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Relationships of migratory (hybrid) rainbow trout spawning life histories to risk of Myxobolus cerebralis infection in the Blackfoot River Basin, Montana

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Abstract - The middle Blackfoot River Basin in western Montana is the site of a low-elevation whirling disease epizootic among rainbow trout (*Oncorhynchus mykiss hybrids*) caused by a recent invasion of the exotic parasite *Myxobolus cerebralis*. To assess exposure of Blackfoot River rainbow trout to the parasite, we investigated the spawning life histories of adult rainbow trout with respect to the distribution and severity of disease in spawning and rearing areas in two distinct reaches of the Blackfoot River. Radio-telemetry confirmed Blackfoot River rainbow trout migrate from wintering sites within the Blackfoot River to spawning tributaries. Over 90% of telemetered rainbow trout in the middle Blackfoot River spawned in a low-gradient, infected stream where fry emerged in early July during the vulnerable, highly infectious period. By contrast, rainbow trout spawning of lower Blackfoot River fish was dispersed among smaller, colder, higher gradient tributaries, most of which fell below disease detection levels. For fluvial rainbow trout risk of exposure varies at a sub-basin scale and relates to the geographical arrangement and properties of tributaries, including the longitudinal relationship of disease to spawning and early rearing areas. Prior to the invasion of *M. cerebralis*, the middle Blackfoot River was identified with recruitment-limitations caused by winter mortality and anthropogenic activities. Management implications suggest that riparian restoration and habitat enhancement with emphasis on migratory native fish within and upstream of infected waters may buffer effects of the disease.

Key words: Blackfoot River, rainbow trout, whirling disease, migration, movement patterns, tributary, and population risk

Introduction

Whirling disease (WD *hereafter*), a parasitic infection caused by the myxosporean *Myxobolus cerebralis*, has been associated with significant declines in wild rainbow trout (RBT *hereafter*) populations in certain streams in the western United States (Nehring and Walker 1996; Vincent 1996). WD was first detected in Montana in 1994 within the renowned Madison River following large and unexplained declines in RBT abundance. Soon thereafter, WD was described as one of the single greatest threats to wild trout (MWDTF 1996). Yet with time and the expansion of WD, it appears the influences of WD on interior populations of RBT are highly variable among watersheds (Nehring and Walker 1996, Modin 1998, Sandell et al. 2001). *M. cerebralis* has a complex, two-host life cycle involving the aquatic oligochaete worm *Tubifex tubifex*, and most salmonids, which include trout, whitefish and salmon. Susceptibility to disease depends on species (MacConnell and Vincent 2002), fish age and size (Ryce et al. 2005), and parasite dose at time of exposure (Vincent 2002). Young trout, particularly RBT, are most vulnerable when infected at less than nine weeks of age (Ryce et al. 2004). Coincidence between this vulnerable period and the release of the infective triactinomyxon (TAM) stage of the parasite largely determines the degree of exposure for young fish and, ultimately, the magnitude of population-level effects. High mortality and recruitment collapse can occur in highly exposed populations (Nehring and Walker 1996, *but see* Sandell et al. 2001).

Environmental conditions play an important role in the distribution of infection and level of severity within and among streams of the Blackfoot Basin (Pierce et al. In review). In Cottonwood Creek, a tributary to the middle Blackfoot River, Smith (1998) initially identified a longitudinal distribution with *tubifex* worms and WD absent from upper glacial valleys but abundant *tubifex* worms and a high severity of disease present in lower-valley stream reaches. More recently, water temperature, channel gradient and fine sediment were primary environmental predictors of WD presence within basin-fed streams of the Blackfoot Basin like Cottonwood Creek (Pierce et al. In review).

Although WD has resulted in very large population declines of RBT in certain Montana (Madison River - Vincent 1996, Baldwin et al. 1998) and Colorado Rivers (Nehring and Walker 1996), population effects are regionally variable. Previous studies of RBT vulnerability to WD in Montana have focused on the tailwater fishery of the Madison River where trout spawn in side-channels (Downing et al. 1999, Krueger et al 2006).

In western Montana, significant declines in RBT in Rock Creek, a large tributary to the upper Clark Fork River near Missoula, followed the introduction of WD (Montana Fish, Wildlife and Park, unpublished data). Likewise, RBT in lower Cottonwood Creek have declined 50% from pre-WD estimates (Peters 1990, Smith 1998, Pierce et al. 2006). Both Rock Creek and lower Cottonwood Creek have experienced community-level shifts toward brown trout (Montana Fish, Wildlife and Parks, unpublished data), a species with partial WD resistant. These observations suggest some risk of population, or community-level, changes in the middle Blackfoot River where RBT declines are now being detected near infected RBT spawning tributaries. Predicting WD effects on RBT populations in western Montana requires assessing the juxtaposition of streams with high vulnerability to infection and the location of spawning and rearing sites.

Previous studies identify Blackfoot River RBT reproduction within tributaries (Peters and Spoon 1989, FWP unpublished data); however, the relative importance of tributary stocks has not been evaluated, nor has the influence and spatial extent of possible WD effects upon fluvial RBT of the Blackfoot River. To investigate these questions, we assessed the overlap of fluvial RBT spawning sites with *M. cerebralis* infection in 10 spawning streams. Our study objectives were to: 1) identify the spawning life-histories of fluvial adult RBT of the Blackfoot River; 2) identify the relative use of spawning tributaries by fluvial stocks of the Blackfoot River; and 3) identify disease severity in spawning streams using sentinel exposures of age-0 RBT. Our purpose is to assess disease risk for migratory RBT stocks for two reaches of the Blackfoot River, to gain a better understanding of fluvial RBT and to identify management measures that could buffer possible RBT declines within rivers of western Montana.

