

2018 WHIRLING DISEASE PROGRAM REPORT



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Executive Summary

Since the discovery of whirling disease in 2016, Alberta Environment and Parks (AEP) created a Whirling Disease Program with an action plan based on three core elements of management: Distribution/Monitoring, Education, and Mitigation.

Highlights of the Whirling Disease Program are outlined below:

Distribution/Monitoring	Mitigation	Education
<p>In 2018, the Whirling Disease Program monitored parasite distribution in wild populations using fish, worm and sediment samples throughout the province, conducted an angler survey on the Bow River, assessed fish populations in four positive watersheds, and launched a large-scale stream temperature monitoring project.</p> <p>A key finding of the 2018 surveillance efforts was the presence of whirling disease clinical signs in Rainbow Trout and Mountain Whitefish in the Lower Crowsnest River. These observations in conjunction with test results from previous years have raised concerns about Rainbow Trout population health in the Lower Crowsnest River and surrounding watersheds.</p> <p>Ongoing efforts are in place to test private ponds that were stocked with potentially infected fish from private facilities.</p> <p>The Government of Alberta committed \$1.35 million to build the Whirling Disease Laboratory in Vegreville, Alberta that will expand capacity for in-house molecular testing. This capital upgrade will expedite disease testing and allow the Whirling Disease Laboratory to provide services to other jurisdictions.</p> <p>AEP partnered with local and international agencies on a number of research projects studying whirling disease in Alberta. Allocating funds to research and development projects will generate novel methodologies to inform effective management of whirling disease in Alberta.</p>	<p>The Government of Alberta created a mandatory Decontamination Protocol for government personnel working in or near waterbodies to mitigate the spread of the parasite. Dedicated decontamination technicians were hired to support the implementation of the protocol and establish two permanent decontamination stations in Edmonton and Lethbridge.</p> <p>Following confirmation of whirling disease in some private aquaculture facilities, a new condition stipulating mandatory fish testing prior to stocking was added to the annual licencing requirements of commercial fish producers to ensure infected fish are not stocked in Alberta.</p>	<p>In the past year, educational efforts reached nearly 8000 individuals at 55 public events to promote a deeper understanding of whirling disease and to encourage action. The ultimate goal of education and outreach is for Albertans to not only undertake AEP's recommended actions to "Clean, Drain, Dry" to help prevent the spread, but to also report observations of fish exhibiting clinical signs of whirling disease, help raise awareness and encourage others to take action.</p>

Alberta has focused on using a broad suite of management tools and techniques to predict risk, prevent the spread of *M. cerebralis* and mitigate the impacts on Alberta's trout and whitefish populations. While every effort is being made to prevent the spread of the parasite, the intent is that these tools, techniques and learnings will be available for other jurisdictions in the event that *M. cerebralis* is discovered in other regions of Canada.

Whirling Disease Program Report 2018

Background

In August 2016, whirling disease was confirmed in fish from Johnson Lake in Banff National Park and the Bow River Watershed in Alberta. This was the first confirmed occurrence of whirling disease in Canada. Whirling disease has subsequently been detected in other areas within the east slopes of Alberta, in particular the Bow River, Oldman River, Red Deer River, and North Saskatchewan River watersheds. These areas are home to sensitive trout populations, including species at risk such as Athabasca Rainbow Trout, Westslope Cutthroat Trout and Bull Trout. Whirling disease presence in Alberta is an additional threat to the sustainability of Alberta's trout and whitefish populations.

Alberta Environment and Parks (AEP) is investing considerable effort and resources to understand the impacts of whirling disease on Alberta's susceptible trout and whitefish species. The Whirling Disease Program includes five components that work collaboratively to understand and manage the impacts of whirling disease in Alberta. These components are:

- Aquaculture,
- Surveillance,
- Laboratory Diagnostics,
- Decontamination, and
- Education and Outreach.

Additionally, the Whirling Disease Program has initiated a number of research projects related to whirling disease in Alberta.

What is Whirling Disease?

- Whirling disease is caused by the parasite *Myxobolus cerebralis* (*M. cerebralis*) that has a two-host lifecycle, alternating between an oligochaete worm, *Tubifex tubifex* (*T. tubifex*), and a salmonid fish host, such as trout or whitefish (Figure 1)¹. Whirling disease causes clinical signs in fish such as cranial and skeletal deformities, darkened tail, and “whirling” behaviour².
- Whirling disease has no known impact on human health³.
- Whirling disease has only been detected in some salmonid species such as Rainbow Trout and Cutthroat Trout⁴ and is not known to impact non-salmonid species⁵.
- In impacted waterbodies, whirling disease may cause high mortalities of up to 90% of wild trout populations^{4,6}.
- Whirling disease can spread both naturally and through human activities. Fish movement is the primary source of parasite transmission⁴, however, it may also be spread through the movement of contaminated mud or water on equipment used for both recreational and industrial activities⁷.

- Confirming whirling disease requires laboratory testing as not all infected fish show clinical signs of the disease⁴. However, the parasite can also be detected in the obligate worm host and sediment using advanced molecular techniques.
- Contrary to previous belief, recent studies have found that the myxospore stage of the parasite, which is released from infected fish, is only viable for one year rather than up to 30 years⁸. The triactinomyxon (TAM) stage of the parasite which is released from infected worms is only viable for one to two weeks^{4,9}.
- Work in the United States has indicated that it is possible to break the life cycle of the parasite in wild streams and eliminate parasite infection¹⁰. However, breaking the life cycle requires the complete removal of the fish host for at least three years, which is exceptionally difficult in flowing water¹⁰. Therefore, it is prudent to focus on stopping the spread of the parasite on the landscape to mitigate its impacts.

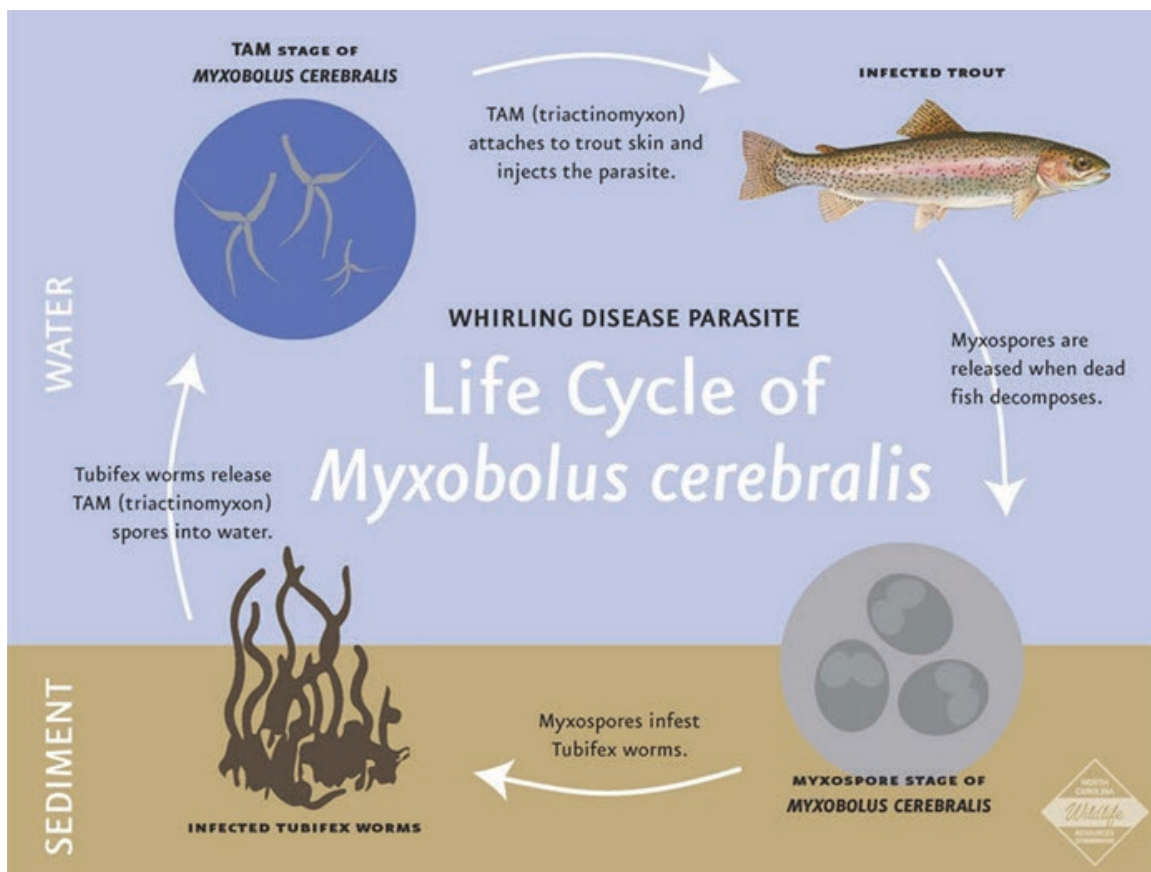


Figure 1: Lifecycle of the Whirling Disease parasite, *Myxobolus cerebralis* (credit: North Carolina Wildlife Resources Commission)

Timeline of Whirling Disease in North America and Alberta

- Whirling disease is native to Europe and was first detected in the United States in the 1950s¹¹. The disease was primarily associated with fish culture practices until the 1990s, at which time it was confirmed to be contributing up to 90% declines in wild trout populations in Colorado and Montana^{4,6}. Currently, whirling disease has been detected in 25 states in the United States⁴.
- Due to the documented trout population declines attributed to whirling disease in the United States, Alberta first conducted whirling disease testing from 1997-2001. During this time, 3,857 wild salmonid fish and 3,721 hatchery fish were tested for *M. cerebralis* in the province. Wild fish were collected from waterbodies within the South Saskatchewan River and Red Deer River watersheds. Additionally, 599 uninfected fish were held in sentinel cages in natural waters and tested for parasite infection. Whirling disease was not detected in Alberta from 1997-2001.
- In August 2016, whirling disease was confirmed in fish from Johnson Lake in Banff National Park and the Bow River watershed in Alberta.
- Alberta's provincial Whirling Disease Program was initiated in 2016 in an effort to monitor and manage whirling disease in Alberta.

Management of Whirling Disease in Alberta

Federal Management–Canadian Food Inspection Agency

The Canadian Food Inspection Agency (CFIA) is the federal agency responsible for reportable diseases in Canada, including whirling disease. On August 25, 2016, the CFIA officially announced the presence of whirling disease in Johnson Lake in Banff National Park. As of January 2019, the CFIA, in partnership with AEP, has confirmed the presence of *M. cerebralis* within the Bow River, Oldman River, Red Deer River and North Saskatchewan River watersheds and declared these watersheds infected with whirling disease (Figure 2). The rest of Alberta is considered a buffer zone (Figure 2) in an effort to protect undeclared watersheds from parasite infection. Legally, a CFIA domestic movement permit is now required to move any aquatic animal or related thing from a declared infected area to a buffer area (remainder of the province), or from a buffer area to other areas of Canada, as whirling disease has only been detected in Alberta. Permits from CFIA are not required to move aquatic animals or things within a declared zone, from a buffer area to an infected area, or from the rest of Canada to Alberta.

Despite these large-scale declarations by the CFIA, the parasite remains undetected in the majority of waterbodies within these declared watersheds. The Province is taking a more cautious approach by managing the parasite at a smaller watershed-scale in an effort to protect trout and whitefish populations. For more information on the federal management of whirling disease, please see the “For More Information” section (p.61).

