Multiscale Prediction of Whirling Disease Risk in the Blackfoot River Basin, Montana: a Useful Consideration for Restoration Prioritization?

L. A. Eby\textsuperscript{a}, R. Pierce\textsuperscript{b}, M. Sparks\textsuperscript{ac}, K. Carim\textsuperscript{a} & C. Podner\textsuperscript{b}

\textsuperscript{a} Montana Fish, Wildlife and Parks, 3201 Spurgin Road, Missoula, Montana 59804, USA
\textsuperscript{b} Wildlife Biology Program, College of Forestry and Conservation, University of Montana, 32 Campus Drive, Missoula, Montana 59812, USA
\textsuperscript{c} Present address: University of Alaska Fairbanks, Post Office Box 75700, Fairbanks, Alaska 99775, USA

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Abstract
Habitat restoration for inland trout (family Salmonidae) is common across western North America, but planners rarely consider disease risk when prioritizing restoration sites. Whirling disease is a parasitic infection caused by the invasive myxosporean parasite *Myxobolus cerebralis* and has been implicated in declines of wild trout populations across western North America. For planners to consider disease, disease risk needs to be predictable across the landscape and influence restoration outcomes. We collated the history of whirling disease infection severity scores on the MacConnell–Baldwin scale from sentinel cage studies for hatchery Rainbow Trout *Oncorhynchus mykiss* in the Blackfoot River basin from 1998 to 2009. At these same sites, we performed reach-scale geomorphic assessments, derived landscape variables from GIS data layers, and assembled fish composition data. We examined relationships between the severity of infection and several landscape-scale and reach-scale variables for 13 basin-fed streams in the Blackfoot River basin of west-central Montana using classification and regression tree analyses. In our data set, valley slope and forest cover were the best predictors of fine sediment. Both spring creeks and gently sloping alluvial basin-fed tributaries to the Blackfoot River basin with higher proportions of fine sediment (particle size < 0.85 mm) were associated with a high severity (≥ grade 3) of infection. Additionally, we explored differences in trout species composition (i.e., susceptible versus resistant species) before and after the whirling disease enzootic using seven basin-fed streams and two spring creeks. We did not detect trout community shifts from susceptible to disease-resistant salmonids in basin-fed disease-positive streams. However, spring creeks showed a negative trend in disease-susceptible salmonids after the whirling disease enzootic. Disease risk appears to be predictable across the landscape and may limit possible restoration outcomes by influencing species composition in spring creeks.
The prioritization of habitat restoration efforts for inland wild trout (family Salmonidae) is often based on the distribution of focal species (i.e., species of special concern), cooperation of landowners or land availability (Sudduth et al. 2007), feasibility, costs, benefits, and available funding (Aitken 1997; Roni et al. 2002; Beechie et al. 2008; Pierce et al. 2008). When prioritization frameworks are developed, they rarely consider disease risk. But if the presence of disease constrains restoration outcomes, it would be useful to consider disease when prioritizing and planning restoration activities. Whether disease should be integrated into restoration planning depends on whether we can predict the risk of disease across landscapes and whether the presence of disease influences restoration outcomes.

Whirling disease is a parasitic infection caused by the invasive myxosporean parasite *Myxobolus cerebralis* (Hoffman 1990; Bartholomew and Wilson 2002), which coevolved on the Eurasian continent with Brown Trout *Salmo trutta* (Bartholomew and Reno 2002). *Myxobolus cerebralis* was introduced on the East Coast of North America in the 1950s, then spread rapidly westward (Bartholomew and Reno 2002). The parasite is now widely established across western North America; however, the prevalence and severity of whirling disease infection is highly variable across river systems (e.g., Hiner and Moffitt 2001; De la Hoz and Budy 2004; Neudecker et al. 2012). As part of its life cycle, *M. cerebralis* requires an oligochaete worm (such as the sludge worm *Tubifex tubifex*) to develop tricactinomyxon (TAM) actinospores, which are released into the water typically when stream temperatures are between 10°C and 15°C (El-Matboui et al. 1999; De la Hoz and Budy 2004; Kerans et al. 2005). Young salmonids (<9 weeks posthatch) are most susceptible to infection by TAM actinospores (MacConnell and Vincent 2002). If exposed to TAMs at this susceptible age, severe infection can occur (Ryce et al. 2004). Clinical signs of whirling disease may include discoloration of the tail, whirling behavior, and skeletal deformities (MacConnell and Vincent 2002).

Whirling disease has been implicated in the decline of several wild trout populations in western North America (Nehring and Walker 1996; Vincent 1996; Nehring 2006), including various rivers in western Montana (MacConnell and Vincent 2002; Granath et al. 2007; McMahon et al. 2010). These declines have been implicated in shifting trout communities dominated by susceptible species (e.g., Rainbow Trout *Onchorhynchus mykiss*) to ones dominated by resistant species, such as Brown Trout (Granath and Vincent 2010). Thus, restoring trout habitat where whirling disease is present may create an ecological sink for susceptible trout, while favoring resistant species (Granath et al. 2007; McMahon et al. 2010). Unfortunately, forecasting the impacts of whirling disease on local salmonid communities is especially difficult given the complex nature of the multitub host life cycle, the environmental preferences of both the hosts and the parasite (Hedrick et al. 1999; Kerans and Zale 2002), and the lack of long-term field studies examining fish populations in streams with and without whirling disease (Karr et al. 2005; Hansen and Budy 2011).

