Journal of Aquatic Animal Health 14:191–203, 2002

Relation of Spawning and Rearing Life History of Rainbow Trout and Susceptibility to *Myxobolus cerebralis* Infection in the Madison River, Montana

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Abstract.—The Madison River, Montana, is the site of a whirling disease epizootic among rainbow trout *Oncorhynchus mykiss* caused by the parasite *Myxobolus cerebralis*. We investigated how the timing and location of the spawning and rearing of rainbow trout influences the risk of exposure to the parasite. Sentinel fish exposures were used to assess the spatial and temporal variation in disease severity in 1998 and 1999. Redd counts, radiotelemetry of spawning rainbow trout, and electrofishing estimates of fry abundance indicated that during the critical 2–3-month posthatch period, when rainbow trout are most susceptible to whirling disease, the majority of fry in the Madison River population are concentrated within a relatively small portion of the upper drainage. Disease severity ranged from low to very high in this area of the river. Large temporal variation in disease severity was also evident: severity was significantly higher in the spring than in the fall and in 1999 than in 1998. These data indicate that the effects of whirling disease on rainbow trout populations are governed by a complex interaction between the timing and location of key life history events (spawning, emergence, and early rearing) and the spatial and temporal variation in the presence of the infectious stages of *M. cerebralis*. Life history diversity within rainbow trout populations may therefore help buffer the effects of whirling disease and help explain why population-level effects are so variable among infected populations.

Whirling disease, a parasitic infection caused by the nonindigenous myxozoan *Myxobolus cerebralis*, has been documented throughout the western United States over the past decade (Bergersen and Anderson 1997). The disease has led to major declines in prized wild rainbow trout *Oncorhynchus mykiss* fisheries in some locales (Nehring and Walker 1996; Vincent 1996). Why some infected rainbow trout populations exhibit severe declines while others show little adverse effect is a perplexing question. For example, 46 of 230 sites sampled in Montana have tested positive for the parasite (Baldwin et al. 1998), yet only 6 sites thus far have experienced disease outbreaks and subsequent population declines (E. R. Vincent, unpublished data). In California, *M. cerebralis* infections have been observed since 1965, but few population-level impacts have been reported (Modin 1998). Similarly, *M. cerebralis* has been detected in two drainages in Oregon, but no population declines have been observed to date (Sandell et al. 2001). In contrast, severe population declines from the disease are widespread throughout Colorado drainages (Nehring and Walker 1996; Nehring et al. 1998). Studies of salmonid susceptibility show rainbow trout to be among the most vulnerable to whirling disease, and no significant resistance has been found in any North American rainbow trout stock tested (Hedrick et al. 1998; Hedrick et al. 1999; Thompson et al. 1999; Densmore et al. 2001; Ryce et al. 2001; Vincent 2002).

When and where wild salmonids spawn and rear may greatly influence their level of exposure to *M. cerebralis*. Though rainbow trout can become infected by the parasite at any time from the free-swimming larval to the adult stage (Markiw 1991, 1992), they are most susceptible to disease during the first 9 weeks posthatch (756 degree-days of development; E. Ryce, Montana State University, personal communication).

Fish in this stage of the life cycle are the most vulnerable because *M. cerebralis* attacks cartilage in the cranial and skeletal regions, which is most prevalent during this stage (Hedrick et al. 1998).

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Received October 12, 2001; accepted June 12, 2002

In individual rainbow trout, severe infections at this life stage typically cause blackened tails, cranial deformities, whirling behavior, and mortality (Hedrick et al. 1998). At the population level, severe infections may lead to the recruitment collapse of age-0 fish (Nehring and Walker 1996; Vincent 1996). The extent of overlap between this vulnerable period and the release of the infective triactinomyxon stage of the parasite could therefore determine the degree of exposure for young fish and, ultimately, the magnitude of population-level effects.

Recent studies with sentinel fish exposures indicate that the susceptibility of young rainbow trout to *M. cerebralis* infection may vary widely over space and time, even in highly infected systems (Zendt and Bergersen 2000; Hiner and Moffitt 2001; Sandell et al. 2001). The purpose of our study was to assess how salmonid life history influences the risk of infection. Life history correlations between hosts and their parasites can play a vital role in the severity of disease (Agnew and Koella 1999). The role that salmonid life history plays in influencing disease severity has not been explored in detail. Rainbow trout and other salmonids exhibit variation in spawning and rearing life history traits within and among populations as a result of local adaptation to environmental conditions (Biette et al. 1981; Quinn et al. 2000). Life history differences that could influence the exposure to *M. cerebralis* during the vulnerable period include the timing and location of spawning (Webb and McLay 1996; Henderson et al. 2000), the timing of emergence (Holtby et al. 1989; Webb and McLay 1996), and migration and rearing life history patterns after emergence (Rosenau 1991; Knight et al. 1999).

Rainbow trout in the Madison River, Montana, have experienced a severe population decline from whirling disease. This nationally renowned river has been managed as a wild rainbow and brown trout *Salmo trutta* fishery since the cessation of stocking in the 1970s (Vincent 1987). From 1979 to 1990, age-2 and older rainbow trout averaged 2,500/km, but since 1991 their numbers have declined to about 10–25% of the 1979–1990 mean (Vincent 1996; P. Byorth, Montana Fish, Wildlife, and Parks, personal communication). Whirling disease was confirmed as the cause of the decline in 1994 (Vincent 1996). Disease severity has remained high since 1991, and recruitment of age-0 rainbow trout was very low in 8 of the last 10 years (Vincent 1996; Byorth, personal communication).

Why the effects of whirling disease have been so severe in the Madison River is unknown. Conversely, given the lack of evidence for disease resistance, how some rainbow trout survive is also uncertain. We investigated the role of rainbow trout life history in influencing the risk of exposure to the pathogen by measuring the degree of overlap between certain life history characteristics of rainbow trout (the timing and location of spawning and the timing of fry emergence and early juvenile rearing) and the spatial and temporal variation in the presence of the infectious stages of *M. cerebralis*. A greater understanding of how host and parasite life histories interact may offer options for mitigating population declines among wild rainbow trout due to whirling disease.

