Status of Whirling Disease in the Crowsnest River

Technical Report | 2019

Status of Whirling Disease in the Crowsnest River, 2019: Technical Report
Marie Veillard and Clayton James

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Reviewer 1 is a University Professor of Biological Sciences with a Ph.D. in ecology and evolution of parasite-host interactions. The reviewer is a parasitologist with 25 years experience leading an active research program with focus on fish-parasite interactions.

Reviewer 2 holds a Ph.D. in fisheries science and has substantial experience in salmonid disease investigations, in particular, studies of *Myxobolus cerebralis*. The reviewer is a recognized expert in the field, holds a scientific position with a state agency, and has published extensively on the subject.

Reviewer 3 holds a Ph.D. in population ecology and has been working as an aquatic ecologist within western Canada for over 25 years. The reviewer's work has encompassed the fields of conservation biology, community restoration, non-native species invasions, population ecology and river ecology. The reviewer uses quantitative techniques to aid in understanding ecological processes and has worked with the Theoretical Population Dynamics Group (University of Amsterdam), the Fisheries Centre (University of British Columbia) and the University of Calgary.

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Acronyms and Abbreviations

ACA	Alberta Conservation Association
AEP	Alberta Environment and Parks
CFIA	Canadian Food Inspection Agency
Mc	*Myxobolus cerebralis*
qPCR	Quantitative polymerase chain reaction
RNTR	Rainbow Trout
TAM	Triactinomyxon
U of A	University of Alberta
YOY	Young-of-the-Year

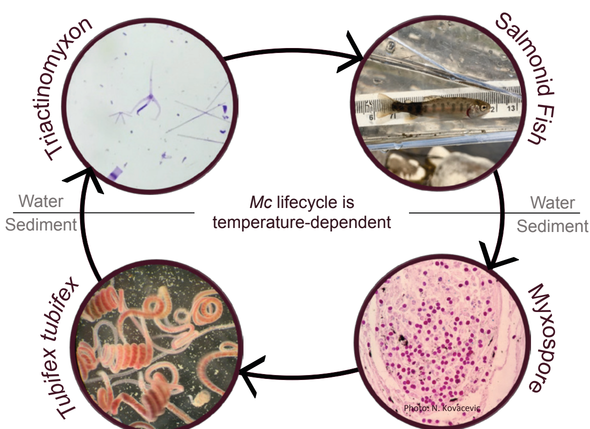
Executive Summary

Whirling disease is a salmonid fish disease caused by the parasite *Myxobolus cerebralis* (*Mc*). It has been implicated in major trout declines in Colorado and Montana and was confirmed in Alberta in 2016 prompting wide-scale surveillance for the parasite. At that time, 100% of fish samples tested from the Crowsnest River were positive for *Mc* infection in the lower section. By 2018, clinical signs of the disease were present in Rainbow Trout and Mountain Whitefish, while yearling Rainbow Trout appeared to be largely absent in the lower Crowsnest River. These results prompted a comprehensive study on the Crowsnest River in 2019. The objectives of this study were to monitor each part of the *Mc* lifecycle and document impacts to fish populations at six sites along the Crowsnest River from upstream (CRR-6) to downstream (CRR-1) and a control site on the Oldman River (OMR-1). We found the average triactinomyxon (TAM) density in the Crowsnest River was 0.06 TAMs / L, with the majority of TAMs detected in the lower watershed (CRR-1 and CRR-2). These results are comparable to TAM densities from the Colorado River (0.05 TAMs / L), a waterbody that experienced greater than 90% declines in Rainbow Trout. Oligochaete worm densities spiked at CRR-3 and CRR-2; worms were actively shedding TAMs in all four of the furthest downstream sites (CRR-4 to CRR-1). Water temperature at all sites spent between 74 to 96% of time within the optimal thermal regime (10°C to 17°C) for parasite development and transmission during the time of year when Rainbow Trout young-of-the-year (YOY) were most susceptible to impacts from *Mc* infection (i.e., nine weeks post-hatch). Sentinel cages were installed at each site and *Mc* negative Rainbow Trout were introduced to the cages for two separate exposure trials. After one week of exposure, 100% of fish tested positive for *Mc* at the furthest downstream site (CRR-1). By the second week, both CRR-2 and CRR-3 were 100% positive. In the second phase of the sentinel cage exposures, Rainbow Trout in the lowest three sites (CRR-1 to CRR-3) had reduced survival compared to other sites and evidence of sporogony in examined cartilage (pre-myxospore stage development). Clinical signs of whirling disease were elevated in wild fish captured in the lower watershed (up to 84% at CRR-2) compared to the upper watershed (as low as 8% at CRR-5). An individual wild fish captured near CRR-2 was ranked as severely infected on the MacConnell-Baldwin scale for histopathological damage, indicating a high likelihood of mortality. Yearling and older fish were largely absent at sites CRR-1 through CRR-4 for the second consecutive year, indicating at least two successive year class failures of Rainbow Trout YOYs in the lower watershed likely due to whirling disease. This study provides strong evidence that the *Mc* lifecycle is well established in the Crowsnest River, especially in the lower reaches, with high TAM densities capable of causing Rainbow Trout population declines. Evidence from the fish host studies suggest *Mc* is likely causing significant mortality of YOY Rainbow Trout in the lower sections.

Introduction

Whirling disease is a salmonid fish disease caused by the parasite *Myxobolus cerebralis* (*Mc*) (Wolf & Markiw 1984). *Mc* has a two-host lifecycle, alternating between an aquatic oligochaete worm host, *Tubifex tubifex*, and a salmonid fish host (Hedrick et al., 1998; Wolf and Markiw, 1984) (Figure 1). The parasite transfers between each host through two infectious spore stages, the myxospore stage (released by the fish host) and the actinospore stage (released by the worm host), otherwise known as a triactinomyxon (TAM). Myxospores are commonly released into the environment as fish hosts decay but can be shed by living hosts as well (Nehring et al. 2002). Oligochaete worms, including *T. tubifex*, consume myxospores found in the sediment or by directly feeding on infected fish carcasses. Within the worm host, myxospores develop into the TAM stage in approximately three to four months. When released from the worm host, the semi-buoyant TAMs drift in the water column and attach to the fins, skin and gills of fish. Upon contact, TAMs inject their sporoplasm into the fish and myxospore development begins. During myxospore development, *Mc* targets and feeds on cartilaginous tissue throughout the fish host (Hoffman et al. 1962; O'Grodnick 1979; Goater et al. 2014). It takes approximately three to four months for mature myxospores to develop in fish.

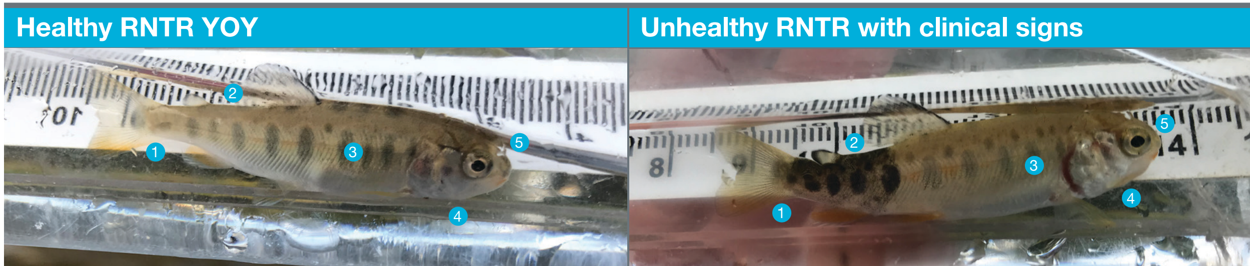
Figure 1: Simplified lifecycle of the whirling disease parasite, *Myxobolus cerebralis*. Development stages of the myxospore and triactinomyxon occur for three to four months in the fish and worm host, respectively.



The development of whirling disease is dependent on many factors including species susceptibility, infection intensity, and parasite development, which is correlated with water temperature (Gilbert & Granath 2003; Elwell et al. 2009). Susceptible fish species are most vulnerable to deleterious impacts if infected prior to nine weeks of age (Ryce et al. 2005) when the skeletal system is completely cartilaginous. Severe infections can disrupt the normal development of the skeletal system (Goater et al., 2014) and cause an inflammatory immune response that creates pressure and constrictions on the brain stem and spinal cord (Rose et al. 2000). In such cases, *Mc* infection may cause whirling disease, which is considered the manifestation of outward signs of infection such as a tail-chasing (or whirling) swimming pattern, blackened tail, spinal and cranial deformities, shortened opercula, and bulging eyes (Table 1). Survival of juvenile fish severely impacted by whirling disease is considered rare as severe infections substantially hinder an individual's ability to feed and avoid predation (DuBey et al. 2007; Goater et al. 2014). Fish that are lightly infected or resistant to the parasite, such as Brown Trout (O'Grodnick 1979; Andree et al. 1999; Thompson et al. 1999), may not be overtly affected by whirling disease, but can still serve as carriers of the parasite thereby continuing its lifecycle.

Table 1: Comparison of a healthy Rainbow Trout (RNTR) young-of-the-year (YOY) and a YOY exhibiting clinical signs due to whirling disease.

Healthy RNTR YOY	Unhealthy RNTR with clinical signs



❶ Spinal deformities can appear as a crooked spine or bent tail.
❷ Black pigmentation can occur throughout the body, but typically occurs in the tail.
❸ Edges of the operculum may appear shortened and frayed with gill filaments exposed.
❹ Cranial deformities can include distortions of the jaw.
❺ Other obvious cranial deformities can include bulging eyeballs and a shortened snout.

Mc is thought to originate in Europe and has been linked to negative economic, ecological, and social impacts to freshwater environments in North America (Elwell et al. 2009). The first confirmed occurrence of *Mc* in North America was in 1956 in a hatchery system in Pennsylvania (Hoffman et al. 1962). By the mid 1990s whirling disease had contributed to declines over 90% in Rainbow Trout populations in the Madison and Colorado Rivers, two blue-ribbon trout fisheries in the intermountain west (Nehring & Walker 1996; Vincent 1996). To date, *Mc* has been detected in 25 states with varying impacts on fish populations (Elwell et al. 2009).

In response to the whirling disease outbreaks in the United States, biologists in Alberta initiated a large-scale monitoring program spanning from 1997 to 2003 to test for *Mc* in the province (ACA unpublished data). The most vulnerable species to *Mc* infection in Alberta are thought to be Rainbow Trout (*Oncorhynchus mykiss*), Cutthroat Trout (*Oncorhynchus clarkii*), Brook Trout (*Salvelinus fontinalis*), Mountain Whitefish (*Prosopium williamsoni*), and Brown Trout (*Salmo trutta*) (Goss unpublished data) (O'Grodnick 1979; Hedrick et al. 1999; Thompson et al. 1999; Schisler 2010). Throughout the 1997 to 2003 monitoring program, over 3800 wild trout and over 3700 hatchery-raised trout were collected for testing from the South Saskatchewan and Red Deer River watersheds, including fish from the Crowsnest River. Fish were tested by microscopic examination of wet mount preparations and fixed slides stained using the Giemsa method to visually identify myxospores following a Pepsin-Trypsin digestion method (ACA unpublished data). A minimum of 100 fields were scanned for each wet mount & stained slide; the parasite was not detected in wild or hatchery-raised trout during this timeframe.

Sentinel cage studies were completed in 2000 and 2003 in the Crowsnest River, Castle River, Oldman River, Bow River, and Elbow River basins (Derksen 2001, 2004). In total, over 1800 known negative trout were exposed to stream conditions and subsequently tested for *Mc* using both microscopy, to visually identify myxospores, and a molecular polymerase chain reaction (PCR) assay, designed to detect the 18S rDNA gene of the parasite (Derksen 2004; John & Derksen 2005). *Mc* was not detected in any fish in the sentinel cage exposures in 2000 or 2003 (John & Derksen 2005). The studies concluded that while the parasite was not present in the surveys, the Crowsnest River had ideal thermal conditions and *Tubifex tubifex* host availability for the establishment and outbreak of the parasite should it be introduced (Derksen 2004). Despite recommendations for continuous monitoring as a means for early detection and rapid response, monitoring for *Mc* in Alberta ceased following the 2003 sentinel cage exposures.

After thirteen years with no surveillance efforts, Alberta confirmed its first case of whirling disease in Johnson Lake, Banff National Park in August 2016 (CFIA 2019). As such, it is likely that *Mc* first established in Alberta at some point between 2003 and 2016. Since the first detection, *Mc* has now been confirmed in waterbodies within the North Saskatchewan River, Red Deer River, and South Saskatchewan River watersheds (AEP 2017, 2019; CFIA 2019). Surveillance results from 2016 showed a high prevalence of *Mc* in the lower Crowsnest River where 100% of pooled fish samples tested positive (AEP 2017, 2019). Fourteen percent of worms collected in 2017 tested positive for *Mc*, and clinical signs were first observed in Rainbow Trout and Mountain Whitefish in 2018 (AEP 2019). Electrofishing surveys conducted in 2018 suggested that in the lower Crowsnest River, young-of-the-year (YOY) Rainbow Trout did not appear to be surviving to age 1+ (AEP 2019). This is a similar trend to what was observed in Colorado in the mid-1990s when whirling disease was first suspected as the cause of major declines in Rainbow Trout populations in the Colorado River (Walker & Nehring 1995).