Study Area

The Blackfoot River, a 5th order tributary (Strahler 1957) of the upper Columbia River, lies in west-central Montana and flows west 212 km from the Continental Divide to its confluence with the Clark Fork River in Bonner, Montana (Figure 1). The River drains a 5,998-km2 heterogeneous watershed through 3,038 km of perennial streams and generates a mean annual discharge of $44.8m^3s$ (United States Geological Survey 2006). The Blackfoot River flows freely to its confluence with the Clark Fork River where Milltown dam, a run-of-the-river hydroelectric facility, has blocked upstream fish passage to the Blackfoot River since 1907. The physical geography of the watershed is geo-structurally controlled and regionally variable with subalpine forests dominating the high mountains, montane woodlands at the mid-elevations and semi-arid glacial (pothole and outwash) topography on the valley floor. Primary tributaries of the upper Blackfoot River (upstream of the Clearwater River) flow through a broader upper valley and alluvial bottomlands. Downstream of the Clearwater River, mountains constrict the Blackfoot River

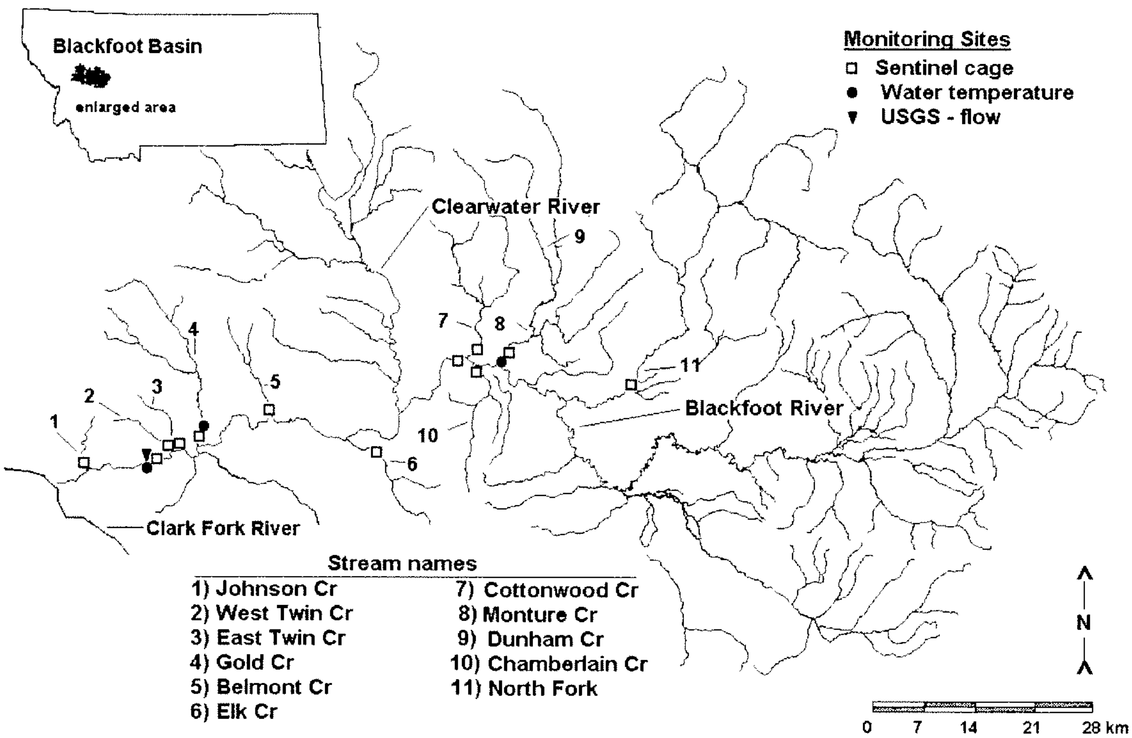

Figure 1. Study area: Blackfoot River Basin with sentinel cage, water temperature and Blackfoot River (USGS) discharge monitoring sites.

to a narrow canyon. With some exceptions, tributaries enter the lower Blackfoot River through a mountainous area with confined channels, steeper gradients and colder summer temperatures.

WD was first detected in the middle Blackfoot Watershed in 1995 in lower Cottonwood Creek. Since then, the disease has increased in distribution and severity. WD now infects the entire mainstem Blackfoot River and lower reaches of many tributaries (Pierce et al. 2006). Tributaries to the Blackfoot River provide spawning and rearing for migratory RBT, as well as other WD susceptible salmonids including bull trout (*Salvelinus confluentus*), westslope cutthroat trout (*O. clarki lewisi*), mountain whitefish (*Prosopium williamsoni*), brown trout (*Salmo trutta*) and brook trout (*Salvelinus fontinalis*) (Pierce et al. 2006).

Montana rivers are managed for a diversity of self-sustaining *wild trout* populations. Within the Blackfoot Basin, wild RBT are present at low-elevations, and population densities increase in the down-river direction (Pierce et al. 2006). Although RBT occupy only about 15% of the Blackfoot Basin, they comprise ~ 70% of the trout community in the lower Blackfoot River. Below the mouth of the North Fork, RBT contribute to a high-value recreational fishery for the mainstem Blackfoot River supporting an estimated 26,817 anglers in 2005 (Montana Statewide Angling Pressure Estimates 2005).

For this study, we telemetered fluvial RBT from the Blackfoot River and examined life history and disease relationships up-and downstream of the mouth of the Clearwater River. The lower Clearwater River flows through a series of natural lakes causing high summer water temperatures (>27°C), and seems to support very little, if any, RBT reproduction (Peters 1990, Pierce et al. 2002). This river demarcates the mid-point of rainbow trout distribution within the Blackfoot River RBT distribution, and separates the Blackfoot Basin into two general sub-basins based on physical differences of tributaries. The lower reach of the Blackfoot River (R1 *hereafter*) extends from Clearwater River confluence 55.8 km downstream to the Blackfoot River confluence with the Clark Fork River. Except for the upper-most tributary to R1 (Elk Creek), RBT spawning tributaries originate in a mountainous region and tend toward smaller (second and third-order), higher gradient streams with colder summer temperatures. Conversely, Elk Creek, a low-gradient stream within an agricultural valley, supports elevated summer temperatures and high instream levels of fine sediment (Pierce et al. 2006). The Blackfoot River between the confluence of the Clearwater River and North Fork Blackfoot River (R2 *hereafter*) has RBT spawning tributaries that are fewer but generally larger (third and fourth-order), flow within wider channels, have broader floodplains with lower gradients and support warmer summer temperatures. An exception is the North Fork, a stream of wilderness origin that is larger and colder than all other R2 tributaries. (Pierce et al. 2006).