Methods

Study area.—The study was conducted on a 46-km section of the Madison River from the Quake Lake outlet to McAtee Bridge near the town of Ennis, Montana (Figure 1). Flows in this section are regulated by releases from Hebgen Reservoir. Average daily discharge during base flow ranges from 30 to 50 m^3/s, with peak flow (80–100 m^3/s) occurring during May and June (Figure 2). The majority of the study section is characterized by fast-water habitat, with main channels dominated by cobble substrate and side channels by gravel and cobble and low fine-sediment deposition. The lower sections of tributary streams experience dewatering and elevated summer temperatures due to irrigation withdrawal. The West Fork tributary is characterized by low gradient and moderate-to-high sediment deposition in its lower reaches.

The main river section was divided into five study reaches based on differences in geomorphology, gradient, and the extent of side channels. The Raynolds reach was characterized by high gradient, an unstable stream channel, and two long side channels with low velocity and abundant spawning gravels. The Three Dollar reach was predominantly high gradient with fewer side channels but numerous boulders with localized spawning gravel deposits. The Pine Butte reach was characterized by moderate gradient and numerous side channels. The Lyons and (particularly) the Palisades reaches had moderate gradients and few side channels. Study reaches ranged in length from 5.3 to 15.1 km (Figure 1; Table 1).

Spawning timing and location.—Spawning timing and location were assessed using a combination of redd surveys and radiotelemetry. Redd surveys indicated the duration of the spawning period

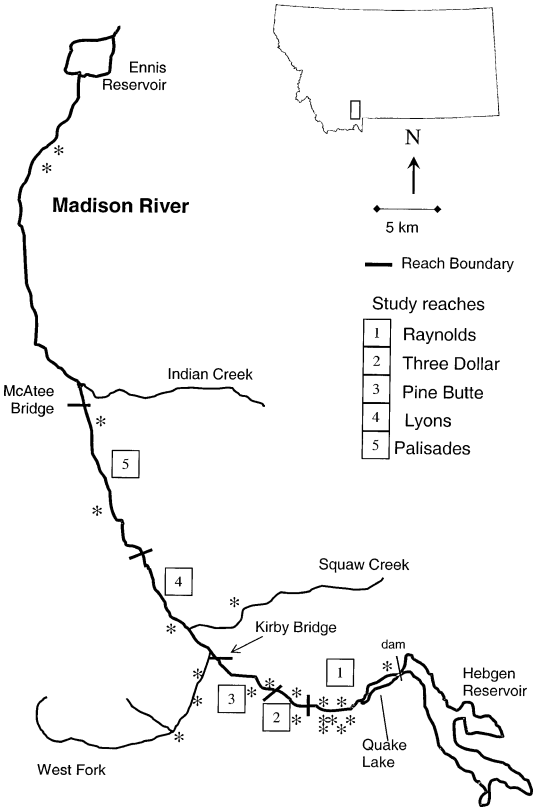

FIGURE 1.—Map of study area along the Madison River, Montana, showing the study reach boundaries and spawning locations (asterisks) of radiotagged rainbow trout.

and allowed quantitative estimation of the relative importance of spawning areas. Radiotelemetry data revealed the location and timing of spawning that was not detected in the redd surveys, which were sometimes limited by high flow and turbidity.

Redd counts.—Redd counts were conducted every 4–9 d in each study reach from April 16 to June 9, 1998, and from February 21 to June 9, 1999. Because actual spawning sites were initially unknown, the 1998 surveys were conducted over a greater proportion of each reach so as to determine spawning distribution. In 1999, a smaller proportion of each reach was sampled in known spawning areas, but the surveys occurred over a longer period to better assess spawning timing. Redd surveys were conducted by hiking the riverbank and wading the river while wearing polarized sunglasses. Deep (>1 m), swift areas of the main channel could not be effectively surveyed, but because most of these areas had large, embedded substrates that would make spawning difficult, we believe that the majority of redds in the main stem were observed. Redd surveys were attempted in tributaries, but high flow and turbidity made counts difficult; therefore, we relied on radiotelemetry to assess spawning at these sites.

Redds were identified by the characteristic pit and tailspill formation and lighter color than the surrounding undisturbed substrate. Each redd location was marked using painted rocks on the riverbed and flags on the adjacent riverbank to avoid multiple counts of the same redd. To assess observer accuracy of redd identification and the number of false redds, about 10% each year were partially excavated to check for the presence of ova and sac fry.

The timing of spawning was assessed by comparing the number of new redds found between surveys. Peak spawning was identified as the median spawning date. Because the majority of redds were observed in side channels, the total number counted in each reach was divided by the side channel area surveyed to compute a redd density (Table 1). Redd density was then multiplied by the total side channel area for each reach to estimate the proportion of total spawning and total number of redds. The side channel area was calculated using the length and mean width, which were determined from equidistant transects made along each side channel.

Radiotelemetry.—Radio transmitters were implanted into 32 adult rainbow trout to determine spawning and migration during the 1999 spawning season. To ensure that the monitoring period encompassed prespawning movements, fish were tagged in October 1998. Fish were captured by electrofishing and transmitters surgically implanted following the procedures outlined by Hart and Summerfelt (1975) and Garrett and Bennett (1995) and released within 0.5 km of their capture site. Fish size ranged from 343 to 460 mm total length (mean = 396 mm) and from 408 to 998 g (mean = 667 g). Larger adults were chosen to increase the likelihood that radiotagged fish were sexually mature. About one fish was tagged every 3 km from the Quake Lake outlet downstream to the inlet of Ennis Reservoir (Figure 1) to ensure that adults throughout the main stem were represented. The external antenna transmitters (model 10–28, Advanced Telemetry Systems, Isanti, Minnesota) weighed 8.2 g and had frequencies of 150.011–150.764 MHz. To maximize battery life and provide data both pre- and postspawn, the transmitters were programmed to operate 4 d on and 26 d off