Angling on the Crowsnest River generates social and economic value for southern Alberta and is a popular fly-fishing stream for Rainbow Trout (Carlson 2000; Bergman 2017). Rainbow Trout represent the third most valuable recreational fish species in Alberta and in total, recreational angling contributes in excess of $1.1 billion annually to Alberta's economy including full and partial expenses (DFO 2018). Due to the social and economic value generated by angling on the Crowsnest River, the presence of whirling disease is cause for concern.

The Crowsnest River became a provincial priority to understand the impact of whirling disease on Rainbow Trout populations, prompting a comprehensive study of the watershed in 2019. The objectives of this study were to: 1) determine the spatial distribution, infection prevalence and severity of *Mc* in the Crowsnest River, 2) measure triactinomyxon (TAM) density in free-flowing water, 3) characterize oligochaete relative abundance throughout the Crowsnest River, 4) measure the timing and location of optimal water temperatures for parasite development and transmission, and 5) estimate density and age-class structure of juvenile Rainbow Trout.

Methods

Sample Sites

Sites were selected based on previous *Mc* test results, geographic features on the landscape that may alter *Mc* prevalence or fish movement, and ease of access (Table 2, Figure 2). Six sites were selected spanning the geographic extent of the Crowsnest River. An additional site was selected on the Oldman River to serve as a control for cage effects.

In 2016, 207 fish were grouped into 29 composite samples and tested for *Mc*. All groups (29/29) tested positive for *Mc* in the lower Crowsnest River, below Frank slide. Samples from the lower Crowsnest River were taken above and below Lundbreck Falls, a natural barrier to fish movement, indicating high infection prevalence above and below the falls. All composite samples in the upper Crowsnest River watershed, above Frank slide, (in Allison Creek) tested negative for *Mc* during the same period (n = 6 composite samples containing 33 fish). With no barriers to fish movement, parasite establishment in the upper Crowsnest River was considered likely to occur over time. The Oldman River above the reservoir is a nearby watercourse that previously tested negative for *Mc* in 2016 (n = 10 composite samples containing 60 fish). The Oldman River was selected as a control site, with the assumption that the parasite had not spread to the watercourse between 2016 and 2019.

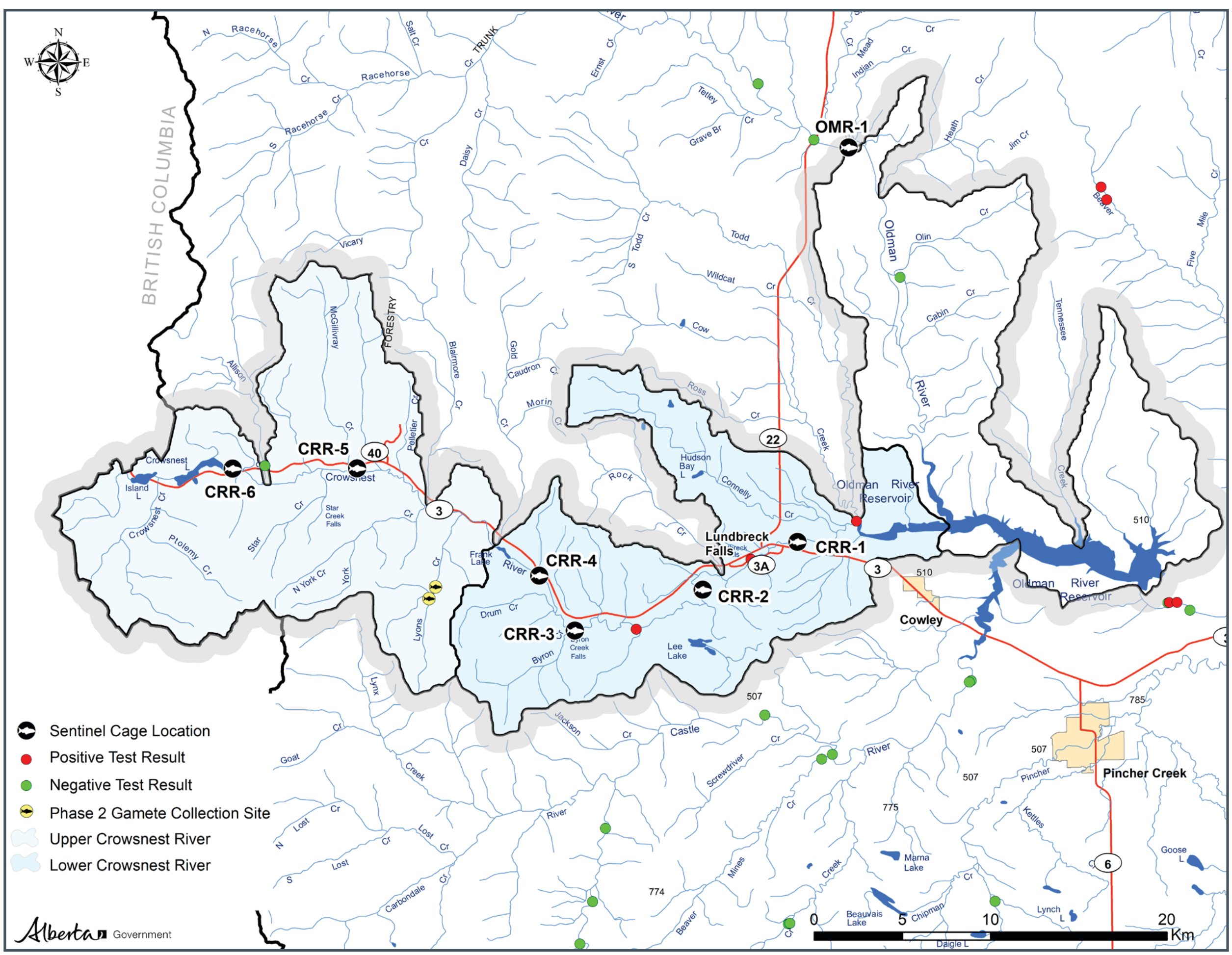
Sites were located immediately downstream of Crowsnest Lake (CRR-6), upstream and downstream of Frank Lake (CRR-5, CRR-4), as well as upstream and downstream of Lundbreck Falls, a natural fish barrier, (CRR-2, CRR-1) (Table 2, Figure 2). Road proximity and land access were considered in site selection for ease of access (CRR-3).

Table 2: Summary of site locations and previous *Mc* test results from 2016. Crowsnest River sites are listed from upstream (CRR-6) to downstream (CRR-1).

Site	Latitude	Longitude	Sub Watershed	Previous Test Result	Access	Location Description
OMR-1	49.794520	-114.126694	Lower Oldman River above Reservoir	Negative [a]	Waldron Flats Road	Upstream of Callum Creek Confluence, Downstream of Hwy 22 Bridge
CRR-6	49.633244	-114.613580	Upper Crowsnest River	Negative [b]	25 Street Bridge, Sentinel AB	Downstream of Crowsnest Lake
CRR-5	49.632921	-114.515786	Upper Crowsnest River	Untested	131 Street Walking Bridge, Coleman AB	Upstream of Frank Lake
CRR-4	49.577533	-114.373401	Lower Crowsnest River	Untested	9th Avenue Bridge, Hillcrest Mines AB	Downstream of Frank Lake
CRR-3	49.549415	-114.345875	Lower Crowsnest River	Positive	East Hillcrest Bridge, Hillcrest Mines AB	Downstream of Byron Creek
CRR-2	49.569593	-114.245196	Lower Crowsnest River	Positive [c]	Private Land	Upstream of Lundbreck Falls
CRR-1	49.593464	-114.170413	Lower Crowsnest River	Positive	Twp Rd 74C Bridge	Downstream of Lundbreck Falls

[a] Test result from location approximately 2 km upstream of OMR-1, near Hwy 22 bridge.
[b] Test result from Allison Creek, a tributary of the upper Crowsnest River.
[c] Test result from location approximately 4 km downstream of CRR-2, above Lundbreck Falls.

Figure 2: Site locations for the 2019 sentinel cage study (black fish circles). Red (positive) and green (negative) dots indicate 2016 and 2017 *Mc* fish test results based on qPCR analysis. Rainbow Trout gametes were collected from two sites on Lyons Creek for the second phase of sentinel exposures (yellow fish circles).



Water Filtration Sampling

Water filtrations were conducted weekly at each location from 18 June 2019 until 24 September 2019 following the methods described in Thompson and Nehring (2000). For each sampling event, approximately 1900 L (V_W) of river water was bucketed from the main flow of the river. Water was filtered through a 20 µm polyester screen material fitted inside a heavy plastic utility tub (51 cm x 66 cm x 20 cm) with 13 mm drilled holes to allow water flow. Samples were rinsed into a 500 mL container using a funnel and plastic wash bottle then kept in a cool, dark place until they could be processed off site.

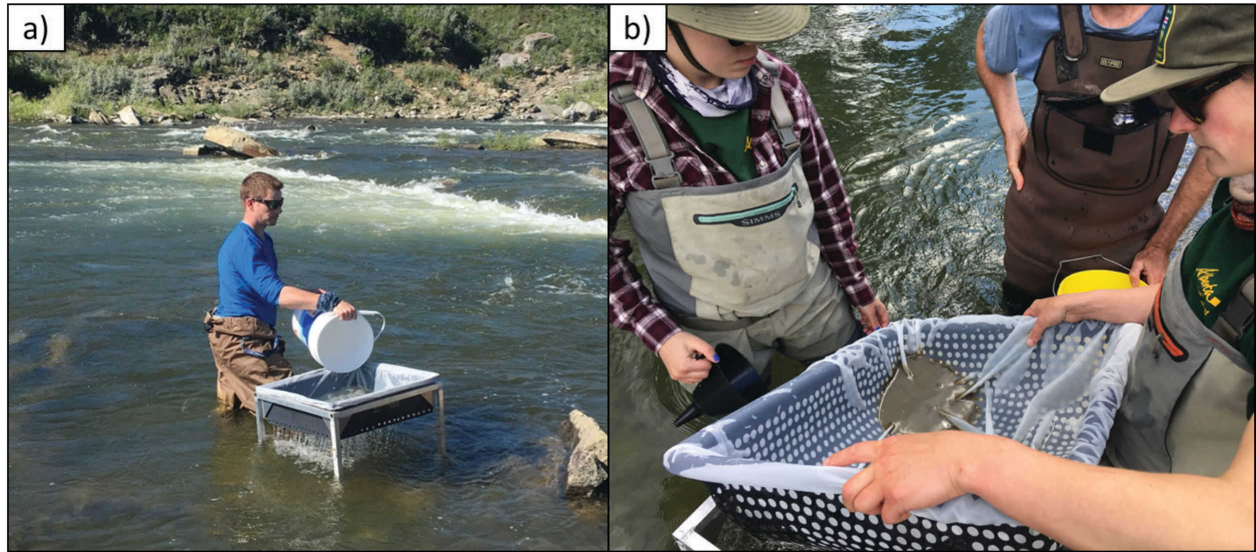
Each sample sat for a minimum of 2 hours to allow sediment to settle out of the water column. Due to the semi-buoyant nature of TAMs, sample containers were gently swirled to re-suspend TAMs in the retentate. The retentate was decanted from the sediment into a graduated cylinder and the volume of the retentate (V_R) was recorded. A subsample of ten 100 µL aliquots (1 mL total) of filtrate were stained with crystal violet solution and then pipetted separately onto a 2 mm x 2 mm gridded petri dish. Each petri dish was examined under a light microscope to enumerate TAMs. Site identification was withheld during

microscopy to eliminate potential bias during the screening process. The average number of TAMs per L was calculated using the following formula:

$$\text{TAMs / L} = \frac{\text{Sum of TAMs in 10 aliquots (i.e. TAMs / 1 mL)} \times V_R \text{ (mL)}}{V_W \text{ (L)}}$$

Where; V_R = volume of retentate, and V_W = volume of original sample.

Figure 3: Field water filtrations were used to assess TAM density in the Crowsnest River. Crews poured approximately 1900 L of stream water through a 20 µm polyester screen (a) and rinsed the filtrate into a 500 mL container (b).