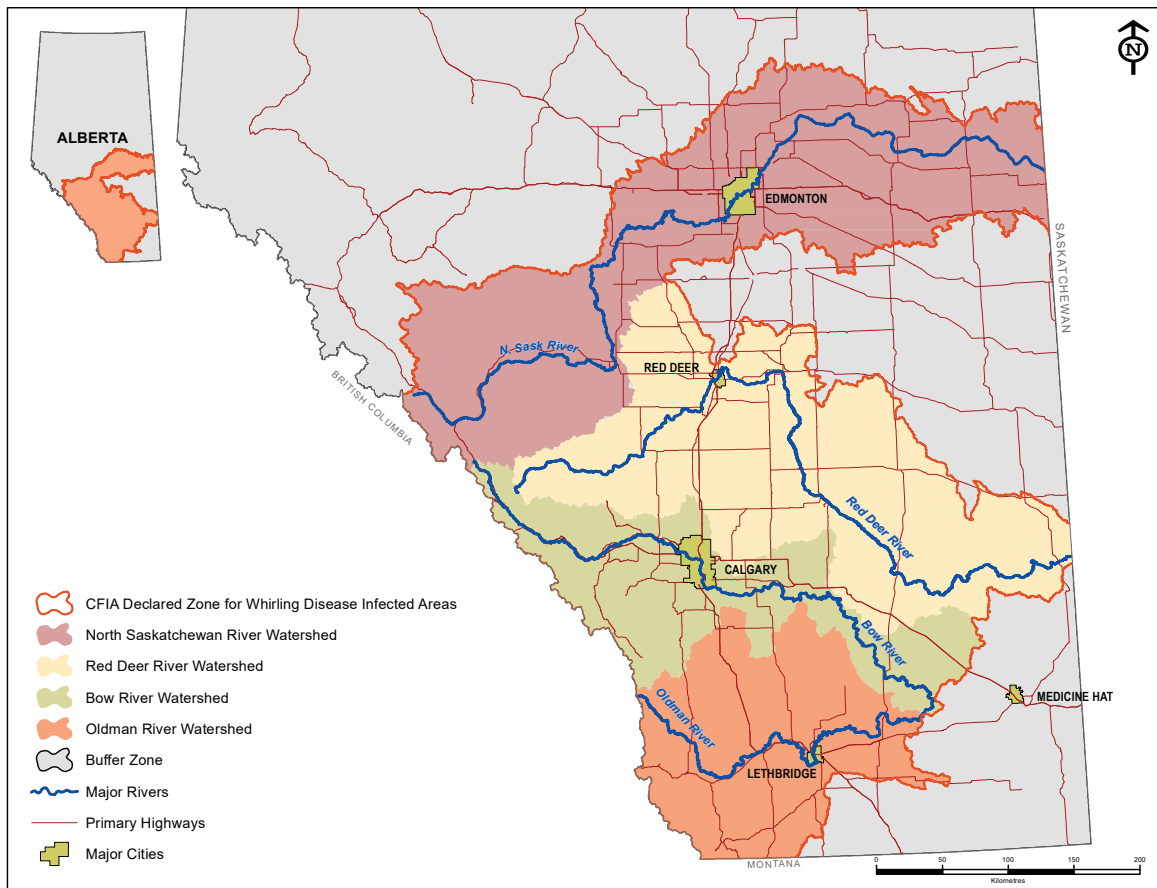


Figure 2: As of January 2019, the CFIA has confirmed the presence of whirling disease (infection with *M. cerebralis*) in the Oldman River, Bow River, Red Deer River, and North Saskatchewan River watersheds.

Provincial Management- Alberta Environment and Parks

Alberta Environment and Parks is responsible for ensuring the conservation of healthy, productive fish habitats and sustainable fish populations. Fish diseases, notably whirling disease, pose a serious risk to fish populations and the sustainability of fisheries. Whirling disease has become an emerging issue in Alberta since its discovery in 2016 due to the impacts it can have on trout and whitefish populations. AEP created the Whirling Disease Program, which operates under the Fish and Wildlife Policy Branch to manage whirling disease using the three core elements outlined below:

- **Distribution/Monitoring** – Define the distribution of the whirling disease parasite in Alberta and monitor current impacts and future risks to fish populations.
- **Education** – Deliver public messaging to provide both information and direction (e.g. location of infected areas, how to prevent the spread, how to report suspect fish, etc.).
- **Mitigation** – Limit the spread of the disease and implement management strategies for the disease where it exists in Alberta.

Following the first detection of *M. cerebralis* in Alberta, AEP led a broad-scale sampling program to delineate the extent of the parasite in susceptible fish species in salmonid-bearing waters within Alberta. As whirling disease has primarily been associated with aquaculture practices and stocking of sub-clinically infected fish, private and public aquaculture facilities were tested for *M. cerebralis* prior to allowing any fall stocking in 2016. AEP continues to manage whirling disease in an effort to prevent the spread of the whirling disease parasite and implement management strategies to reduce the risk to susceptible fish populations.

Potential Impacts of Whirling Disease

Environmental Impacts of Whirling Disease

Alberta is home to both native and naturalized fish species in the family Salmonidae (e.g., trout, whitefish, char, and grayling). These species experience high angling pressure in conjunction with habitat degradation and other stressors, which contribute to declines in populations. Westslope Cutthroat Trout, Athabasca Rainbow Trout and Bull Trout populations have been federally listed under the *Species at Risk Act*.

Whirling disease is an additional stressor to these important salmonid populations, and in affected waters, can cause declines of up to 90% in trout populations^{4,6}. Whirling disease has only been observed in salmonids and is not known to affect other fish species. Within the Salmonidae family, species and strains of species vary in their susceptibility to the disease. In Alberta, the most susceptible species are thought to be Rainbow Trout, Cutthroat Trout, and Brook Trout; while Brown Trout and Bull Trout may be less susceptible and Arctic Grayling is thought to be completely non-susceptible. Mountain Whitefish are highly susceptible to the physical parasite attachment until they develop scales, at which point they become less susceptible¹³. AEP is committed to minimizing the impact of whirling disease on provincial trout and whitefish populations.

Economic Impacts of Whirling Disease

The market value of recreational angling in Alberta is approximately \$660 million¹⁴. Protection of this valuable sport fishing industry will require investment in:

- Increased biosecurity protocols and improved infrastructure in provincially and privately operated fish culture facilities;
- Closure of some infected private fish culture facilities that cannot be decontaminated;
- Investment to develop an accredited fish disease laboratory in Alberta;
- Increased disease surveillance of wild and stocked fish;
- Decontamination protocols for government and industry working in aquatic environments; and,
- Education and outreach activities.

Trout stocking contributes approximately \$165 million annually to the market value of recreational angling in Alberta. One of the largest operational risks to facilities and the provincial

fisheries management program is disease. Disease related risks include: the introduction of a disease into a facility resulting in a hatchery epizootic event, the transmission of a disease between facilities, or the transfer of a disease from a facility to wild fish populations¹⁴.

In 2006, the value of the private aquaculture industry was estimated at \$10 million¹⁵. Sixty percent of this revenue is generated from table fish sales, which include salmonids. Forty percent of this revenue is from operators that raise trout for sale to recreational licence holders, other commercial fish farmers, bioassay labs and wholesalers. These fish sales include u-fishing opportunities, government contracts, and private pond stocking¹⁵.

Social Impacts of Whirling Disease

Alberta's fisheries are managed to ensure healthy and productive populations for future generations of Albertans; for Indigenous people's food and culture; and, to support recreational angling opportunities¹⁶. Many Albertans use this fisheries resource for consumptive or non-consumptive purposes, and over 300,000 recreational angling licences are sold annually¹⁴. Stewardship is the responsibility of all Albertans as everyone benefits from healthy ecosystems. The presence of whirling disease in the province highlights the need for all Albertans to follow "Clean, Drain, Dry" practices while angling or recreating in Alberta waters. Whirling disease may result in reduced angling or harvest opportunities as some publicly and privately stocked waters may experience closures or may continue to be managed for catch and release angling opportunities.

Updates on the Whirling Disease Program

The Whirling Disease Program works in the areas of aquaculture, surveillance, laboratory diagnostics, decontamination, and education and outreach to collaboratively understand and manage the impacts of whirling disease in Alberta. Updates on each area of the Whirling Disease Program are outlined below. Overview maps containing a complete summary of site work conducted within Alberta from 2016 to 2018 by watershed (Figures 27 to 31) can be found in the "Overview Maps" section (p.53). Additionally, the Whirling Disease Program has initiated a number of research projects related to understanding the impacts of whirling disease in Alberta that are found described in the "Research" section (p.47).

Aquaculture

The movement of live fish from private and provincial fish culture facilities was halted immediately after the confirmation of whirling disease in Johnson Lake in August 2016. The following sections describe the actions taken by AEP in provincial hatcheries, private facilities, recreational ponds and other stocked waterbodies in 2018 in response to whirling disease. For more information on AEP's initial aquaculture response, please see the 2017 Whirling Disease Program Report¹⁷.

Provincial Hatcheries

The causative agent of whirling disease, *M. cerebralis*, has not been detected at any of Alberta's four provincial hatcheries. However, the risk of parasite transmission into provincial aquaculture facilities still exists through the movement of infected fish, mud and water containing infectious spores. In recognition of these risks of parasite transmission, preventive control plans have been developed at the provincial fish hatcheries to prevent disease-causing agents from entering or leaving the fish production buildings and to minimize the risk of spreading these agents. The Whirling Disease Program has contracted Environmental Dynamics Inc. to work with hatchery and fisheries management personnel to develop enhanced biosecurity protocols and procedures for each facility. Three of the four provincial hatcheries are working to attain CFIA compartmentalization status, wherein a facility has separate 'clean' and 'dirty' compartments and rigorous protocols governing the movement of fish, eggs, and personnel between the compartments. The fourth facility is unlikely to attain compartmentalization status due to the age and nature of the facility. Attaining compartmentalization status allows Alberta's facilities to ship aquatic animals out of a whirling disease positive zone without a CFIA permit for each shipment.

Class A Facilities

Class A Commercial Aquaculture facilities are private facilities that are licenced to rear and distribute live salmonids in Alberta. As of April 01, 2019, all Class A Commercial Fish Culture facilities will be required to provide fish for *M. cerebralis* testing as a condition of their annual licence. This new condition on Class A licences reflects the Government of Alberta's commitment to prevent the spread of whirling disease by ensuring that private facilities are not moving infected fish in Alberta.

Class C Licences, Recreational Licences and Public Waters

Assessment of High and Moderate Risk Ponds

Class C Commercial and Recreational Aquaculture licence holders are authorized to keep live trout, but cannot sell live fish. Following a CFIA epidemiological assessment in 2016, 684 privately stocked ponds in Alberta were identified as having been stocked sometime within the previous three years from private aquaculture facilities that were deemed infected with *M. cerebralis* in 2016. In 2017, all 684 Class C and Recreational ponds that were stocked with fish from a facility that tested positive for *M. cerebralis* were initially assessed according to a Risk Management Framework to evaluate the risk of spreading or perpetuating whirling disease to public waters¹⁷. The framework was based on stocking history, known fish locations, and hydrological connection to natural water bodies or floodplains. Three hundred and twenty nine (329) private ponds and 11 stocked public ponds were determined to be at moderate to high risk of spreading or perpetuating *M. cerebralis*, and these private licences were suspended. Stocking of the 11 public ponds was also suspended.

In 2018, all ponds with suspended licences underwent a finer scale review of hydrological connectivity and assessment of the likelihood of escapement of the *M. cerebralis* parasite into waters containing wild trout populations. The hydrological review conditionally reinstated a number of licences based on distance from watercourses and a lack of hydrological connectivity. Currently, licences remain suspended for 94 ponds, preventing the stocking of trout until the pond has been confirmed to have a low likelihood of establishment of the whirling disease life cycle.



*AEP staff collecting samples for *M. cerebralis* testing from a privately stocked pond*

Aquaculture Field Component – 2018

Management of potentially infected stocked ponds is an important aspect in preventing the spread of whirling disease. Ponds with established *M. cerebralis* lifecycles may create hotspots on the landscape where wild trout populations may be at a higher risk of disease transfer. In 2018, the aquaculture component prioritized sampling from ponds with suspended licences and ponds with reinstated licences within areas of known salmonid presence as these ponds had a higher risk of spreading the parasite to wild populations. Field personnel collected aquatic worms and sediment samples from ponds to help determine if there was a high likelihood that the parasite lifecycle had established in these waterbodies.