Within and among watersheds, the prevalence (percent infected) and severity of whirling disease infection depends upon the abundance and presence of susceptible fish hosts (MacConnell and Vincent 2002), the strains of *T. tubifex* present and overall oligochaete community composition (Beauchamp et al. 2005; Nehring et al. 2013, 2014), and the physical environment (e.g., Anlauf and Moffitt 2008; Neudecker et al. 2012). Environmental conditions, such as substrate composition, stream temperature, and velocity, can influence the abundance of *T. tubifex*, the production of TAMs, and the susceptibility of different salmonid hosts to infection (e.g., Allen and Bergersen 2002; Kerans et al. 2005; Hallett et al. 2009). Other studies highlight the specificity of this parasite to particular strains of *T. tubifex* and the potential role of resistant strains serving as a filter and reducing the number of spores that complete their life cycle (Beauchamp et al. 2005; Nehring et al. 2013, 2014), as well as the potential for the development of resistance in certain fish populations (Baerwald et al. 2008; Miller and Vincent 2008).

Given these complexities, predicting high-quality habitat for *T. tubifex* may be the most practical approach for determining areas of highest risk for *M. cerebralis* infection (e.g., Allen and Bergersen 2002; Schisler et al. 2006; Anlauf and Moffitt 2010). The relationship between the occurrence of *T. tubifex* and fine substrate is well established (Lazim and Learner 1987; Kaeser and Sharpe 2006; Anlauf and Moffitt 2008), and *T. tubifex* distribution is positively associated with organic matter and nutrients (Sauter and Gude 1996; Arndt et al. 2002). Previous studies found that incorporating variables from both the reach scale (e.g., amount of slow habitat such as pools) and landscape scale (e.g., watershed size and land cover) were the best approach to predicting fine sediment but highlighted that these predictors of fine substrate needed to be validated across multiple drainages to generalize broader trends (Anlauf and Moffitt 2010). At the reach scale, characteristics such as stream slope, depth, channel sinuosity, bank stability, flow regime, and riparian livestock damage can also impact local substrate composition. While, at the landscape scale, habitat formation and substrate characteristics are influenced by variation in basin hydrology linked to climate, valley slope, lithology, and properties of the soils (e.g., Frissell et al. 1986; Poff and Ward 1989), as well as anthropogenic changes to land cover (i.e., reduced forest cover [Allan et al. 1997]).

To evaluate our ability to predict the risk of whirling disease across a river basin, we must validate whether landscape-scale and reach-scale variables can predict fine sediment (e.g., Anlauf and Moffitt 2008). To understand the potential for restoration to alter the spatial patterns of whirling disease presence, we must also understand whether disease severity is associated with fine sediment and the significant physical predictors of fine sediment. In addition, we need a better understanding of whether whirling disease can influence community
composition and, therefore, limit restoration outcomes. In the Blackfoot River basin, Montana, sites were periodically monitored for whirling disease prevalence and severity (Table 1; Pierce et al. 2009, 2012; McMahon et al. 2010; Neudecker et al. 2012) between 1998 and 2009, allowing us to investigate whirling disease risk across the basin. Our study addresses three questions: First, how well do landscape-scale characteristics (valley slope, sinuosity, stream order, and percent forest cover) and reach-scale characteristics (bank-full width and depth, channel slope, and entrenchment ratio) predict fine sediment? Second, do the same variables that predict fine sediment also predict whirling disease infection severity? Finally, does the presence of whirling disease result in a community dominated by more resistant species thus limiting recovery of more susceptible species? Answering these questions will help us understand how the presence of whirling disease may alter the range of possible restoration goals that are achievable at a given site.

METHODS

Study Area

The Blackfoot River, a fifth-order tributary (Strahler 1957) of the upper Columbia River, lies in west-central Montana and flows west 211 km from the Continental Divide to its confluence with the Clark Fork River in Bonner, Montana (Montana DNRC 1984). The geography of the watershed is a physically diverse, glacial landscape with alpine and subalpine mountains at the upper elevations, montane forests at the middle elevations, and semiarid glacial pothole and outwash topography on the valley floor. Larger tributaries of the Blackfoot River, located in the mid to upper basin, typically begin in glacial valleys, flow through steep headwaters, and then transition to meandering streams in broad valleys with gentle relief on the floor of the Blackfoot Valley. Conversely, smaller tributaries in the lower Blackfoot River basin flow through confined steeper channels before directly entering the lower Blackfoot River (Alt and Hyndman 1986). The Blackfoot River contains diverse self-sustaining wild trout populations, many of which have migratory behavior and reproduce in tributaries (Swanberg 1997; Schmettering 2001; Pierce et al. 2009). Many of the Blackfoot River basin tributaries have migratory behavior and reproduce in tributaries (Swanberg 1997; Schmettering 2001; Pierce et al. 2009). Many of the Blackfoot River basin tributaries have been targets for a variety of restoration activities, including all the tributaries used in this study (Pierce et al. 2008, 2013). Typical restoration activities include a mix of improved fish passage, reduction of entrainment, active channel restoration, grazing changes, removal of streamside feedlots, and increased instream flows in each tributary. Even though all of the tributaries in this study have received some efforts towards habitat improvement, restoration activities occurred upstream of the whirling disease monitoring sites.