Remaining retentate was preserved in 70% ethanol or submitted as raw water samples for testing by a provincial laboratory in Edmonton, Alberta. Samples were tested to confirm the presence or absence of *Mc* using a qPCR-based test developed in partnership with the University of Alberta targeting the 18S gene of the parasite. A qPCR-based approach was chosen for its high sensitivity, ability to provide a quantitative measure of parasite intensity where needed, and for the convenience of eliminating post-amplification processing. In the laboratory, water filtration samples were concentrated down to 6 mL by centrifugation in 50 mL conical tubes at 4200 rpm for 5 minutes. Further concentration was completed in 1.5 mL microfuge tubes to recover all particulates in the water sample or 250 µL for samples rich in organic matter or algae. This cut-off was implemented to ensure volume compatibility with the DNA isolation columns used and to minimize clogging of the filters. Concentrated water filtrate samples were re-suspended in 300 µL nuclease-free water from which total DNA was isolated using DNeasy Blood and Tissue kit (Qiagen Inc.). Five µL of eluted DNA was used as template for quantitative PCR. Probe (5'-/56-FAM/AGTGTTGGA/ZEN/GTAGTGTGCCGTCTT/3IABkFQ/-3'), forward (5'- GCTGATCGAATGGTGCTACTAA-3') and reverse (5'- TCAACTGCCATCCTTACGC-3') primers were each used at 250 nM per reaction. Primers, probe and mastermix (PrimeTime® Gene Expression Mastermix) were procured from Integrated DNA Technologies Inc. (IDT). Cycling parameters were 20 seconds at 95°C, then 40 cycles of 95°C for 1 second and 60°C for 20 seconds. Testing was completed in triplicate 20 µL reactions using standard curve and hydrolysis probe chemistry. All samples with cycle threshold (Ct) values in all triplicates after thermal cycling were interpreted as positive, while those with

undetermined Ct in all triplicates were interpreted as negative. Otherwise, samples were retested to increase the number of replicates used to interpret the results.

Alignment of microscopy and qPCR test results was assessed using a chi-squared test for independence. To compare the parasite density in the Crowsnest River to waterbodies with known whirling disease impacts, we summarized TAM filtration data from 2001 to 2003 in Colorado (Nehring & Thompson 2003). Data from Colorado was excluded from our summary if water filtrations occurred in areas of hatchery influence, such as in effluent channels. All relevant data was summarized by sample number, number of TAMs observed, average TAM density (TAMs / L), minimum TAM density (TAMs / L), and maximum TAM density (TAMs / L).

Worm Community Assessment

Aquatic worms were sampled at a total of five 1 m^2 quadrats within 300 m of each site between 17 July 2019 and 29 August 2019. Quadrats were selected by targeting ideal worm habitat such as slow flowing or stagnant sections of river characterized by high organic debris and sedimentation. At each quadrat, the uppermost 10 cm of sediment was disturbed and collected using a 500 µm benthic kick net for a total 30 seconds. Samples were transferred to a 20 L pail for sorting. Samples from all five quadrats were combined, thoroughly mixed, and then sorted for worms until either 300 individual worms were collected or 1 h of sorting time had been reached (e.g. two persons sorting for 30 minutes = 1 h of sorting time), whichever came first (Alexander et al. 2011). Worm relative abundance was calculated as the number of worms collected per minute.

The remaining unsorted worm sample was retained and transported to a cool dark place (10-17°C) for at least 24 hours to test if worms were actively releasing TAMs at each site. Aeration was added to pails to keep worms alive. After a minimum of 24 hours, water was decanted from the pail and filtered through a 20 µm filter. The resulting filtrate was examined for TAMs using the same laboratory methods as the water filtration sampling. Water samples were subsequently confirmed for the presence of *Mc* using qPCR.

Stream Temperature Monitoring

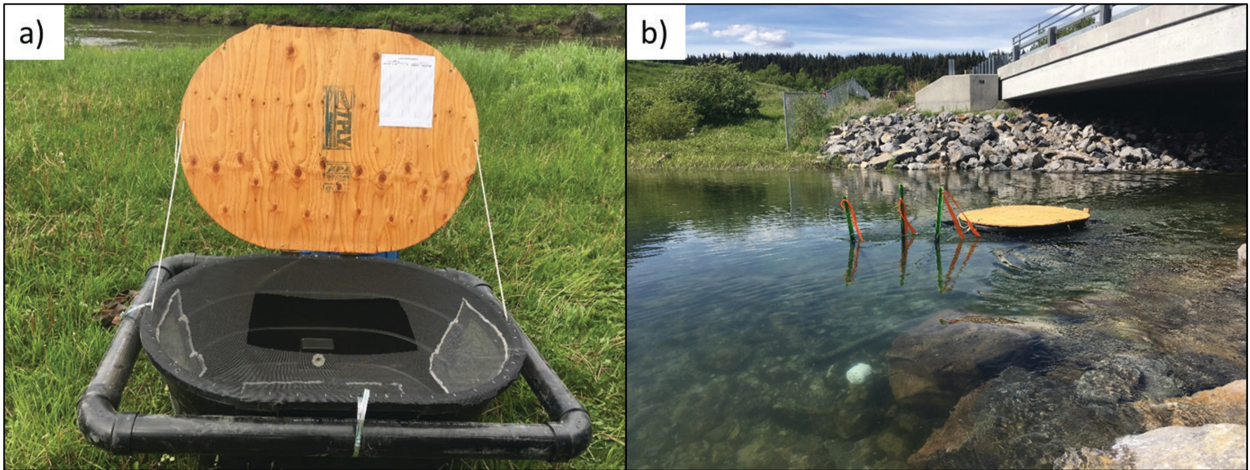
Two HOBO MX Tidbit 400 temperature loggers were installed at each site, one inside each sentinel cage and one within the thalweg of the river near each cage, to monitor both stream and cage water temperatures. Temperature loggers were set to record water temperatures every half hour at the top and bottom of the hour for the duration of the study. Differences between cage and stream temperatures were assessed using linear regression analysis. The proportion of time spent within the preferred thermal window (10-17°C) of *Mc* (El-Matbouli et al. 1999) was calculated for each site during the most susceptible time frame for YOY infection (nine weeks post-hatch). Rainbow Trout hatch was estimated based on published temperature cues for spawning and egg development (McPhail 2007) and compared to average water temperatures taken from four sites in the Crowsnest River in 2019. We estimate that approximately 50% of Rainbow Trout eggs hatched around 19 June 2019, at which point they were highly susceptible to *Mc* for the following nine weeks. Approximately 50% emergence of fry from the gravel was estimated to occur by 24 July 2019. To account for variability in hatch and emergence times, we extended the nine week susceptibility window until 30 September 2019. Therefore, the overlap of time spent in the optimal thermal window of *Mc* during the susceptible time period for Rainbow Trout YOY was calculated between 19 June and 30 September 2019. The same timeframe was applied to the Oldman River site as the thermal profiles are similar between the two rivers and the objective is to understand conditions necessary for whirling disease in the Crowsnest River. Exceedance of critical temperatures for parasite development (20°C) and parasite purging from the worm host (25°C) were assessed at each site (El-Matbouli et al. 1999).

Fish Exposures

Sentinel Cage Design

Sentinel cages were constructed using a 280 L poly oval stock tank (1.2 m x 0.8 mx 0.5 m). A 0.3 m^2 window was cut from both the front and the back of the cage and covered with 12 cm hardware cloth on the outside and aluminum window screen on the inside to prevent escapement and to allow the flow through of water (Figure 4). An additional window was placed in the floor to allow excrement and fine debris to settle out. A water-sealed PVC pipe rectangle (20 cm diameter) was constructed and attached to the tank using pipe clamps to provide floatation. Cages were secured in place by fastening the cage to three 2 m fence t-posts using airline cable. Wooden lids were hinged to the top of the tank and locked to prevent vandalism and predation.

Figure 4: Sentinel cage design (a) and installation (b) on the Crowsnest River, 2019. Installation photograph is from site CRR-6. A water temperature logger was installed upstream of the cage inside a white PVC housing.



Phase One – Infection Prevalence

Approximately 630 triploid (3N) Rainbow Trout fingerlings were purchased from a whirling disease negative private hatchery, Smoky Trout Farm Ltd., located near Red Deer, Alberta. Fish were approximately 15 weeks post-hatch when delivered to AEP staff in Blairmore, Alberta on 26 June 2019. Hatchery staff separated the fish into seven bags ranging from 72 to 137 fingerlings per bag. Fingerlings were immediately transferred to the sentinel cages by AEP staff. Every two to three days from 26 June 2019 to 7 August 2019 cages were thoroughly cleaned and siphoned to remove excess debris and fish were fed dry feed. If mortalities were found, they were immediately removed and either frozen or preserved in 95% ethanol and submitted for *Mc* testing using qPCR analysis.

Beginning on 3 July 2019, 12 to 15 fish were removed from each cage weekly for a total of six weeks (Jovani & Tella 2006). Fish were euthanized using a clove oil solution (Borski & Hodson 2003) and then tested for the presence of *Mc* using the qPCR test described in the water filtration section. All remaining fish (n = 8 – 60) were euthanized and sent for testing on 7 August 2019. All sentinel cage fish were processed as homogenates to capture the pre-spore stages of *Mc* as fish were not held for sufficient degree-days for myxospores to fully develop. Whole-bodies were homogenized in Dulbecco's

Phosphate-Buffered Saline (DPBS, Corning Inc.), adding 10 mL of DPBS per gram of tissue. For fish weighing less than 1 g, 2 mL of buffer was used to ensure compatibility with the homogenization equipment. To isolate DNA, 200 µL of the homogenized tissue was used as the starting material. The Qiagen DNeasy Blood and Tissue kit was used following the manufacturers recommendations except that elution of DNA was done in only 100 µL of elution buffer with prior incubation at room temperature for 5 minutes. Five µL of eluted DNA was used as template for quantitative PCR. Testing was completed in triplicate 20 µL reactions using standard curve and hydrolysis probe chemistry described in the water filtration section.

Upon completion of the first sentinel exposure phase, cages were removed, cleaned of organic residue, repaired and replaced back into the river in preparation for the second phase. Full decontamination of equipment was not required as equipment was not being moved between sites.

Phase Two – Fish Survival and Histopathology

Rainbow Trout gametes (milt and eggs) were collected from 11 adult females and 13 adult males on 22 and 24 June 2019 from Lyons Creek, a tributary of Crowsnest River (Figure 2), by the consulting company Hemmera. Eggs were fertilized in the field by Hemmera and an aquaculture consultant. A total of 1125 fertilized eggs were delivered to Nautilus Environmental Company Inc., in Calgary, AB where they were hatched and raised until 22 August 2019 (approximately 23 days post hatch). On 22 August 2019, approximately 450 fry were transferred to AEP staff and evenly distributed into each cage. Fish were fed dry feed and cages were cleaned thoroughly every one to three days from 22 August until 30 October 2019.

All observed mortalities were recorded, removed, and preserved in 95% ethanol for qPCR testing. Not all fish were accounted for and reported due to possible escapement or accidental removal during cleaning and siphoning. On 30 October 2019, all remaining fish were removed, euthanized in clove oil, preserved in 10% buffered formalin, and sent to the University of Alberta (U of A) to score *Mc* sporogony (myxospore development) using histopathology. All fish submitted for histopathology testing were identified by a random number sequence to eliminate potential human bias during the screening process.

Supplemental Wild Rainbow Trout Cage

On 25 September 2019, a second sentinel cage (CRR-2W) was installed parallel to the cage located at site CRR-2. The purpose of this second cage was to house 30 wild YOY Rainbow Trout captured immediately downstream of site CRR-2 via electrofishing to monitor differences in clinical signs and histopathology between wild caught fish and sentinel cage fish sourced from Lyons Creek. Only one out of thirty individuals from CRR-2W remained alive at the end of the study. This individual was sent to the U of A for histopathology testing.

Histopathology

With the exception of CRR-2W (n = 1), three fish from each cage were selected randomly from each site, embedded in paraffin wax, sectioned and stained with hematoxylin and eosin. In each individual, the gills, skull, and spine were examined using light microscopy for evidence of sporogony. Where sufficient time had elapsed for spore development, cartilage damage was ranked on the MacConnell-Baldwin scale to assess the severity of infection. The MacConnell-Baldwin scale is a widely used method to categorize infection severity into six qualitative groups: (0) no infection, (1) minimal, (2) mild, (3) moderate, (4) high, and (5) severe. Scores > 3 cause cartilage damage in juvenile fish and result in elevated mortality (Hedrick et al. 1999; Baldwin et al. 2000; Ryce et al. 2005). Due to weather and resource constraints, we were unable to hold fish from the sentinel cages long enough to develop myxospores, therefore only the single wild fish from CRR-2W was ranked on this scale.

Fish Sampling

Backpack Electrofishing Surveys

Backpack electrofishing surveys were conducted from 11 September 2019 to 17 October 2019 using adapted methods described in the provincial sampling standard (AESRD 2013) to sample wadeable areas of the Crowsnest River. Surveys were conducted in early fall to optimize the chance of detecting overt clinical signs of whirling disease in wild caught fish. Single pass surveys were conducted within 500 m of each sentinel cage location on the Crowsnest River, with the exception of CRR-6, which lacks a well-defined channel. Fish were examined for clinical signs of whirling disease including head and spinal deformities, shortened opercula, a distinct black tail, and whirling (tail-chasing) behaviour (Table 1). Signs of whirling disease were recorded and photographed. As whirling disease affects the survival of YOY fish, catch per unit effort (CPUE = fish captured per 100 seconds of effort) was assessed for both YOY and juvenile (1+ year olds) Rainbow Trout. Catch rates were summarized into binned fish lengths (10 mm bins) and presented as modified length-frequency distribution to assess population structure and relative abundance of size classes.