Methods

Radio-telemetry – We assessed migration patterns, relative use of tributaries, timing of migration events and location of RBT spawning using radio-telemetry. Twenty-five RBT were captured in the lower Blackfoot River, phenotypically identified as RBT and implanted with continuous (12 hour on/off) Lotek™ radio transmitters on 8 March 2004 (*n*=4), between 28 February – 8 March 2005 (*n*=10) and 7 – 22 March 2006 (*n*=11) and tracked to spawning areas within tributaries. These fish ranged from 34.0 to 49.0 cm in total length (mean, 41.4) and from 408 to 1,270 g in weight (mean, 680). We selected larger "plump" female fish (based on absence of a kype) to increase the likelihood that telemetered fish were sexually mature, and to more accurately identify the timing and location of spawning events. Visual identification was later verified for the 21 of the 25 fish collected in 2005-06 through genetic analysis of fin clips using 17 fragments of nuclear DNA at the University of Montana, Trout and Wild Salmon Genetics Laboratory (Boecklen and Howard 1997).

Transmitters were evenly distributed among fish in the lower 35.4 km of R1 (*n*=12); whereas telemetered fish were captured only in a 6.4-km section in R2 (*n*=13) due to shelf ice and limited river access. Fish were captured prior to spawning migrations (by electro-fishing) in suspected wintering pools. Individually coded transmitters weighed 7.7 g, had an estimated life of 450 days, emitted an individual coded signal, did not exceed 2 percent of fish weight (Winters 1997), and were implanted following standard surgical methods (Swanberg 1999).

Technicians located telemetered fish on foot using a hand held three-element Yagi antenna or by truck using an omni-directional whip antenna. We located fish weekly prior to migrations, 2-3 times per week during migrations and spawning, once per week following spawning and generally once per month thereafter.

We recorded upstream movements by river kilometer. We assumed fish spawned if they ascended a stream with suitable spawning habitats during the spring spawning period, and the upper-most location was the assumed spawning site. We estimated spawning dates as the median date between two contacts for a given event (i.e. spawning or migration) (Swanberg 1997). Peak spawning among spawners was identified as the median spawning date. We assumed the reach influenced by whirling disease extended from wintering locations to spawning sites.

Water temperature and flows - Water temperatures and flows were measured in the Blackfoot River to assess their influences on RBT migrations. Thermographs (Onset™) were placed (2005-06) at rkm 12.7 at the U.S. Geological Survey gauging station (guage number: 12345000). We used both mean daily discharge and temperature to examine potential relationships with RBT movements. Onset thermographs were placed in lower Gold (2005-06) and Monture creeks (2004-06) where mean daily temperatures were calculated to identify relationships of tributary movements and spawning. To predict the timing of fry emergence for Gold and Monture Creeks, we calculated the incubation period using a 350°C degree-day span (Piper 1982), beginning at the estimated spawning date for each individual fish that spawned in Gold and Monture Creeks, and emergence was estimated at three weeks post-hatch. All thermographs recorded at 48-minute intervals.

Analyses of life histories – To test the potential influence of introgression on movement patterns, we compared the start date of migration and the total pre-spawning migration distance between hybrids, and "pure" RBT using Mann-Whitney rank sum tests. For the total group, we used linear regressions to assess potential associations between the start date of migration and distance to spawning sites; the total duration (days) and total distance (km) of migrations ; and the date spawners returned to the River and the total migration period (days). We used Kruskal-Wallis (ANOVA) on ranks to assess tributary size (i.e. stream-order) and the date of entry to a spawning stream and days spent within a tributary. For reach-stratified spawners, we used Mann Whitney rank sum tests to analyze the start of migration, dates RBT entered tributaries and the upstream distance to spawning sites upon entering a tributary, estimated spawning dates and dates RBT exited tributaries. All tests were evaluated at the $\alpha = 0.05$ level of significance.

WD infection and severity – We conducted sentinel exposures of 50 hatchery RBT fry (age-0 cohorts) at known RBT spawning sites in 10 streams to identify WD severity in individual streams and the spatial variation of *M. cerebralis* among tributaries (Figure 1). These fish were exposed at 98-103 days post-hatch at mean length of 36mm in 2005 and 45mm in 2006. Exposures were completed in July within 9 weeks of the estimate post-hatch period for wild fish. This timing coincides with high RBT susceptibility (Ryce et al. 2004), estimated emergence of wild RBT fry and the corresponding peak TAM production period within rivers of western Montana (Vincent 2000) including the Blackfoot Basin (Fish, Wildlife and Parks, unpublished data). The exposure period for each live cage was standardized at 10 days. At the end of that time, fry were transferred to Pony, MT, where they were held for an additional 80 days at a constant 10 °C to ensure that WD, if present, would reach maximum intensity (Vincent 2000). At the end of the holding period, all surviving fish were sacrificed and sent to the Washington State University Animal Disease Diagnostic Laboratory at Pullman, WA. At the lab, fish heads were examined histologically and scored using the MacConnell-Baldwin grading scale, which ranks whirling disease from 0 (absent) to 5 (severe) (Baldwin et al. 2000). Sentinel exposures were considered severe if a majority (%) of exposed RBT had histological (lesion) scores of ≥ 3 on the MacConnell-Baldwin scale. Lesion scores ≥ 3 are determined by severe cartilage damage and a dispersed inflammatory response that occurs in infected fish (Baldwin et al. 1998).

Results

Migratory life histories and spawning - For 25 telemetered RBT, we made a total of 1,594 contacts with an average of 64 contacts (range: 12-129) per fish. All 25 RBT were successfully tracked to spawning tributaries from March 2004 to December 2006 (Table 1). Fourteen of twenty fish that underwent genetic analysis tested as post-F_1 RBT hybrids with westslope cutthroat trout having a predominant rainbow trout genetic contribution; the remaining six tested as genetically unaltered rainbow trout (Leary 2005, 2006). Four migrants captured in 2004 that later entered Monture Creek (n=3) and the North Fork (n=1) were untested. There were no significant differences between hybrid and "pure" RBT for either start (date) of migration (Mann Whitney, P=0.78) or the total pre-spawning distance moved (Mann Whitney, P=0.56).

River temperatures and flows incrementally increased during the (2004-2006) RBT pre-spawning migrations. In these years, migrations began between 19 March and 15 April on the rising limb of the hydrograph as mean daily temperatures approached 5°C (Figure 2). With the onset of migration, twenty-four RBT moved up-river and one moved down-river. In nine days telemetered RBT traveled a median of 6.8 rkm to their respective spawning tributary. RBT from R1 moved a (median) distance of 10.0 rkm (range 0.5 – 56.8) compared to 6.6 rkm (range 2.7-21.4) for R2.