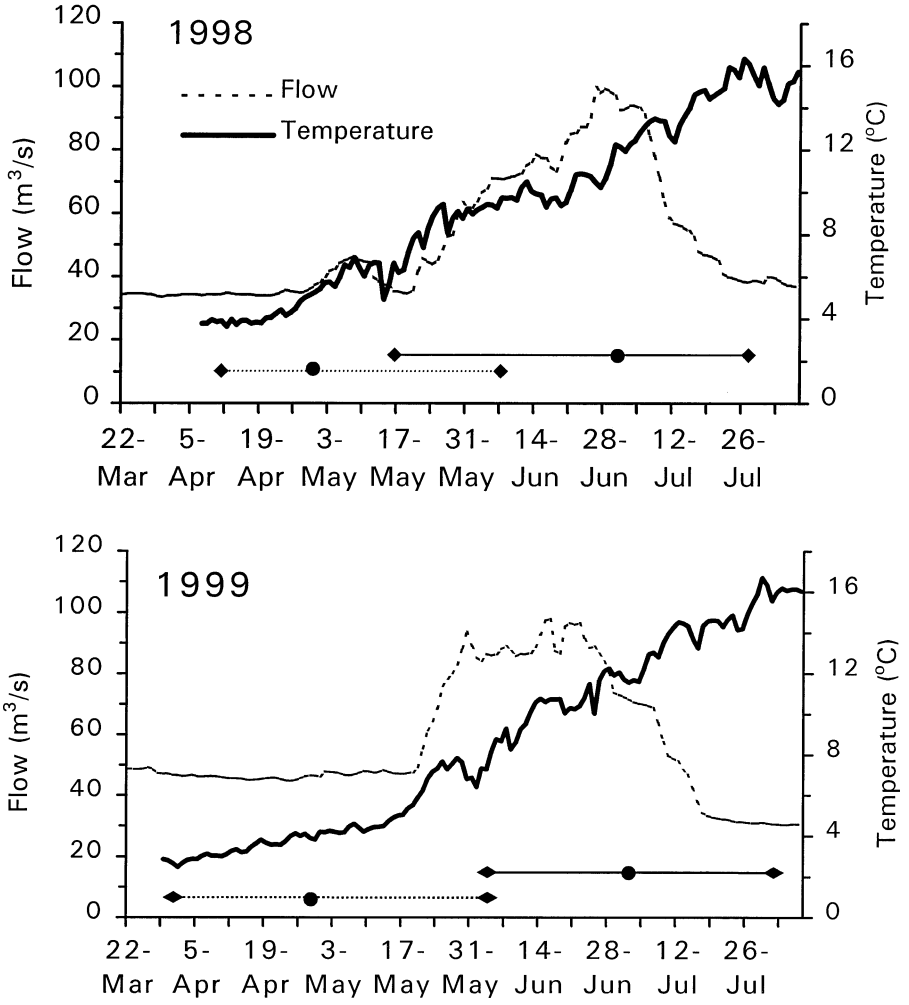

FIGURE 2.—Mean daily flow (left scale) and temperature (right scale) during rainbow trout spawning and emergence, 1998 and 1999. The duration and peak dates of spawning are shown by the dotted lines and diamonds in the lower part of each panel; the duration and peak dates of emergence are shown by the solid lines and diamonds in the lower part of each panel.

TABLE 1.—Estimated number of rainbow trout redds by study reach in the Madison River in 1998 and 1999. Redd density is the mean number of redds observed per hectare of side channel (SC) surveyed.

Study reach	Reach length (km)	Total SC area (ha)	Number of redds		SC area surveyed (ha)		Redd density (number/ha)		Estimated total redds		Proportion of total spawning (%)	
			1998	1999	1998	1999	1998	1999	1998	1999	1998	1999
Raynolds	5.3	5.3	495	649	4.7	4.7	106	138	564	734	58	59
Three Dollar	5.3	3.1	141	77	3.0	1.3	47	61	144	187	15	15
Pine Butte	7.6	6.5	126	89	5.4	2.8	23	32	149	207	15	17
Lyons	12.9	2.4	53	16	2.4	0.5	22	33	54	81	5	6
Palisades	15.1	3.1	38	21	1.7	1.5	22	14	69	44	7	3
Total	46.2	20.4	853	852	17.2	10.8	50	79	980	1,253	100	100

from September 21, 1998, to February 19, 1999, and every day thereafter.

Radiotagged fish were located monthly from October 1998 through February 1999 and twice weekly from March to July 1999. Initial locations were ascertained from roads paralleling the river using a whip antenna mounted on a truck except for two occasions (March 30 and May 8), when an airplane was used to locate tagged fish that could not be located from the ground. Fish positions (±5 m) were then obtained by triangulation from the riverbank using a directional handheld H antenna. The locations of all tagged fish were recorded on 1:24,000 quadrangle maps.

We estimated the timing and location of spawning based on a characteristic movement pattern. That is, spawning was characterized by a period of rapid movement in the spring, followed by a 1–2-week period of little movement, followed by another period of more extensive movement (Swanberg 1997; Henderson et al. 2000). Spawning time was estimated as the median date during the period of little movement and spawning location as the site where fish remained at least 5 d during this period. All radiotagged fish that moved into a tributary or side channel where spawning gravel or redds were observed were assumed to have spawned.

Emergence timing.—Emergence timing was measured with fry emergence traps placed over redds (Fraley et al. 1986). Traps consisted of nets measuring 1 m × 2 m that were tapered toward a perforated polyvinyl chloride cap at the downstream end. Emergence trapping took place from early May through August. Traps were spread among study reaches in proportion to the total number of redds observed. Traps were cleaned and checked for emergent fry every 1–7 d. Emergent fry were identified to species (Weisel 1966) and released. The emergence date was defined as the first date when fry were captured; most emergence occurred within 1 week of initial fry emergence. We included only those redds that yielded at least 10 fry.