Results

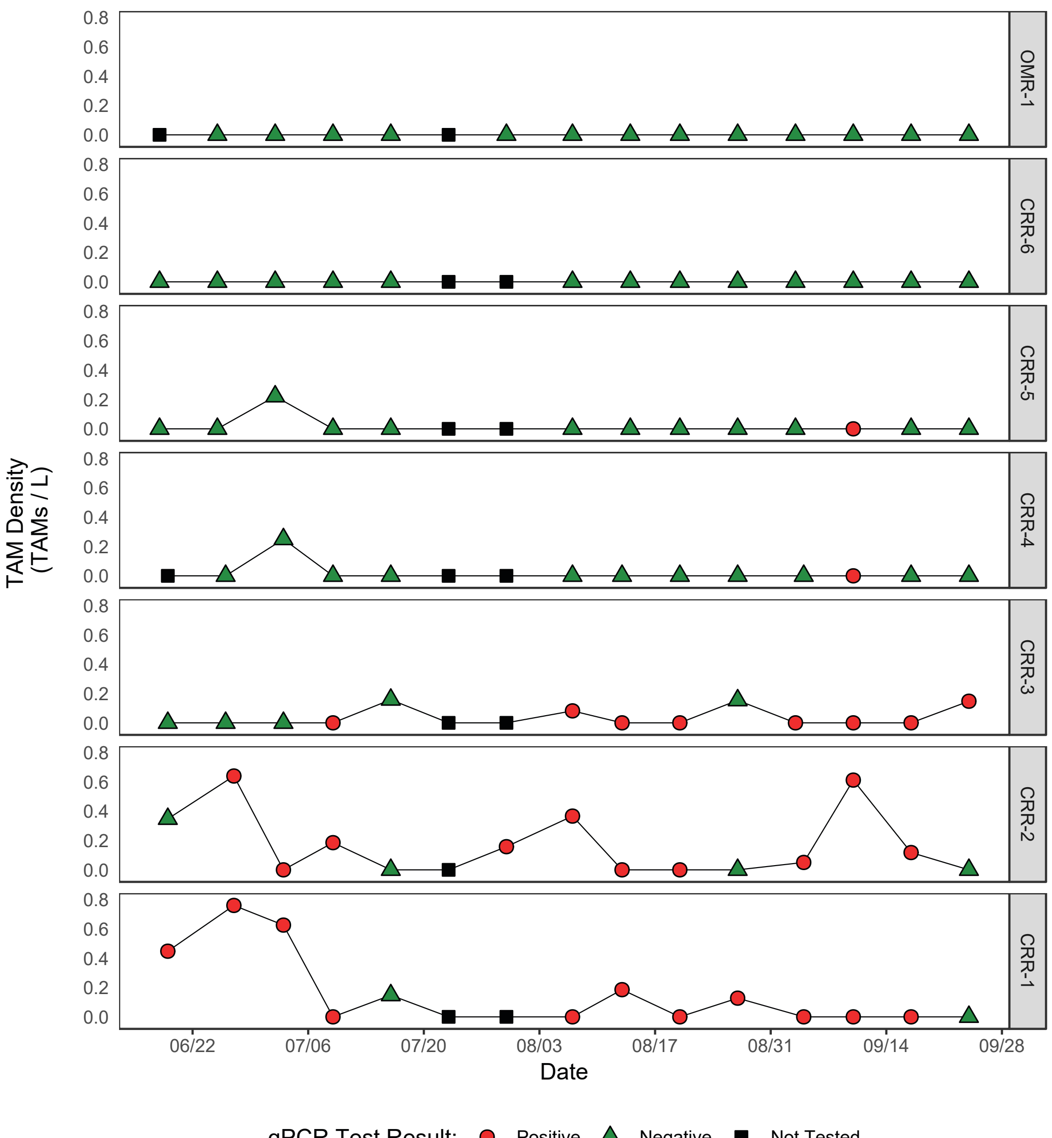
Water Filtration Sampling

With the exception of OMR-1 and CRR-6, *Mc* TAMs were detected in water filtration samples at all sites by both microscopy and qPCR over the duration of the study (Table 3, Figure 5). At site CRR-5, a single TAM was detected on 2 July 2019, and a *Mc* positive qPCR test result occurred on 10 September 2019. Similarly, at site CRR-4, a single TAM was observed using microscopy on 3 July 2019, and a positive qPCR result occurred on 10 September 2019. These single TAM detections resulted in average TAM densities of 0.01 TAMs / L and 0.02 TAMs / L at CRR-5 and CRR-4, respectively. At site CRR-3, single TAMs were detected microscopically on four separate sampling events for an average of 0.04 TAMs / L. At site CRR-3, *Mc* was detected using qPCR during eight sampling events. Site CRR-2 had the highest frequency of physical TAM detections during the study, where TAMs were observed in eight weekly sampling events, ranging from one to seven TAMs per event. CRR-2 had the highest average TAM density at 0.17 TAMs / L. At site CRR-2, *Mc* was detected using qPCR on 10 separate sampling occasions. At CRR-1, TAMs were observed during six weekly sampling events using microscopy ranging from one to five TAMs per sampling event and an average of 0.15 TAMs / L. At this site, *Mc* was confirmed in 11 weekly sampling events using qPCR. The single highest density of TAMs observed in this study was 0.76 TAMs / L and occurred at CRR-1 on 27 June 2019. In total, 40 TAMs were observed on the Crowsnest River in 20 out of 89 sampling events. On average, the TAM density in the Crowsnest River was 0.06 TAMs / L.

Table 3: Summary of weekly water filtration results by site and river using both microscopy and molecular testing from 18 June to 24 September 2019. Average, minimum and maximum TAM densities are reported as TAMs / L.

Site	Microscopy					Molecular Testing (qPCR)	
	Sampling Events (Count)	Samples with TAMs Observed (Count)	Total TAMs Observed	Avg. Estimated TAMs per Sample (min. – max.)	Avg. TAM Density, TAMs / L (min. – max.)	Tested Samples (Count)	Mc Positive Results (Count)
Site Summary							
OMR-1	14	0	0	0	0.00	13	0
CRR-6	15	0	0	0	0.00	13	0
CRR-5	15	1	1	28 (0–420)	0.01 (0–0.22)	13	1
CRR-4	14	1	1	32 (0–475)	0.02 (0–0.25)	12	1
CRR-3	15	4	4	68 (0 -300)	0.04 (0–0.16)	13	8
CRR-2	15	8	20	314 (0–1215)	0.17 (0–0.64)	14	10
CRR-1	15	6	14	290 (0–1443)	0.15 (0–0.76)	13	11
River Summary							
Oldman River	14	0	0	0	0.00	13	0
Crowsnest River	89	20	40	122 (0- 1443)	0.06 (0–0.76)	78	31

Figure 5: Weekly TAM detections calculated from microscopy and molecular testing at sentinel cage sites in the Crowsnest and Oldman Rivers between 18 June and 24 September 2019. Horizontal axis represents date of sample collection; the vertical axis represents TAM density (TAMs / L) calculated for each sample based on microscopy results. Coloured points represent the qPCR test result at each sampling event separated into three categories: positive (red circles), negative (green triangles), and not tested (black squares).



There was a significant relationship between results from microscopy and qPCR testing (X^2 =12.757, df = 1, $p < 0.001$). When TAMs were detected microscopically, *Mc* qPCR test results agreed in 14 of 20 samples, indicating a true positive alignment in 70% of cases (Table 4). When no TAMs were detected using microscopy, qPCR results agreed in 54 of 71 samples, indicating a true negative alignment in 76% of cases. More positives were returned using qPCR testing than microscopy (17 / 71 samples = 24%), likely due to the lower limit of detection for molecular testing. Instances where TAMs were detected

microscopically but not molecularly (6 / 20 samples = 30%), may be due to low TAM densities in the sample where, by chance, we found a physical TAM but there was not sufficient DNA to detect the parasite via molecular tests. It is also possible, that these instances represent the presence of non-*Mc* TAMs identified through microscopy. However, this scenario is less likely due to distinct morphological features of *Mc* TAMs.

Table 4: Comparison of test results from TAM microscopy and molecular qPCR testing. Values are reported as observed counts where both microscopy and qPCR testing were completed; percentages in brackets represent the percent alignment of molecular test results with microscopy.

		Molecular (qPCR)		
	Test Result	Positive	Negative	Total
Microscopy	Positive	14 (70%)	6 (30%)	**20**
	Negative	17 (24%)	54 (76%)	**71**
	Total	**31**	**60**	**91**
$X^2 = 12.757$, df = 1, $p < 0.001$				

Compared to rivers in Colorado where TAM densities were assessed in 16 watercourses from 2001 to 2003, the Crowsnest River ranked the fifth highest for average TAM density (Table 5). It had a comparable average to the Colorado River, Fraser River, and South Cottonwood River, all of which experienced up to or greater than 90% declines in Rainbow Trout populations over a five to ten year period. In Colorado, TAM densities as low as 0.01 TAMs / L resulted in up to 90% declines in Rainbow Trout populations in Beaver Creek and Fryingpan River; a similar TAM density was measured at CRR-5 in the Crowsnest River in 2019. CRR-2 had the highest average TAM density of 0.17 TAMs / L, which was comparable to the second highest transmission rates measured in Colorado from Clear Creek. In Clear Creek, Brook Trout populations became extirpated over a five to ten year period.

Table 5: TAM density in the Crowsnest River (2019) compared to Colorado waterbodies (2001 to 2003) (Nehring & Thompson 2003) ranked from highest to lowest average TAM density (TAMs / L).

Waterbody	Sample Events	Total TAMs Observed	Avg. TAMs / L	Min. TAMs / L	Max. TAMs / L
Gunnison River[a], 2002–2003	24	228	0.30	0	2.09
Clear Creek[b], South Platte River, 2002–2003	26	198	0.17	0	2.27
Spring Creek[c], 2002–2003	54	177	0.13	0	2.41
Middle Fork South Platte River[b], 2002–2003	29	123	0.11	0	0.90
Crowsnest River, 2019	**89**	**40**	**0.06**	**0**	**0.76**
Big Thompson River[d], 2002-2003	55	66	0.05	0	0.48
Fraser River[a], 2002–2003	24	36	0.05	0	0.29
Colorado River[a], 2002–2003	85	109	0.05	0	0.89
South Cottonwood Creek[ab], 2002–2003	24	47	0.05	0	1.03
South Boulder Creek, 2001–2002	32	50	0.05	0	0.63
Taylor River[c], 2002–2003	23	12	0.02	0	0.17
Quartz Creek[c], 2001–2003	25	12	0.02	0	0.20

Waterbody	Sample Events	Total TAMs Observed	Avg. TAMs / L	Min. TAMs / L	Max. TAMs / L
South Platte River[a], 2002–2003	22	10	0.02	0	0.09
Beaver Creek[a], 2002-2003	21	7	0.01	0	0.08
Fryingpan River[a], 2001–2002	297	82	0.01	0	0.27
Dolores River[c], 2002–2003	24	2	0.00	0	0.03
Clear Creek[c], Arkansas River, 2002–2003	24	1	0.00	0	0.01

[a] Wild Rainbow Trout population declines ≥ 90% over a five to ten year period;
[b] Wild Brook Trout extirpated in five to ten years;
[c] Only wild Brown Trout present before *Mc* parasite was enzootic;
[d] Wild Rainbow Trout thriving but worm microhabitat is limited except in and immediately below a mainstem reservoir. (Nehring Pers. Comm. 2020)

Worm Community Assessment

Worm relative abundance was highest in sites CRR-3 and CRR-2 measuring 10.0 and 11.5 worms / min, respectively. Relative abundance was lowest at sites CRR-6 and OMR-1, measuring 0.2 and 0.7 worms / min, respectively. At sites CRR-1, CRR-4, and CRR-5, moderate worm relative abundances were measured ranging from 1.1 to 2.9 worms / min (Table 6). However, sites were sampled over a wide timeframe (17 July 2019 to 29 August 2019), which may confound the results as the presence of juvenile worms within a sample may fluctuate over time following spring breeding events. Future work should aim to standardize sample timing to limit the possible temporal effect.