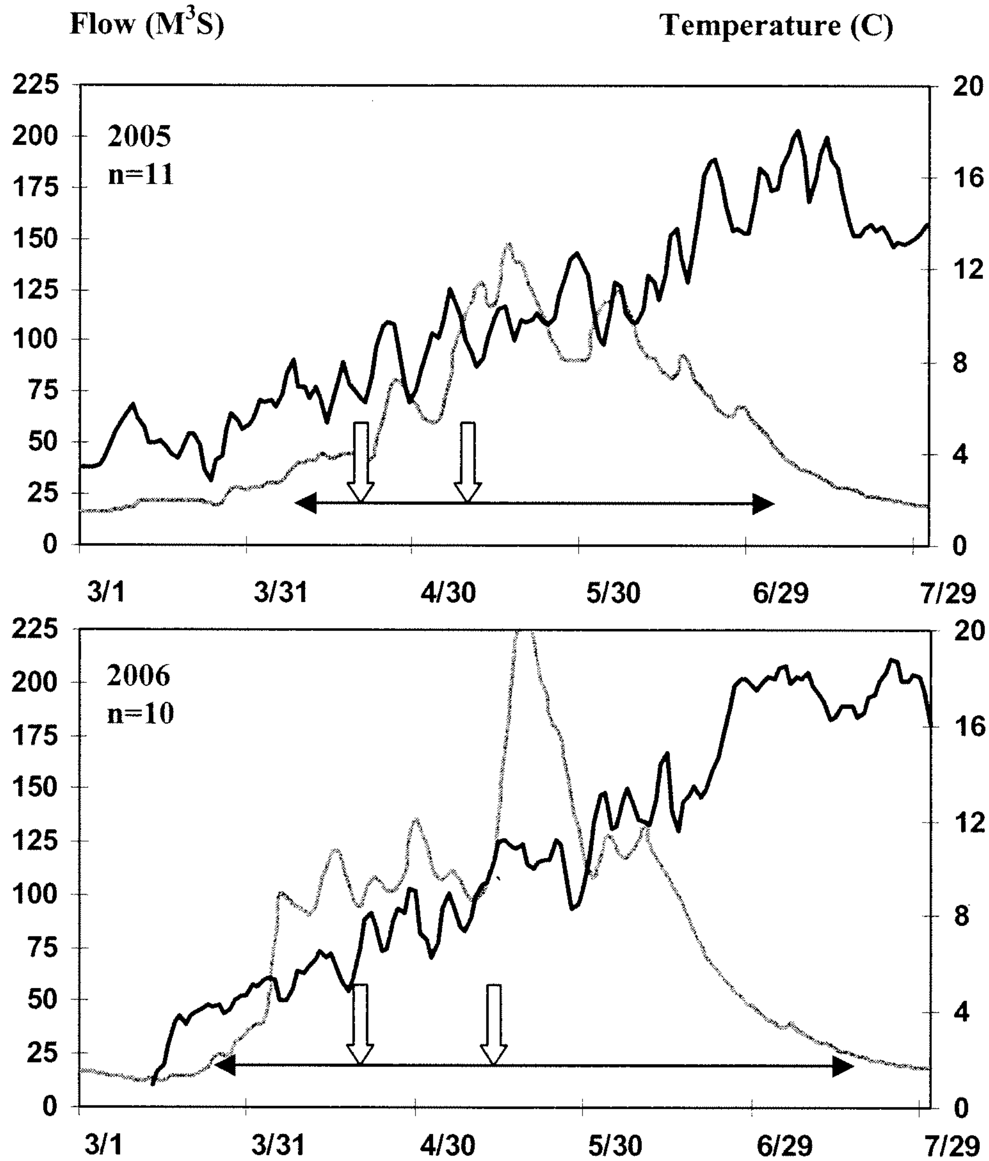

Figure 2. Blackfoot River: Mean daily flow (left axis - gray line) and mean daily water temperatures (right axis - dark line) during rainbow trout spawning migration. The total migration period is shown by the arrowed horizontal lines. The vertical arrows show median dates spawners entered and exited tributaries.

For the total group, there was no relationship between the date migrations began and the total distance to spawning sites (linear regression, R2 =0.008, P=0.89). However, RBT with longer pre-spawning distance (start locations and spawning sites) underwent migrations of longer duration (linear regression, R^2= 0.20, P=0.04), and RBT returned to the river later than fish exhibiting movements of shorter duration (linear regression, R^2=0.36, P=0.003).

Spawners spent on average of 17 days (range, 3-63) in tributaries and ascended a median of 3.0 km (range, 0.2-19.8) to their spawning grounds where they held for an average of six (range 1-14) days before returning to the Blackfoot River. We observed R2 fish migrated significantly farther up tributaries (median, 7.1 versus 1.0 km) to spawning sites than R1 fish (Mann Whitney, P=0.005). Based on the distance between winter pools and spawning sites, fish moved a (median) distance of 12.1 rkm for the total group, and a median of 10.6 (range 1.1 – 63.2) rkm for R1 fish compared to 12.6 (range 6.0 – 27.5) for R2 fish.

Migration events began slightly earlier and ended later for R2 fish although these differences were not statistically significant. RBT from the R2 began their migrations eight days earlier (median, 9 April versus 17 April; Mann Whitney, P=0.17), entered tributaries nine days earlier (median, 17 April versus 26 April; Mann Whitney, P=0.10) and spawned six days earlier (median, 28 April versus 4 May; Mann Whitney, P =0.40). However, the duration of tributary use was five days longer for R2 fish (median, 17 days versus 12 days), and fish exited tributaries six days later (median, 15 May versus 9 May; Mann Whitney, P=0.24) than R1 fish.