Privately stocked ponds were sampled between January and October, 2018. Samples were sent to the University of Alberta for analysis. See Table 1 for an overview of the number of ponds sampled in 2018 and see the “Overview Maps” section (p.53) for test results.

Pond Breakdown	Ponds Sampled	Total Number of Ponds
Moderate and High Risk Ponds	179	329
Ponds with Suspended Licences	80*	94

Table 1: Aquaculture Field Sampling Overview, 2018

*All pond owners with suspended licences were contacted for sampling. Eleven licence holders could not be contacted due to out of date contact information, two owners are no longer stocking fish, and one owner sold their property.

Management Actions for Stocked Ponds

A Decision Framework was developed to support structured and transparent decision-making based on the results of *M. cerebralis* testing from stocked waterbodies (Figure 3). Ponds are sampled using an Eckman grab from multiple locations and depths on the pond, including samples from the middle and the shores of the pond. The minimum number of samples collected is dependent on the size of the pond, as well as crews' abilities to collect *T. tubifex* from sites. In an average sized pond (up to 500 m² in diameter), crews collect a minimum of three separate *T. tubifex* and sediment samples for qPCR testing for *M. cerebralis*. In larger ponds, additional samples are collected to ensure thorough coverage of the pond bottom. At sites where no worms were found, crews are required to collect a minimum of three additional sediment grab samples to check for the presence of worms. However, the number of additional samples is dependent on the size of the pond. All collected sediment and *T. tubifex* samples are tested for *M. cerebralis* using qPCR. The Decision Framework provides direction for management actions when test results are returned. The following is a break down of three potential decisions based on worm and sediment test results:

1. Continue to allow stocking of the waterbody in the following scenarios:

- a. If *T. tubifex* worms and sediment samples are both negative for *M. cerebralis*, then the likelihood of the parasite's lifecycle becoming established is low, as the parasite was not detected in the waterbody. The waterbody can continue to be stocked.
- b. If sediment samples are negative for *M. cerebralis*, and no worms are collected during the initial sampling, crews will return to perform confirmatory sampling, focusing solely on searching for worms throughout the waterbody and not collecting sediment samples. If no worms are collected in the waterbody following confirmatory sampling, then the waterbody can continue to be stocked. In this scenario, the waterbody will be sampled at least two times with intensified sampling protocols to ensure there are no worms present. This indicates that the likelihood of the parasite's lifecycle becoming established is low as the parasite was not detected and one of the obligate hosts (i.e., the worm host) of the lifecycle is likely not present in the waterbody.
- c. If sediment samples are positive for *M. cerebralis* and no worms were collected in the waterbody following confirmatory sampling, then the waterbody can continue to be stocked. In this scenario, the waterbody will be sampled at least two times with intensified sampling protocols to ensure there are no worms present. The lack of the obligate worm host in the waterbody indicates that the likelihood of the parasite's lifecycle becoming established is low.

2. Conduct further assessments in the following scenarios:

- a. If *T. tubifex* worms are positive for *M. cerebralis* this indicates that there is potential for the parasite's lifecycle to become established with the addition of the fish host. Further assessment of the waterbody is required to determine if the parasite lifecycle has become established and can be spread to the natural environment.
- b. If *T. tubifex* worms are negative for *M. cerebralis* and sediment samples are positive for *M. cerebralis*, then further assessment is required. The presence of both the obligate worm host and the myxospore, as indicated by positive sediment samples, indicate

that there is potential for the parasite's lifecycle to become established with the addition of the trout host. Further assessment of the waterbody is required to determine if the parasite lifecycle has become established.

In both of these scenarios, we consider ponds 'positive' and at risk of spreading whirling disease to native trout populations, and as such, any sediment or worm samples that test 'positive' for *M. cerebralis* result in a suspension of the fish stocking licence for that pond. Many pond owners have not stocked their ponds, or had fish in them, for three years. For those who have had ponds fallow of trout for three years or more and are interested in stocking, their pond can undergo further assessment for the presence of *M. cerebralis*.

The following two methods can be used for further assessments:

- **Sentinel Study:** A sentinel study can be used to evaluate if susceptible fish exposed to a pond environment become infected with *M. cerebralis*. In this assessment, uninfected juvenile trout will be held in the waterbody for a set period of time to coincide with the ideal temperatures at which *T. tubifex* release the highest abundance of triactinomyxons (TAMs). If fish become infected in the waterbody, then this is a positive indication that *T. tubifex* worms are releasing TAMs and the parasite's lifecycle is established. In this scenario, the addition of the trout host is not recommended and mitigation options would be considered. Conversely, if the fish are negative for *M. cerebralis* then the likelihood is low that the parasite's lifecycle is established in the waterbody and stocking options can be considered.
- **TAM Release:** *T. tubifex* worms are collected from a waterbody that tested positive for *M. cerebralis*. The worms are assessed in a laboratory setting to determine if the infected worms release the TAM stage of the parasite, which is responsible for infecting the fish host. The release of TAMs by *T. tubifex* worms is a positive indication that the parasite's lifecycle can be established in a waterbody. The introduction of the trout host would complete the lifecycle, therefore the addition of the trout host is not recommended. In this scenario, mitigation options are considered.

3. Consider mitigation options: In ponds where there is a high likelihood that the parasite lifecycle is established, the direction is to consider mitigation options. The following mitigation methods are proposed as research studies with the objective to break the lifecycle of *M. cerebralis*:

- a.** Remove all fish from the waterbody and leave fallow (i.e., fish-free) for three years. This is based on research findings that the myxospore stage is only viable for approximately one year⁸ and the *T. tubifex* worm host has been kept alive in presumably optimal (laboratory) settings for approximately three years¹. The waterbody would be reassessed after three years using one of the above methods to verify that the lifecycle has been broken.
- b.** Drain waterbody, till the sediment and allow the sediment to freeze. In mid-winter, till the sediment a second time and allow to refreeze. This freezing and tilling cycle should eliminate the worm host, thereby breaking the parasite lifecycle. The waterbody would be reassessed to determine if the *T. tubifex* host has been eliminated. Alternately, a drain-till-till cycle may be used during the hottest time of the summer, when air temperatures are greater than 20°C, as exposure to high temperatures and UV light may kill both the worm hosts and the parasite.

- c. Apply an aquatic agent that would eliminate the *T. tubifex* worm host from the waterbody (i.e., Lampricide), thereby breaking the parasite lifecycle. The waterbody would be assessed post-exposure to determine if the *T. tubifex* host has been eliminated.

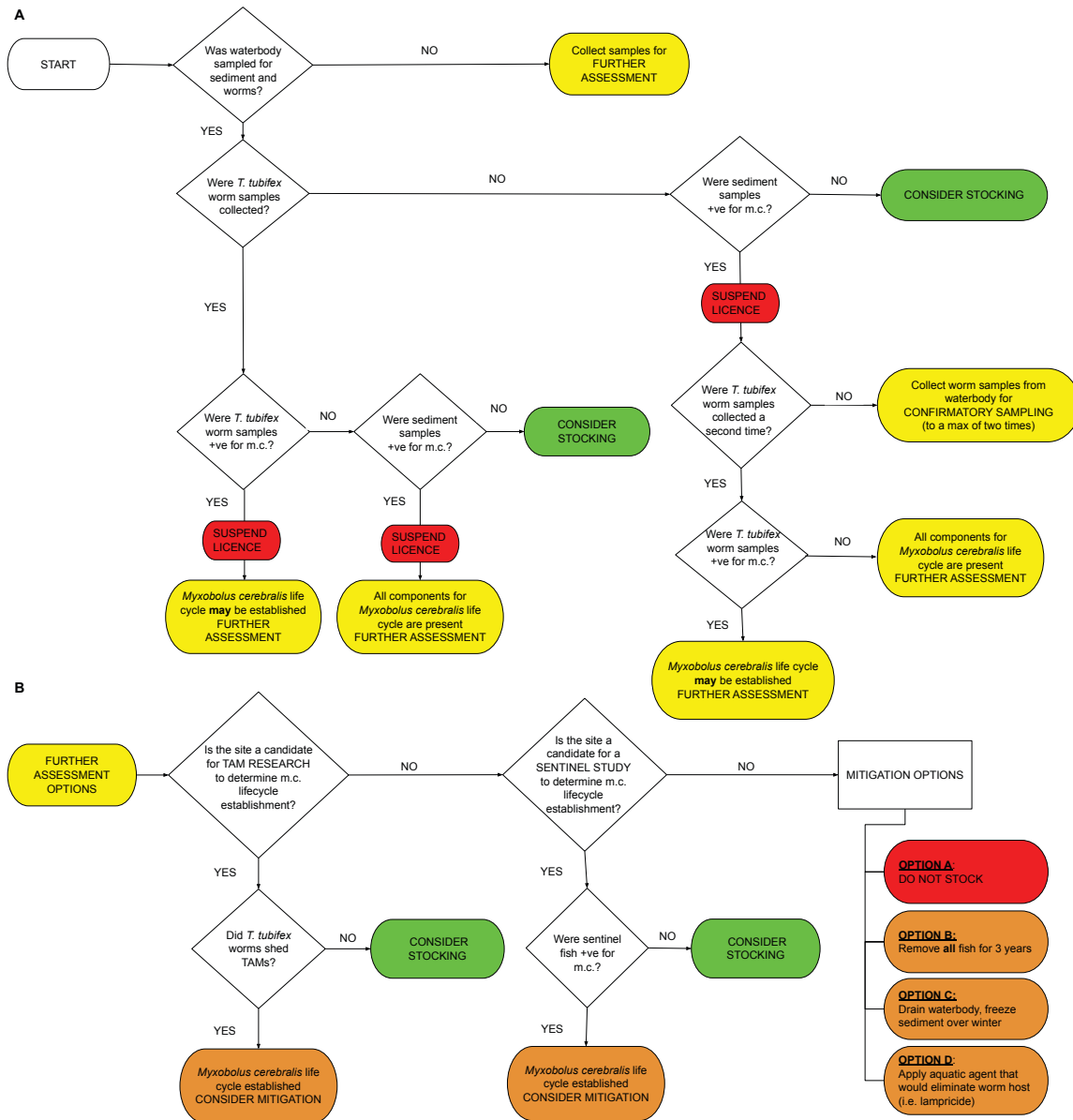


Figure 3: The Decision Framework provides direction for management actions when test results are returned. When reviewing results management actions will be either to consider stocking the waterbody or to conduct further assessment and determine if the lifecycle is established (A). Further assessment options include conducting research on TAM release from worms, conducting a sentinel cage study, or considering mitigation options (B).

Surveillance

A key objective for whirling disease management in Alberta's wild fish populations is to determine the current distribution of *M. cerebralis*. As the distribution of *M. cerebralis* is not likely to be static, ongoing monitoring efforts are essential for effective management. However, this is only one part of the surveillance component of the Whirling Disease Program. Once the general distribution of the parasite has been established in Alberta, the next step is to understand the potential impacts that the parasite may have or is currently having on Alberta's fish populations. Finally, evaluating the risk of further parasite spread, establishment, and population impacts will provide insight into effective mitigation and management strategies to help limit impacts on fish populations throughout Alberta.

The information presented here outlines four projects conducted by the surveillance component, including:

1. Parasite distribution monitoring using fish, worm and sediment samples;
2. An angler survey conducted on the Bow River;
3. Fish population impacts from four watersheds; and
4. A large-scale stream temperature monitoring project to identify important temperature regimes associated with the establishment and outbreak of *M. cerebralis*.

Parasite Distribution Monitoring

Fish Testing Results

Testing for *M. cerebralis* in fish is the standard to confirm the establishment of the parasite in wild populations of salmonids. Using fish testing, *M. cerebralis* has been confirmed in several locations in the Bow River, Oldman River, Red Deer River, and North Saskatchewan River watersheds from 2016-2018 (see the "Overview Maps" section, p.53). These areas support susceptible trout and mountain whitefish that may be at risk of population declines due to whirling disease.

