Rainbow Trout Spawning Characteristics and Relation to the Parasite *Myxobolus cerebralis* in the Missouri River, Montana

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Abstract

The *myxosporean* parasite *Myxobolus cerebralis* is responsible for significant declines of rainbow trout (*Oncorhynchus mykiss*) populations in several western states, including Montana. Despite a high prevalence of the parasite in Montana's Missouri River, there have been no apparent impacts to the rainbow trout population. This study examined long-term *M. cerebralis* monitoring data from the Missouri River system below Holter Dam and evaluated rainbow trout spawning characteristics such as migration distance, spawning location, site fidelity and amount of spawning in the Missouri River and tributaries over three years in an attempt to explain why the population has not declined in the presence of *M. cerebralis*. Over 13 years of monitoring, a mean 5.3 percent of rainbow trout handled during population estimates had clinical signs of *M. cerebrali*s infection. In experiments using sentinel fish 53 percent of the spawning habitat had high severity of *M. cerebralis*, 38 percent had low to moderate severity, and 9 percent had no infection. Radio telemetry showed spawning locations varied among years and tagged fish lacked spawning site fidelity. The distance that radio-tagged rainbow trout migrated to spawning locations was significantly different among river sections of the study area. Twenty-eight percent of the spawning redds were found in the Missouri River and 72 percent in the tributaries. Relative to previous studies, we found less tributary spawning and an increase in Missouri River spawning, where *M. cerebralis* infection severity is lower. Our findings suggest that diverse spawning behaviors may contribute to rainbow trout population stability by spreading the risk of *M. cerebralis* impact over spawning locations that have a broad range of infection severity.

Key words: rainbow trout, whirling disease, *Myxobolus cerebralis*, radio telemetry, redds, spawning

Introduction

The Missouri River-Holter Dam tailwater fishery is one of the most productive trout fisheries in Montana. Out of 1,200 fisheries monitored for angler use statistics in Montana it consistently ranks as one of the top four most heavily fished waters (Montana Fish, Wildlife and Parks (MFWP) unpublished data). From 1995 through 2009 anglers spent an average 97,430 (range 75,000-123,000) days per year fishing this reach of river. The trout assemblage is approximately 83 percent rainbow trout (*Oncorhynchus mykiss*) and 17 percent brown trout (*Salmo trutta*). Brown trout and rainbow trout stocking began in 1928 and 1933, respectively, and continued intermittently though 1973. At that time, Montana instituted a statewide wild fish management policy and discontinued stocking trout in most rivers and streams. This trout fishery has been sustained by wild reproduction ever since. From 1980 through 2010 MFWP has conducted annual boat-mounted electrofishing mark-recapture population estimates of trout greater than 25 cm long in two sections of this river.

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Median estimates for number of trout 25 cm and longer per km in the 8.9 km-long Craig section are 1,793 (range =1,096-3,185) for rainbow trout and 306 (range =91-827) for brown trout (Fig. 1: Grisak et al. 2010). In the 6.6 km-long Pelican Point section median estimates are 885 (range =389-2,360) for rainbow trout and 193 (range =84-427) for brown trout (Fig. 1: Grisak et al. 2010).

In 1995, *Myxobolus cerebralis*, a myxosporean parasite that causes whirling disease in rainbow trout, was discovered in Little Prickly Pear Creek; one of the most productive trout spawning tributaries of the Missouri River below Holter Dam (Leathe et al. 2002, Munro 2004). Within three years, clinical signs of whirling disease, such as black tail and whirling behavior, were observed in wild age-0 rainbow trout in Little Prickly Pear Creek (Grisak 1999). The parasite's range expanded into the Missouri River and some additional spawning tributaries over the next four years (Grisak 1999, Leathe 2001). This development raised great concern among fishery managers because from 1991 to 1994 the Madison River, Montana and the Colorado River, Colorado experienced sharp declines of rainbow trout, approaching 90 percent, due to *M. cerebralis* (Vincent 1996, Nehring and Walker 1996). Fearing similar population declines in the Missouri River, fishery managers investigated the relative recruitment of rainbow trout from tributaries where the parasite was established to total rainbow trout production (Leathe 2001, Munro 2004). Monitoring *M. cerebralis* in the Missouri River and its tributaries began in 1997 to evaluate the spread of the parasite and to develop population risk assessments. This monitoring has continued for 13 years.

Historically, fishery managers hypothesized that rainbow trout life history in this area was locally adapted, believing that fish in the upstream portion of this section spawned in Little Prickly Pear Creek, and fish in the downstream portion spawned in the Dearborn River (Horton et al. 2003, Horton and Tews 2005, Horton and Hamlin 2006). The presence of *M. cerebralis* in both of these spawning tributaries fueled speculation that the adult populations would ultimately become recruitment limited, resulting in population declines (McMahon et al. 2001, Leathe et al. 2002, Munro 2004). Despite 15 years of *M. cerebralis* presence in the most productive spawning tributaries, there has been no measurable reduction of adult rainbow trout in the Missouri River (Grisak 2010). We hypothesized that rainbow trout spawning behavior may be responsible for the stable populations.