Worms collected from Crowsnest River were found to be actively shedding *Mc* TAMs after a minimum of 24 hours at the four furthest downstream sites (CRR-4, CRR-3, CRR-2, and CRR-1). Worms held from CRR-6 and CRR-5 were not found to be actively shedding TAMs (Table 6). Molecular tests confirmed that the TAMs observed in actively shedding colonies were *Mc* (Table 6)

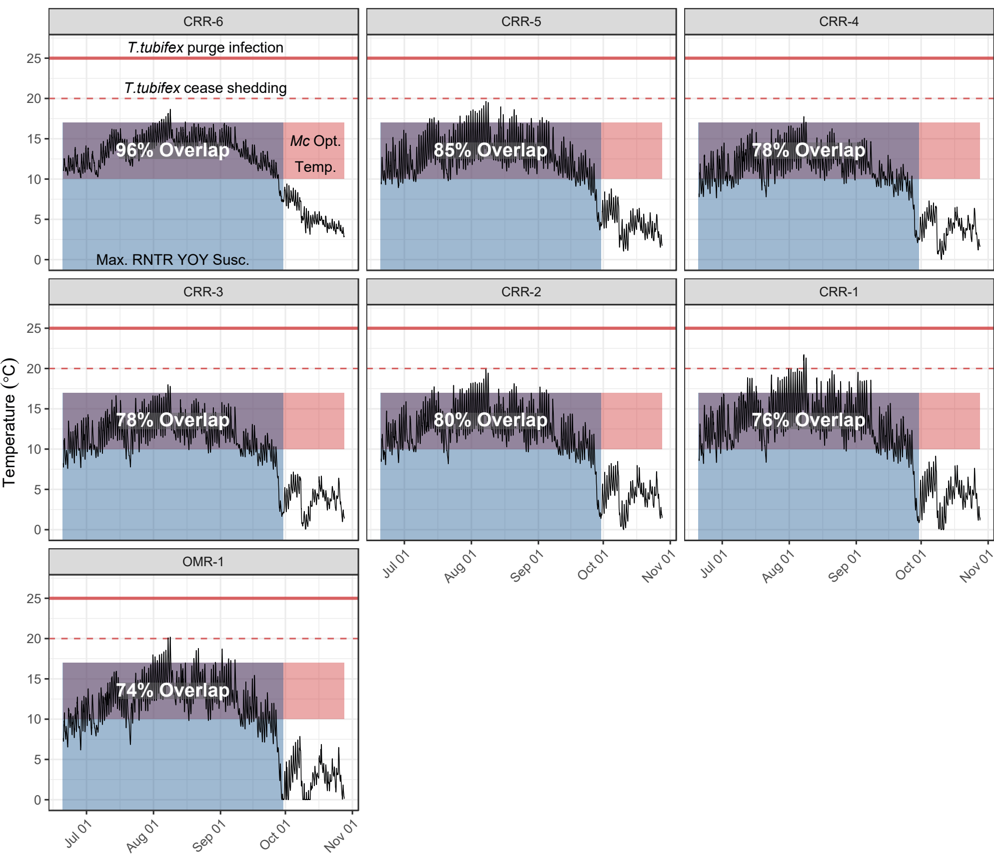
Table 6: Worm relative abundance and TAM release at each site sampled in 2019. Molecular tests were used to confirm *Mc* in actively shedding worm colonies. Dashes indicate no result.

Site	Sampling Date	No. of Worms Collected	Worm Density (worms/min)	Worms Actively Shedding *Mc* TAMs	qPCR Test Result for *Mc*
OMR-1	1-Aug-19	314	0.7	-	-
CRR-6	29-Aug-19	86	0.2	No	-
CRR-5	29-Aug-19	321	1.1	No	-
CRR-4	20-Aug-19	392	2.9	Yes	Positive
CRR-3	17-Jul-19	418	10.0	Yes	Positive
CRR-2	18-Jul-19	420	11.5	Yes	Positive
CRR-1	24-Jul-19	313	1.4	Yes	Positive

Stream Temperature Monitoring

Cage temperatures were highly correlated with stream temperatures outside of the cage ($R^2 = 1.0$; p-value < 0.001; x = stream temp., y = cage temp.; x = 0.02 + y). The proportion of time water temperatures fell within the optimal thermal regime (10°C to 17°C) for *Mc* from 24 July to 30 September 2019 was 74% in the Oldman River and ranged from 76% to 96% on the Crowsnest River (Figure 6). The upper Crowsnest River site (CRR-6) spent the most time in the optimal thermal regime during the most vulnerable time for YOY infection. Water temperatures increased downstream and the bottom of the watershed (CRR-1) spent the least amount of time in the optimal thermal regime for parasite development. However, temperatures at CRR-1 were still within the optimal range for parasite development and transmission 76% of the time. CRR-1 and OMR-1 both exceeded the 20°C temperature threshold, however the duration of these exceedances were not sufficient to prevent TAM shedding from the oligochaete host (El-Matbouli et al. 1999). No site exceeded 25°C, the temperature threshold in which there is evidence that *Mc* is purged from the oligochaete host.

Figure 6: **Cage temperature (°C) data collected every half hour at all sites over the duration of the 2019 sentinel cage study. Pink rectangles represent the optimal thermal regime (10°C to 17°C) for *Mc* development and transmission. Blue rectangles represent the estimated duration of time that YOY Rainbow Trout are most susceptible to *Mc* infection in the Crowsnest River. Purple rectangles represent the overlap between optimal parasite transmission and maximum susceptibility of Rainbow Trout YOY. Red dashed line represents temperature at which *Mc* TAM development is inhibited and *T. tubifex* cease shedding TAMs (El-Matbouli et al. 1999). Red solid line represents temperature lethal to *Mc* TAMs and *T. tubifex* are purged of infection (El-Matbouli et al. 1999).**

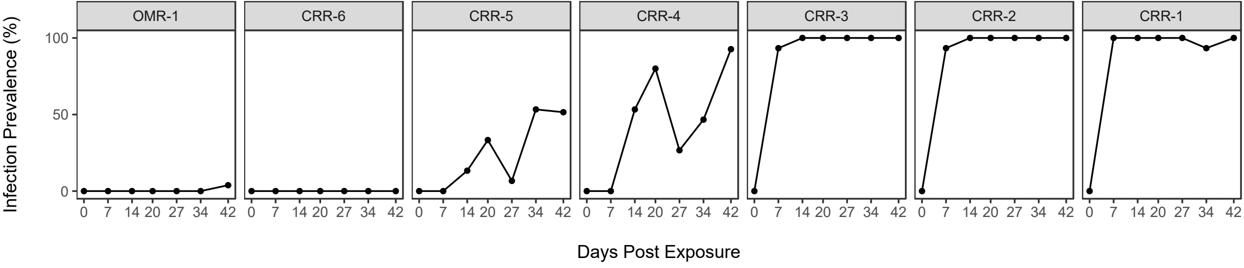


Fish Exposures

Phase One – Infection Prevalence

At seven days post exposure, *Mc* was detected in 100% of fish tested from cage CRR-1 (Figure 7). At CRR-2 and CRR-3, infection prevalence was 94% and 93% at seven days post exposure, respectively (Figure 7). *Mc* was undetected in fish collected from cages OMR-1, CRR-6, CRR-5, and CRR-4 after seven days of exposure. Following the second week of field exposures, CRR-2 and CRR-3 reached 100% infection prevalence. All three lower cages (CRR-1, CRR-2, and CRR-3) remained at 100% prevalence for the duration of the study with one exception; a single fish tested negative for *Mc* (93% prevalence) at site CRR-1 at 34 days post exposure but the cage returned to 100% prevalence in week 6. Cages CRR-4 and CRR-5 had their first detections of *Mc* after two weeks post exposure with infection prevalence of 53% and 13%, respectively. By week three, infection prevalence rose to 80% and 33% in CRR-4 and CRR-5, respectively. Week four saw a drop in infection prevalence at CRR-4 to 27% and CRR-5 to 7%, but rebounded to 47% and 53% in week five, respectively. At six weeks post exposure, CRR-4 reached 92% prevalence, its highest result throughout the study whereas CRR-5 appeared to stabilize around 52%. Sentinel cage site CRR-6 tested parasite-free for the full duration of the study. OMR-1 caged fish tested negative for the first five weeks but one fish tested positive at week six. This was a novel detection in the Oldman River watershed above the reservoir. Subsequent testing was completed which confirmed this positive result; the Oldman River above the reservoir is now considered positive for *Mc* establishment.

Figure 7: Infection prevalence of Rainbow Trout over time from phase one of the sentinel cage study conducted from 26 June to 7 August 2019. Known negative fish were introduced to cages at day zero. Each week, 12 to 15 fish were removed from each cage for infection prevalence testing.



Phase Two – Fish Survival and Histopathology

Fish Survival Rates

Lyons Creek Rainbow Trout had a 36% survival rate in the Oldman River and ranged from 14% to 64% in the Crowsnest River (Table 7). Average survival amongst all sentinel cages was 31%, with the lowest survival rates for fish occurring in the four furthest downstream sites (14% to 25%). Site CRR-5 exhibited the highest survival rates at 64%, whereas site CRR-2 had the lowest survival with only 14% of individuals surviving until the end of the study (Table 7). Fish that did not survive were categorized into two groups: observed mortalities and unobserved losses. Observed mortalities were individual mortalities observed by crew members and removed from cages. Cause of death cannot be ascertained for these individuals, however, it is assumed that mortality rates are a culmination of a variety of causes, including development of whirling disease, especially at sites with high TAM abundance (CRR-1 and CRR-2). Unobserved losses included all fish that were unaccounted for, likely due to a combination of

escapement, potential cannibalism, and loss of mortalities. Due to the small size of individuals and rapid decomposition of bodies from water mould (*Saprolegnia sp.*), mortalities that were difficult to identify and collect may have been accidentally siphoned from cages during cleaning.

Table 7: **Survival analysis of Lyons Creek fish placed in sentinel cages from 22 August to 30 October 2019 during phase two of the sentinel cage study.**

Site	Total Fish	Observed Mortality (Count)	Unobserved Loss (Count)	Survival (Count)	Observed Mortality (%)	Unobserved Loss (%)	Survival (%)
OMR-1	64	24	17	23	37.5%	26.6%	35.9%
CRR-6	64	29	12	23	45.3%	18.8%	35.9%
CRR-5	63	15	8	40	23.8%	12.7%	63.5%
CRR-4	64	23	25	16	35.9%	39.1%	25.0%
CRR-3	64	33	18	13	51.6%	28.1%	20.3%
CRR-2	64	35	20	9	54.7%	31.3%	14.1%
CRR-1	62	36	13	13	58.1%	21.0%	21.0%

Supplemental Wild Rainbow Trout Cage

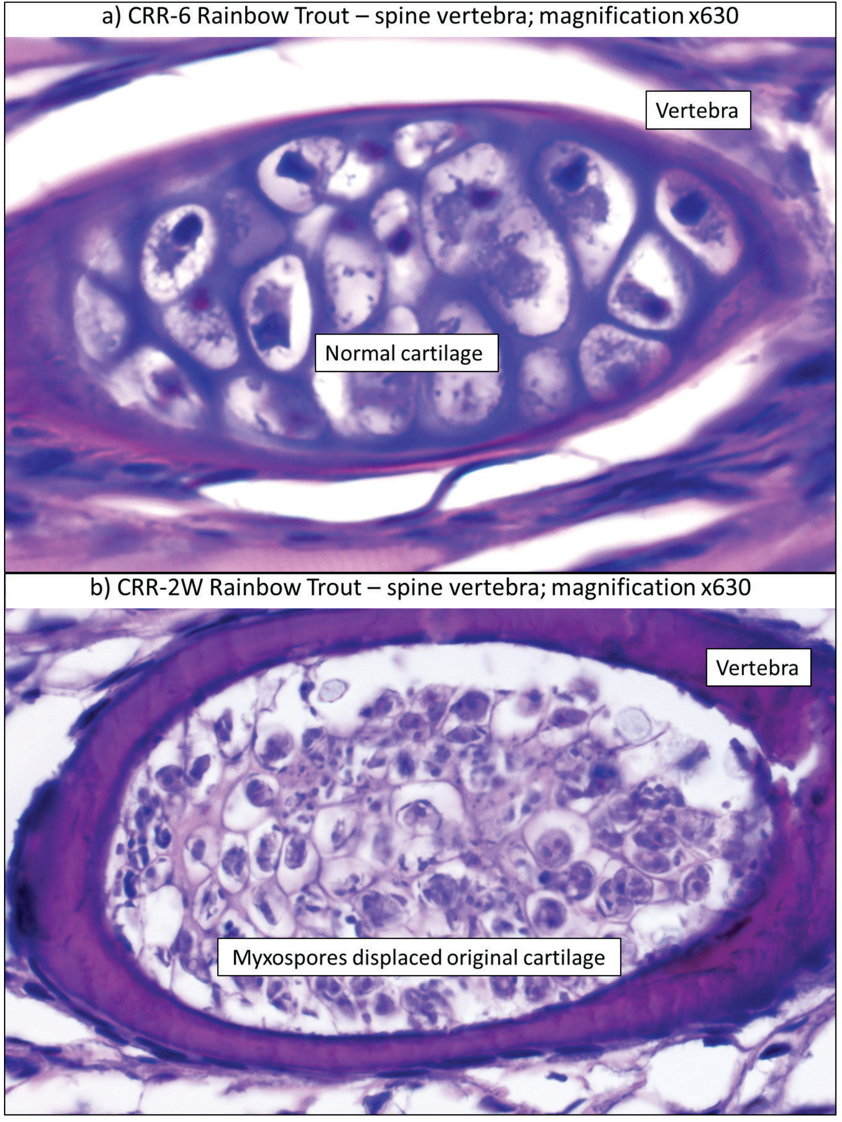
Of the original 30 wild Rainbow Trout placed in the supplemental cage at site CRR-2W on 25 September 2019, only 70% of fish were alive after approximately two weeks. By 16 October 2019, only 11 fish (36%) remained in the cage. At that time, 100% of the remaining 11 fish exhibited whirling behaviour and had obvious skeletal deformities. Possible escapement may have occurred as a result of a snow storm that began on 10 October 2019 where large amounts of snow weighed down cages and possibly submerged them for a period of time. On 27 October 2019, only a single fish remained in the cage and there was evidence that suggested a small mammal had accessed the cage and likely consumed the other remaining fish.