Native salmonids of the Blackfoot River basin include Westslope Cutthroat Trout _O. clarkii lewisi_, a Montana Species of Special Concern (Shepard et al. 2005), native Bull Trout _Salvelinus confluentus_, a char designated as threatened under the Endangered Species Act (USFWS 2010), and Mountain Whitefish _Prosopium williamsoni_, a species common to the Blackfoot River (Pierce et al. 2012). Nonnative trout include Rainbow Trout, Brook Trout _S. fontinalis_, and Brown Trout (Pierce et al. 2012). Other native nongame fishes are present in the main stem but those occasionally captured in the tributaries in low numbers include Slimy Sculpin _Cottus cognatus_ and Longnose Dace _Rhinichthys cataractae_.

Based on laboratory exposures, Rainbow Trout, Brook Trout, Westslope Cutthroat Trout, Bull Trout, and Mountain Whitefish possess high or intermediate susceptibility to whirling disease (MacConnell and Vincent 2002), whereas nonnative Brown Trout are the only fish naturally more resistant to the parasite due to their coevolution with _M. cerebralis_ in Eurasia (Bartholomew and Reno 2002). Considering the spatial and temporal overlap of young, small, vulnerable fish and the production of TAM actinospores can help link susceptibility and exposure to predict vulnerability and highlight where whirling disease may be most likely to cause population-level impacts. In basin-fed streams, the emergence of Rainbow Trout and Cutthroat Trout fry overlaps with the peak of TAM production in the early to midsummer (Vincent 2000; Downing et al. 2002; Pierce et al. 2009). Fall-spawning susceptible fishes (Brook Trout and Bull Trout) in basin-fed streams have lower exposure to _M. cerebralis_ (MacConnell and Vincent 2002) due to hatching periods that do not overlap with the seasonal peak in TAM production (Pierce et al. 2009; Neudecker et al. 2012). In spring creeks TAM production begins in late fall and lasts longer, resulting in exposure of young Brook Trout spawned in low to middle elevation sites (Neudecker et al. 2012). Overall, Bull Trout make up a small component of the catches in the streams included in this dataset and they typically spawn higher in the watershed (higher slopes, bigger substrate, and cooler temperatures), where the presence of whirling disease is less frequently observed.

Whirling Disease Exposures

Sentinel cage exposures of hatchery Rainbow Trout (50 age-0 diploid cohorts) were used to determine disease prevalence and severity within each of the 17 study streams (Figure 1). Streams had between two and nine exposure events over the 12-year study period. As described in prior studies (Pierce et al. 2009, 2012; Neudecker et al. 2012), cages were placed in flowing water and exposures were completed in July within 9 weeks posthatch to coincide with fry emergence in Blackfoot River tributaries, periods of susceptibility (Ryce et al. 2005), and the known seasonal peak of TAM production within many rivers in western Montana (Vincent 2000). Spring-fed systems have a more protracted period of peak TAM production from late fall through spring, which results
**TABLE 1.** Histological scores indicating the severity of infection summarized as both the mean score from sentinel cages and the percent of exposed individuals scoring above a 3 for severity of infection on the MacConnell-Baldwin scale (0 = nondetect; 5 = severe); scores are presented as follows: mean cage score; percent with >3 infection severity. Stream ID and name relate to the location on the study area map (Figure 1). When no data was available for a year, it is indicated with “nd.” The Presence column indicates whether the stream was considered disease positive (1) or negative (0) for the analyses. Fourteen streams were basin-fed streams and two streams (Rock and Kleinschmidt creeks) were spring creeks. Thirteen streams were included in the physical assessments (indicated with a P) and nine streams were used in fish assessment (indicated with an F).