Water temperature and flow were measured to assess their relationship to spawning and emergence timing. Water temperature was measured from a thermograph placed in the Raynolds study reach. Flow was recorded at the U.S. Geological Survey's Kirby gauging station (river km 18, measuring from the outlet of Quake Lake).

Fry distribution and abundance.—Trends in the distribution and abundance of age-0 rainbow trout fry over time in the five study reaches were determined for the 3-month posthatch period of peak susceptibility to *M. cerebralis* infection. The relative abundance of fry (catch per 300 s) was measured by backpack electrofishing a 1-m-wide band along shallow stream margins of side channels and the main channel (Bozek and Rahel 1991). In 1999, eight monitoring sites, including at least one per study reach, were sampled every 2–3 weeks from July through September. Within each site, four 15-m-long sections were randomly sampled each time to estimate average fry abundance.

M. cerebralis infection.—Sentinel fish were used to assess the spatial and temporal variation of *M. cerebralis* infection in relation to rainbow trout spawning and rearing. Three strains of rainbow trout were used for sentinel exposures: the domestic-strain Arlee and Erwin and the wild-strain Eagle Lake. All three strains have equally high susceptibility to whirling disease (Vincent 2002). Fish were raised at 10–12°C in parasite-free water in a hatchery prior to placement in sentinel fish cages at a size of 35–60 mm and an age of 1–2 months posthatch. The size, age, and strain of test fish were similar within an exposure group but varied somewhat between exposure times due to the long time period over which exposures were conducted. Thus, the severity of disease that developed among exposed fish provided an indirect measure of the presence and abundance of the infectious stages of *M. cerebralis*. A sentinel cage consisted of a screen-covered cylinder 0.5 m wide × 0.6 m high that was placed along the stream bank. Sixty test fish were randomly selected and held in a cage for 9–11 d, then transported to a laboratory and held in aquaria at 10–12°C for an additional 80 d to ensure full development of *M. cerebralis* spores (Baldwin et al. 1998). At the end of this period, fish were sacrificed with an overdose of anesthetic and the heads removed. Individual heads were fixed with Davidson's solution (72 h) followed by isopropyl alcohol and sent to the Washington Animal Disease Diagnostic Laboratory (Pullman) for histological examination. Disease severity was scored on a scale of 0 (none) to 4 (severe) using a lesion rating system (Baldwin et al. 2000). Histological data for each exposure group were summarized by the percent of fish infected and a mean lesion score.

Ten sites in 1998 and 21 sites in 1999 were sampled to assess the spatial variation in disease severity in the main-stem Madison River (Table 2). Sites were located in side channels near likely or known spawning and fry rearing areas. The number of cages varied from one to eight per reach

TABLE 2.—Range of mean lesion scores and percentages of fish infected by *Myxobolus cerebralis* in exposure groups of sentinel rainbow trout in the Madison River and West Fork tributary, 1998 and 1999. Dates of exposure are given in the text, and study reach locations are shown in Figure 1. No sentinel fish cages were present in the Three Dollar reach. River kilometers are measured downstream from the Quake Lake outlet.

Study reach and site	River kilometer	Score (% infected)	
		1998	1999
Raynolds			
North Slide Upper	2.4		0.1–0.2 (5–15)
North Slide Lower	2.6	0.1–1.0 (11–64)	0.1–1.7 (6–68)
South Slide Upper	2.7		1.9–2.5 (77–91)
South Slide Lower	2.9	0.02–3.5 (2–90)	1.6–2.5 (80–83)
Raynolds Bridge	5.3	0.1–1.2 (11–56)	0.6 (40)
Pine Butte			
Pine Butte Upper	14.6	0.1–1.3 (6–50)	0.9–1.1 (51–61)
Pine Butte Lower	14.8	0.2–1.4 (10–62)	1.1–1.2 (55–68)
Eagle Nest Upper	14.9		1.9–2.5 (78–86)
Eagle Nest Lower	15.0		1.5–1.8 (74–82)
Grizzly Upper	17.1		3.7–3.9 (96–98)
Grizzly Lower	17.2		3.71–3.72 (97–98)
Kirby Bridge Upper	17.9		2.3–3.1 (85–94)
Kirby Bridge Lower[a]	18.0	0.2–3.6 (15–93)	0.1–3.0 (4–97)
Lyons			
Lyons Bridge	19.7	0.6–1.8 (33–74)	1.1 (72)
Lyons Upper	20.3		0.7–2.7 (55–91)
Lyons Lower	20.4		2.9–3.9 (93–98)
Sun West Upper	25.6		2.7–3.3 (92–98)
Sun West Lower	25.7		2.8–2.9 (95–98)
Palisades			
Palisades Upper	32.1	0.1–3.2 (4–94)	2.4–2.6 (88–89)
Palisades Lower	32.2	0.2–3.4 (10–88)	2.6–3.1 (91–94)
McAtee Bridge	46.2	0.1–1.7 (4–70)	1.4 (86)
West Fork tributary			
Upper		0.0–0.2 (0–10)	
Lower		0.0–1.0 (0–54)	

[a] Successive exposure site.

depending on access and the spatial variability within reaches. No cages were deployed in the Three Dollar reach due to a lack of road access. Sampling periods (May 25–June 4, July 8–18, and September 28–October 8, 1998; and May 25–June 4 and June 24–July 5, 1999) corresponded to the spring and fall peaks in infection observed in the Madison River and other Montana rivers. Sentinel cages were deployed in the West Fork tributary during three periods in 1998 (June 14–24, July 9–17, and September 17–25) to assess tributary disease severity. The temporal variation in disease severity was assessed by successive 10-d exposure trials run in 1998 (May 5–July 25, $n = 8$; and September 16–October 18, $n = 4$) and 1999 (April 15–July 15, $n = 9$) at Kirby Bridge, which is located at about the midpoint of the study area (river km 18).