RBT spawned in six tributaries ranging from 2^{nd} to 4^{th} order with the Monture Creek watershed and Gold Creek supporting the highest proportion of spawners (*n*=12 or 48%) and (*n*=5 or 20%) respectfully (Table 1). Fish from R1 spawned in four tributaries: Gold Creek (*n*=5), Belmont Creek (*n*=4), East Twin (*n*=2) and

Monture Creek (n=1); whereas R2 fish spawned in Monture (n=10) and its tributary Dunham Creek (n=2), and only one spawning outside of the Monture Creek Basin, within the North Fork. Based on stream-order, spawners entered larger tributaries earlier than smaller tributaries (ANOVA, P=0.02). However, there was no significant difference with stream-order and time spent in tributaries (ANOVA, P=0.20),

During the period of pre-spawning river migration (19 March to 15 April), mean water temperatures in the Blackfoot River were higher in 2005 (5.6°C) than 2006 (4.9°C). Thirteen RBT entered Monture Creek and five entered Gold Creek at mean water temperatures of 5.6 (range 3.6-8.1°C), and RBT spawned at mean temperatures of 5.2 (range 3.4-8.0 °C) in these drainages.

After spawning, all fish with active radios (n=24) exited the tributaries. Three of 24 (12%) post-spawners (fish: 2, 9 and 25) moved downstream of Milltown Dam into the Clark Fork River during peak flow (May and July), including two spawners from Gold Creek and one that moved downriver >74-km after spawning in Monture Creek. However, the majority (n=18 or 76%) either returned to (n=9), or were within 1.6 km (n=9) of their original start locations; three (12%) moved downriver a mean of 14.0 km (range, 4.3-23.7) from their starting locations.

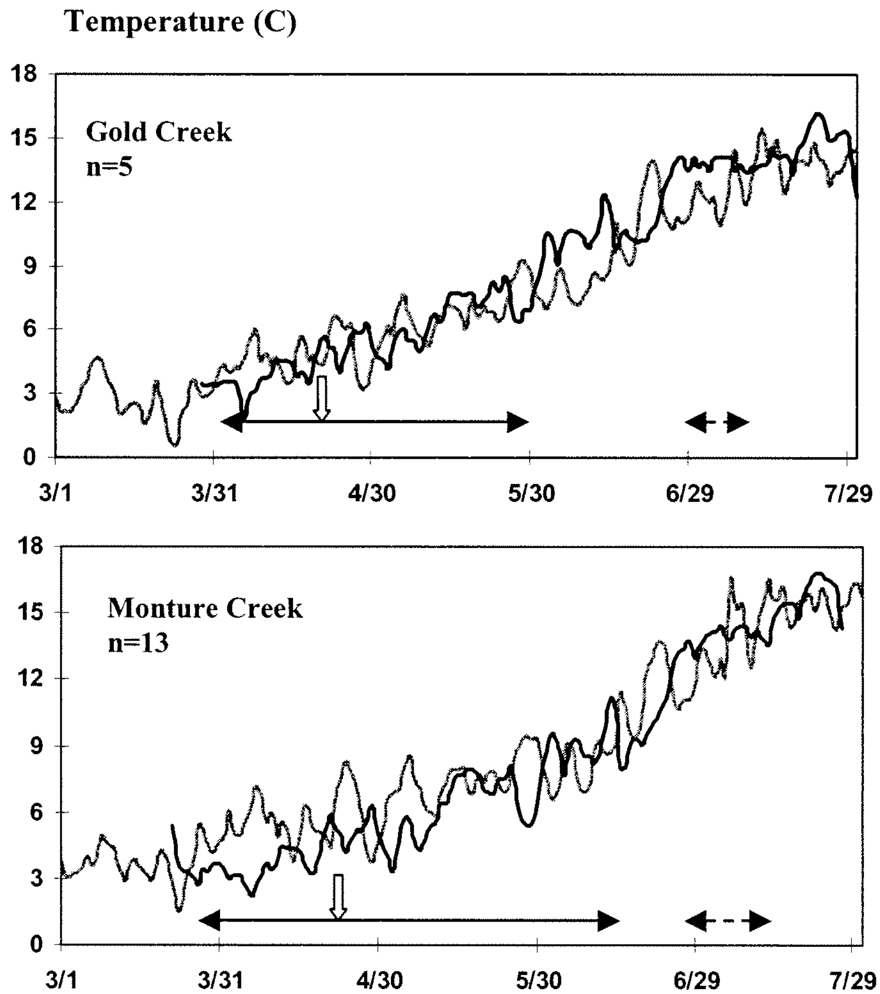

Figure 3. Water temperatures for Gold (top) and Monture Creeks in 2005 (gray) and 2006 (black). Duration within tributaries (2004-06) and the estimated emergence periods for 18 spawners are shown by arrowed left and right horizontal lines, respectively. The median spawning date for the tributary is shown by vertical arrows.

We monitored 17 fish at summering sites within the Blackfoot River. A majority of these (n=11 or 65%) showed either no movement (n=5) or remained within 1.6 km (n=6) of starting locations; six (35%) summered an average of 9.3 km (range, 2.6-23.7) from their original starting sites. Of 15 fish tracked into winter, all remained within 0.3 km of summering locations. We also observed a few rainbow trout moving laterally to the margins of the shoreline and into flooded vegetation during high spring runoff, an apparent refuge-seeking response to high river flows.

Emergence and WD severity- Estimated fry emergence was complete by July 11 (2005 and 2006) for both Gold and Monture Creeks. Sentinel exposures were completed for six tributaries in R1, four in R2 and both study reaches of the Blackfoot River. Histological examinations identified infection rates ranging from 0 – 100% and mean lesion scores ranging from 0-4.82 on the MacConnell-Baldwin scale (Table 2). Of the six RBT streams within R1, five streams recorded low severity (majority < grade 3), and of those most (n=4) detected no infection despite the near proximity (within 0.3 km) to infected waters of the Blackfoot River (Table 2). Conversely, sentinel exposures in three of four spawning streams in R2 recorded high severity (majority ≥ grade 3) and identified only the North Fork with a low severity. The percent of Blackfoot River fish with high severity (≥ grade 3) was 43% in R1 compared with 66% in R2.