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<td>1. Johnson Creek</td>
<td>P</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>0.0;0</td>
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<td>P</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
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<td>0.0;0</td>
<td>0.0;0</td>
<td>0.0;0</td>
<td>nd</td>
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<td>3. East Twin Creek</td>
<td>P</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
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<td>nd</td>
<td>0.0;0</td>
<td>0.0;0</td>
<td>nd</td>
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<td>0.0;0</td>
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<td>5. Gold Creek</td>
<td>P, F</td>
<td>nd</td>
<td>0.2;0</td>
<td>0.0;0</td>
<td>nd</td>
<td>0.2;0</td>
<td>0.4;4</td>
<td>1.5;27</td>
<td>2.5;49</td>
<td>0.3;2</td>
<td>3.4;76</td>
<td>2.9;63</td>
<td>4.4;89</td>
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<td>6. Belmont Creek</td>
<td>P, F</td>
<td>nd</td>
<td>0.0;0</td>
<td>0.0;0</td>
<td>nd</td>
<td>0.0;0</td>
<td>2.8;64</td>
<td>4.3;100</td>
<td>4.8;98</td>
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<td>nd</td>
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<tr>
<td>7. Elk Creek</td>
<td>P, F</td>
<td>nd</td>
<td>0.0;0</td>
<td>0.0;0</td>
<td>nd</td>
<td>0.0;0</td>
<td>2.8;64</td>
<td>4.3;100</td>
<td>4.8;98</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
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<tr>
<td>8. Blanchard Creek</td>
<td>P</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
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<td>nd</td>
<td>nd</td>
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<tr>
<td>9. Cottonwood Creek</td>
<td>P</td>
<td>3.7;94</td>
<td>4.5;98</td>
<td>nd</td>
<td>4.5;96</td>
<td>nd</td>
<td>3.8;100</td>
<td>4.0;81</td>
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<td>10. Chamberlain Creek</td>
<td>P, F</td>
<td>0.2;0</td>
<td>2.7;64</td>
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<td>1.9;44</td>
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<td>11. Monture Creek</td>
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<td>nd</td>
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<td>4.8;97</td>
<td>4.6;95</td>
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<td>12. Rock Creek (spring)</td>
<td>F</td>
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<td>0.0;0</td>
<td>2.3;47</td>
<td>3.9;77</td>
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<td>3.4;82</td>
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<tr>
<td>13. Kleinschmidt Creek (spring)</td>
<td>F</td>
<td>nd</td>
<td>3.6;78</td>
<td>4.5;86</td>
<td>3.8;80</td>
<td>nd</td>
<td>4.9;98</td>
<td>4.7;93</td>
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<td>14. Arrastra Creek</td>
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<td>15. Poorman Creek</td>
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<td>nd</td>
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<td>nd</td>
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<td>nd</td>
<td>nd</td>
<td>0.8;12</td>
<td>nd</td>
<td>4.7;95</td>
<td>nd</td>
<td>nd</td>
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</tr>
<tr>
<td>16. Landers Fork</td>
<td>P</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
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<td>0.0;0</td>
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*Spring creek sentinel cage exposures were completed in April. All basin-fed stream exposures were completed in July.*
in parasite exposure for fall-spawning species (i.e., Brook Trout) during the most susceptible early life stage (Neudecker et al. 2012). In spring creeks, sentinel cage exposures occurred in April. All cages were placed in known areas of spawning and rearing for wild trout. Following field exposures and a holding period to allow the infection to develop, fish were sacrificed and their heads were histologically examined and scored using the MacConnell–Baldwin rating scale (Hedrick et al. 1999; Baldwin et al. 2000; Ryce et al. 2004), which categorically ranked the severity of infection into six qualitative groups: (0) no infection, (1) minimal, (2) mild, (3) moderate, (4) high, and (5) severe. In infections with severity scores >3, *M. cerebralis* digest and destroy cartilage of susceptible young fish, causing inflammation and lesions in the spine and cranium and skeletal damage, which can ultimately elevate mortality (Hedrick et al. 1999; MacConnell and Vincent 2002; Ryce et al. 2004). Based on these impacts to survival, we categorized streams as those with (positive) and without (negative) expected disease population impacts. Specifically, streams in our study were considered disease negative if the average histological score for the sentinel cage exposures was <1.5 for the severity of infection in any year tested and disease positive if the average histological score for the exposure group was ≥3 severity and the majority of the fish scored grade ≥3 severity (Table 1).

**Physical Variables as Predictors of Fine Sediment and Whirling Disease Severity**

*Tributary selection.*—Landscape-scale and reach-scale variables, as well as disease presence and severity in trout in sentinel cage studies, were collected for 13 basin-fed tributaries to the Blackfoot River (Figure 1; Table 1). Reach-scale field assessments occurred once at each of the whirling disease monitoring reaches near known spawning areas for *Oncorhynchus* spp. (Rainbow Trout, Westslope Cutthroat Trout, or hybrids). All sites in this dataset had the potential for direct invasion by *M. cerebralis* because they are connected to the Blackfoot River in areas where fish infected with *M. cerebralis* occur (Pierce et al. 2009, 2012).

*Physical assessments.*—At each reach, we examined the substrate for the amount of fine sediment (defined as a particle size <0.85 mm) by extracting a McNeil core sample from six separate riffles using modified methods first described by McNeil and Ahnell (1964). For this assessment, the hollow cone of a McNeil core sampler was pushed 10 cm into the...
streambed. Substrate was then extracted, dried, and sieved following standardized methods (Shepard et al. 1984). The turbid water within the sampler was measured for fine-sediment content utilizing an Imhoff cone as described in Shepard and Graham (1982) and Shepard et al. (1984). The estimated dry weight of the sediment within the Imhoff cone was added to the weight of material <0.85 mm. We calculated the percent of the sample that was <0.85 mm of particle size to quantify fine sediment (clay, silt, and fine sands) for each sample.