In this study we (1) describe annual timing of spawning migrations and locations to test the hypothesis of localized spawning behavior, (2) estimate the amount of spawning that occurs in the Missouri River compared to its tributaries, (3) describe spawning migration behavior over multiple years, (4) describe the magnitude of spawning migrations over multiple years, (5) estimate the scale at which repeat spawners return to previous spawning sites as a measure of spawning site fidelity, and (6) summarize the *M. cerebralis* monitoring data collected over the past 13 years.

Study area

The study area was located in central Montana and consisted of a 41.6-km reach of the Missouri River and its tributaries extending from Holter Dam downstream to the Pelican Point Fishing Access Site (Fig. 1). At the lower end of the study area, the Missouri River drains 47, 187 km^2 and had a gradient of 0.88m/km. Three upstream hydroelectric dams (Canyon Ferry, Hauser and Holter) regulate flows. Mean annual discharge measured below Holter Dam from 1946 to 2010 ranged from 85 to 241 m^3/s (USGS unpublished data for station 06066500) and mean annual water temperature was 8.4^{o}C (SE: 0.17). The principal spawning tributaries in the study area were Little Prickly Pear Creek (which included Lyons Creek and Wolf Creek), the Dearborn River, and Sheep Creek (Fig. 1). Little Prickly Pear Creek originates near the Continental Divide and flows easterly from the Rocky Mountains for 57 km before entering the Missouri River 3.8 km downstream of Holter Dam. Little Prickly Pear Creek basin drains 1,026 km^2. Mean

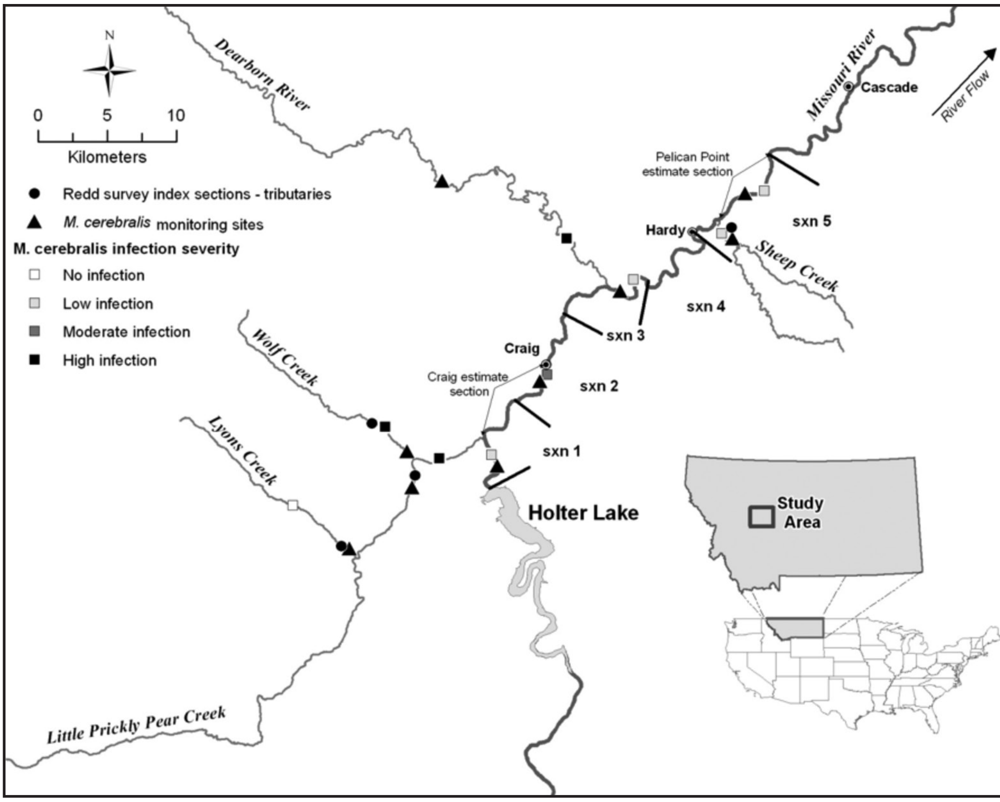

Figure 1. Missouri River-Holter Dam tail-water study area with population estimate sections, *M. cerebralis* monitoring sites, and *M. cerebralis* infection severity.

annual discharge from 1963 to 2010 ranged from 1.25 to 5.06 m^3/s (USGS unpublished data for station 6071300) and mean annual water temperature was 7.7°C (SE: 1.03). The Dearborn River originates on the east slope of the Rocky Mountain front, near the Continental Divide, and flows easterly for 107 km before entering the Missouri River 25.3 km downstream of Holter Dam. This basin drains 1,418 km^2 and the mean annual discharge from 1946 to 2010 ranged from 1.7 to 10.3 m^3/s (USGS unpublished data for station 6073500). Mean annual water temperature was 7.7°C (SE: 0.18). The North and South forks of Sheep Creek originate in the Big Belt Mountains and each flows for approximately 10 km before forming Sheep Creek. Sheep Creek flows westerly for 3.3 km before entering the Missouri River 37.8 km downstream of Holter Dam. The Sheep Creek basin drains 96 km^2. Temperature data were not available for Sheep Creek.