Discussion

A similar study east of the Continental Divide in Montana investigated RBT spawning life history and risk to juvenile survival within an infected "tailwater" section of the Madison River (Downing et al. 1999). By contrast, our study, undertaken west of the Continental Divide within a headwater basin of the upper Clark Fork drainage, examined fluvial life history within a "free-flowing" river system. Common to both areas are predictable migratory strategies involving pre-spawning migrants holding within wintering areas prior to upriver movement; the fidelity of most post-spawners to their initial tagging location; upstream migrations of similar distances (mean, 14.5 versus 18.7 km) to spawning grounds; and fry emergence by early July during the vulnerable, highly infectious period (Downing et al. 1999, FWP unpublished data). Life history differences

Reach and fish #	Start of river migration		Pre-spawning river migration		Tributary spawning					End of Migration		
	rkm	start date	Total rkm	Total # days	Tributary name	km to spawning site	Estimated spawning date	Days in trib.	Date exited	Date migration ended	Date returned to migration starting location	River km at the end of migration
1--1	4.7	4/4/06	17.5	5	Gold Cr	0.3	4/16/06	14	4/23/06	4/23/06	4/23/06	4.7
1--2	5.5	4/13/06	16.7	11	Gold Cr	0.3	5/1/06	12	5/6/06			
1--3	6	4/22/06	11.4	24	East Twin Cr	0.2	5/18/06	12	5/28/06	6/17/06		6.8
1--4	14	4/27/06	8.2	4	Gold Cr	1	5/15/06	22	5/23/06	6/17/06	5/28/06	22.7
1--5	16.9	5/2/05	0.5	1	East Twin Cr	0.6	5/6/05	6	5/9/05	5/9/05		16.6
1--6	17.1	4/9/05	56.8	14	Monture Cr	6.4	4/26/05	63	6/25/05	7/7/05	7/7/05	17.1
1--7	17.1	4/30/06	18.2	20	Belmont Cr	0.2	5/23/06	8	5/28/06	7/23/06		19.6
1--8	17.7	4/2/06	4.5	2	Gold Cr	3.1	4/18/06	23	4/27/06	4/29/06	4/29/06	17.4
1--9	19	4/7/05	3	9	Gold Cr	0.5	4/18/05	11	4/27/05	5/4/05		
1--10	24	4/11/06	11.3	16	Belmont Cr	3.4	5/15/06	31	5/28/06	5/28/06	5/28/06	23.8
1--11	26.6	5/15/06	8.7	1	Belmont Cr	0.2	5/17/06	5	5/20/06	5/31/06		2.9
1--12	38.8	4/7/05	3.4	17	Belmont Cr	1.5	4/26/05	5	4/28/05	5/7/05	5/7/05	38.8
2--13	65.5	4/7/04	21.4	3	NFBlkft	1.9	4/13/04	6	4/16/04	4/23/04		
2--14	66	4/25/05	7.9	10	Dunham Cr	19.6	5/20/05	20	5/25/05	6/26/05	6/26/05	66.0
2--15	66	4/7/05	5.5	11	Monture Cr	3.2	4/29/05	17	5/5/05	5/6/05	5/6/05	66.0
2--16	66.8	3/19/04	7.1	5	Monture Cr	7.1	4/1/04	13	4/6/04	4/28/04		52.8
2--17	67.1	3/30/04	6.8	6	Monture Cr	5.3		11				
2--18	67.1	3/24/05	6.8	10	Monture Cr	7.1	4/17/05	20	4/23/04	4/28/04		62.8
2--19	67.1	4/26/05	6.8	7	Monture Cr	6.9	5/11/05	11	5/14/05	5/26/05	5/26/05	67.1
2--20	67.3	4/23/04	6.6	4	Monture Cr	0.3	4/29/04	3	4/30/04	5/6/04		66.9
2--21	69	4/25/05	4.8	10	Dunham Cr	19.8	5/16/05	17	5/22/05	6/12/05	6/12/05	69.0
2--22	70.3	3/25/06	3.5	15	Monture Cr	5.6	4/23/06	22	5/1/06	5/15/06		72.9
2--23	70.3	4/10/06	3.5	10	Monture Cr	2.4	4/28/06	16	5/6/06	5/6/06	5/6/06	70.3
2--24	70.3	4/8/06	3.5	8	Monture Cr	7.2	5/7/06	29	5/15/06	6/17/06		55.0
2--25	71.1	4/8/05	2.7	9	Monture Cr	9.8	4/28/05	17	5/4/05			

Table 1. Summary of migration events including: start of river migration, migration time and distance, tributary spawning (dates and locations) and total migration distance for 25-telemetered RBT in two reaches of the Blackfoot River. Fish # (1-25) relates to migration start and spawning locations on Figure 4

between the Madison and Blackfoot sites involve primarily mainstem spawning within the Madison River compared to tributary spawning within the Blackfoot Basin. Although WD has been shown to vary within the mainstem of the Madison River (Downing et al. 1999, Krueger et al 2006), our study identifies large differences in histological scores among spawning tributaries.

Like the Madison River, TAM production in the Blackfoot River express a seasonal peak in June and July, followed by declines by September (Downing et al. 1999, Montana Fish, Wildlife and Parks, unpublished data). This seasonal pattern of high TAM release overlaps with the emergence of wild RBT fry at the critical early life stage. In Blackfoot tributaries prone to high TAM production and high incidence of infection, the epizootic has been both rapid and severe. At category 3.0 severity, granulomatous lesions can be large and severely impact bone, causing distortion and breakage, which leaves the fish weak, less able to compete for food and habitat and ultimately increases chances of mortality. Mean lesion scores of >2.75 have been associated with significant levels of mortality in wild rainbow trout populations (Vincent 2002). Infections in Elk Creek increased from non-detectable to a mean lesion score of 2.8 within a single year (2002 to 2003) before increasing to 4.8

identified in this study. Likewise, infections in Monture Creek increased from non-detect to a mean lesion score of 3.2 between 1999 and 2002 (Pierce et al 2006) before increasing to 4.8 in this study. *M. Cerebralis*