In addition to fine sediment, a related suite of physical stream assessments were conducted at both the landscape and reach scales to obtain our predictor variables. For landscape variables, we used 1:24,000 scale topographic maps and aerial photos in ArcView GIS version 3.3 (http://nris.mt.gov/) to calculate valley slope, sinuosity, stream order, and percent forest cover (Table 2). For reach-scale variables, we performed geomorphic surveys across varying reach lengths (295–3,270 m) to ensure a sampling reach of at least 30 bank-full widths in each stream using methods described by Rosgen (1996). These surveys included bank-full width and depth, and width/depth ratios at riffles, as well as percent channel slope and an entrenchment ratio for a reach (Table 2). We also performed a visual categorical assessment of streambank stability and animal damage as described in Stevenson and Mills (1999).

**Analyses.**—To identify how well landscape-scale and reach-scale characteristics predict fine sediment, we first

### Table 2

<table>
<thead>
<tr>
<th>Habitat variable</th>
<th>Description of measure</th>
<th>Data source</th>
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<tbody>
<tr>
<td><strong>Landscape variables</strong></td>
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<tr>
<td>Valley slope</td>
<td>Average stream slope calculated upstream of the sampled reach</td>
<td>1:24,000 digitized stream layer and U.S. Geological Survey (USGS) topographic map</td>
</tr>
<tr>
<td>Sinuosity</td>
<td>Average sinuosity calculated upstream of the sampled reach</td>
<td>1:24,000 digitized aerial photos and USGS topographic map</td>
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<tr>
<td>Percent forest</td>
<td>Area classified as forest, typically includes Douglas fir <em>Pseudotsuga menziesii</em> and lodgepole pine <em>Pinus contorta</em></td>
<td>1:24,000 USGS topographic maps and quad aerial photos</td>
</tr>
<tr>
<td>Stream order</td>
<td>Headwaters are first order and the confluence of two streams of order <em>n</em> forms a stream of order <em>n+1</em></td>
<td>1:24,000 digital stream layer from USGS topographic maps</td>
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<td><strong>Reach variables</strong></td>
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<tr>
<td>Percent reach slope</td>
<td>Longitudinal profile</td>
<td>Measured in field (Rosgen 1996)</td>
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<tr>
<td>Bank-full width/depth ratio</td>
<td>Ratio of bank-full width and bank-full depth measured at a riffle</td>
<td>Measured in field (Rosgen 1996)</td>
</tr>
<tr>
<td>Bank-full depth</td>
<td>Average depth of the thalweg in a representative riffle along the reach</td>
<td>Measured in field (Rosgen 1996)</td>
</tr>
<tr>
<td>Entrenchment ratio</td>
<td>A measure of floodplain connectivity and vertical containment</td>
<td>Measured in field (Rosgen 1996)</td>
</tr>
<tr>
<td>Bank stability (rock and vegetative cover)</td>
<td>Reach visually classified between 5 (very stable) indicating &gt; 90% vegetative cover or &gt; 65% large boulders to a rank of &lt; 1 (no or low stability) evidenced by no or low vegetative cover and banks composed of gravel and fines with no cover from large boulders or other features that would provide resistance to erosion</td>
<td>Measured in field (Stevenson and Mills 1999)</td>
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<tr>
<td>Animal damage</td>
<td>Reach visually classified into one of four categories ranging from undamaged (4) to excessive damage (1) with 76–100% of the reach length impacted as evidenced by erosion</td>
<td>Measured in field (Stevenson and Mills 1999)</td>
</tr>
</tbody>
</table>
examined Pearson’s correlation coefficients of the variables to ensure significantly correlated variables were not in the same analyses. We constructed scatterplots of each potential predictor variable versus fine sediment (<0.85 mm) to look for outliers and nonlinearities. We used classification and regression trees (Venables and Ripley 1997) to examine whether the fine sediment differed in response to any of the landscape-scale predictor variables. Classification and regression trees partition a dataset (categorical or continuous data) by recursively partitioning the data into subsets using either continuous or categorical dependent variables (Breiman et al. 1984). Because of the small data set, we set the required minimum node size to three but reduced the number of potential splits of the dataset (“pruned the tree”) and set the required minimum deviance explained to 0.05 to prevent overfitting the data. We used this same analytical approach to examine reach-scale stream features that best predict fine sediment. Reach-scale predictors included the bank-full width and depth, channel slope, and entrenchment ratio, as well as rankings for animal damage and streambank stability. We combined the significant predictors from both the landscape-scale and reach-scale analyses to examine whether combining predictors across scale improved our results for predictions of fine sediment.

After establishing which landscape-scale and reach-scale variables predicted fine sediment, we then examined whether fine sediment was associated with the presence of whirling disease at a site. First we conducted a t-test to compare differences in fine sediment at sites with disease absence and disease presence. Then we used the same analytical approach as above (classification and regression trees) but limited predictor variables to those relevant for predicting fine sediment at the landscape and reach scales. To better illustrate the relationship between whirling disease infection severity and landscape and reach variables, we plotted the significant predictor variable or variables resulting from the classification and regression tree analysis with the most recent histological results from sentinel cage results for each site reported in Table 1.