Disease severity within a study reach was calculated by pooling the mean lesion scores for all exposure groups within that reach. Statistical differences in disease severity among reaches were determined using Kruskal–Wallis nonparametric analysis of variance. Differences in severity among sampling periods and years were assessed by pooling the mean lesion severity scores for all exposure groups within a particular time period and comparing the mean scores via a Mann–Whitney nonparametric test. The level of significance for statistical testing was 0.05.

Results

Spawning Timing and Location

Redd counts.—A total of 1,705 redds were counted during the two spawning seasons. Despite the large number of redds, superimposition was low, with 15 or less incidences recorded each year. The proportion of false redds also was low; in 1998, 86% (68 of 79) of the redds sampled contained ova or sac fry, and in 1999 the figure was 100% (77 of 77). Generally, conditions upstream of the West Fork were suitable for redd counts except for brief (1–2-d) periods of high flow and

turbidity. Redd visibility was low in the lower two study reaches (Lyons and Palisades) after May 11, 1998, and May 24, 1999, because of turbid runoff from the West Fork tributary, but most spawning had occurred prior to these dates. Therefore, we believe that the majority of redds in the study area were located.

Rainbow trout spawned over extended periods (about 2 months) in both 1998 and 1999 (Figure 2). In 1998, 208 redds were found during the first week of redd surveys, indicating that spawning had begun before this period. The median spawning date was estimated as April 30, but new redds were found as late as June 9. In 1999, redds were observed from late March to early June, with a median spawning date of May 1.

Spawning timing was similar among the study reaches. Water temperature during spawning was similar in both years, ranging from 5°C to 10°C in 1998 and from 4°C to 9°C in 1999 (Figure 2). Most spawning occurred prior to the spring rise in the hydrograph (Figure 2).

Most spawning occurred in the upper study reach, with the number of redds declining sharply downstream (Table 1). The Raynolds reach comprised 12% of the total study area but contained nearly 60% of the total redds. The proportion of redds by study reach varied little between years despite differences in total counts. In both years, the majority (95%) of redds were observed in side channels.

Radiotelemetry.—We obtained spawning data on 21 of 32 radiotagged adult rainbow trout in 1999. The remaining radiotagged fish were lost to predation ($n = 9$) or transmitter malfunction ($n = 2$).

Tagged fish generally remained within 0.5 km of their initial tagging location prior to spawning migration beginning in late March, when movement increased markedly. Spawning migration averaged 18.7 km (range, 0–66.3 km). The majority (81% or 17 of 21) spawned in side channels within the main stem, whereas 19% (4) spawned in tributaries (Figure 1). Main-stem spawners spawned over an 82-km stretch ranging from 9.5 km upstream to 72.5 km downstream of the Quake Lake outlet (Figure 1). Spawning of radiotagged trout was concentrated in the upper study reach, with fish tagged throughout the river moving into this area to spawn. Three of the tributary spawners spawned in the West Fork and one in Squaw Creek. The median spawning date for main-stem spawners (April 30) was about 1 month earlier than that for tributary spawners (May 27). Following spawning, most (75%) fish moved back to within 2 km of their original tagging location.

Emergence

We collected emergent rainbow trout fry from 12 of 20 (60%) redds trapped in 1998 and from 31 of 45 (69%) of those trapped in 1999. Despite a protracted (2-month) spawning period, most emergence occurred within a relatively narrow 3-week window from late June to early July (Figure 3). The median emergence date was July 2 (range, May 19 to July 21) in 1998 and July 6 (range, June 3 to August 3) in 1999. Emergence coincided with the early summer decline in the hydrograph in both 1998 and 1999 (Figure 2). Water temperature at the time of emergence averaged 11.8°C in 1998 and 12.5°C in 1999.

Fry Distribution and Abundance

Like the spawning distribution, the distribution of age-0 rainbow trout was concentrated in the upper study reaches (Figure 4). In 1999, an average of 78% (range, 74–84%) of all age-0 rainbow trout captured in each sampling period occurred in the Raynolds and Three Dollar study sections. September fry abundance and redd density were positively correlated ($r = 0.95$, $P = 0.01$), suggesting that most fry remained near their spawning areas the first 2–3 months after emergence.

M. cerebralis Infection

The mean lesion scores used to assess disease severity in exposed groups of sentinel trout in the main-stem Madison River ranged from 0.1 to 3.6 (4–94% prevalence of infection) in 1998 and from 0.1 to 3.9 in 1999 (4–98%; Table 2). The differences in mean lesion scores among study reaches during June and July for each year (Figure 5) were not statistically significant. However, all study reaches had localized sites of high disease severity, and lesion scores varied widely over short distances. For example, the mean lesion scores of exposed groups located in different side channels about 100 m apart in the Raynolds study reach (North and South Slide Lower, Table 2) were 0.1 and 3.5 during the same sampling period in 1998 (May 25–June 4). Similarly, during the same sampling period in 1999 (May 25–June 4), exposure groups 2.4 km apart in the Pine Butte study reach had mean lesion scores of 0.9 (Pine Butte Upper) and 3.9 (Grizzly Upper). In the only tributary site sampled (West Fork), the mean lesion scores were low (0.0–1.0) and infection prevalence was low to moderate (0–54%).