In 2017, extensive sampling for *M. cerebralis* was undertaken in watersheds occupied by trout along the eastern slopes of the Rocky Mountain range. Sampling efforts were focused on filling data gaps from the 2016 emergency response where data was insufficient or did not exist¹⁷. Sites were selected based on the following criteria:

1. Abundance of susceptible non-threatened salmonid species: Rainbow Trout, Cutthroat Trout, Brook Trout, Mountain Whitefish and Brown Trout;
2. High risk areas for whirling disease based on stream gradient and temperature;
3. Location of known parasite vectors such as: high risk stocked ponds, irrigation canals, popular fishing locations or other known vectors;
4. Geographic breaks restricting the risk of whirling disease spread (e.g., barriers to fish movement such as dams and waterfalls); and
5. Accessibility to sites.

Targets for sampling were based upon surveillance criteria developed by the CFIA. A total of 175 fish (40-200 mm in total length) within a connected population (i.e., no fish barriers) were required to ascertain ‘disease freedom’ at the desired diagnostic confidence (corresponds to 95% confidence of detecting the parasite if present at an assumed 2% prevalence in fish). Populations were assessed at the same watershed scale as the provincial Fish Sustainability Index (FSI)¹⁸. A total of 4,199 individual wild fish were sampled for *M. cerebralis* testing in 2017.

Individual fish collected in 2017 from the Peace River, Athabasca River and North Saskatchewan River watersheds, were pooled for *M. cerebralis* testing. The testing utilized both tissue homogenates and pepsin trypsin digests for spore release and concentration; both samples were then tested by molecular methods (qPCR). There were no positive detections for *M. cerebralis* in fish collected from the Athabasca River (849 fish tested) or Peace River watersheds (181 fish tested) (Figure 4). However, new positive detections for *M. cerebralis* were confirmed within the North Saskatchewan River watershed at sites in Clear Creek, Prairie Creek, Mud Creek, Alford Creek, and Swan Creek (Figure 5). These new positive findings prompted a declaration from the CFIA confirming the presence of *M. cerebralis* in the North Saskatchewan River watershed on March 9th, 2018. In the North Saskatchewan River watershed, positive detections of *M. cerebralis* were primarily in Brown Trout and Brook Trout, while fewer Mountain Whitefish were infected.

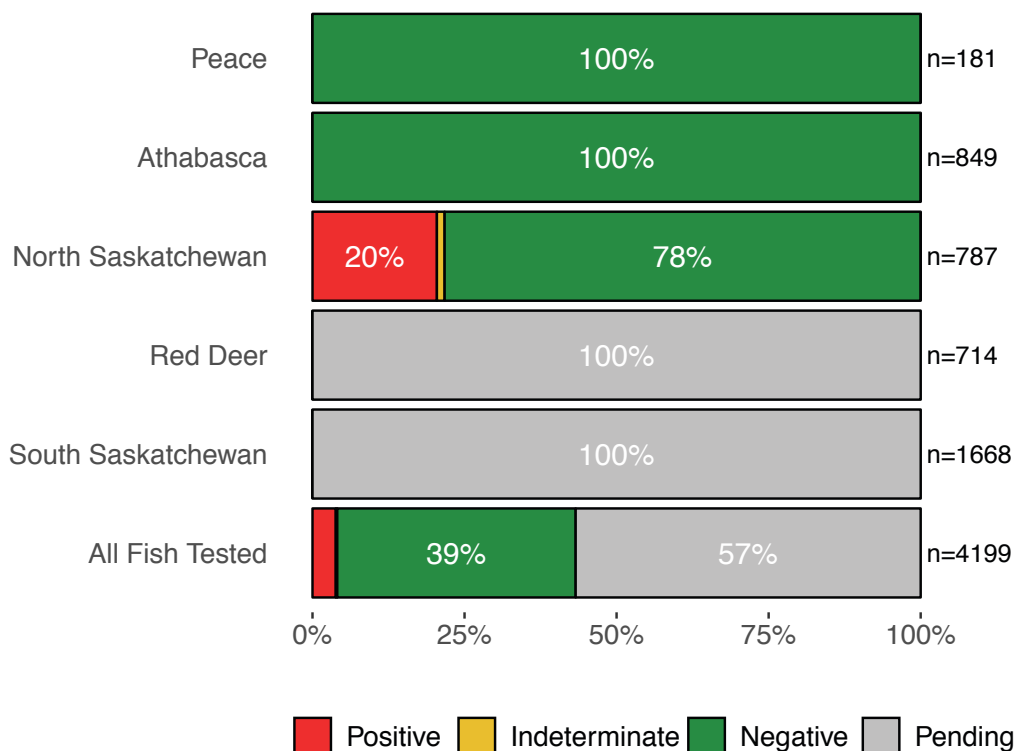


Figure 4: Test results for *M. cerebralis* for each watershed from 2017 fish sampling. Sample size (n) is displayed to the right of each watershed bar. All samples collected from the Peace River and Athabasca River watersheds tested negative for *M. cerebralis*. Up to 20% of fish collected in

the North Saskatchewan River watershed tested positive for the parasite. Results from the Red Deer River and South Saskatchewan River watersheds are pending due to the change in testing from pooled to individual fish samples.

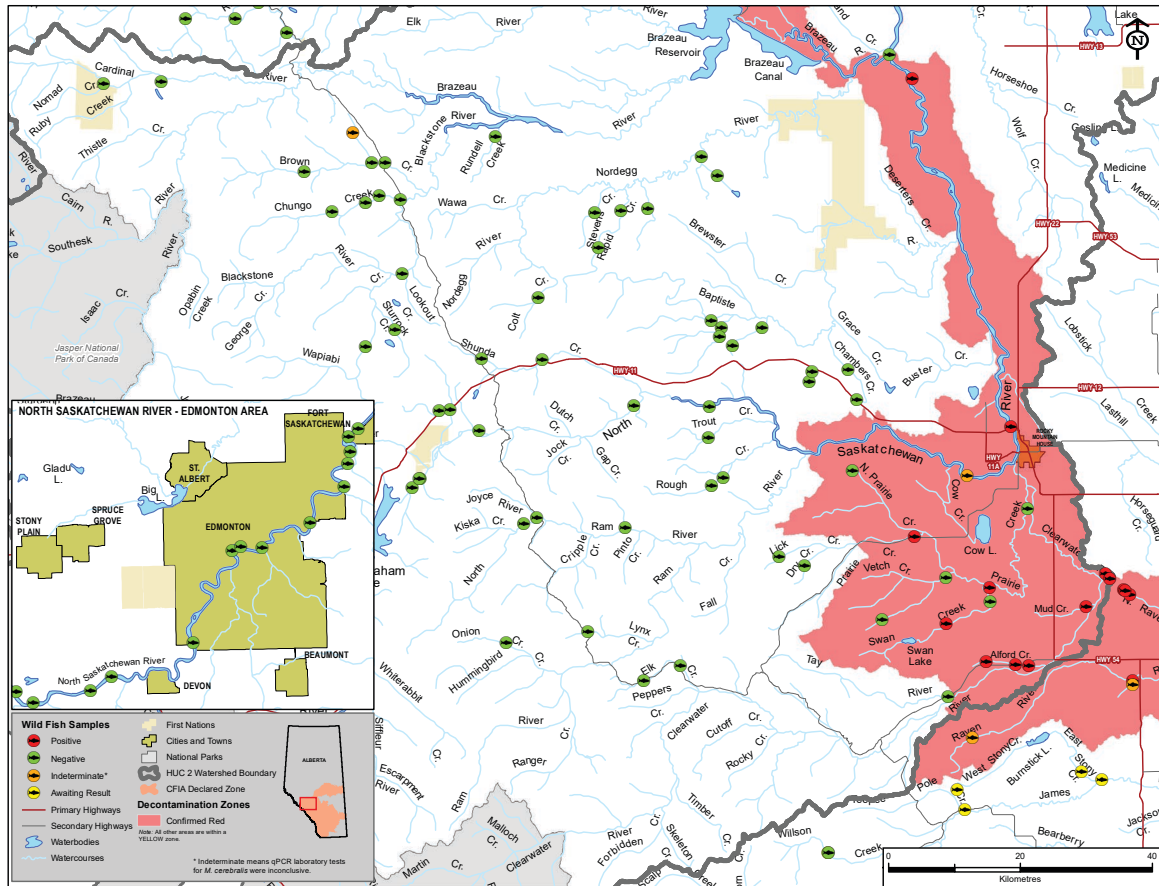


Figure 5: North Saskatchewan River fish sample sites from 2016 and 2017 showing positive (red circles) and negative (green circles) test results.

In the fall of 2018, the Whirling Disease Program shifted from testing pools of fish to testing individual fish to understand changes in parasite prevalence over time within waterbodies. Consequently, results are pending for fish sampled in 2017 in the Red Deer River and South Saskatchewan River watersheds (Figure 4).

Additional fish samples were collected in 2018 if there was reason to suspect whirling disease infection (e.g., visible clinical signs, public concerns, etc.) in waterbodies in which the parasite was not previously detected. Sites where fish were collected in 2018 included Elbow Lake and the Ram River. Additional samples were collected for individual testing to establish prevalence estimates in watersheds of interest including the Lower Crowsnest River watershed and the Lower St. Mary River watershed. Results are pending.



Tubifex tubifex worms collected for *M. cerebralis* testing from the Crowsnest River

Worm and Sediment Testing Results

In an effort to reduce the impacts of lethal fish sampling on Alberta's trout populations, 2017 and 2018 surveillance efforts focused on developing a parasite detection method using worm and sediment samples in partnership with the University of Alberta. The ability to accurately detect the parasite in either the environment or within the second obligate host, *T. tubifex*, can help locate point sources of fish infection in positive watersheds and provide an alternate method to test for parasite establishment in areas in which the parasite has not yet been detected. Using worm and sediment samples for surveillance is both time and cost effective and reduces the harm on fish populations by lessening the amount of lethal sampling required to detect the parasite. However, lethal fish samples will continue to be used as the standard to confirm *M. cerebralis* establishment in a watershed. Due to extensive sampling efforts conducted by AEP, the University of Alberta has successfully validated a more accurate molecular diagnostic technique to detect the presence of *M. cerebralis* found in fish, *T. tubifex* worms, and sediment.

Aquatic worms and sediment samples were collected concurrently at all 2017 sample sites as well as at a selection of sites sampled in 2016 for fish testing (see "Overview Maps" section, p.53). Worm and sediment samples were collected throughout 2018 as part of all surveillance component field work (see "Overview Maps" section, p.53).

In 2017 and 2018, sediment samples were collected from a total of 322 wild sites (Table 2). Only five of these samples tested positive for *M. cerebralis* from sites in the Red Deer River and South Saskatchewan River watersheds. All sediment samples collected in the Peace River, Athabasca River, and North Saskatchewan watersheds in 2017/2018 tested negative for *M. cerebralis*.

Test Result	Peace	Athabasca	North Saskatchewan	Red Deer	South Saskatchewan	Total
Positive	0	0	0	2	3	5
Negative	26	42	66	37	140	311
Indeterminate	0	0	1	2	3	6
Total	26	42	67	41	146	322

Table 2: Sediment sample results by watershed, 2017-2018:

Composite invertebrate samples were collected from over 300 sites and aquatic worms were found in 143 of those samples. In total, 1,231 worms were collected and tested for *M. cerebralis* (Table 3). The number of worms collected at each site varied from one to over 100 worms. Positive *M. cerebralis* detections were found in the Peace River, Athabasca River, and South Saskatchewan River watersheds but not in the North Saskatchewan River and Red Deer River watersheds (Table 3). Approximately 40% of all collected worms were identified as *T. tubifex* using genetic testing (Figure 6). The other 60% of collected worms consisted of over 20 other species (e.g., *Limnodrilus* sp.) (Figure 6). From the nine positive detections of *M. cerebralis* in worms that were collected from the Athabasca River watershed, only five from the Athabasca River main stem were identified to family. Results indicate that four worms were *Limnodrilus* sp. and one was *Haplotaxida* sp. The single positive detection in the Peace River was identified as *Nais bretscheri* and was found in the Simonette River.