Effects of Whirling Disease on Fish Species Composition

To examine the possible whirling-disease-related shifts in susceptible species, we compared trout community composition before and after disease detection in disease-positive and disease-negative streams (as defined above). Even though other species are present in the Blackfoot River basin, salmonid fishes dominate the catch at these tributary sites.

Tributary selection.—To examine the potential changes to species composition, we selected disease-positive tributaries with at least three fish population monitoring sites that had two or more years of fish data collection before the detection of whirling disease and several years of fish monitoring after whirling disease exceeded a histological score of 3.0. We limited our fish dataset to monitoring sites in the lower reaches of tributaries (0.32–6.44 km upstream from the mouth) to maintain proximity with sentinel cage study sites and to avoid confounding trends associated with longitudinal changes in trout community composition. The fish data were averaged across all three sites for each year. This would ensure a robust estimate of the community composed of susceptible species before and after substantial disease impacts in streams that became disease positive. We used the sentinel cage field exposures to estimate the year in which whirling disease was above our disease-negative threshold. For the two streams (Cottonwood and Kleinschmidt creeks) that exceeded our threshold at first testing, we used 1994, the year that whirling disease was detected in the state. For our disease-negative tributaries, we selected tributaries with a similar fish data structure in time and space that also had sentinel cage data indicating low to no exposure to whirling disease. Ultimately, there were seven basin-fed tributaries (four were disease positive) and two spring-fed tributaries (both disease positive) that met these criteria for our analyses.

Fish population data collection.—To determine the relative abundance of wild trout, we performed a single-pass survey using a backpack electrofishing unit during base flow in the summers between 1989 and 2010. Pierce et al. (2013) demonstrated that single-pass estimates were linearly related to population estimates for the same watershed with the same sampling procedures. All fish were identified to species, counted, and measured (total length in millimeters). Due to sampling inefficiencies for age-0 trout, we removed age-0 trout for our analyses (using length-frequency histograms) and used ≥ age-1 fish in our analyses.

Analyses.—To define our high and low susceptible-species groups, we considered susceptibility among species (MacConnell and Vincent 2002) and the seasonality of high parasite exposure in basin-fed streams versus spring-fed streams. For basin-fed streams, all spring spawners (Oncorhynchus spp.) were combined to examine trends in the abundance of highly susceptible fish because of both the susceptibility of Oncorhynchus spp. to whirling disease (Vincent 2002) and the overlap in emergence of fry during the height of TAM production in the early to midsummer (Vincent 2000; Downing et al. 2002; Pierce et al. 2009). Conversely, fall-spawning fish in basin-fed streams are less vulnerable to M. cerebralis (MacConnell and Vincent 2002) because fry emergence occurs at periods that do not overlap with the seasonal peak in TAM production (Neudecker et al. 2012). In the two spring creeks, we included Brook Trout with Oncorhynchus spp. into a category of susceptible species because of their susceptibility in laboratory studies (Vincent 2000) and the overlap of young fish with TAM production. Because of the species differences in exposure to TAM basin-fed and spring-fed streams, we analyzed trends in community composition for these stream types separately.

To examine whether whirling disease influenced the proportion of the community composed of susceptible species, we examined the proportion of the total catch composed of
sustainable species for each sampling event. We standardized the proportion of total catch composed of susceptible species within each tributary by transforming them to z-scores and fit a linear mixed model ($Z_{\text{susceptible}} \sim \text{Time}, \text{Stream}$) to examine trends across time. The fixed variable “Time” refers to the monitoring year (as opposed to calendar year), with the first year of monitoring as year 0. Stream was included in the mixed model as a random variable (similar to blocking by stream). We examined trends in basin-fed disease-positive and disease-negative streams, as well as spring creek disease-positive streams, separately. We ensured our analytical assumptions were met and examined residuals for trends. Statistical analyses were conducted in R Statistical Software (R Development Core Team 2012).

RESULTS

Physical Variables as Predictors of Fine Sediment

The percent of fine sediment (particle size <0.85 mm) measured in Elk Creek was 2.5 times that of any other stream in our study. Therefore, we examined the associations of landscape-scale and reach-scale variables with and without the inclusion of Elk Creek to ensure that this site did not have undue influence on our results.

Among the landscape variables, Pearson’s correlation coefficients of all variables predicting fine sediment were less than 0.6 and not significant. As a result, all landscape-scale variables were included in this analysis. Valley slope was the primary explanatory variable predicting fine sediment with and without Elk Creek included in the analyses. With Elk Creek in the analyses, valley slope (breaks <0.8 and <1.75) was the only variable in the model, with a residual deviance of 21.65. Without Elk Creek in the analyses, valley slope (<1.75) and percent forest cover (<86.85%) remained in the final model, with a final residual mean deviance of 2.04. In this final model, the majority of variance in fine sediment was explained by valley slope. Within more gentle-sloping valleys, less forest cover was also associated with higher fine sediment in the substrate.

There were no significant correlations among the reach-scale variables and all Pearson’s correlation coefficients were less than 0.65. As a result, all reach-scale variables were included in this analysis. With Elk Creek included in the analyses, regression tree results indicated slope (<0.00775) and depth (<1.37 m) were important explanatory reach-scale variables of fine sediment, with a residual mean deviance of 29.05. Lower-gradient channels and sites with greater bank-full depth had more fine sediment. With the removal of Elk Creek, bank-full depth was the best explanatory variable, with an initial break at <1.50 m and then <1.02 m, and a model residual mean deviance of 3.11. Not surprisingly, deeper sites, again, had more fine sediment.