Methods

Radio Telemetry

We used radio telemetry to describe rainbow trout spawning migrations from 2008 through 2010 and estimate spawning site fidelity among years. We divided the Missouri River portion of the study area into five sections (each 8.3-km long) and dedicated an equal number of transmitters for each section. We tested two hypotheses relating to the scale at which trout migrate to spawn. The first hypothesis, derived from previous managers, was whether fish from the upper half (sections 1 and 2) of the study area would preferentially spawn in Little Prickly Pear Creek and fish from the lower half (sections 3, 4 and 5) would spawn in the Dearborn River. The second hypothesis we tested was whether all fish would spawn within the 8.3 km-long section of the Missouri River in which they were tagged.

Three of the sections (1,3,5) had tributaries that were considered part of the section.

Fish were captured using boat electrofishing (smooth DC, 300 V, 4 A). We selected individual rainbow trout ranging from 40 to 60 cm total length (TL) to ensure fish were sexually mature, young enough to spawn in the next two seasons, and large enough to carry the radio transmitter (<6.7 % total body weight) without influencing behavior (Brown et al. 1999). We surgically implanted radio transmitters (Lotek MCFT-3A, 148 MHz, 6.7g in-water weight) in the abdomen of six trout in each section following the external antennae procedure described by Cooke and Bunt (2001). Fish were tagged approximately six months before the spawning season to allow them to acclimate to transmitters. Because of predation by osprey (*Pandion haliaetus*), bald eagle (*Heliaeetus leucocephalus*), and great blue heron (*Ardea herodias*) and angler transmitter returns, four radios were recovered and re-implanted in new fish. We tagged 23 female and 7 male rainbow trout (range 42.4-51.1 cm TL). In the upper half of the study area we tagged 14 female and 4 male fish and in the lower half we tagged 9 female and 3 male fish. Mean body weight loading of the transmitters was 1.65 percent (SE: 0.08).

Tagged fish were detected using a mobile radio-telemetry receiver (Lotek SRX 400 W5) and a truck, jet boat, or airplane. Stationary radio receivers (Lotek SRX 400 W7AS) were positioned at the mouths of Sheep Creek, Dearborn River and Little Prickly Pear Creek, and at the Craig Bridge (Fig. 1). Receivers at tributary mouths provided precise dates and times when fish entered or exited the tributaries.

Mobile surveillance was increased during spawning seasons in order to locate spawning sites. We had a priori knowledge from previous spawning studies in this area that radio-tagged rainbow trout ascended tributaries and stayed at redd sites for a mean 4.7 days (range 1-22) before moving downstream (Grisak 1999). Given the difficulty in determining exact spawning sites, we estimated spawning locations and times by documenting a fish's furthest movement during the spawning season and assuming it spawned in the area at that time (Grisak 1999, Burrell et al. 2000, Henderson et al. 2000, Pierce et al. 2009).

Locations of tagged fish were recorded on a map with longitudinal distances of the Missouri River and its tributaries marked to the nearest 0.1 km. The beginning reference point (river kilometer [rkm] 0.0) in the Missouri River was Holter Dam, located on the upstream end of the study area (Fig. 1). For tributaries, the beginning reference point (rkm 0.0) was the mouth of each stream and longitudinal measurements continued upstream.

Redd Counts

We counted spawning redds in the tributaries and the mainstem Missouri River from 2007 to 2010 to estimate the spatial distribution and relative magnitude of rainbow trout spawning each year. Redd counts were conducted weekly by surveying index sections of tributary streams starting on 15 March (Fig 1). The peak of the spawning run for each stream was calculated using the mean value of all redds counted in the index sections and determining the dates of the peak. At the point when few additional redds and few or no spawning fish were observed in the index sections, we conducted an expanded basin-wide count in each stream within one week (Grisak 1999). Redd counts on the Missouri and Dearborn rivers were conducted using a Bell OH-58 helicopter with the doors removed flying ≈75 meters above the water's surface. Two observers wearing polarizing eyeglasses counted redds and recorded the locations with a GPS unit. The spatial distribution of redds in the rivers was determined using ArcGIS. Two-way communication between the observers reduced the likelihood double-counting redds in the center of the rivers.