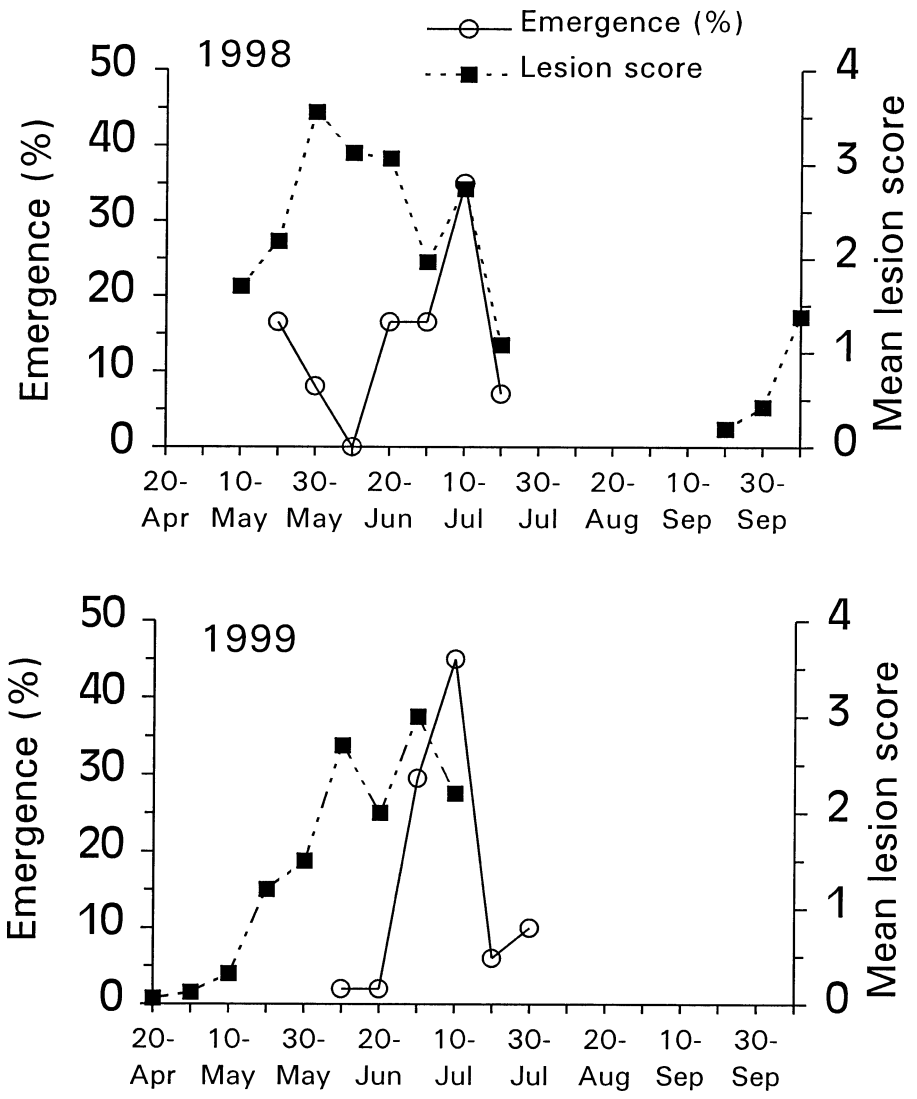

FIGURE 3.—Percent emergence (circles; left scale) of rainbow trout fry from spawning redds in 1998 (n = 12) and 1999 (n = 31) in relation to the mean lesion score (squares; right scale) of exposure groups of sentinel rainbow trout. Percent emergence is defined as the proportion of redds with emergence traps that contained emergent fry. The mean lesion score is based on successive 10-d exposures at the Kirby Bridge site.

Large temporal variation in disease severity was also evident. In 1998, the mean lesion scores in June and July were significantly higher than those in October (Figure 5; Mann–Whitney tests, $P < 0.01$). Furthermore, the mean lesion score of all exposure groups in June–July 1998 was significantly lower than that during the same period in 1999 (1.5 versus 2.1; $U = 2.0$, $P = 0.046$). The highest mean lesion score was 2.75 or more at 36% (4 of 11) of exposure sites in 1998 and at 72% (13 of 18) of exposure sites in 1999. Lesion scores of 2.75 and above have been associated with significant levels of mortality in wild rainbow trout populations (Vincent 2002).

Successive sentinel fish exposures revealed a difference in the time of highest disease severity between the 2 years. In 1998, the highest mean lesion scores occurred during the May 25–June 4 exposure period; in 1999, the highest scores occurred about 1 month later, during the June 24–July 5 exposure period (Figure 3). This difference in timing apparently led to a greater risk of exposure to high infection for emergent rainbow trout fry in 1999 than in 1998 (Figure 3).

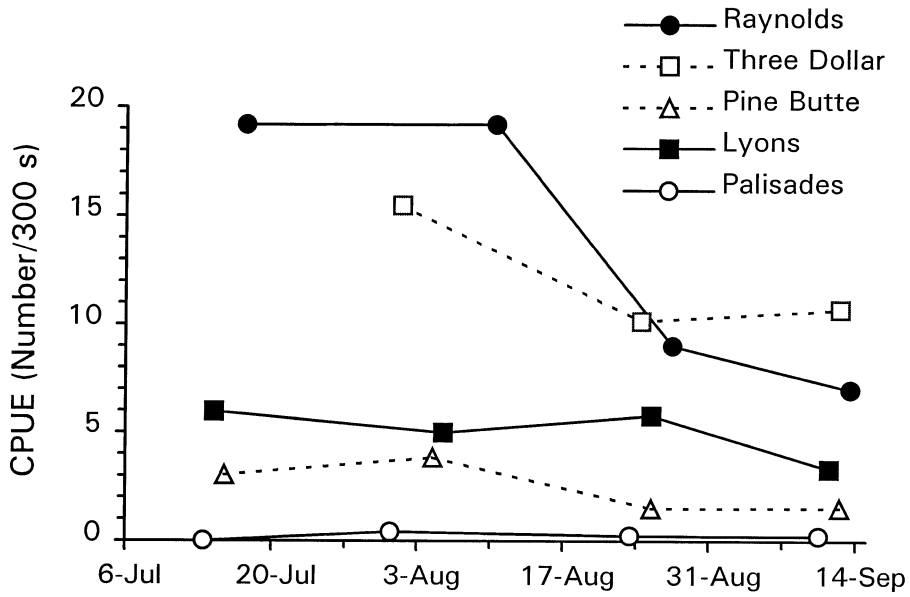

FIGURE 4.—Electrofishing catch per unit effort (CPUE) of age-0 rainbow trout in each study reach from July to September 1999.