Figure 8: **Wild Rainbow Trout held in sentinel cage CRR-2W for observation. Obvious clinical signs include black tail, skeletal deformities, bulging eyes and a shortened opercula.**

Histopathology

Fish from phase two of the sentinel study lacked sufficient time to fully develop myxospores, however, clear evidence of sporogony (early stages of myxospore development) were observed in all fish examined from CRR-3, CRR-2, and CRR-1. Evidence of sporogony was not detected in fish from the control cage at OMR-1, nor was it detected in the remaining Crowsnest River sites, CRR-4, CCR-5, or CRR-6. In the single wild fish from CRR-2W, large numbers of myxospores had partially or fully displaced the cartilage in all skeletal regions examined (gills, skull, and spine) (Figure 9). The individual from CRR-2W was considered severely infected with *Mc* and was ranked as a five on the MacConnell-Baldwin rating scale (Hedrick et al. 1999; Baldwin et al. 2000; Ryce et al. 2005).

Figure 9: Histopathology sections of Rainbow Trout vertebra from fish collected from CRR-6 (a) and CRR-2W (b), viewed at 630x magnification. Fish at CRR-6 showed no signs of histopathological damage from myxospore development (a), whereas myxospores displaced cartilage throughout the head, spine and gills of the individual fish from CRR-2W (b). Photo credit: Edyta Jasinska, University of Alberta.



Fish Sampling

Backpack Electrofishing Surveys

In total, 151 Rainbow Trout were captured during the 2019 study, of which, 95% were considered YOY (< 85 mm FL). The average fork length across all sites was 53 mm and ranged from 32 to 167 mm. These results are similar to what was observed in 2018 where 121 Rainbow Trout were captured, of which 96% were YOY. In 2018, the mean fork length of Rainbow Trout was 52 mm and ranged from 25 to 180 mm.

High numbers of YOY Rainbow Trout were captured at all sample sites with the exception of CRR-1, where only four YOY were captured. This equates to a catch-per-unit-effort (CPUE) of 0.24 fish / 100 s (Table 8). The CPUE of YOYs at site CRR-2 to CRR-5 ranged from 1.60 to 4.01 fish / 100 s. Age 1+ Rainbow Trout were not captured in any of the three furthest downstream sites and only one individual was captured at CRR-4 (Table 8, Figure 10). At site CRR-5, nine age 1+ Rainbow Trout were captured that survived past their first year with a CPUE of 0.37 fish/100 s.

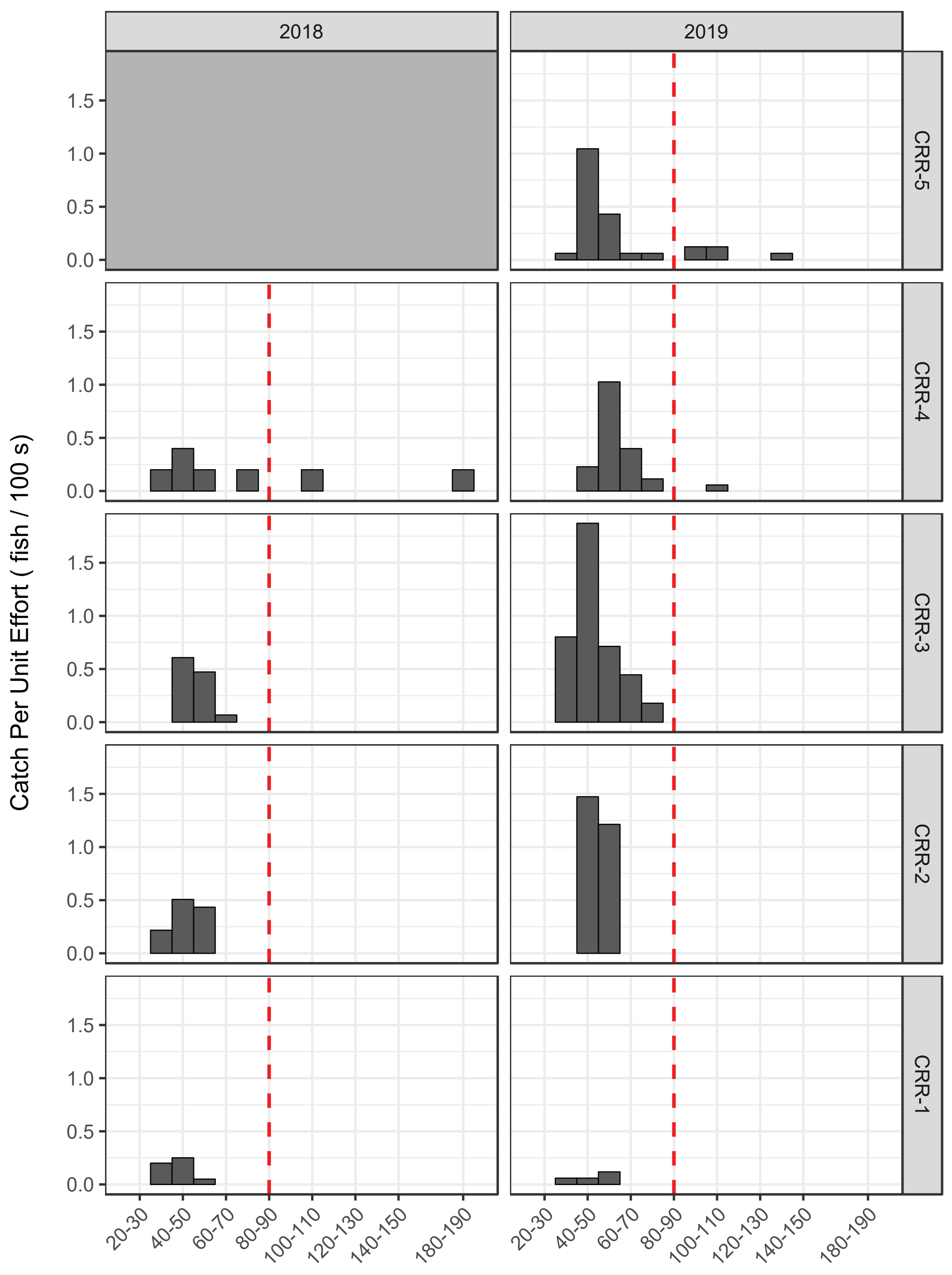
Clinical signs of whirling disease were observed in Rainbow Trout at all sampled sites (CRR-1 to CRR-5) and ranged from 8% of captured individuals at CRR-5 to 84% at CRR-2 (Table 8). The most common clinical signs observed were shortened opercula and black tail, however, slumping foreheads, spinal deformities, and whirling behaviour were also observed.

Table 8: Backpack electrofishing results on the Crowsnest River, sampled from 12 September to 17 October 2019.

Site	Sample Date	Raw Catch (No. Individuals)			Catch Per Unit Effort (fish / 100 s)			Clinical Sign (%)		
		Total RNTR	RNTR YOY	RNTR Juveniles (Age 1+)	Total RNTR	RNTR YOY	RNTR Juveniles (Age 1+)	Total	RNTR YOY	RNTR Juveniles (Age 1+)
CRR-5	17-Oct-19	32	26	6	1.97	1.60	0.37	8%	8%	0%
CRR-4	12-Sep-19	32	31	1	1.83	1.77	0.06	19%	19%	0%
CRR-3	11-Sep-19	45	45	0	4.01	4.01	0.00	36%	36%	0%
CRR-2[a]	25-Sep-19	31	31	0	2.69	2.69	0.00	84%	84%	0%
CRR-1	12-Sep-19	4	4	0	0.24	0.24	0.00	50%	50%	0%

Notes: [a] Captured fish were placed in supplemental CRR-2W cage.

Figure 10: Rainbow Trout catch rates (fish / 100 s) by size (bins = 10 mm) using backpack electrofishing in 2018 and 2019. Red dashed line indicates the likely transition from YOY to age 1+ (85 mm). Site CRR-5 was not surveyed in 2018 (grey box).



Discussion

The results of this study represent the first evidence that whirling disease is impacting self-sustaining Rainbow Trout populations in Alberta and in Canada. Triactinomyxon densities in the lower Crowsnest River were comparable to levels observed in numerous Colorado waterbodies where declines in Rainbow Trout populations occurred due to whirling disease. Nehring and Thompson (2003) found that the Colorado River, known for its dramatic collapse of Rainbow Trout (> 90% declines), averaged 0.05 TAMs / L (range 0 to 0.89) when measured across all sites from 2002 to 2003. The Crowsnest River had a comparable average of 0.06 TAMs / L (range 0 to 0.76) across all sites in 2019. Compared to the state-wide assessment of TAM density in 16 whirling disease positive waterbodies in Colorado, the Crowsnest River ranked fifth highest amongst TAM densities on record. TAM densities in the Crowsnest River were highest at the furthest downstream sites likely due to sediment accumulation in areas with lower water velocity along the streambanks, backwater eddies, and pockets that create optimal microhabitats for aquatic oligochaetes. Worm microhabitats tend to increase in downstream sections as stream gradients decrease and water moves from mountainous areas towards wider valleys.

Worm relative abundance was highest at sites CRR-3 and CRR-2 compared to all other sites. This spike in worm abundance may be a result of the natural patchiness of worm populations or may be influenced by human-induced nutrient enrichment in the watershed. For example, the wastewater treatment facility is located immediately upstream of site CRR-3. While nutrient data was not collected as part of this study, previous studies have associated oligochaete density with point sources of organic enrichment (Kaeser et al. 2006; Kaeser & Sharpe 2008; Marcogliese et al. 2009). In Quebec, enrichment from wastewater outflow contributed to high oligochaete densities and elevated myxozoan infection in Spottail Shiners (*Notropis hudsonius*) (Marcogliese et al. 2009). Therefore, future studies should assess the relationship between point and non-point source organic enrichment on oligochaete communities in the Crowsnest River.

The highest abundances of worms detected in this study were located upstream of sites where the highest densities of TAMs occurred (CRR-2 and CRR-1), supporting a relationship between worm relative abundance and TAM density. Worms collected from sites in the lower portion of the watershed (CRR-1 to CRR-4) were confirmed to be actively shedding TAMs throughout the study. Where *Mc* is well established, approximately 2% of the worm population may be actively shedding TAMs (Rognlie & Knapp 1998). Therefore, it is not surprising to find elevated TAM densities in areas downstream of high worm abundance, however, this relationship is not always clear as one worm can produce between 400 to 88,000 TAMs based on a variety of confounding factors (Gilbert & Granath 2001; Nehring et al. 2013). TAMs are thought to remain viable for approximately three to four days when held at 12.5°C (Markiw 1992), however, Kallert and El-Matbouli (2008) found that only 43% and 13.5% of TAMs were viable after two and three days, respectively when held at 12°C. Despite the discrepancy, at least a portion of TAMs have a high likelihood of remaining viable long enough to reach downstream sites in the lower Crowsnest River, therefore, TAM densities are considered an accumulation of TAMs released from multiple worm hotspots upstream.

In the Cache la Poudre River, Colorado, sites with up to 94% fish infection with *Mc* had no TAMs detected via microscopy (Allen & Bergersen 2002). This was likely due to an underestimation of true TAM density using the water filtration methodology. Due to the known underestimation, Nehring and Thompson's (Nehring & Thompson 2003) state-wide assessment concluded that a single detection of a TAM over 12 separate sampling events (~8% detection rate) was sufficient to cause declines to wild self-sustaining Rainbow Trout populations. Throughout our study, a total of 40 TAMs were found in 20 of 89 separate sampling events on the Crowsnest River, representing a 22% detection rate over all sites. Using

a molecular assay, 35% (31/89) of water samples tested positive for *Mc*. In the lower portions of the Crowsnest River, TAMs were detected regularly throughout the open-water season, with peaks in TAM production occurring in late June and mid September. This suggests there is some seasonality of TAM release occurring in the Crowsnest River that is similar to previous findings in Colorado and Montana (Gilbert & Granath 2001; Allen & Bergersen 2002; Downing et al. 2002; Pierce et al. 2009). This dual peak in TAM production may be due to the presence of spring and fall spawning salmonids in the Crowsnest River as it does not appear to be associated with optimal stream temperatures coming available in the spring and fall. Stream temperature throughout the Crowsnest River spent the majority of the open water season within the desired thermal range for TAM production and release. Temperature did not appear to be a limiting factor for *Mc* in the Crowsnest River, as TAM release occurred throughout the entire open water season as confirmed by both microscopy and qPCR.