M. cerebralis monitoring

Monitoring of *M. cerebralis* was conducted according to protocols described by Baldwin et al. (2000) and Vincent (2002). Caged sentinel fish (60 age-0 rainbow trout) were placed in streams for 10-d periods when water temperatures were conducive to promote infections in rainbow trout (9-17°C: Vincent 1999). An Onset temperature data recorder attached to the cage recorded water temperatures on 30 minute intervals during the exposure period. In order to measure the peak and range of infection severity at many of the sites, multiple 10d sequential sentinel fish experiments were conducted when mean daily water temperature ranged from 9 to 17°C. After exposure, the fish were moved to a laboratory facility where the parasite was allowed to mature in infected fish over a 90-d period. The fish were euthanized with tricane methanesulfonate (MS-222) and prepared for histological analyses by first removing and then preserving the head and gill arches in formalin. Histological analyses were performed on median plane sections of fish heads, which involved quantifying the abundance and severity of *M. cerebralis* lesions in cranial tissue.

The severity of infection was rated on a 0-5 scale for each fish and histological scores were averaged for each lot (Hedrick et al. 1999, Baldwin et al. 2000, Vincent 2002). Population risk assessments were developed based on reports that mean histological score of 2.75 and higher usually cause declines in wild rainbow trout populations (Vincent 2002). From this we developed three risk categories based on mean histology score (>2.75 = high, < 2.74 = moderate, 0.00 = no risk).

We also measured clinical signs of whirling disease such as deformed mandible/maxilla, shortened operculum, dolphin head and spinal deformity (Vincent 2002) while handling fish during annual rainbow trout population estimates.

Data Analysis

Because we evaluated fish movements over multiple years, it was necessary to define a starting point for fish movements each year. For the first year, the starting point was the 8.3 km-long section of the Missouri River where each fish was collected and implanted with a radio transmitter. For each successive year the starting point was the section where each fish was located on 1 January. We used chi-square goodness-of-fit tests ($\alpha<0.05$) to determine whether the predicted number of fish that spawned within their annual starting section or the upper and lower half of the study area was significantly different from that observed.

We hypothesized that the distance rainbow trout moved to spawning sites would be similar among the five groups. Migration distances from annual starting sections to spawning locations were evaluated using single factor analysis of variance (ANOVA: $\alpha<0.05$) to determine whether magnitudes of movements were significantly different among sections.

Histopathology and water temperature data were analyzed using logistic regression (Peng et al. 2002). Mean histology scores and mean daily water temperature were the ranked categories. Histology scores were ranked according to risk of population impacts (>2.75 = high, <2.74 = low). A maximum likelihood estimate was used to determine if there was a significant ($\alpha<0.05$) relationship between water temperature and risk category and an odds ratio estimate was used to determine the odds of sentinel fish test lots being in one of the risk categories and what influence temperature had on the odds ratio. Tests were performed using the proc logistic procedure in SAS.

Results

Radio Telemetry

We relocated the 30 radio-tagged rainbow trout a total of 1,577 times and mean relocations per fish was 34 (range 1-134).

Rainbow trout spawning was not localized to the half of the study area in which they were tagged ($\chi^2=9.76$, df=1, $P=0.009$). We found no significant difference between the number of fish that spawned within or outside of the five sections ($\chi^2=0.08$, df=1, $P=0.39$). The distance that rainbow trout migrated to spawning locations was significantly different among the five sections (ANOVA:

F=3.44; df=3,20; *P*=0.03). Fish from the uppermost section (Section 1) of the Missouri River moved the greatest mean distance (28.9 km, SE: 9.0) to spawning locations. Fish from each section entered the Dearborn River to spawn, yet 36 percent of the Dearborn spawners migrated downstream from section 1.

In 2008, 88 percent of the radio tagged rainbow trout made spawning migrations and 62 percent of these moved outside of their starting section to spawn. Of the tagged fish that made spawning migrations, 73 percent spawned in the Missouri River and 27 percent spawned in the Dearborn River. Tagged fish migrated a mean 17.0 (SE: 4.0) km to reach spawning sites (Table 1).

In 2009, 81 percent of the radio-tagged rainbow trout migrated to spawning sites and only 20 percent of these moved outside of their starting section to spawn. The mean distance fish traveled to reach spawning sites was 13.1 (SE: 6.3) km (Table 1). Only one fish from each section traveled outside of their respective section to spawn in 2009. Fish from sections 2 and 4 moved the greatest distance to spawning sites (81.6 km and 59.6 km, respectively).

In 2010 only half of the radio tags were transmitting and only 20 percent of the fish with active tags moved outside of their home section to spawn. The mean distance fish traveled to reach spawning sites was 20.8 (SE: 13.9) km (Table 1), but this mean estimate is heavily influenced by one fish that left the study area in late 2009 and resided near the town of Great Falls over the winter. In early 2010, it traveled 116.8 km from its starting section to spawn in the Dearborn River for the second time in three years. Excluding this fish, the remaining fish traveled a mean distance of 7.1 (SE: 2.7) km to spawning sites.

Overall, radio-tagged rainbow trout were presumed to have spawned, based on their movements, over a 79 d period from 3 March to 5 May. Over the three-year period, 69 percent of rainbow trout spawned only in the Missouri River, 31 percent spawned in a tributary at least one time, 12 percent spawned in tributaries multiple times, 73 percent spawned in consecutive years, 27 percent spawned only once and, 15 percent spawned in a different river or stream. Tagged fish traveled a mean 16.4 (SE: 3.7) km to reach spawning sites (Fig 2).