Discussion

The location and timing of rainbow trout emergence in the Madison River had a significant effect on the degree of exposure to *M. cerebralis*. Redd surveys and radiotelemetry indicated that the majority of these fish spawned in a relatively small portion of the upper river. Therefore, at the time of hatching and emergence, when rainbow trout are most susceptible to infection from *M. cerebralis* (Markiw 1991), the majority of fry in the Madison River population are concentrated within a relatively small area of the drainage. Exposure studies with sentinel fish cages indicated that the risk of infection within this area ranged from low to very high during this period.

Although the majority of redds were concentrated in a small area, the time of spawning stretched over a protracted period of about 2 months (April–May). The median spawning date, however, was about the same in both 1998 and 1999 (May 1), and most fry emerged within a relatively narrow 3-week window from late June to early July in both years. The timing of hatching and emergence among salmonid fry is closely regulated by temperature (Kwain 1975; Piper et al. 1982), and we believe that intragravel temperature differences among redds (Downing 2000) led to different development rates and a convergence of emergence timing. The emergence of fry within relatively narrow time windows is common among salmonids and is likely an adaptive feature (Holtby et al. 1989). Despite similar times of emergence, differences in the timing of the highest disease severity between the 2 years of our study suggest that emergent rainbow trout fry had a higher risk of infection in 1999 than in 1998.

We found that after emergence rainbow trout fry remained in close proximity to redd sites in the side channels of the two upper study reaches, at least during their first summer. An apparent lack of dispersal has also been documented in other salmonid populations (Bozek and Rahel 1991; Magee et al. 1996). Though the fry catch rate decreased over time in all study reaches, we found no evidence for a long-distance migration downstream, as has been reported in other studies (Rosenau 1991; Knight et al. 1999). Rather, the decline in abundance was likely due to mortality or a shift into deeper, swifter water as fish grew (Knight et al. 1999). Given the propensity of salmonids to home to their spawning grounds, the migration of some adult rainbow trout up to 66.3 km to spawn in the upper study reaches suggests that there is wide dispersal of trout recruited from the upper river spawning area throughout the entire main stem. Our results suggest that this movement occurs sometime after the time of peak vulnerability to *M. cerebralis* infection. However, the wide variation in disease severity in the upper study reaches indicates that the movement of fry over even short

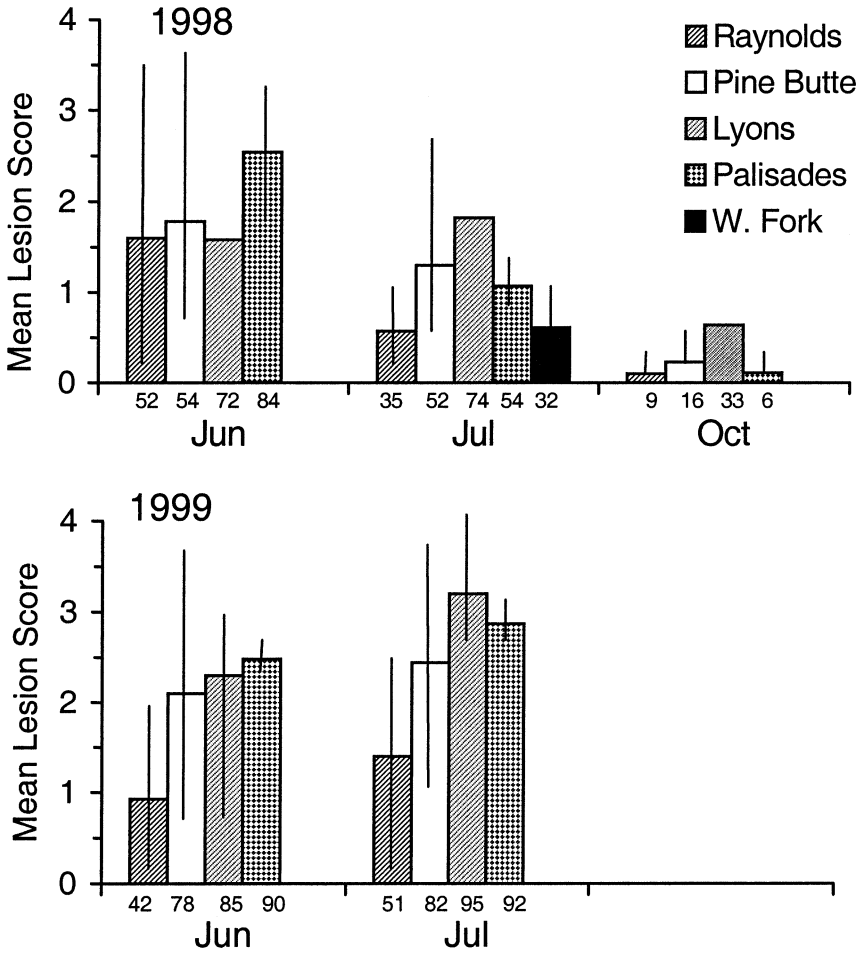

FIGURE 5.—Mean lesion scores of the exposure groups of sentinel rainbow trout placed in each study reach, 1998 and 1999. The thin vertical lines indicate ranges; the numbers below each bar indicate the percentages of infection.

distances (100 m) could greatly influence exposure to *M. cerebralis* infection. Previous investigations found that even short-term exposure (0.5–2 h) to a high dose of *M. cerebralis* triactinomyxons could cause severe whirling disease in rainbow trout fry (Markiw 1991; Ryce et al. 2001).

The large variation in disease severity that we observed in exposure groups within close proximity was surprising. Previous drainagewide studies of *M. cerebralis* infection using sentinel fish exposures have shown either a relatively uniform severity or variation in disease severity on much larger spatial scales. Hiner and Moffitt (2001) examined four sites along a 25-km stretch of the Boise River, Idaho, and found that mean lesion scores varied from 1.0 at the uppermost site to more than 3.5 at the lowermost site. In a 28-km section of the Lostine River, Oregon, the prevalence of infection varied from less than 10% at upstream sites to 75% in the lowermost site (Sandell et al. 2001). In contrast, mean lesion scores averaged more than 3.4 at all eight sites along a 30-km reach of Little Prickly Pear Creek, Montana (Vincent, unpublished data). In the Madison River, we found that disease severity typically differed by at least two orders of magnitude within the four study reaches, which ranged in length from 5.3 to 15.1 km.