High TAM production throughout the open-water season suggests that Rainbow Trout in the lower Crowsnest River are exposed to high doses of *Mc* immediately upon hatch and throughout their most vulnerable life stages (up to nine weeks post hatch) (Ryce et al. 2005). The prevalence of infection in the first phase of the sentinel study mirrored the same pattern observed in TAM densities with fish at the lower three sites experiencing higher infection rates compared to fish in the upper sites. After only seven days of exposure in June, 100% of fish in CRR-1 were infected with *Mc*. From week two to six, with one exception (a single fish in CRR-1 in week five), all fish collected from sites CRR-1, CRR-2, and CRR-3 tested positive for *Mc*. High TAM density at these sites is ideal to infect Rainbow Trout YOYs immediately upon hatch, which causes the most detrimental impacts to their survival. Site CRR-4 exhibited fluctuating infection prevalence rates throughout the study but eventually peaked at 93% in week six. In the upper portion of the watershed, where the parasite was not previously detected, individuals at CRR-5 tested positive for *Mc* two weeks post-exposure with generally mild infection rates throughout the study, peaking in week five at 53%. The observed decline in infection prevalence at CRR-4 and CRR-5 in week four may be due to bias in fish sampling where infected fish were more likely to be netted and removed from the cage in the earlier weeks. Infection with *Mc* has been shown to reduce swimming performance (DuBey et al. 2007) and stamina (Ryce et al. 2005), which could result in an inability to avoid threats. This may also explain why the only negative fish sampled at CRR-1 occurred late in the study. It is possible the negative fish at CRR-1 had some natural immunity to the parasite or it may have avoided parasite infection prior to sampling despite high densities of *Mc* TAMs in water filtration samples. There is evidence that some fish species, including Rainbow Trout, may alter their behaviour to avoid inconspicuous parasite life cycle stages (Karvonen et al. 2004; James et al. 2008). The uppermost site on the Crowsnest River (CRR-6) remained negative throughout the duration of this study. After six weeks of exposure, a single fish at OMR-1 tested positive for *Mc*. This was the first detection of the parasite in the Oldman River above the reservoir, therefore additional testing was completed to confirm these findings. The Oldman River above the reservoir is now considered positive for *Mc* establishment.

Survival and tissue damage of caged fish showed a similar pattern with reduced survival and elevated tissue damage in the furthest downstream sites. A maximum of 25% of fish survived at sites CRR-1 to CRR-4 compared to 36%-64% survival in the upper Crowsnest River (CRR-5 and CRR-6) and the Oldman River (OMR-1). Fish in the second phase of the study were only allowed two months of parasite development prior to collection due to weather and resource constraints. As it generally takes a minimum of three to four months for myxospores to fully develop, fish in the second phase were not given sufficient time to develop the full extent of whirling disease by the end of this study. Yet, early results show obvious signs of sporogony in fish from the lower cages (CRR-3, CRR-2, and CRR-1) compared to fish in the upper watershed and the control site (CRR-4, CRR-5, CRR-6 and OMR-1). Large numbers of myxospores were found in the individual wild fish captured and held in CRR-2W. In this individual, severe infection occurred throughout the body in the head, gills and spine where myxospores

had fully displaced cartilage in some regions indicating that the survival of this individual would have been very unlikely. The difference in timing of spore development between caged and wild fish is likely because the lab-reared caged fish spent their first three weeks post-hatch unexposed to *Mc*, whereas wild fish would have been exposed to the parasite immediately upon hatch. In the wild, hatch likely began as early as six weeks prior to lab-reared fish, therefore, wild YOY Rainbow Trout had sufficient time to fully develop myxospores and display signs of whirling disease by the end of this study.

During backpack electrofishing surveys in mid-September, 84% of wild fish had obvious clinical signs of whirling disease near site CRR-2. Thirty of these wild individuals were held in a separate sentinel cage and by mid-October, only 36% of individuals had survived. All remaining fish displayed a 'whirling' swimming behaviour and physiological signs associated with whirling disease. Only 8% of fish had observable whirling disease signs at our uppermost sampled site (CRR-5) and there was a general trend of increasing severity of clinical signs further downstream. Only 50% of wild Rainbow Trout captured at our lowest site (CRR-1) had clinical signs, however, it should be noted that only four fish were captured at CRR-1. Low capture rates of YOY Rainbow Trout at CRR-1 (below Lundbreck Falls) may indicate a lack of recruitment in 2019 and elevated mortality of YOYs compared to sites above Lundbreck Falls. Wild Rainbow Trout likely hatched in late June, 2019. At CRR-1, 85% (11/13) of water filtration samples taken between 20 June and 24 September 2019 tested positive for *Mc* using qPCR. TAM density averaged 0.15 TAMs / L with a peak of 0.76 TAMs / L. From these results we know that newly hatched fish were likely exposed to high levels of TAMs immediately upon emergence. As demonstrated by Markiw (1991), this may result in high mortality of YOYs as quickly as 12 days post-hatch. Markiw (1991) found that 2-day old sac-fry had survival rates of only 32% and 9% when exposed to 10 and 100 TAMs, respectively. When exposed to 1000 TAMs, all sac fry died within 12 days of exposure. Using the average measured TAM density, newly hatched fish need only come in contact with 66 L of river water to be exposed to approximately 10 TAMs. During peak TAM release, as little as 14 L of river water carried approximately 10 TAMs at CRR-1. Moreover, the lack of YOY found below the falls could be due to the length of time *Mc* has been established in the lower drainage. As the disease appears more severe moving downstream, whirling disease may have impacted fish populations below Lundbreck Falls for a longer period of time compared to upper sites and low recruitment success may be the result of impaired adult age classes.

The strongest line of evidence that whirling disease is currently having a population-level impact on Rainbow Trout in the Crowsnest River is the absence of age 1 and older juveniles. During backpack electrofishing surveys in 2018, a large cohort of YOY Rainbow Trout were present in the lower sections of the Crowsnest River, while very few yearlings or older were captured (AEP 2019). The large YOY cohort that was captured in 2018 appeared to be absent in 2019 (i.e., lack of age 1+ fish) and likely did not survive past their first summer. These findings parallel the well documented recruitment failure of YOY Rainbow Trout in the Colorado River in 1993 and should be cause for concern (Walker & Nehring 1995). Taken together, the high infection prevalence, elevated clinical sign, high TAM density and reduced survival in the lower sections of the Crowsnest River in 2019 suggest at least two successive year class failures likely due to whirling disease. Whereas in 2010, many juvenile Rainbow Trout were captured using a backpack electrofisher in the Crowsnest River (AEP 2019). The large numbers of YOY Rainbow Trout captured in both 2018 and 2019 indicates that adult fish are still present and abundant in the watershed, with the potential exception of CRR-1. Adult fish rarely succumb to whirling disease but still serve as carriers of the parasite. Older fish contribute to increased myxospore loads in the watershed as they die. The lack of YOY recruitment into older age classes as shown in this study illustrates that few fish will be available to replace older adults as they die, which may lead to a collapse of Rainbow Trout populations in the Crowsnest River. This may already be the case at CRR-1 as only four YOY were captured in 2019, putting into question the status of adult fish in this reach.

The timing of this study suggests we are documenting whirling disease as an emerging threat in the Crowsnest River before full population effects have occurred. Westslope Cutthroat Trout critical habitat is directly connected to the Crowsnest River and at-risk populations may be currently experiencing similar impacts from whirling disease. However, we lack sufficient testing to confirm. Despite the proximity of known high parasite infections to sensitive salmonid populations, whirling disease is still not considered a major threat to at-risk salmonid populations in Alberta (Sinnatamby et al. 2019). This is comparable to how Colorado viewed sensitive Cutthroat Trout populations in the context of whirling disease outbreak in the early years of its detection (Nehring 2006). Nehring (2006) concluded that this assertion was the result of a lack of systematic effort to monitor the parasite distribution and impacts in habitats capable of supporting at-risk salmonids. Results of an eight year study in Colorado from 2003 through 2010 were not encouraging (Nehring 2010). *Mc* was enzootic in numerous high elevation lake and stream habitats (up to 3,700 m) that supported Cutthroat Trout. A high percentage of those Cutthroat Trout populations were heavily infected with *Mc* and experiencing population-level impacts (Nehring 2010). Given the large geographical extent of *Mc* and the relatively short duration of its known presence in Alberta, little can be concluded about the impacts of the parasite on at-risk trout species at this time. While further investigations are required to study the impacts of whirling disease on species at risk, this report clearly demonstrates that whirling disease should be considered a serious and ongoing threat to susceptible salmonid populations in Alberta, particularly in watercourses that are hydrologically connected to the Crowsnest River.

Recommendations

This study documents the first evidence of an epizootic outbreak of whirling disease in Alberta and Canada. To date, monitoring and surveillance efforts have emphasized detection and distribution of *Mc* throughout the province but lacked empirical evidence of whirling disease. This is an important distinction as the presence of *Mc* does not always imply whirling disease will occur or that fish populations will be impacted by the disease. As such, our first recommendation is to broaden the focus of *Mc* surveillance in Alberta from a predominately presence/absence-based testing to evaluating *Mc* positive watersheds for potential whirling disease outbreaks using the tools outlined in this study. Continued testing for the parasite in watersheds where *Mc* has not previously been detected but that possess favourable conditions for outbreak (i.e., vulnerable salmonid rearing areas, presence of the worm host, and suitable thermal conditions) is also recommended. Priority should be given to watersheds where ecological (i.e., species at risk such as Westslope Cutthroat Trout or Athabasca Rainbow Trout) or economic (i.e. blue-ribbon fisheries such as the Bow River) impacts from whirling disease will have the greatest effect.

We offer a simplified framework to evaluate risk of whirling disease outbreak in a watershed by assessing thermal regimes, worm communities, parasite presence, and potential fish impacts (Figure 11). This framework provides a systematic assessment for all watersheds that risk either the establishment or outbreak of whirling disease in Alberta. Based on the framework, our findings in the Crowsnest River suggest management actions should aim to reduce fish impacts. To achieve this objective, the recommended action is to develop an adaptive management strategy to balance social, environmental, and economic impacts from Rainbow Trout die-offs.

It is essential to understand that once *Mc* has established in flowing-water it is not feasible to eradicate other than through extreme and costly measures (Nehring et al. 2018). As it is an untenable option to attempt to eradicate the parasite from the Crowsnest River, mitigation strategies should focus on the hosts and their environment in an effort to minimize the prevalence and intensity of infection. Generally,

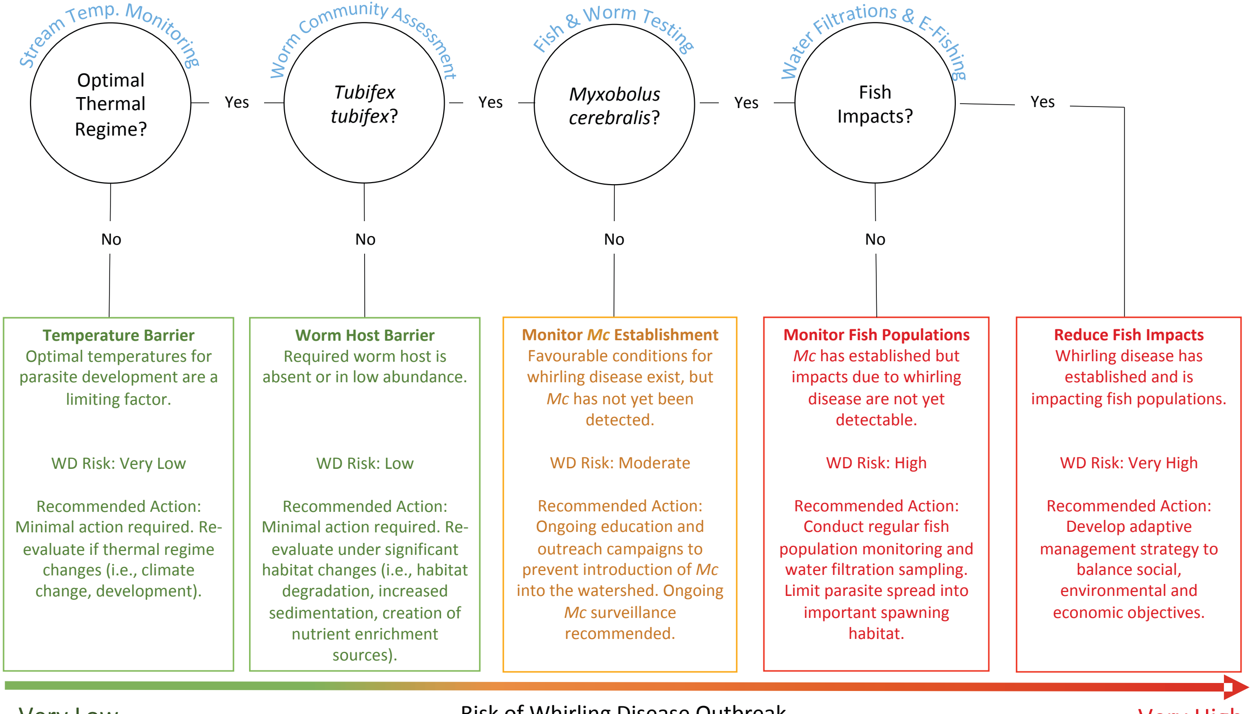
restoration and mitigation approaches can use the two host stages and their particular environment as starting points. Riparian habitat restoration is a practical management action which can be used at any severity of whirling disease outbreak, by reducing available habitat for oligochaete worms through sedimentation, erosion, and nutrient-enrichment control (Thompson 2011). However, the success of this method in reducing whirling disease prevalence varies based on watershed features as well as the scale and location of restoration activities. Furthermore, actions targeting worm host resistance to whirling disease have largely proven unsuccessful in the United States (Nehring et al. 2018) and therefore, host-specific actions should target the fish host.