Test Result	Peace	Athabasca	North Saskatchewan	Red Deer	South Saskatchewan	Total
Positive	1	9	0	0	19	28
Negative	9	275	102	111	658	1155
Indeterminate	0	25	4	1	18	48
Total	10	308	106	112	695	1231

Table 3: Individual worm sample results by watershed, 2017-2018

Positive detections of worm species in the Athabasca River and Peace River watersheds are in contrast to results of fish testing to date. While there is always the potential for false positives, the improved test developed through the University of Alberta has increased specificity and should not cross react with any known myxozoan species based on current DNA sequences. The Athabasca River and Peace River drainages have had 1,443 and 715 fish tested to date, respectively. Positive worm samples indicate that *M. cerebralis* may be present in these watersheds where it has not previously been detected in fish. However, it cannot be confirmed that the parasite lifecycle has established since none of the positive worms were *T. tubifex* and there have been no positive fish detected. These findings demonstrate the advantage of incorporating worm testing into a surveillance plan to potentially detect the parasite before its lifecycle is established. This reduces the need to sacrifice large numbers of fish as a first step of detecting *M. cerebralis*. In addition, these findings have prompted the Whirling Disease Program

to increase sampling efforts in both the Peace River and Athabasca River watersheds in the 2019 field season to proactively monitor the spatial distribution of the parasite.

Throughout Alberta, 35-56% of individual worms collected from invertebrate samples were *T. tubifex* (Figure 6). However, efforts were biased towards determining the species of individuals that most closely resembled *T. tubifex*, therefore, these numbers may not represent the actual proportion of *T. tubifex* in worm communities. A standardized approach for identifying *T. tubifex* density in the field is required to assess the worm community composition and the risk of parasite perpetuation within any given site. It is important to note that while *T. tubifex* is the only worm species known to complete the lifecycle of *M. cerebralis*, it is not the only worm species to ingest *M. cerebralis* myxospores. Therefore, the parasite detection can be increased by testing all collected worms for the presence of *M. cerebralis*, as indicated by the Peace River and Athabasca River watershed detections.

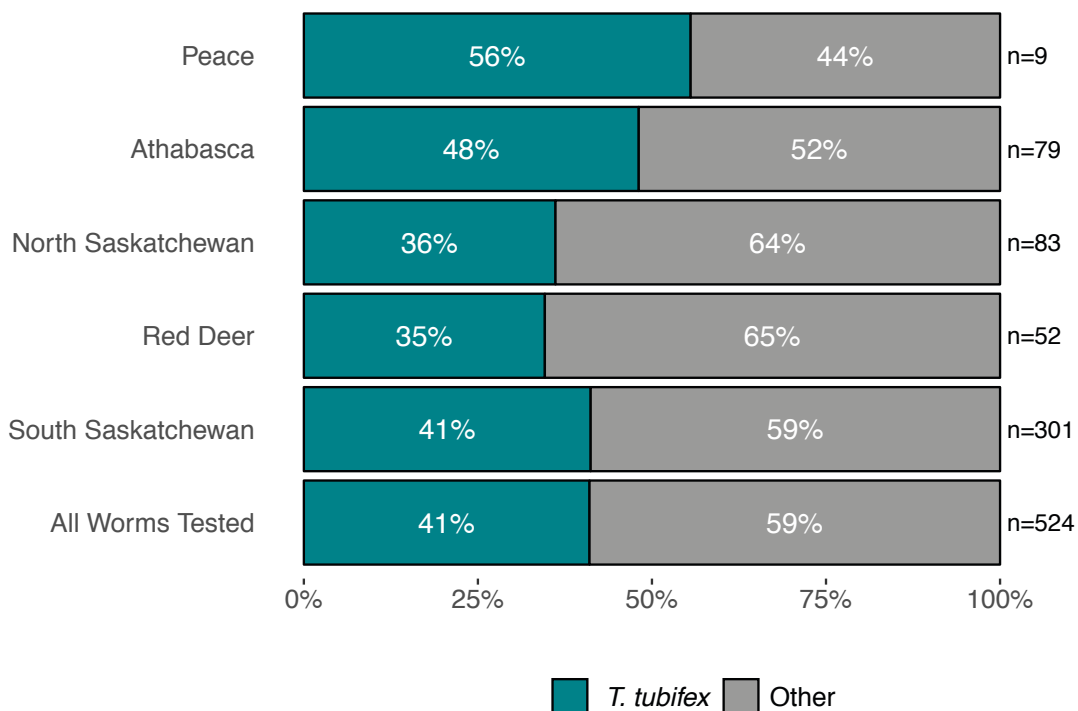


Figure 6: Percentage of collected and barcoded worms that were *T. tubifex* or other species per watershed. Sample size (n) is displayed to the right of each watershed bar.



AEP staff conducting an angler survey on the Bow River in 2018

Bow River Angler Survey

In 2016, the Bow River and several of its headwater tributaries tested positive for *M. cerebralis*. The Bow River is considered a world-class trout fishery, popular among local, national, and international anglers. The lower mainstem of the Bow River runs through the city of Calgary, the most densely populated city in Alberta. Recently, whirling disease was identified as one of several potential stressors responsible for declines in Rainbow Trout populations in the Bow River since 2003¹⁹.

Angler surveys are one of several tools used by fisheries managers to assess angling effort and catch rates of sportfish populations. Given positive detections of *M. cerebralis* in the Bow River and the implication of the parasite as a potential cause for Rainbow Trout population declines¹⁹, the Whirling Disease Program opted to conduct an angler survey in 2018 to investigate potential impacts of whirling disease on susceptible trout and whitefish species in the Bow River (e.g., Mountain Whitefish, Rainbow Trout,

and Brown Trout). To make valid comparisons, the 2018 study was conducted using consistent methods as the Bow River angler survey conducted in 2006²⁰.

The primary objectives of the 2018 survey included:

1. Understanding potential impacts of whirling disease on the Bow River, including the presence of clinical signs of the disease and catch rates of susceptible trout and whitefish species.
2. Assessing recreational users' current knowledge and understanding of whirling disease in Alberta (see the Education section, p.36); and
3. Educating the general public on whirling disease and how to help prevent spreading the parasite.

Due to the large size of the Bow River, the 2018 angler survey study was stratified spatially into four separate reaches (Figure 7) and by time (AM vs. PM, and weekday vs. weekend).

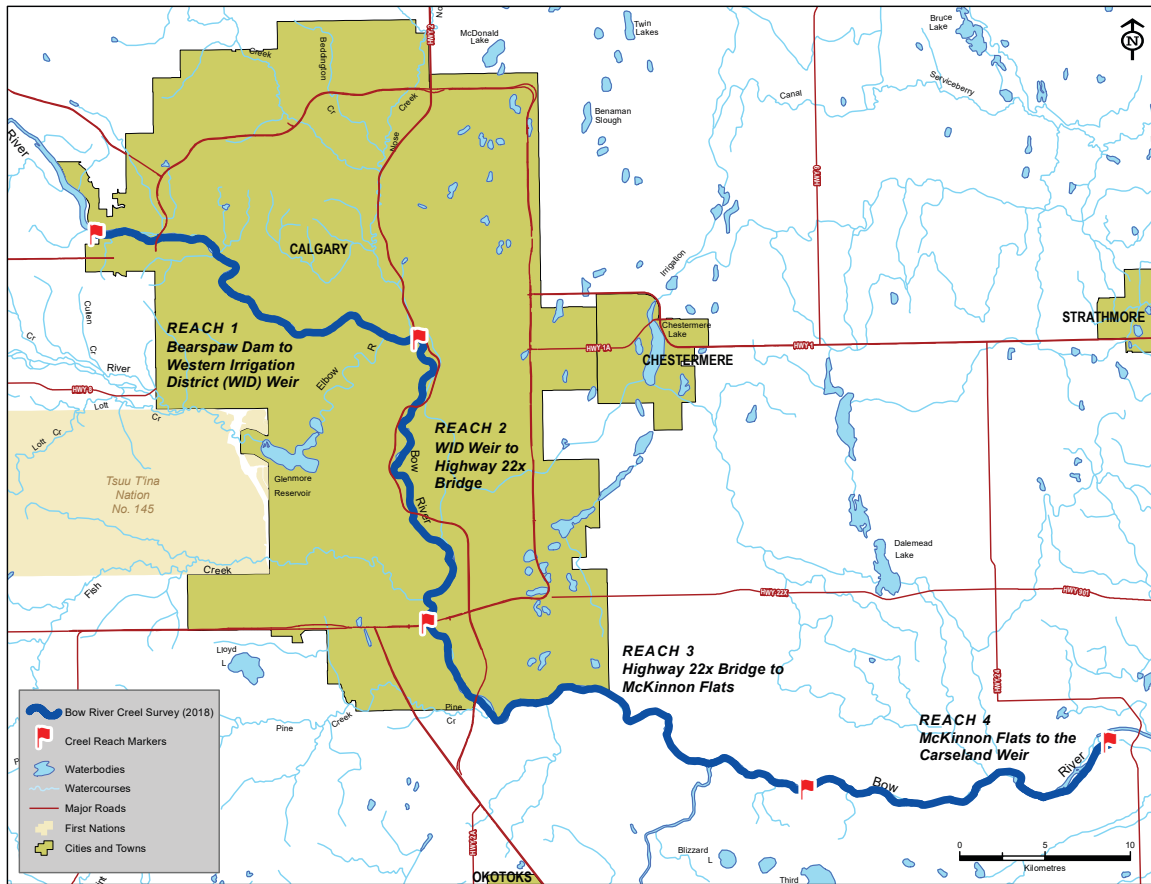


Figure 7: Illustration of the four reaches of the Bow River for the 2018 Angler Survey. Reach 1 was the furthest upstream and spanned from the Bears paw dam to the Western Irrigation District (WID) weir; Reach 2 was from WID weir to Highway 22X bridge; Reach 3 was from the Highway 22X bridge to McKinnon Flats; and Reach 4 was the furthest downstream from McKinnon Flats to the Carseland Weir.

Throughout the duration of the survey period, the Whirling Disease Program did not observe or receive any reports of fish exhibiting clinical signs of whirling disease despite encountering over 5000 anglers from June to November 2018. Angler catch rates for Rainbow Trout and Brown Trout have remained stable since 2006 and no observable clinical signs of whirling disease were reported throughout the survey period or throughout the Bow River population survey. At this point, we cannot confirm that whirling disease has played a significant role in the declines of Rainbow Trout populations observed in the Bow River¹⁹. However, due to the presence of the parasite throughout the watershed and its recreational value to the province, it is important for the Whirling Disease Program to continue monitoring the Bow River and its tributaries for impacts of the whirling disease parasite on fish populations.

Fish Population Impact Assessment

Whirling disease has been implicated in major trout declines in the United States in a relatively short timeframe^{6,21}. Following the original discovery of *M. cerebralis* in 1986 in the Colorado

River, Walker and Nehring⁶ found a complete collapse of Age 1+ and Age 2+ Rainbow Trout surveyed in 1993 and 1994. This collapse was not observable in older age classes⁶. Therefore, to determine if *M. cerebralis* is currently impacting Alberta's fish populations, four watersheds were selected for assessment (Figure 8).

Watersheds were selected using the following criteria:

1. *M. cerebralis* was previously detected in the watershed (with the exception of the Lower St Mary River);
2. Juvenile (age 0+, 1+, and 2+) susceptible trout species of concern (either Rainbow Trout and/or Cutthroat Trout) were present and relatively high in historical abundance;
3. Watersheds could be successfully sampled using backpack electrofishing; and,
4. Waterbodies were popular angling destinations.

Watersheds selected for assessment that previously tested positive for *M. cerebralis* included the Upper Jumpingpound River, the Upper Belly River, and the Lower Crowsnest River (Figure 8). The Lower St. Mary River has not tested positive for the parasite but was selected on the basis that it may serve as a control and baseline to compare with positive sites. Additionally, the Lower St. Mary River is connected through irrigation canals to both the Waterton and Belly Rivers. Both rivers have previously tested positive for the parasite thereby increasing the likelihood of future establishment of *M. cerebralis* in the St. Mary River.