After spawning, 21 percent of the rainbow trout returned to within 1.6 km of their starting point for that year, but the remaining fish moved to new locations after spawning. Only 8 percent of the rainbow trout displayed repeat tributary spawning in two successive years. The mean distance between spawning locations for repeat tributary spawners was 6.5 (SE: 0.2) km. Missouri River mainstem spawners accounted for over half (58%) of the sample. Mean distance between spawning locations for repeat mainstem spawners was 6.1 (SE: 2.1) km. The average total distance rainbow trout traveled over the three year period was 68.5 km (SE: 17.4). Only 11 percent traveled more than 160 km in any one calendar year. Radio- tagged rainbow trout spawned in each of the five sections in the Missouri River and in the lower 52.8 km of Dearborn River (Fig 2.). None of the radio tagged fish entered the Little Prickly Pear drainage (Little Prickly Pear Creek, Lyons Creek, Wolf Creek) or Sheep Creek during the study. Over half (52%) of rainbow trout traveled downstream to reach spawning locations.

Table 1. Spawning migration characteristics of radio-tagged rainbow trout in the Missouri River, Montana, 2008-10.

Year	% of tagged fish that spawned	Mean km traveled to spawn	Standard error (km)	Range (km)
2008	88	17.0	4.0	0.1-64.4
2009	81	13.1	6.3	0.1-81.6
2010	20	20.8	13.9	0.3-116.8
All years	---	16.4	3.7	---

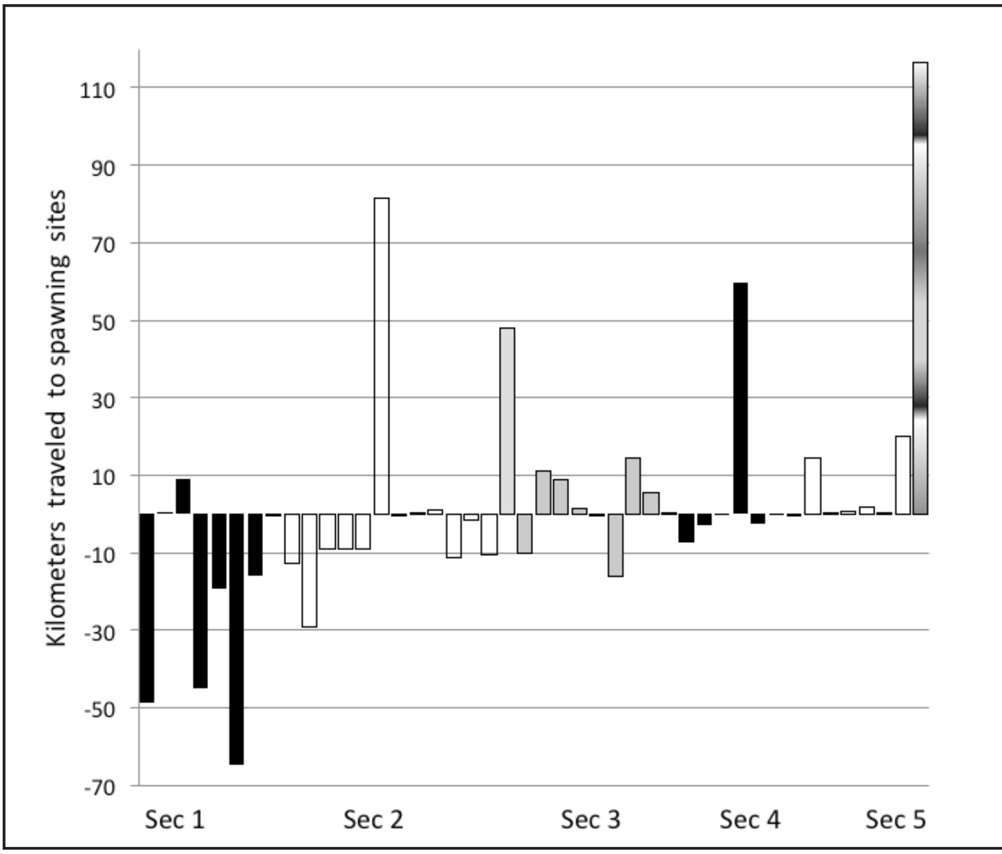

Figure 2. Distance radio-tagged rainbow trout traveled to spawning sites upstream (+km) and downstream (-km) by section, Missouri River, Montana 2008-2010. Gradient filled bar represents a fish that left the study area and then returned to spawn.

Redd Counts

In April 2007, the Missouri River portion of the study area was surveyed to identify the spatial distribution of redds. The mean distance between redds in the Missouri River was 1.89 (SE: 0.21) km. Spawning redds were distributed throughout the entire longitudinal reach of the Missouri River.