As whirling disease generally has a substantial effect on Rainbow Trout reproductive success, fisheries managers need to carefully consider social, environmental and economic consequences of the spread of the parasite, as well as the long-term effect of implemented mitigations strategies. Changes in management objectives for the Crowsnest River, specifically, can focus on combinations of two major mitigation approaches, 1) the long-term establishment of whirling disease resistant strains of Rainbow Trout through natural selective processes or stocking and 2) management towards a change in fish community composition of whirling disease tolerant species. Any combination of these options need to be carefully weighed against ecological risks to the connected environments as well as the social and economic impacts to the communities that benefit and rely on the associated industries.

The Crowsnest River is a highly valued fishery. Maintaining the economic value that it brings to the communities is as much of a concern as the fact that the Crowsnest River watershed is also home to Westslope Cutthroat Trout, a federally listed species at risk. Cutthroat Trout are susceptible to whirling disease and are threatened by hybridization with Rainbow Trout. While they are effectively extirpated from the Crowsnest River mainstem due, in part, to historic stocking of Rainbow Trout into the system, Cutthroat Trout persist in many of the tributaries as well as in the connected Oldman and Castle River watersheds. Any modification of trout strains or species compositions will have to consider these factors.

AEP is currently working on viable options to maintain a sport fishery in the Crowsnest River, while at the same time reducing the impact and spread of whirling disease and protecting the other ecological interests in the watershed. AEP is exploring several options and will consult with experts on whirling disease, stakeholders and the general public on best options available before implementation. Additional details on the development of management strategies as well as how to get involved can be found in the associated summary document (alberta.ca/whirling-disease.aspx). While management strategies are developed and implemented, anyone using the watershed for recreation can do their part to mitigate and reduce the spread by following the provincial recommendations to clean, drain and dry all gear that is exposed to water and sediment, never move live or dead fish or fish parts between waterbodies, and use fish cleaning stations or put fish parts in the garbage.

Figure 11: Simplified framework to evaluate risk of whirling disease outbreak in wild populations of susceptible salmonids. Data gathered from each watershed using suggested monitoring tools (blue text) will help categorize risk and provide recommended actions. The monitoring tools associated with each risk category may be used on an ongoing basis to re-assess changes in the risk category over time. Recommended actions and monitoring tools outlined in previous risk categories may be used at any point for ongoing monitoring to assess finer-scale changes in the watershed.



Literature Cited

AEP. 2017. Whirling Disease Program Report (2017). Government of Alberta. Edmonton, AB.

AEP. 2019. Whirling Disease Program Report (2018). Government of Alberta. Edmonton, AB.

ACA. Unpublished data. Surveillance for *Myxobolus cerbralis*, the causitive agent of whirling disease, in salmonids in Alberta, 1997-2001 (Draft Report).

Alberta Environment and Sustainable Resource Development (AESRD). 2013. Standard for sampling of small streams in Alberta (public version). Government of Alberta. Edmonton, AB.

Alexander JD, Kerans BL, Koel TM, Rasmussen C. 2011. Context-specific parasitism in *Tubifex tubifex* in geothermally influenced stream reaches in Yellowstone National Park . Journal of the North American Benthological Society **30**:853–867.

Allen MB, Bergersen EP. 2002. Factors influencing the distribution of *Myxobolus cerebralis*, the causative agent of whirling disease, in the Cache la Poudre River, Colorado. Diseases of Aquatic Organisms **49**:51–60.

Andree KB, El-Matbouli M, Hoffman RW, Hedrick RP. 1999. Comparison of 18S and ITS-1 rDNA sequences of selected geographic isolates of *Myxobolus cerebralis*. International Journal for Parasitology **29**:771–775.

Baldwin TJ, Vincent ER, Silflow RM, Stanek DR. 2000. *Myxobolus cerebralis* infection in Rainbow Trout (*Oncorhynchus mykiss*) and Brown Trout (*Salmo trutta*) exposed under natural stream conditions. Journal of Veterinary Diagnostic Investigation **12**:312–321.

Bergman V. 2017. Trout town–Crowsnest Pass, Alberta. Available from https://www.vicbergman.com/trout-town-crowsnest-pass-alberta/ (accessed February 5, 2020).

Borski RJ, Hodson RG. 2003. Fish research and the institutional animal care and use committee. ILAR journal / National Research Council, Institute of Laboratory Animal Resources **44**:286–294.

Carlson L. 2000. A tool for monitoring the effectiveness of V-weirs for the over-wintering salmonids in the Crowsnest River. University of Calgary.

CFIA. 2019. Confirmed detections of whirling disease – Alberta.

Derksen J. 2001. Whirling disease sentinel study Alberta 2000. Lethbridge, AB.

Derksen J. 2004. Whirling disease sentinel study Alberta 2003. Lethbridge, AB.

DFO. 2018. Survey of recreational fishing in Canada, 2015. Ottawa, ON.

Downing DC, McMahon TE, Kerans BL, Vincent ER. 2002. Relation of spawning and rearing life history of Rainbow Trout and susceptibility to *Myxobolus cerebralis* infection in the Madison River, Montana. Journal of Aquatic Animal Health **14**:191–203.

DuBey RJ, Caldwell CA, Gould WR. 2007. Relative susceptibility and effects on performance of Rio Grande Cutthroat Trout and Rainbow Trout challenged with *Myxobolus cerebralis*. Transactions of the American Fisheries Society **136**:1406–1414.

El-Matbouli M, McDowell TS, Antonio DB, Andree KB, Hedrick RP. 1999. Effect of water temperature on the development, release and survival of the triactinomyxon stage of *Myxobolus cerebralis* in its oligochaete host. International Journal for Parasitology **29**:627–641.

Elwell LCS, Stromberg KE, Ryce EKN, Bartholomew JL. 2009. Whirling disease in the United States: A summary of progress in research and management 2009:61.

Gilbert MA, Granath WO. 2003. Whirling disease of salmonid fish: Life cycle, biology, and disease. The Journal of Parasitology **89**:658–667.

Gilbert MA, Granath WO. 2001. Persistent infection of *Myxobolus cerebralis*, the causative agent of salmonid whirling disease, in *Tubifex tubifex*. American Society of Parasitologists **87**:101–107.

Goater TM, Goater CP, Esch GW. 2014. Parasitism: the diversity and ecology of animal parasites. Second. Cambridge University Press, Cambridge.

Goss G. Unpublished data. Susceptibility study of three species of native salmonids to whirling disease 2018 – 2019.

Hedrick RP, El-Matbouli M, Adkison MA, MacConnell E. 1998. Whirling disease: Re-emergence among wild trout. Immunological Reviews **166**:365–376.

Hedrick RP, McDowell TS, Mukkatira K, Georgiadis MP. 1999. Susceptibility of selected inland salmonids to experimentally induced infections with *Myxobolus cerebralis*, the causative agent of whirling disease. Journal of Aquatic Animal Health **11**:330–339.

Hoffman GL, Dunbar CE, Bradford A. 1962. Whirling disease of trouts caused by *Myxosoma cerebralis* in the United States. U.S. Fish and Wildlife Service Special Scientific Report Fisheries **427**:1–14.

James CT, Noyes KJ, Stumbo AD, Wisenden BD, Goater CP. 2008. Cost of exposure to trematode cercariae and learned recognition and avoidance of parasitism risk by fathead minnows *Pimephales promelas*. Journal of Fish Biology **73**:2238–2248.

John J, Derksen J. 2005. Testing Rainbow sentinel Trout from southern Alberta rivers for whirling disease (2004). Lethbridge, AB.

Jovani R, Tella JL. 2006. Parasite prevalence and sample size: misconceptions and solutions. Trends in Parasitology **22**:214–218.

Kaeser AJ, Rasmussen C, Sharpe WE. 2006. An examination of environmental factors associated with *Myxobolus cerebralis* infection of wild trout in Pennsylvania. Journal of Aquatic Animal Health **18**:90–100.

Kaeser AJ, Sharpe WE. 2008. The ecology of *Tubifex tubifex* in two *Myxobolus cerebralis* enzootic streams in Pennsylvania. Journal of Freshwater Ecology **23**:575–588.

Kallert DM, El-Matbouli M. 2008. Differences in viability and reactivity of actinospores of three myxozoan species upon ageing. Folia Parasitologica **55**:105–110.

Karvonen A, Seppala O, Valtonen ET. 2004. Parasite resistance and avoidance behaviour in preventing eye fluke infections in fish. Parasitology **129**:159–164.

Marcogliese DJ, Gendron AD, Cone DK. 2009. Impact of municipal effluents and hydrological regime on myxozoan parasite communities of fish. International Journal for Parasitology **39**:1345–1351.

Markiw ME. 1991. Whirling disease: earliest susceptible age of Rainbow Trout to the triactinomyxid of *Myxobolus cerebralis*. Aquaculture **92**:1–6.

Markiw ME. 1992. Experimentally induced whirling disease II. Determination of longevity of the infective triactinomyxon stage of *Myxobolus cerebralis* by vital staining. Journal of Aquatic Animal Health **4**:44–47.

McPhail JD. 2007. The freshwater fishes of British Columbia. University of Alberta Press, Edmonton, AB.

Nehring BR, Thompson KG, Taurman KA, Shuler DL. 2002. Laboratory studies indicating that living Brown Trout *Salmo trutta* expel viable *Myxobolus cerebralis* myxospores. Pages 125–134 AFS Symposium.

Nehring BR, Walker PG. 1996. Whirling disease in the wild: The new reality in the intermountain west. Fisheries **21**:28–30.

Nehring BR. 2006. Whirling disease investigations federal aid project F-237-R13. Colorado Division of Wildlife Job Progress Report. Fort Collins, CO.

Nehring BR. 2010. Whirling disease investigations federal aid project F-237R-17. Colorado Division of Wildlife Job Progress Report. Fort Collins, CO.

Nehring BR, Alves J, Nehring JB, Felt B. 2018. Elimination of *Myxobolus cerebralis* in Placer Creek, a native Cutthroat Trout stream in Colorado. Journal of Aquatic Animal Health **30**:264–279.

Nehring BR, Hancock B, Catanese M, Stinson MET, Winkelman DL, Wood JS, Epp J. 2013. Reduced *Myxobolus cerebralis* actinospore production in a Colorado Reservoir may be linked to changes in *Tubifex tubifex* population structure. Journal of Aquatic Animal Health **25**:205–220.

Nehring BR, Thompson KG. 2003. Whirling disease investigations federal aid project F-237-R10. Colorado Division of Wildlife Job Progress Report. Fort Collins, CO.

O'Grodnick JJ. 1979. Susceptibility of various salmonids to whirling disease (*Myxosoma cerebralis*). Transactions of the American Fisheries Society **108**:187–190.

Pierce R, Podner C, Davidson M, Vincent ER. 2009. Correlation of fluvial Rainbow Trout spawning life history with severity of infection by *Myxobolus cerebralis* in the Blackfoot River basin, Montana. Transactions of the American Fisheries Society **138**:251–263.

Rognlie MC, Knapp SE. 1998. *Myxobolus cerebralis* in *Tubifex tubifex* from a whirling disease epizootic in Montana. Journal of Parasitology **84**:711–713.

Rose JD, Marrs GS, Lewis C, Schisler G. 2000. Whirling disease behavior and its relation to pathology of brain stem and spinal cord in Rainbow Trout. Journal of Aquatic Animal Health **12**:107–118.

Ryce EKN, Zale AV., MacConnell E, Nelson M. 2005. Effects of fish age versus size on the development of whirling disease in Rainbow Trout. Diseases of Aquatic Organisms **63**:69–76.

Schisler GJ. 2010. Early development of Mountain Whitefish: Effects of whirling disease (*Myxobolus cerebralis*) exposure on juvenile Mountain Whitefish (*Prosopium williamsoni*). Colorado Division of Wildlife, Aquatic Research Section. Fort Collins, CO.

Sinnatamby RN, Cantin A, Post JR. 2019. Threats to at-risk salmonids of the Canadian Rocky Mountain region:1–18.

Thompson KG. 2011. Evaluation of small-scale habitat manipulation to reduce the impact of the whirling disease parasite in streams. Aquatic Ecosystem Health & Management **14**:305–317.

Thompson KG, Nehring BR. 2000. A simple technique used to filter and quantify the actinospore of *Myxobolus cerebralis* and determine its seasonal abundance in the Colorado River. Journal of Aquatic Animal Health **12**:316–323.

Thompson KG, Nehring BR, Bowden DC, Wygant T. 1999. Field exposure of seven species or subspecies of salmonids to *Myxobolus cerebralis* in the Colorado River, Middle Park, Colorado. Journal of Aquatic Animal Health **11**:312–329.

Vincent ER. 1996. Whirling disease and wild trout: The Montana experience. Fisheries **21**:32–33.

Walker PG, Nehring BR. 1995. An investigation to determine the cause(s) of the disappearance of young wild Rainbow Trout in the Upper Colorado River, in the Middle Park, Colorado:152.

Wolf K, Markiw ME. 1984. Biology contravenes taxonomy in the Myxozoa: New discoveries show alternation of invertebrate and vertebrate hosts. Science **225**:1449 LP – 1452.