Electrofishing sites were chosen using the Generalized Random Tessellation Stratification (GRTS) methodology²². The GRTS method randomly selects electrofishing sites throughout a watershed of interest using a spatially balanced sampling design²². Target sample sizes within a watershed range from 10 to 15 sites and are based on the ability to detect a 50-90% decline in juvenile fish abundance over 5 years. In some cases, impacts observed from whirling disease in Colorado have resulted in a complete collapse of young-of-the-year (YOY) age classes⁶. If such changes are occurring in Alberta, this change in fish abundance should be detectable using the suggested target sample size of 10 to 15 sites and clinical signs (e.g., black tail, spinal and cranial deformities, whirling behaviour) should be observable in populations experiencing impacts.

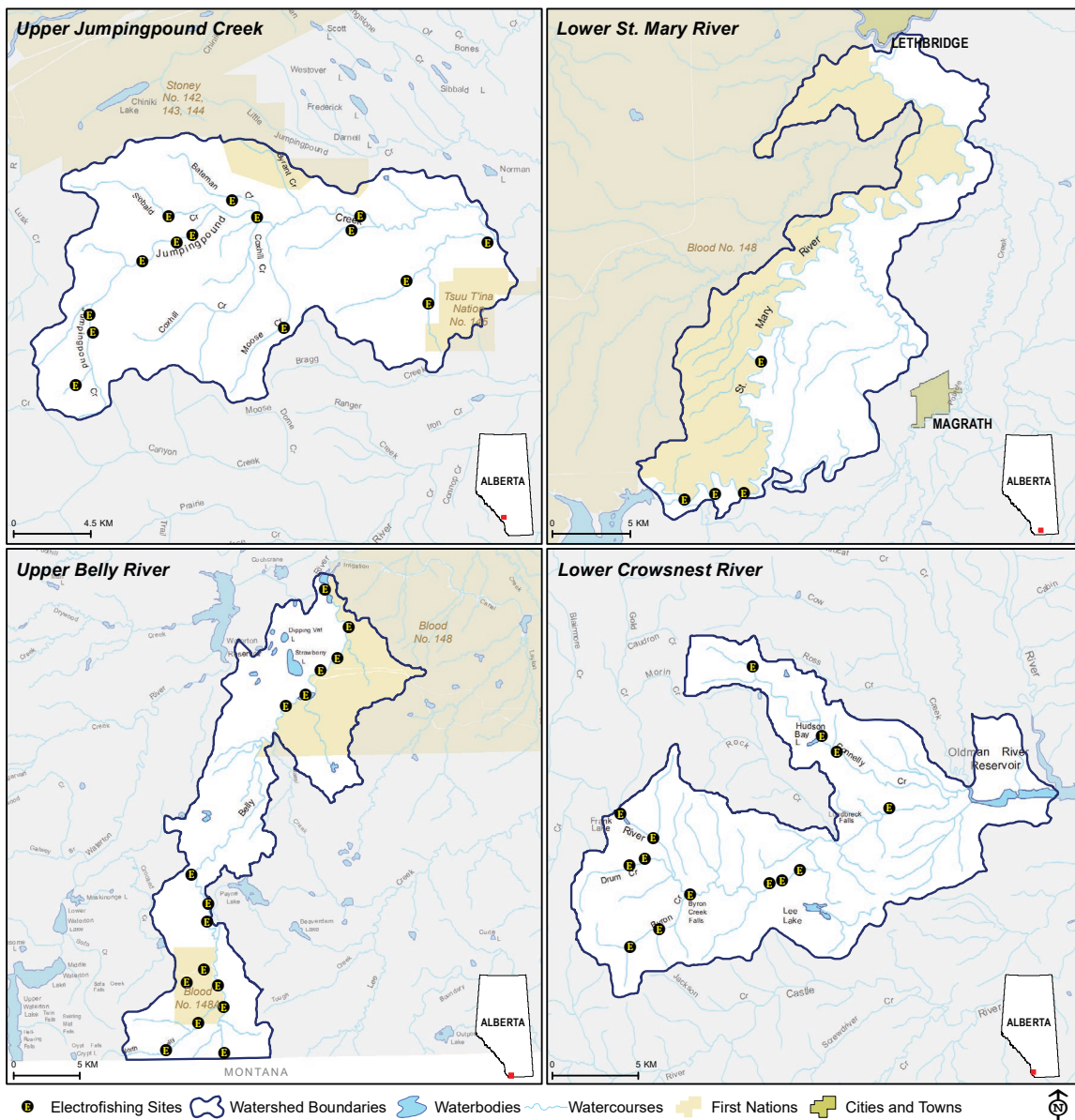


Figure 8: Electrofishing sites in four watersheds sampled in 2018. Upper Jumpingpound River watershed, Upper Belly River watershed and Lower Crowsnest River watershed have all tested positive for the whirling disease parasite, *M. cerebralis*. The whirling disease parasite has not been detected in the Lower St. Mary River watershed, however it is connected to two positive waterbodies through irrigation canals.

Summary of Electrofishing Findings

This is the first year that AEP has examined the potential impacts of whirling disease on native or naturalized trout populations. In addition, this is not considered a complete population assessment of each watershed as the use of backpack electrofishing units limits our capacity to collect and assess larger size classes. Therefore, a more comprehensive watershed approach may be required to draw conclusions about the present population status and long-term effects. However, in this first year of sampling we were able to identify the following results:

Lower St. Mary River

Sites on the Lower St. Mary River watershed were sampled on August 21 and September 26, 2018. The Lower St. Mary River watershed is a tailwater fishery flowing out of the St. Mary Reservoir and in 2018 it was determined that the GRTS method was not an effective design for monitoring Rainbow Trout in the St. Mary River watershed. As a result, only four sites were sampled on the main stem downstream of the St. Mary Reservoir (Figure 8). Future sampling efforts on the Lower St. Mary River will require a revised sampling area to reflect where juvenile Rainbow Trout are located within the watershed. Despite these limitations, 20 juvenile Rainbow Trout were captured and retained for testing for the presence of *M. cerebralis*. Results are pending.

Upper Jumpingpound Creek

The Upper Jumpingpound Creek watershed was sampled from August 14 to September 25, 2018. Catch rates on the Upper Jumpingpound Creek indicated that there was healthy recruitment of Cutthroat Trout and Brook Trout as a large portion of fish had made it past the most vulnerable size class of infection and successfully recruited into older, less vulnerable size classes²³. Strong cohorts of multiple age classes were present in both populations. Conversely, Rainbow Trout showed a strong pulse of YOY but were lacking abundant fish 1-year or older. Despite the lack of older size classes of Rainbow Trout in Jumpingpound Creek, no clinical signs of whirling disease were observed throughout the survey and all size classes of Cutthroat Trout and Brook Trout were present. Both species are known to be susceptible to whirling disease. As such, whirling disease may not have had obvious population level impacts in the watershed at this point, but continued monitoring is required.

Upper Belly River

The Upper Belly River watershed was sampled from August 22 to September 12, 2018. Rainbow Trout catch rates in the Upper Belly River watershed were low compared to the other watersheds sampled. The presence of both YOY and a cohort of fish likely age 1+ or 2+ (61% of total catch) indicate that fish are surviving past the vulnerable size of infection. Moreover, Brook Trout appear to have a healthy size class distribution including YOY's and a strong cohort of older fish. Bull Trout are considered relatively non-susceptible to the parasite²⁴ and appear to have strong recruitment and survival beyond fish a year or older. A lack of clinical signs in either Rainbow Trout or Brook Trout combined with good YOY survival, indicate that at this point, whirling disease has not likely had a negative population level impact in the Upper Belly River watershed.

Lower Crowsnest River

The Lower Crowsnest River watershed was sampled from August 23 to August 30, 2018. Rainbow Trout catch rates in the Lower Crowsnest River watershed were the highest of any species in this study. However, YOY comprised 96% of the total catch of Rainbow Trout, while older size classes were largely lacking. Only 4% of Rainbow Trout were captured in the less vulnerable size class of fish (age 1+ and 2+) indicating YOYs may not be surviving into older age classes (Figure 9). A lack of Rainbow Trout captured that were at least a year old is concerning considering the high abundance of YOYs captured during the survey. This pattern resembles what Walker and Nehring⁶ found in the Colorado River in 1993 and 1994. Given the larger size of the river, backpack electrofishing may have introduced some sampling bias due to a lower catchability of larger fish. However, historical data, accessed from the Government of Alberta's Fish and Wildlife Management Information System (FWMIS), on backpack electrofishing surveys conducted in August 2000, indicate that Rainbow Trout were most abundant between 90 and

120 mm, a size class that was largely lacking in the 2018 survey (Figure 9). Sites sampled in 2000 were located on the mainstem of the Crowsnest River immediately upstream of the sites sampled in 2018 and habitat conditions were similar between sites (Figure 10).

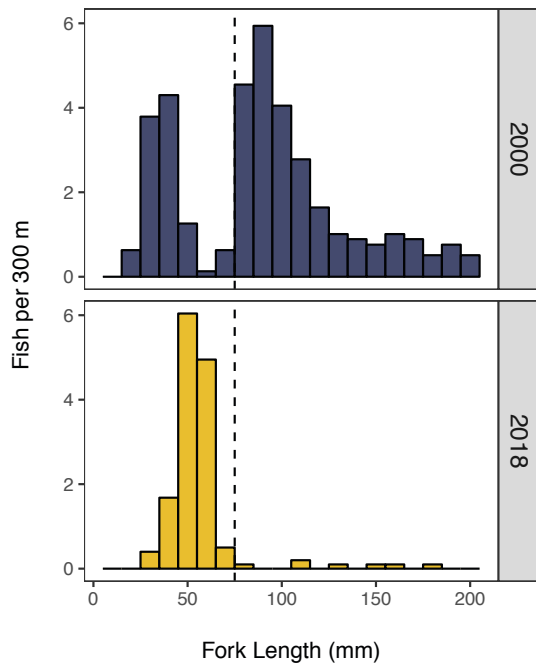


Figure 9: Length-frequency plots of juvenile Rainbow Trout captured by backpack electrofishing in the month of August from 2000 and 2018 in the Crowsnest River watershed.

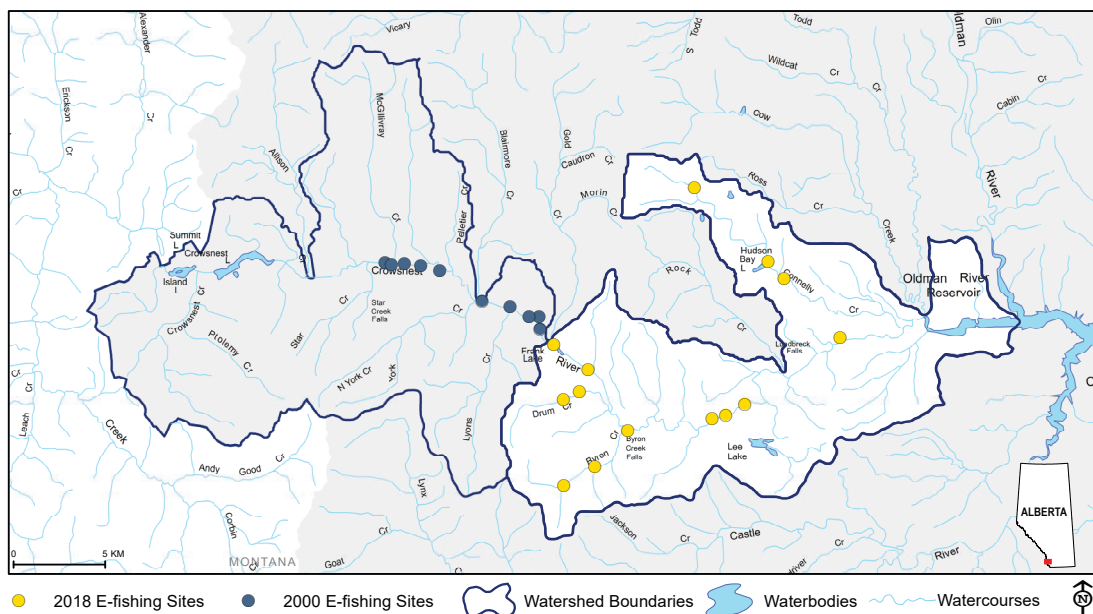


Figure 10: Electrofishing sites from 2000 and 2018 sampling on the Crowsnest River.