During the Spring of 2010 weather conditions (wind, overcast, rain) and flow (122 m3/s) in the Missouri River were favorable for observing redds from the air and 39 percent (1,644) of the redds we counted occurred in the Missouri River (Table 2). We observed redds in deep water (> 2 m deep) at six locations in the Missouri River. The greatest number of deep water redds was 47 which occurred at rkm 3.5, followed by 24 redds at rkm 4.9, 11 redds at rkm 14.2, 2 redds at rkm 16.9, 2 redds at rkm 18.4 and 6 redds at rkm 22.7. From 2008-10 the spatial distribution of redds spanned 122 km of river and stream annually. Overall, 28 percent of the mean number of annual redds occurred in the Missouri River and 72 percent occurred in tributaries (Table 2). The mean annual number of redds for the entire system was 4,071 (SE: 378) (Table 2). Little Prickly Pear Creek had 44-47 percent of the total annual redds, the highest mean number of redds and the highest mean density (92/km) of redds. The Dearborn River had the lowest mean density at 21/km (Table 2).

M. cerebralis monitoring

Little Prickly Pear Creek had the highest *M. cerebralis* infection scores in 11 of 13 years (Table 3). Other streams

Table 2. Rainbow trout redds counted during the basin wide surveys and redd density (redds/km) for the Missouri River and tributaries, Montana 2007-10.

Stream	Dist (Km)	Number of redds (redds per km) 2007	2008	2009	2010	Mean	Rel %
Missouri River	41.6	---	b	b	1644 (40)	1644 (40)	28
Dearborn River	30.4	---	b	b	632 (21)	632 (21)	10
Little Prickly Pear Cr.	19.5	2125 (109)	1461 (75)	b	b	1793 (92)	29
Lyons Creek	11.2	847 (76)	897 (80)	b	386 (34)	710 (63)	11
Wolf Creek	12.5	1289 (103)	678 (54)	b	1451 (116)	1139 (91)	18
Sheep Creek	3.2	282 (88)	286 (89)	b	234 (73)	267 (83)	4
Total		**4543**	**3322**		**4347**	**4071**	

b - high turbid flows precluded counting redds

with multiple high (> 2.75) scores over the monitoring period were Wolf Creek (58%) and the Dearborn River (50%). In the Missouri River, the Craig monitoring site had high scores in 6 of 13 (46%) years and was the only Missouri River site with multiple scores greater than 2.75. Overall, 53 percent of the habitat used by rainbow trout for spawning had a high severity of *M. cerebralis*, 38 percent had low to moderate severity, and 9 percent had no infection, as detectable by sentinel fish. Lyons Creek remains the only stream in our study area where the parasite has not been detected.

From 1997 to 2009, 5.3 percent (range 1.7-8.9%) of the rainbow trout sampled during the annual population estimates displayed clinical signs of *M. cerebralis* infection (jaw deformity, shortened operculum, dolphin head and spinal deformity: Table 4). Dolphin head was the deformity that occurred the most. The highest numbers of deformed fish occurred in 2001, when 8.9 percent of the fish handled during the population estimate displayed clinical signs of *M. cerebralis* infection.

Myxobolus cerebralis infection in sentinel fish occurred from 6 May to 3 July (median = 31 May). The dates of exposure for test lots with mean infection scores of 2.75 and greater ranged from 15 May to 3 July (median = 30 May) and the dates of exposure for tests lots with mean infection scores of 2.74 and less ranged from 5 May to 3 July (median = 1 July).

There was a significant relationship between histopathology rank and temperature (maximum likelihood estimate, P=0.02). The mean daily water temperature in test lots with infection scores 2.75 and greater was 11.6°C (range = 8.7-16.5), whereas it was 12.9°C (range = 9.6-18.1) for test lots with scores 2.74 and less. The odds of sentinel fish test lots being classified as low risk was higher by 1.097 for each degree temperature increase and the odds increased by a factor of 2.519 for every 10 degree increase in temperature.

Discussion and Conclusions

A variety of factors may influence the relative impact that *M. cerebralis* has on rainbow trout populations. Among these are life history and spawning behaviors (Downing et al. 2001, Pierce et al. 2009), differential infection susceptibility among strains of fish (Vincent 2002), habitat conditions (Granath et al. 2007) and water temperature at time of emergence and during early-life rearing (Baldwin et al. 2000).

Where native, rainbow trout have demonstrated discrete spawning behaviors and site fidelity for spawning, feeding and overwintering (Meka et al. 2003). The data from this study did not support the hypotheses that radio-tagged fish would spawn within their annual starting section or that spawning was localized to either half of the study area. The location of rainbow

Table 3. Mean cranial lesion histopathology scores (0-5) from sentinel rainbow trout exposed to *M. cerebralis* at select sites in the Missouri River below Holter Dam and its tributaries. Values represent the highest mean infection severity measured for any 10 d period for each year.