To combine information across landscape and reach scales, we examined correlations between all landscape-scale and reach-scale variables and found only one significant correlation—between valley slope and channel slope (0.88; $P < 0.01$). As valley slope was a key landscape predictor, we retained the landscape valley slope variable in the multiscalar analysis. The resultant regression tree model only contained landscape variables (valley slope and forest cover; Figure 2), as including the reach-scale variable of depth did not improve our model.

Physical Variables as Predictors of Whirling Disease Severity

As expected, we found that fine-sediment levels were higher in disease-positive than disease-negative streams (Figure 3A; $t = -2.01, P = 0.03, n = 13$; one tailed). Classification and regression tree results considering the landscape-scale and reach-scale variables selected above found that valley slope (<0.8) and forest cover (<91.3%) were the best predictors of whirling disease and resulted in 1 misclassification out of 12 streams. Similar to the results examining variance in fine sediment, the landscape-scale variables had a better fit than the reach-scale predictors when combined. We plotted this relationship to better illustrate the association between valley slope and whirling disease (Figure 3B).

Effect of Whirling Disease on Species Composition

We did not observe any trends in trout community composition over time that were associated with whirling disease.
Negative streams (the trend line was not significant in either basin-fed disease-presence in our basin-fed streams (Figures 4, 5). The slope of the trend line was not significant in either basin-fed disease-negative streams ($Z_{\text{susceptible}} = 0.014 \times \text{Time} - 0.19; 95\% \text{CI} = -0.041 \text{ to } +0.07$) or disease-positive streams ($Z_{\text{susceptible}} = -0.025 \times \text{Time} + 0.32; 95\% \text{CI} = -0.08 \text{ to } +0.03$; Figures 4, 5). For the two spring creeks, we detected a negative trend over time in susceptible species ($Z_{\text{susceptible}} = -0.096 \times \text{Time} + 1.27; 95\% \text{CI} = -0.154 \text{ to } -0.038$; Figure 6).

**DISCUSSION**

Our study showed a higher whirling disease risk for gently sloping alluvial valleys and spring creeks in the Blackfoot River basin. For basin-fed tributaries, the landscape variables of valley slope and forest cover were the overall best indicators of fine sediment (which is *T. tubifex* potential habitat), and valley slope was the best predictor of the presence of whirling disease. Streams with higher valley slopes had significantly lower levels of fine sediment and were categorized as disease negative (Figure 3). Spring creeks were not only predisposed to whirling disease, but the disease appeared to be influencing species composition—potentially constraining possible restoration outcomes—in these systems. Given the association of whirling disease risk with stream characteristics and the potential impacts on community composition in spring creeks, managers may want to consider whirling disease risk when prioritizing restoration sites across the landscape or setting restoration goals in spring creeks.

**Predicting Risk (Fine Sediment and Severity of Infection)**

Our analysis of landscape variables found high fine sediment and high infection severity in broad alluvial valleys with gentle, down-valley gradients (e.g., valley type VIII in Rosgen 1996). Here, alluvial floodplains are the most predominant landforms, which typically produce a high fine-sediment supply. Soils are developed over alluvium; thus, meandering streams in alluvial valleys are susceptible to naturally high levels of bank erosion and fine-sediment input. In the upper Blackfoot River basin, broad stream valleys are often utilized for intensive grazing and other land uses (e.g., farming, timber harvest, road construction) that commonly increase instream sediment levels and elevate water temperatures. By contrast, the steeper streams of the lower Blackfoot River basin support lower in-channel sediment levels, lower stream temperatures (R. Pierce, unpublished data), and thus a lower risk of whirling disease.

Similar to Anlauf and Moffitt (2010), our analyses of both landscape-scale and reach-scale features found that natural geomorphic variables and anthropogenic impacts can influence the proportion of fine sediment at a site. Anlauf and Moffitt (2010) found that the amount of slow habitat (pools, backwaters) versus fast habitat (riffles, runs) and riparian land cover type (conifer cover or agriculture) predicted differences in fine sediment at the reach scale. Most of the variation in our data was explained by geomorphology (valley slope), but anthropogenic degradation can create and enhance *T. tubifex* habitat (Waters 1995; Zendt and Bergersen 2000; McGinnis and Kerans 2013), playing a larger role in substrate composition and whirling disease than illustrated by our study. All sites in our data set are impacted to some degree by forest management practices, grazing, or agriculture. However, with the exception of Elk Creek, the riparian areas of the streams in this data set were not severely impacted by heavy grazing. Elk Creek, a disease-positive stream, had 2.5 times more fine sediment than any other site, the most gentle valley slope, the highest sinuosity, the most animal damage, and the lowest stream bank stability. That said, previous literature and our study demonstrated that some streams may be naturally at higher risk of disease because of their geomorphology. The relative role of natural versus anthropogenic drivers of whirling disease is context dependent. Even though we have focused on physical factors, certainly other factors, such as oligochaete community composition (Nehring et al. 2013, 2014), could further explain the variation in whirling disease infection severity among sites with gentle valley slopes.
Though high instream sediment may predispose certain basin-fed streams to whirling disease, water temperatures between 10°C and 15°C may likewise facilitate whirling disease infection by promoting the production of TAMs (El-Matbouli et al. 1999; De la Hoz and Budy 2004; Kerans et al. 2005). Hansen and Budy (2011) showed a short-term reduction in the prevalence of *M. cerebralis* infection in a small stream in a northern Utah watershed where passive restoration (via grazing exclusion) reduced summer stream temperatures below 10°C. This suggests that the potential for restoration to reduce whirling disease risk may be possible when linked with substrate and temperature.