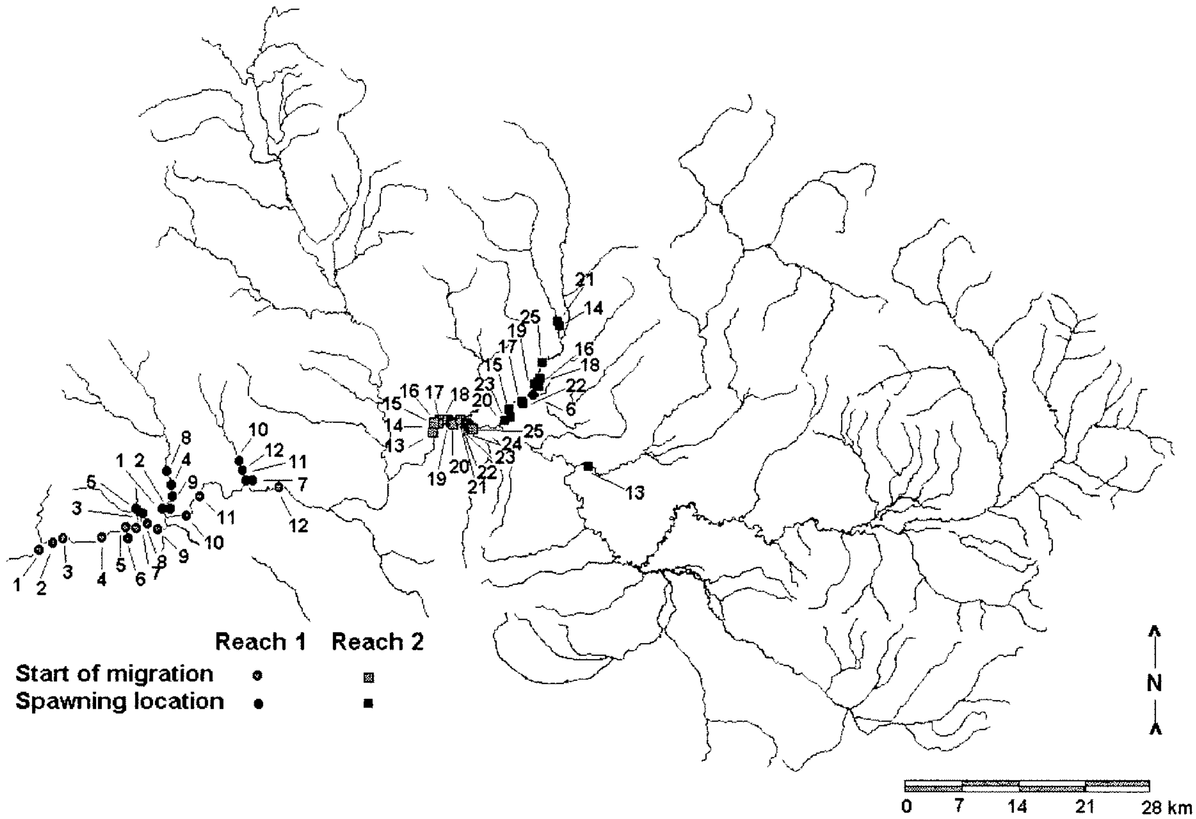

Figure 4. Start of migrations (open symbols) and upstream-most location (closed symbols) of spawning rainbow trout.

infections to high severity in the middle Blackfoot River coincide with a temporal trend (1998-2004) of increased cranial deformities (a sublethal clinical sign of WD infection) and recent declines in RBT abundance in the Blackfoot River downstream of the Monture Creek confluence (Pierce et al 2006).

Similar to spatial variability of infected waters within a Utah watershed (Hoz Franco and Budy 2004), infections within the Blackfoot Basin vary geographically depending on the physical properties and arrangement of tributaries. For basin-fed tributaries within the Blackfoot Basin, conditions correlated with infection include wide alluvial valleys with warm water during summer, lower stream gradients and higher levels of fine sediments (Pierce et al. In review); all are conditions that favor habitat for *T. tubifex* or production of TAMs (Arndt et al. 2002, El-Matbouli et al. 1999).

For R1 fish, spawning was dispersed in lower reaches of three morphologically similar (cold, high-gradient) tributaries to the lower Blackfoot Basin. Within this group of similar streams are three additional tributaries (Johnson, East Twin and Bear Creeks), all of which support known (Peters and Spoon 1989, FWP unpublished data) but limited RBT spawning (based on our telemetry findings) and low to no measurable infection. From Belmont Creek (rkm 35.4) downriver, this concentrated group of relatively "clean" tributaries enters the Blackfoot River at a mean interval of one stream per 5.8 km of river. Of 11 RBT tracked from wintering pools to tributaries within this area, ten-expressed unidirectional (upstream) migration over a 9.2 km median distance to spawning areas including 8.7 km of the lower Blackfoot River. Unlike the upper reach, these movement patterns identify several overlapping spawning stocks cohabit this reach of the Blackfoot River. Sentinel exposures within this group of R1 tributaries consistently test at low levels (< grade 3 or non-detect) of severity compared to R2 tributaries where sentinel exposures consistently rank at high ($\geq$ grade 3) severity (Pierce et al 2006). The RBT densities in the lower Blackfoot River remains stable (Pierce et al. 2006) despite an apparent annual loss of ~15-20% of lower River RBT spawners over Milltown Dam identified in this study.

In contrast to this R1 river area, the 51.5-km reach of the Blackfoot River between Belmont Creek and the North Fork (R2) contain fewer (five) RBT spawning streams - one per 10.3 km of river although most (four) enter within a 17.8-km section of Blackfoot River between Cottonwood Creek (rkm 69.2) and the North Fork (rm 86.9). Consequently, RBT recruitment sources within the 33.8-km section of the Blackfoot River between Belmont and Cottonwood Creek are limited. Elk Creek enters this reach but this stream is water quality (sediment and temperature) impaired (Blackfoot Challenge 2006), supports high severity of WD, and has experienced RBT declines in recent years (Pierce et al 2004). Of the five RBT spawning streams upstream of Belmont Creek, only the North Fork supports a low severity of WD, yet it supports limited RBT reproduction (*this study*) and recruits relatively fewer age-0 RBT to the Blackfoot River than downstream tributaries (Peters and Spoon 1989).

Age-0 RBT abundance has been longitudinally evaluated in all RBT spawning streams during the early rearing mid-summer period (Peters and Spoon 1989, Peters 1990, Pierce et al. 2004, 2006). Juvenile inventories identify relatively high abundance of age-0 RBT within and downstream of all central spawning grounds and concentrated densities extend to the Blackfoot River below the mouths of all spawning tributaries identified in this study (Peters and Spoon 1989), a pattern of rearing consistent with the Madison River (Downing et al. 2002). For the Blackfoot Basin, this pattern of limited early dispersal suggests a higher risk of disease exposure throughout the lower reaches of most R2 tributary and mainstem rearing areas, but conversely low risk in R1 tributaries (except Elk Creek) and those fry dispersing to the mainstem Blackfoot River during the summer period when WD severity ranked high.