Of particular concern is the observation of several fish exhibiting clinical signs of whirling disease in the Lower Crowsnest River. Impacted fish predominantly had black tail and shortened opercula (Figure 11 a & b), and at least one individual had a severely slumping forehead (Figure 11 b & c). Field crews noted that one fish was whirling in a sampling bucket and an angler reported an unidentified fish exhibiting whirling behaviour just below Lundbreck Falls on September 3, 2018, close to our sample sites. These classic signs of whirling disease were primarily observed in YOY Rainbow Trout but were occasionally seen in Mountain Whitefish. Two Rainbow Trout exhibiting clinical signs were retained for diagnostic testing to confirm infection with *M. cerebralis*; both individuals tested positive for the parasite. Field crews additionally captured and retained 21 Rainbow Trout from two sampling sites to determine parasite prevalence in individual fish within the watershed. Results are pending.



Figure 11: Clinical signs of whirling disease found in the Lower Crowsnest River watershed during 2018 electrofishing surveys. Photo A illustrates Rainbow Trout displaying blackened tail and a shortened operculum (red arrows); Photo B illustrates Rainbow Trout displaying blackened tail, a shortened operculum, and cranial deformities as seen by the slumped forehead (red arrows); and Photo C illustrates Mountain Whitefish displaying skeletal deformities in the caudal region (red arrow).

Our findings in 2018, in combination with previous test results, indicate that the parasite is highly prevalent in the Crowsnest River. In 2016, 100% (29/29) of fish pools consisting of 207 fish tested positive for the parasite, in addition to 14% of individual worms and 17% of sediment samples collected in 2017 (Figure 12). Worms collected from the Crowsnest River in 2018 by the University of Alberta were confirmed to be shedding the actinospore stage of the parasite (TAMs) that is infectious to fish. Fish exhibiting clinical signs are likely heavily infected with the parasite and as a result their survival can be severely impacted. An additional study examining all age classes of Rainbow Trout on the Crowsnest River using a float electrofishing unit would provide more detail on the severity of whirling disease to date. However, it is clear that whirling disease is having an impact in the Lower Crowsnest River watershed.

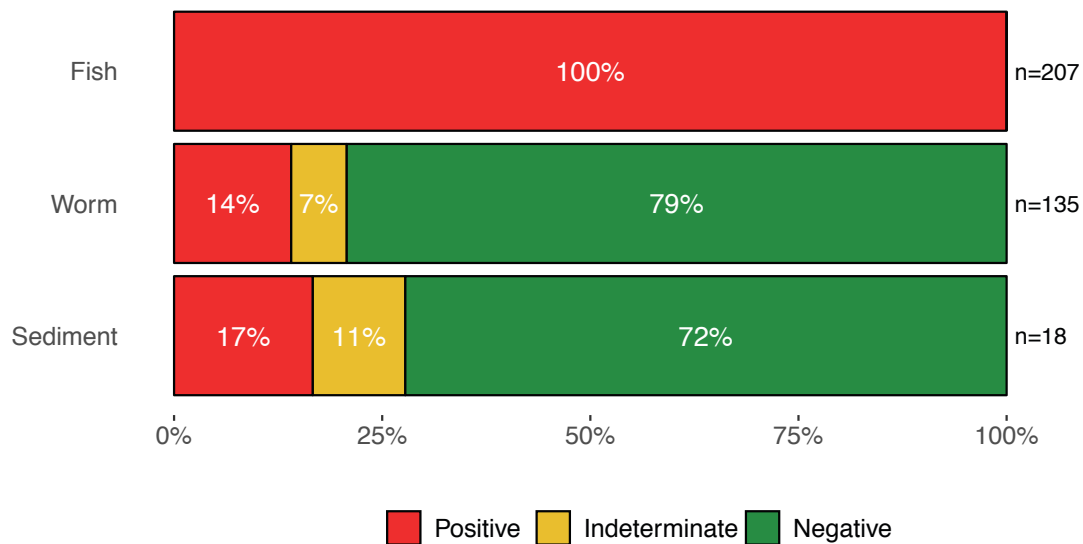


Figure 12: Test results from the Crowsnest River in pooled fish samples, individual worms and sediment samples collected from 2016 to 2018. Sample size (n) is displayed to the right of each bar. A total of 207 individual fish were divided into 29 pools and tested as a composite sample. All 29 pools tested positive for *M. cerebralis*, however, these results do not indicate that each individual fish was infected.

Stream Temperature Monitoring

Stream temperature is a critical factor associated with *M. cerebralis* development⁴, timing of release of the actinospore stage of parasite (TAM) into the environment from the *T. tubifex* worm host²⁶, and elevated mortality of infected fish²⁶. Calculations of stream-based growing degree days and optimal parasite release windows can be used to determine the associated risk of infection on susceptible salmonid populations. In 2018, the Whirling Disease Program initiated a large-scale stream temperature monitoring project to assess water temperature conditions associated with the presence of the parasite in Alberta. Temperature loggers were installed throughout the eastern slopes region of Alberta, ranging from the Athabasca River watershed to the South Saskatchewan River watershed in 2018. The Whirling Disease Program partnered with Trout Unlimited Canada, Canadian Conservation Corps (in association with Alberta Parks) and the Paul Band First Nations to install loggers throughout the study area. AEP provided training sessions, standard operating procedures, and equipment kits to support stream temperature logger deployments within partner groups. In 2018, the Whirling Disease Program and partners deployed 311 loggers throughout the eastern slopes of Alberta (see the "Overview Maps" section, p.53) using marine epoxy and rebar installation methods (Figure 13). Loggers were configured to record temperature every thirty minutes. Of these loggers, 275 were left instream to record temperature data year-round.



Figure 13: Stream temperature logger hardware and installation methods used by the Whirling Disease Program in 2018. HOBO MX2203 TidbiT temperature loggers were secured inside white PVC housings; stickers with logos and contact information were placed on the PVC housing (A). Loggers were installed using rebar (B) or marine epoxy (C-E). To install loggers using marine epoxy, a ring of epoxy was moulded to the back of the PVC housing (C), and installed on the downstream side of a large boulder using a large rock to prop against the housing until the epoxy set (D&E).

This project generated extensive amounts of data, which highlighted the need to create a centralized provincial database to store and share stream temperature logger data. Without a provincial database in place, stream temperature data has been stored locally by many agencies in the province, which limits the ability to share and utilize existing data. In 2018, the Whirling Disease Program initiated a multi-agency provincial stream temperature working group to connect with organizations that currently or historically collect stream temperature data in the province. This group consists of federal and provincial governments, non-profit organizations, universities, and industry. Together, the group has been instrumental in designing requirements for data input into the provincial database as well as sharing expertise in study design, installation methods, data cleaning, and data analysis. Collectively, known agencies in Alberta,

including the Whirling Disease Program, installed approximately 600 stream temperature loggers throughout the province in the summer of 2018, with over 300 loggers remaining instream throughout the winter (Figure 14).

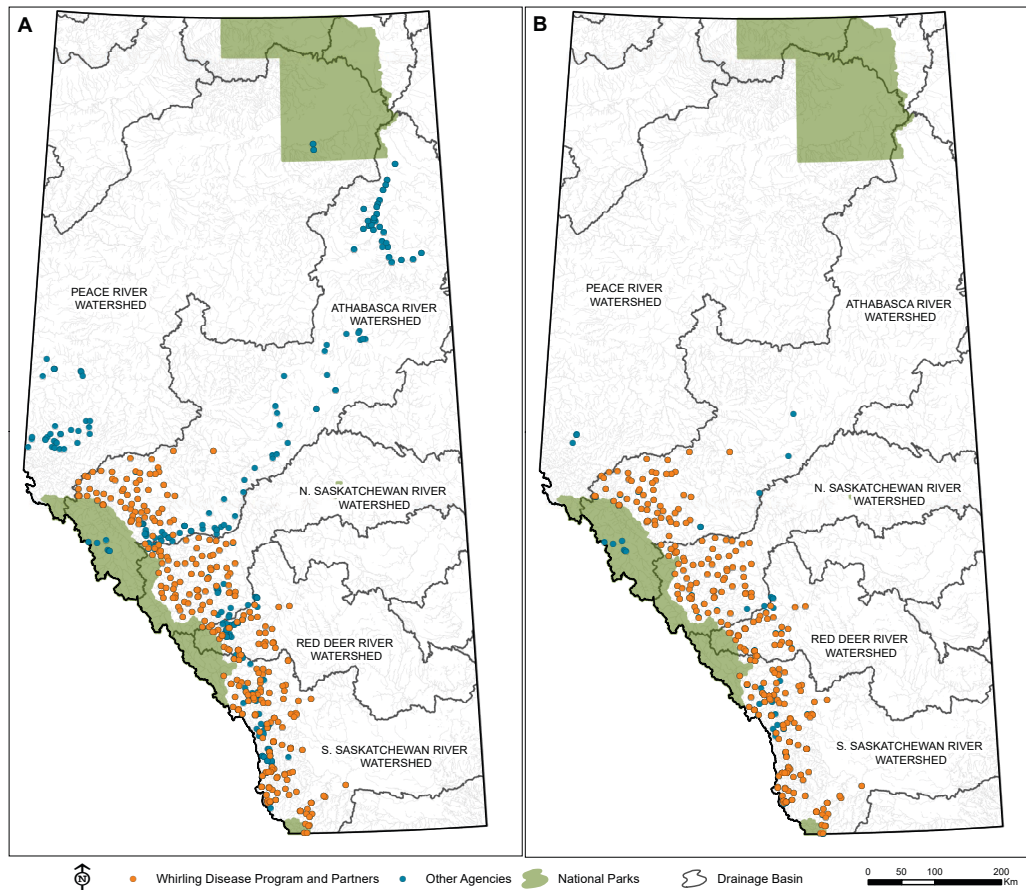


Figure 14: Location of stream temperature loggers installed in 2018 by the Whirling Disease Program and partners (orange dots) and other agencies in the province (blue dots). A total of 591 loggers were installed throughout five major river watersheds, the Peace River, Athabasca River, North Saskatchewan River, Red Deer River, and South Saskatchewan River watersheds, of which the Whirling Disease Program and partners installed 311 loggers (A); a total of 338 loggers were left instream throughout the winter to collect year-round data, of which the Whirling Disease Program and partners contributed 275 loggers (B).

All available stream temperature data within the eastern slopes of Alberta will be analyzed using spatial stream network models²⁷, which are used to model stream temperatures as part of the NorWeST project in the western United States (<https://www.fs.fed.us/rm/boise/AWAE/projects/NorWeST.html>). These models will be used to predict stream temperatures along watercourses throughout the eastern slopes and predict how disease risk may change over time. While this project is an initiative of the Whirling Disease Program, this data encompasses and supports multiple fisheries conservation programs and initiatives.

Surveillance Component Plans – 2019

Sampling conducted in 2017/18 provided valuable information to further understand the distribution of the whirling disease parasite in Alberta. Projects have been implemented to begin assessing the risk of spread of the parasite and the risk of potential impacts to susceptible fish populations. Therefore, the focus for surveillance in 2019 will be to:

1. Continue building a widespread and robust spatial stream temperature network with multiple collaborative agencies;
2. Develop a more comprehensive watershed assessment protocol tailored specifically to whirling disease sampling but following Provincial Standards; and,
3. Continue ongoing surveillance in areas with a high risk of spread or in areas with reported observations of whirling disease throughout the province.