The factors leading to high infection in some areas but not in others are unclear, but variation in the density of *Tubifex tubifex* (the oligochaete host of *M. cerebralis*) and *M. cerebralis* spore loading are likely mechanisms (Hedrick et al. 1999; Zendt and Bergersen 2000; Kerans and Zale 2002).

Myxobolus cerebralis infection may be more severe and *T. tubifex* more abundant in degraded and organically enriched sites (Zendt and Bergersen 2000; Hiner and Moffitt 2001; Sandell et al. 2001). In the Madison River, localized sites of infection occur in side channels characterized by a combination of low flow and a high percentage of fine sediments (Krueger 2002).

Disease severity in the Madison River also varied widely by season, being very low in the early spring, peaking in the late spring, and decreasing in the fall. We did not sample during the summer, as previous work had shown that season to be a time of low infection (MacConnell and Vincent 2002). Seasonal changes in infection are closely linked to seasonal changes in water temperature and are thought to result from the temperature-mediated release of the triactinomyxon stage of the parasite (El-Matbouli et al. 1999). We found that peak infection occurred at temperatures near 12°C, which was similar to those observed in other sentinel fish exposure studies (Baldwin et al. 2000; MacConnell and Vincent 2002). The seasonal pattern in disease severity indicates that spring spawners would have much higher risk of infection than fall spawners. In addition, age-0 rainbow trout would face a much lower chance of infection, even in highly infected waters, if they were to avoid exposure during the first few weeks after hatching and emergence, when they are most vulnerable. For example, at one side channel in the Raynolds study reach where many rainbow trout spawned and reared, the disease severity dropped from 3.5 to 0.02 over a 6-week period in late spring (May 26 to July 8, 1998).

The annual variation in disease severity is also noteworthy. In 1998, the number of exposure groups with mean lesion scores more than 2.75 was half that observed in 1999 (36% versus 72%). Annual differences in infection may be related to flow and temperature differences or to recruitment and turnover in the *T. tubifex* population (Zendt and Bergersen 2000). Although water temperatures were similar during the May–June peak infection periods in both years, in 1998 the peak discharge occurred about a month later and the difference in base and peak flow was about 25% greater than in 1999. High discharge could reduce disease severity by diluting the triactinomyxon concentration (MacConnell and Vincent 2002; but see Thompson et al. 1999) and by scouring of the fine-sediment habitat occupied by *T. tubifex* (Zendt and Bergersen 2000). Annual differences in the timing and severity of infection may have important population effects. The 1998 cohort was the most abundant of any cohort sampled during annual population estimates since 1991, when whirling disease effects were first noted in the population (Byorth, personal communication).

Conclusions and Management Implications

Our results indicate that the risk of exposure of rainbow trout to *M. cerebralis* infection is governed by a complex interplay between the timing and location of key life history events (spawning, emergence, and early rearing) and the spatial and temporal variation in infection risk. This interplay has important implications for disease monitoring. Deployment of sentinel fish exposure groups to monitor *M. cerebralis* infection must be appropriate for the river system and life history traits of the species of concern or biased estimates of the extent and severity of whirling disease may result. For example, we found that disease severity varied widely over distances of less than 2 km and over time periods of just a few weeks. Using sentinel fish cages to identify when the peak infection occurs and where the hot spots of infection are in relation to spawning and rearing areas in a particular drainage is a key feature of effective monitoring.

Differences among populations in spawning and rearing life history in conjunction with spatial and temporal variation in the risk of exposure to *M. cerebralis* infection may partially explain why population-level effects are so variable among infected populations. The life history diversity of introduced populations of rainbow trout has not been well documented, but high diversity in spawning and rearing life history is likely common (Biette et al. 1981; Rosenau 1991; Quinn and Unwin 1993). For example, in contrast to the patterns prevailing in the Madison River, main-stem spawning is rare and tributary spawning and rearing is the predominant life history of rainbow trout in the Missouri River, Montana (authors' unpublished data).

The tendency for the spawning and rearing of rainbow trout to occur at times of high risk of exposure to *M. cerebralis* infection over much of the main stem has likely led to the severe decline of that species in the Madison River. We found little use of tributaries for spawning, and our limited sampling of these sites suggested a lower disease severity. Why tributary spawning is so rare in the Madison River is unknown. However, limited spawning habitat and poor rearing conditions as a result of dewatering from irrigation and the

reduced competitive ability of tributary-reared juveniles due to later spawning (1 month) and colder temperatures (Sloat 2001) are probable causes. However, selection pressure or reduced competition may now favor tributary-spawned fish (Quinn et al. 2000; Allendorf et al. 2001). The use of a tributary-rearing check on the scales of adult recruits is presently being used to test this hypothesis (Byorth, personal communication).

These findings suggest several potential management actions in *M. cerebralis*-positive waters. Specifically, improvements in spawning and rearing habitat quality in areas of low infection risk and the introduction of stocks that differ in their timing and location of spawning and rearing are tools that could combat severe population declines.

Acknowledgments

Funding for this study was provided by the National Partnership on Management of Wild and Native Coldwater Fisheries and Montana Fish, Wildlife, and Parks. We thank Pat Clancey, Pat Byorth, Rob Jakubowski, and Grant Grisak for technical support; and Aemi Herrera, Ryen Anderson, Jill Downing, Mike Enk, Paul Hutchinson, Shane Keep, Rebecca Krueger, Brad Liermann, Adam McMahon, Bill Mercer, Clint Sestrich, Stan Van Sickle, and Kris Whitaker for field assistance. Eileen Ryce, Brad Shepard, Al Zale, and three referees provided helpful comments on earlier drafts of the manuscript.

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