Prior to the invasion of *M. cerebralis,* Peters and Spoon (1989) identified Monture Creek as a primary source of RBT recruitment, but considered the middle Blackfoot River as recruitment limited. Our study confirmed this spawning relationship with >90% of telemetered R2 fish spawning within the lower Monture Basin with a central (median) spawning location of rkm 6.9 (range 0.3-19.8). Although the 2005 Monture Creek sentinel exposure identify a severe (97% $\geq$ grade 3) exposure, the cage was located downstream (rkm 3.2) of the central spawning site. To clarify disease severity within the central spawning area, we further examined *M. cerebralis* exposures at rkm 7.4 and upstream of identified RBT spawning areas (rkm 20.8) with additional sentinel exposures in 2006. Exposure results confirmed the high severity at the central spawning areas (95% $\geq$ grade 3), but detected no upstream infection. The combined 2005-06 exposure results confirm risk of severe exposure within and downstream of primary Monture Creek spawning areas, yet the upstream attenuation to no infection suggests an upper segment of the Monture RBT spawning site at a low level of risk.

The discrepancy between river migration distances in R1 an R2 (6.6 verses 10.0) raises concerns of disease-related recruitment losses in R2. In addition to a reduced level of river use, high lesion scores at the primary RBT spawning site (Monture Creek) indicate potential for a synergistic reduction in R2 recruits, including fish dispersing to downstream waters where trout populations are currently limited by the low number and poor quality of existing spawning streams (i.e. upstream of Belmont Creek).

Oncorhynchus resistance to pathogens such as whirling disease can take many forms such as inherent life history strategies that help avoid exposure of *M. cerebralis* at early life stages, or physiological resistance such as an innate immune response that limit the pathogen from infecting the host (MacConnell and Vincent 2002). Similar to nearby stocks within the Clark Fork Basin, a majority of fish identified as RBT in the Blackfoot Basin were found to be mildly introgressed with westslope cutthroat trout. Physiological resistance of RBT/ westslope cutthroat trout hybrids to WD is untested, but it is possible that F_1 hybrids may have an intermediate level of resistance between the low resistance of non-hybridized RBT and the "moderate" resistance of non-hybridized westslope cutthroat trout (MacConnell and Vincent 2002, Hendrick et al 1999). Within the Blackfoot watershed, the longitudinal distribution from pure westslope cutthroat trout predominant in upper Blackfoot Basin to a more RBT-dominated community downstream of the North Fork suggests an inter-specific reduction in WD susceptibility among the *Oncorhynchus* community, particularly when further considered within a context migratory life histories and environmental factors that influence infection (and severity) along a longitudinal continuum (Smith 1998, Pierce et al. In Review)

Management Implications – Management implications vary by river reach and involve the potential for an additive loss of recruitment to the middle Blackfoot River, and the need to offset this loss by correcting anthropogenic degradation of spawning and rearing streams. The middle Blackfoot River (upstream of the

Belmont Creek) was previously identified with trout recruitment problems brought on by drought and winter mortality, limited spawning areas and degradation of existing spawning and rearing areas caused by agricultural and other land uses. For the middle Blackfoot River, this study and other tributary assessments suggest abundant restoration opportunities even in tributaries that host high levels of disease.

Based the community-level changes in Rock Creek, brown trout clearly have potential for expansion under environments prone to WD. This naturally more resistant species has also shown significant population increases in highly infected spring creeks within the middle Blackfoot Basin once limiting factors related to physical habitat were corrected (Pierce et al. 2006). Like brown trout, native westslope cutthroat trout and bull trout could thrive within certain infected environments. While both westslope cutthroat trout and bull trout possess partial resistance to WD (MacConnell and Vincent 2002), both species also possess life history strategies that help avoid exposure of *M. cerebralis* at early life stages by spawning in headwaters of the Blackfoot Basin (including Monture Creek) where contact with *M. cerebralis* at critical stages (age-0) is reduced. Young cutthroat trout and bull trout migrate to infected waters at more disease-resistant (age-1 and older) stages. Both species migrate extensively within the Blackfoot Basin (Swanberg 1997, Schmetterling 2001, Pierce et al. 2007), including infected sections of the Blackfoot River prone to limited RBT recruitment (Pierce et al 2006).

Even moderate levels of WD resistance for certain native species can temper population effects within waters that support severe WD. One example of this is Chamberlain Creek, a tributary supporting primarily westslope cutthroat trout. Following remediation of dewatering, ditch entrainment, riparian grazing and channel alterations, westslope cutthroat trout densities in lower Chamberlain Creek increased from two to 80 fish/100m by 1994 and remained stable thereafter (Pierce et al. 1997, 2006). After this recovery, telemetered adult fluvial cutthroat from the Blackfoot River identified Chamberlain Creek as an important westslope cutthroat trout spawning stream to the lower Blackfoot River (Schmetterling 2001). Densities of westslope cutthroat trout have remained stable in lower Chamberlain Creek despite a high severity of WD (range of mean lesion scores, 2.7 to 4.3) between 1999 and 2005. Population trends for fluvial westslope cutthroat trout in the lower and middle Blackfoot River have been stable despite the epizootic among rainbow trout.

Conclusions – Although future population (and community) effects are difficult to predict, our study clearly indicates disease risks to Blackfoot River RBT vary from the tributary to sub-basin scales. Our study suggests the middle Blackfoot River is at a higher risk of RBT recruitment loss through WD, perhaps at levels sufficient of affect angling success. Some highly infected valley-floor streams in the middle Blackfoot Valley seem predisposed to high WD because of their low gradient, high water temperatures and high sediment levels and synergistic effects of heavy grazing and other disturbances. By contrast, higher gradient mountain streams are less prone to infection. To offset potential RBT losses in disease prone waters of the middle Blackfoot Basin, stakeholders must 1) better manage riparian areas for channel stability, increased shade and erosion reduction, 2) promote native fish recovery and migratory life histories, and 3) restore (or enhance) habitats favoring salmonid life stages less affected by the WD pathogen.

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