Stream -site	Year												
	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
Little Prickly Pear Creek - Wirth div	2.94	3.98	4.66	4.76	4.43	4.96	4.92	4.87	4.97	0.11	4.72	1.96	4.70
Lyons Creek - mouth	---	0.00	---	0.00	0.00	0.00	0.00	0.00	0.00	0.00	---	0.00	---
Wolf Creek - mouth	---	1.24	0.20	0.78	4.47	3.96	3.98	4.80	4.42	0.15	4.58	2.70	3.94
Missouri River - Holter Dam	0.00	0.00	0.28	0.14	0.16	0.20	0.00	---	---	---	---	0.00	0.00
Missouri River - Craig	0.66	1.83	3.88	2.82	3.47	2.22	1.76	3.33	3.78	0.15	2.02	4.02	0.85
Missouri River - Mid Canon	---	0.00	---	0.30	1.60	0.19	0.34	2.00	3.00	0.13	1.96	---	1.44
Missouri River - Pelican Point	---	0.02	2.35	---	0.26	0.10	0.12	0.97	1.66	0.00	1.80	0.04	1.56
Dearborn River - Hwy 287	---	0.00	0.00	0.00	0.07	1.18	2.58	4.59	4.72	4.13	3.00	4.27	4.13
Sheep Creek - mouth	0.00	0.11	0.00	0.00	0.00	0.00	0.00	---	0.08	0.00	---	0.34	---

trout at the point of tagging or on 1 January was not an indicator of where they spawned. The mean distance and maximum distance that fish traveled to spawning locations was greater than has been reported for other rainbow trout populations (Adams 1996, Lisac 1996, Meka et al. 2003). In many instances fish traveled over suitable habitat, as evidenced by existing redds, to reach eventual spawning destinations. Such behavior suggests a determination to reach a specific area for spawning, but the majority of fish lacked spawning site fidelity between years.

Low site fidelity may be an important life history characteristic that spreads the risk posed by *M. cerebralis* over spawning locations with a broad range of infection severity. There is some speculation of differing virulence of *M. cerebralis* strains based on geographical location (Modin 1998). Other studies in our area showed a high degree of spatial variation and *M. cerebrali*s infection intensity in the primary host *Tubifex tubifex* (McMahon et al. 2001). The variable infection severity in *T. tubifex* may contribute to the variable levels of disease severity seen among stream salmonid populations (Stevens et al. 2001). Relating this to infection in fish or fish population declines has proven difficult in other areas. For example, despite variable *M. cerebralis* infection in *T. tubifex* among varied habitats in the Rock Creek drainage of Montana, lower infections in this host did not necessarily translate to lower infections in fish because the infectious stage of the parasite (triactinomyxon) spreads rapidly in flowing water (Granath et al. 2007).

The low spawning site fidelity observed in our study and the broad spatial scale over which fish redistributed after spawning were in contrast to other research but are behaviors that could facilitate avoidance of unfavorable habitat conditions (Adams, 1996, Adams 1999, Meeka et al. 2003, Pierce et al. 2009). Whether the difference represent behavioral changes in response to *M. cerebralis*, or differences in behavior have been longstanding components of the trout life history, remains unknown.

Table 4. Relative occurrence of clinical signs of *M. Cerebralis* infection in wild rainbow trout sampled during population estimates in the Craig section of the Missouri River, Montana 1997-2009.

Year	BB	SOP	DH	JAW	Total deformed	Total rainbow trout handled	% of deformed in total catch
1997	45	19	18	10	92	5339	1.72%
1998	23	15	107	8	153	5329	2.87%
1999	54	20	116	40	230	5036	4.57%
2000	87	19	111	60	277	4835	5.73%
2001	176	16	158	46	396	4475	8.85%
2002	121	15	143	58	337	4735	7.12%
2003	68	33	84	74	259	4272	6.06%
2004	67	19	97	40	223	3885	5.74%
2005	58	47	80	57	242	4163	5.81%
2006	34	24	71	48	177	3269	5.41%
2007	19	37	52	44	152	4117	3.69%
2008	20	35	55	53	163	2636	6.18%
2009	14	24	55	73	166	3742	4.44%

BB – bent back/spinal deformity
SOP – shortened operculum
DH – dolphin head
JAW – deformed mandible/maxilla

After spawning, the majority (79%) of tagged fish moved to sites other than their pre-spawn locations. In other areas where *M. cerebralis* has caused reductions in rainbow trout populations, 75 to 76 percent of spawners returned to within 1.5 to 2.0 km of their pre spawn location (Downing et al. 20032, Pierce et al. 2009). The fact that some fish spawned only once during the study suggests the possibility of alternate year spawning by a low percentage (27%) of rainbow trout in the Missouri River system. Alternate year spawning has been reported in female rainbow trout in tributaries to the King Salmon River in Alaska (Adams 1999) and is typical of fish with slow metabolism that cannot store the proper amount of energy necessary to develop eggs and make migrations to spawning areas (Palstra et al. 2010).