In the Blackfoot River basin, we have not seen indications that the stream habitat restoration efforts reduced the average histological scores for whirling disease. Several basin-fed tributaries with high severity of infection (Belmont, Cottonwood, Chamberlain, Elk, and Monture creeks) have undergone substantial habitat restoration efforts, including instream channel restoration, riparian vegetation improvement, increased stream flows, and removal of streamside feedlots, during this time period. These restoration actions were designed to improve fish habitat and reestablish movement corridors for migratory native trout, which typically spawn and rear upstream of the sentinel cage sites, and were not designed to reduce whirling disease prevalence or severity. The warmer and less variable seasonal temperature profiles paired with the higher sediment loads in spring creeks influence whirling disease dynamics and result in a higher risk compared with basin-fed streams (Kerans et al. 2005; Neudecker et al. 2012; Pierce et al. 2014a). Kleinschmidt Creek had extensive restoration (channel reconstruction and grazing exclusion), which resulted in a decrease in daily average summer stream temperature from 11.2°C to 10.0°C; however, there were no reductions in the severity of *M. cerebralis* infection at the reach. Infection severity remained high (≥3; Pierce et al. 2014a), thus the natural characteristics of spring creeks may make them more susceptible to whirling disease regardless of typical habitat restoration efforts.

**Restoration Outcomes (Community Composition)**

For basin-fed streams, there were no apparent changes in community composition (susceptible versus disease-resistant species) before and after the whirling disease epizootic (Figures 4, 5). Larger river sections of western Montana, including the main-stem Blackfoot River, have documented
that susceptible species (specifically juvenile Rainbow Trout) declined in the presence of whirling disease (Vincent 1996; Granath et al. 2007; McMahon et al. 2010). The effects of whirling disease may be most apparent in river communities because of their high susceptibility and high levels of exposure, whereas the tributary assemblages may be buffered by contributions from upstream spawning areas where exposure may be lower (Pierce, unpublished data).

Similar to the findings in our two spring creeks in this study (Figure 6, Supplementary Figure S.1 found in the online version of this article), the shift to a community dominated by Brown Trout that was associated with exposure to whirling disease has been observed in other studies. In Kleinschmidt Creek, Brown Trout abundance increased in the presence of whirling disease following full channel restoration in 2001 (Pierce et al. 2015), supporting these study results. Similarly in Rock Creek near Missoula, Montana (a tributary to the Clark Fork River and a different Rock Creek than in this data set), the trout community also shifted dramatically from about 90% Rainbow Trout prior to whirling disease to primarily Brown Trout following the whirling disease epizootic (McMahon et al. 2010).

**Restoration Prioritization**

If managers are prioritizing restoration to support and augment susceptible salmonid populations in the presence of whirling disease, then physical features of the broader landscape, as well as life histories of target salmonids, should be considered. Within the heterogeneity of the Blackfoot River basin, salmonid distributions vary with longitudinal gradients. For example, Brown Trout and Rainbow Trout occupy the Blackfoot River and lower tributary system where _M. cerebralis_ is present (Pierce et al. 2009, 2014b). Westslope Cutthroat Trout are prevalent across tributaries of the Blackfoot River basin from the headwaters to the rivers, with migratory life histories connecting these habitats (Schmetterling 2001; Pierce et al. 2014b). McMahon et al. (2010) found evidence of Rainbow Trout declines in the Blackfoot River but no indication of disease-related Brown Trout increases (McMahon et al. 2010). Additionally, long-term monitoring in the main-stem Blackfoot River (1989–2014) has shown a positive trend in Westslope Cutthroat Trout abundance and an increasing proportion of Westslope Cutthroat Trout within the trout community of the Blackfoot River (Pierce and Podner 2013).
Certainly, habitat connectivity between disease-free headwaters and disease-prone streams on the valley floor could help maintain susceptible trout throughout the basin. Although our spring creek sample size was especially small, our results suggest that spring creeks may be ecological sinks for susceptible species and may promote Brown Trout on the landscape. This may present a special challenge for decision makers given the potential of nonnative Brown Trout to increase predation or competition with more susceptible species of fisheries and conservation value (e.g., Rainbow Trout or Westslope Cutthroat Trout; McHugh et al. 2008). As novel parasites and diseases move across the landscape, the consideration of disease in prioritizing restoration plans is likely to become more critical.

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