	0	0	0	11.5
Jefferson River Upper	8/25-9/4	Fish Lake	24	29.4	80	WTRL	30	0	0	0	0	0	0	0	0	15.8
Jefferson River Lower	8/25-9/4	Fish Lake	24	29.4	80	WTRL	30	0	0	0	0	0	0	0	0	15.9

*indicates replicate cage
WTRL = Wild Trout Research Lab

APPENDIX B

TOTAL AND AVERAGE NUMBER OF OLIGOCHAETES AT EACH SAMPLING SITE IN BEN HART SPRING CREEK, WILLOW SPRINGS SPRING CREEK, AND NELSON SPRING CREEK, MONTANA, FROM 2000.

Sample site	Tubifex tubifex	Rhyacodrilis sp.	Naididae	Limnodrilis sp.	Lumbriculidae + Others
Ben Hart					
1	214	106	8	336	0
2	47	25	344	296	0
3	162	78	112	816	16
4	83	45	0	192	0
5	109	59	96	432	8
6	302	146	160	1408	0
7	26	14	0	192	0
8	213	123	176	512	24
9	624	368	928	1792	0
10	274	142	2168	344	32
Total	**2054**	**1106**	**3992**	**6320**	**80**
Average per sample	**205**	**111**	**399**	**632**	**8**
Nelson					
1	256	88	32	488	0
2	24	8	80	336	72
3	518	170	48	360	8
4	1176	392	80	808	40
5	78	26	40	144	0
Total	**2052**	**684**	**280**	**2136**	**120**
Average per sample	**410**	**137**	**56**	**427**	**24**
Willow Springs					
1	29	115	32	88	0
2	126	1186	256	1104	32
3	33	343	112	192	72
4	27	293	48	416	32
Total	**215**	**1937**	**448**	**1800**	**136**
Average per sample	**54**	**484**	**112**	**450**	**34**