Comparing these results with the limited historic rainbow trout life history information for this area suggest a higher use of the Missouri River for spawning (Munro 2004, Leathe et al. 1988, McMahon et al. 2001). Changes in the number of redds included a 27 to 37 percent decrease in the tributary streams (Little Prickly Pear, Lyons, Wolf creeks: Grisak 1999), 77 percent decrease in the Dearborn River and 64 percent increase in the Missouri River (Leathe et al. 1988). Such changes may contribute to the persistence of rainbow trout in the Missouri River-Holter Dam tailwater fishery because *M. cerebralis* infections measured in the Missouri River are less severe than in its tributaries. By comparison, spawning by rainbow trout in Montana's Madison River system occurs mostly in the mainstem and is concentrated in the upper reaches where the majority of emerging young are susceptible to a high prevalence of *M. cerebralis* (Downing et al. 2002). Other research has shown that life histories of rainbow trout could lead to higher *M. cerebralis* infection if young fish have to migrate through areas with a high density of infected *T. tubifex* (Sandell et al. 2001).

Over the 13-year monitoring period the adult rainbow trout population in the Missouri River remains near the long term median despite high (>2.75 histopathology score) *M. cerebralis* severity measured in sentinel fish in over half (53%) of the available spawning habitat (Table 2: Grisak et al. 2010). Three of the four Missouri River monitoring sites (Holter, Mid Canon, Pelican Point) had relatively low infection severities compared to the Craig site. The high infection severity at the Craig site may be related more to its proximity to Little Prickly Pear Creek, located 7.4 rkm upstream, than to the infection severity in the Missouri River. Granath et al. (2007) reported that infections in sentinel fish may be the result of triactinomyxons produced hundreds or thousands of meters upstream. In essence, localized heavily degraded habitats, suitable to infected *T. tubifex*, may serve as a major source of infection at a broader scale than would be assumed from sampling the distribution of *T. tubifex* worms or from the results of sentinel fish studies. The monitoring site that had the second highest infection severity scores was the Mid Canon site, which is located 2.4 rkm downstream of the Dearborn River.

Histopathology rank was correlated to water temperature. Mean water temperature at sites with high severity infections was slightly less than at sites with low to moderate severity infections suggesting infection severity peaks as water temperature rises and then drops as water temperature increases above 12^{o}C. Other field experiments with rainbow trout showed the highest infection intensities occurred when water temperatures were between 12 and 16^{o}C then declined rapidly as mean daily water temperatures decreased or increased from these optimum water temperatures (Vincent 2002).

There is evidence of differing infection susceptibility among rainbow trout strains (Vincent 2002). However, attributing the stability of rainbow trout populations in the Missouri River to a particular strain would be difficult because rainbow trout are not native to this region and the population was founded by over 40 years of stocking undesignated strains. Since 1973, the population has been influenced by at least 12 strains of rainbow trout (Eagle Lake, Arlee, Erwin, DeSmet, Madison River, Shasta, McConaughy, Bozeman, Lewistown, Winthrop, Beula, Wild) stocked in the upstream reservoirs (Holter, Hauser, Canyon Ferry: MFWP unpublished data). Given the unknown origin of the majority of rainbow trout stocked in this area, as well as the uncertain contribution from 12 different strains there is no way of knowing which strains have succeeded or persisted over time. The consequences of such a stocking history may be a population that has a high level of gene flow and lacks selective pressures for distinct behavior patterns and spawning site fidelity (Allendorf and Phelps 1981, Slatkin 1985, Slatkin 1987), as our results show. These concepts have been studied extensively in Pacific salmon species when evaluating the interaction of wild and hatchery-reared fish and potential changes in local genetic adaptations (Hendry et al. 1996, Quinn 1993, Quinn et al. 2006).

The 79-d rainbow trout spawning period observed in our study indicates the fry emergence period would be broad enough to span the entire thermal range of *M. cerebralis* infection susceptibility. As such, fish of each cohort could be either unaffected or exposed to the parasite at levels which they likely survive. With such a broad spawning period, fry from early and late spawning fish could emerge at times when water temperatures are outside of the range of *M. cerebralis* infection (Vincent 2002), or when high spring flows dilute the concentration of triactinomyxons and flush fry out of tributaries into the Missouri River where the infection severity is less. The timing of outmigration of young rainbow trout can influence *M. cerebralis* infection. Infection can occur for a minimum of 60 to 65 days post hatch (Baldwin et al. 2000, Vincent 2002, Downing et al. 2002). Fish that migrate out of natal streams at age-0 have been shown to have a higher infection severity than fish that migrated at age-1 (Leathe et al. 2002).

This study found that the only observable impact of *M. cerebralis* infection to the adult population was the small number (<9%) of adult rainbow trout that we encountered during population estimates that had clinical signs of *M. cerebralis* infection. Despite no outbreak of whirling disease, a small segment of the rainbow trout population persists with a mild infection. The spawning behaviors we have described likely contribute to the stability of the rainbow trout populations in the Missouri River, even after 15 years of *M. cerebralis* presence in the study area.

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