To achieve the first objective, the Whirling Disease Program will access a subset of temperature loggers in the spring of 2019 to assess overwintering success of the two installation methods. In the fall, data from all loggers installed in 2018 will be downloaded prior to freeze up. Throughout the season, the Whirling Disease Program will continue to provide training sessions to external agencies such as Trout Unlimited Canada chapters and the Paul Band First Nation.

To achieve the second objective, the broader Crowsnest River (HUC 8) will be assessed in partnership with regional biologists using a combination of float and backpack electrofishing to determine the overall status of the fish population in 2019. As the Upper Belly River and Upper Jumpingpound Creek watersheds are of lower concern for population-level impacts associated with whirling disease, sampling will be shifted to a biannual schedule with both watersheds being resampled again in 2020. This will free up resources to sample the upper portion of the Crowsnest River. In addition, a sentinel cage program is being considered on the Crowsnest River to determine ‘hot spots’ for parasite release and the rate of mortality occurring in Rainbow Trout populations.

In addition, supplemental parasite sampling will be conducted in a selection of positive and negative watersheds to provide additional information on the current and future risk of whirling disease. Sampling procedures will include water filtrations to determine TAM abundance; *T. tubifex* collections to determine density, infection prevalence and overall worm community structure; and lethal fish samples to determine parasite prevalence and myxospore burden in fish. Some of these parasite-specific sampling methods may also be used to supplement standardized watershed assessments conducted by regional biologists.

To address the third objective, ongoing testing will be conducted on sediment, worms, and fish throughout the province as needed and where opportunity exists between overlapping programs and initiatives. Specifically, more emphasis will be placed on the Athabasca and Peace River drainages due to the recent *M. cerebralis* findings in worm species in both watersheds.

Laboratory Diagnostics

The Whirling Disease Laboratory is located in Vegreville, Alberta to support the Whirling Disease Program by efficiently and accurately processing fish for whirling disease testing (Figure 15).

Fish samples submitted to the Whirling Disease Laboratory are processed on site and sent to the Molecular Biology Service Unit (MBSU) at the University of Alberta for parasite detection using qPCR. Samples are processed using either homogenization, pepsin-trypsin digestion or a combination of both methods. To date the laboratory has processed over 15,000 wild and cultured fish samples. In 2018, laboratory standard operating procedures were reviewed and updated to increase the resolution of testing results for wild fish. Wild fish are now tested individually rather than in pools in order to determine parasite prevalence. This increased resolution will provide better understanding of the effects of whirling disease on wild fish populations and improve AEP's monitoring and mitigation efforts. Additional techniques including measurement of myxospore burden are currently in development in order to evaluate the severity of infection in wild fish.



Figure 15: Processing fish samples in Vegreville, Alberta. Photo Credit: Gateway Gazette

The Government of Alberta has committed \$1.35 million to build a molecular laboratory in Vegreville, to expand laboratory capacity to include in-house molecular (qPCR) testing. This capital upgrade will expedite disease testing and allow the Whirling Disease Laboratory to provide testing services to other jurisdictions. The laboratory continues to work towards obtaining accreditation under the requirements of International Organization for Standardization (ISO) 17025 and position itself to act as external laboratory for the National Aquatic Animal Health Program (NAAHP). Accreditation will confirm the Whirling Disease Laboratory's capability to provide reliable and consistent results.

In February 2018, the laboratory was assessed by the CFIA and verified to be in compliance with the physical and operational requirements of a Level 2 Aquatic Laboratory (AQC2) in accordance with the CFIA's Containment Standards for Facilities Handling Aquatic Animal Pathogens.

Decontamination

Government of Alberta Decontamination Protocol for Watercraft & Equipment

In August 2017, the Decontamination Protocol became mandatory for the Government of Alberta and partners when completing work in or near provincial waterbodies. The protocol defines three levels of decontamination based on the risk of spreading whirling disease from the work location. In areas of lowest risk (i.e., White Zone) a Level 1 decontamination: Clean, Drain, Dry is required. In high risk areas where whirling disease has not been detected (i.e., Yellow Zone), a Level 2 decontamination: Disinfection treatment is required. In areas in which whirling disease has been detected (i.e., Red Zone), a Level 3 decontamination: Temperature treatment is required. The risk level can be found by referencing the Decontamination Risk Zone Map (Figure 16).

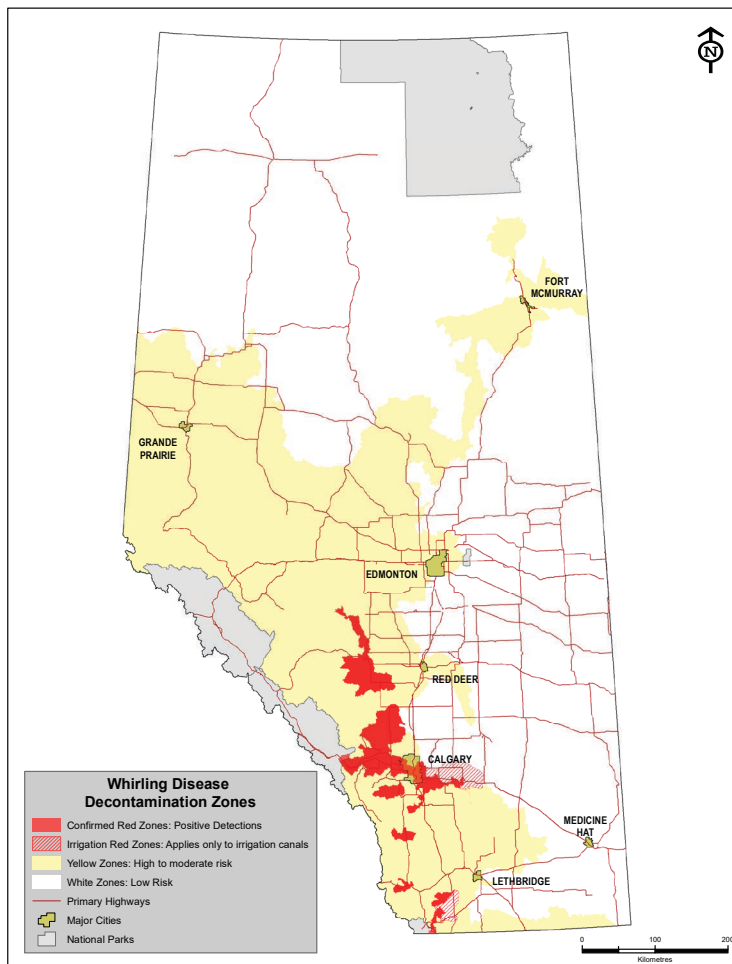


Figure 16: Decontamination Risk Zone Map that categorizes the province into three zones of whirling disease risk. These zones are 1) White Zone: low risk of whirling disease infection, 2) Yellow Zone: high risk of whirling disease infection, but the parasite has not been detected and 3) Red Zone: areas with confirmed detection of *M. cerebralis*, the whirling disease parasite.

Decontamination Risk Zone Map

The Decontamination Risk Zone Map (Figure 16) categorizes the province into the Risk Zones and must be referenced prior to completing work in the field. This map is subject to change depending on whirling disease monitoring results and detections. The current protocol and Decontamination Risk Zone Map can be found at the Stop the Spread website: <https://www.alberta.ca/stop-whirling-disease.aspx>. Decontamination risk zones are available for download as a shapefile, or as a KMZ file compatible with Google Earth. The decontamination boundaries are also in GeoDiscover Alberta—an online searchable catalog of open government data.

Decontamination Risk Zones – Updates

The following updates have been made to the Decontamination Risk Zone Map:

- Nine regions that were previously designated as suspect positive zones were updated to confirmed positive zones.
- Eight regions that were previously in yellow decontamination zones were updated to new confirmed red zones based on the discovery of positive wild fish test results from the 2017 field season.
- All red zones were standardized to a Hydrologic Unit Code 10 (HUC 10) scale. HUC watersheds define nested hydrologic units, or watersheds, that consist of successively smaller watersheds ranging from a coarse scale (HUC 2) to a fine scale (HUC 12). Three previously designated red zones that were defined at a HUC 12 watershed level, were expanded to a red zone at the HUC 10 level to be consistent with the delineation of other red zones.
- In 2019, the main stem of the North Saskatchewan River changed from a suspect red zone to a yellow zone, once all fish downstream of the Brazeau Dam tested negative for *M. cerebralis*.
- Yellow zones were constricted to the known region of susceptible salmonid species in Alberta. The susceptible salmonid region was determined by historical catch data from FWMIS, as well as input from regional fisheries biologists on presence and absence of susceptible salmonid species.

Decontamination Protocol – Policy Updates

In 2018, decontamination technicians provided education, training and resources to multiple ministries throughout the province including Environment and Parks, Agriculture and Forestry, Justice and Solicitor General and Transportation. Additionally, technicians worked with external partners to provide guidance and decontamination resources for voluntary adherence to the Decontamination Protocol. AEP continues to work with the federal government ministries, such as the Department of National Defence and Parks Canada, to incorporate the Decontamination Protocol into their operations within Alberta.

AEP worked closely with stakeholders to develop adherence requirements for the protocol. Table 4 summarizes the completed and ongoing work.

Stakeholder	Information and Timelines
Research Licences	In July 2018, adherence to the protocol became a Best Practice for all Fish Research Licence holders. As of April 1, 2019, the protocol became a mandatory Condition of Licence
Alberta Transportation Contractors	Adherence to the protocol will become a Special Provision for contractors working in or near water and found within the Design Bulletins. <i>Tentative- Fall 2019</i>
Public Lands Act Approvals	Adherence to the protocol will become a Condition of Approvals for all work being completed within the bed and banks of a waterbody. <i>Tentative – Fall 2019</i>
Water Act Approvals	Adherence to the protocol will become a Condition of Approvals for all work being completed within the bed and banks of a waterbody.
Alberta Energy Regulator	The Alberta Energy Regulator is working with Environment and Parks to align the Conditions for Licences and Approvals. <i>In Progress</i>

Table 4: Summary of completed and ongoing adherence requirements for the Decontamination Protocol

In early 2018, Decontamination Instructions for Industrial and Construction Operations were drafted by a multi-stakeholder task team with representatives from AEP, Transportation and external stakeholders representing the Construction and Environmental Consulting industries.

In November 2018, the drafted Decontamination Instructions for Industrial and Construction Operations were reviewed by a variety of external stakeholders and industrial associations. The review was completed through information sessions and TalkAEP online surveys hosted by AEP. More than 40 representatives from various associations and companies attended the information sessions. Over 247 representatives visited the TalkAEP site and 38 provided feedback using the online survey or directly through email. The results from the survey questions indicate:

- Respondents understand the importance of completing the Decontamination Protocol to prevent the spread of whirling disease and aquatic invasive species;
- many operations do not currently have a cleaning protocol established to prevent the spread; and,
- incorporation of feedback into the proposed Decontamination Instructions for Industrial and Construction Operations is necessary to make the Decontamination Protocol easier to implement.

Following the feedback and information for stakeholders the Decontamination Instructions for Industrial and Construction Operations has been revised to limit the decontamination requirements for these users to Level 1 decontamination: Clean, Drain, Dry. A thorough and detailed Clean, Drain, Dry process on all equipment, vehicles and machinery, before leaving work site will assist in ensuring that the vectors for spreading whirling disease and aquatic

invasive species are left on-site where they were collected and not spread between waterbodies. An updated version of the protocol, which will include the Decontamination Instructions for Industrial and Construction Operations as well as other considerations around winter and emergency operations, will be available in 2019.

Decontamination Services

Decontamination Technicians

To support implementation of the protocol, the Whirling Disease Program hired dedicated decontamination technicians that are available to provide decontamination services. In 2018, decontamination technicians were based out of five locations throughout the province and spent over 230 hours completing over 120 decontaminations from May through November (Figure 17). Decontaminations were completed on a large variety of equipment, vehicles and machinery including but not limited to: watercraft, heavy equipment, fish stocking vehicles, scientific sampling gear, forestry/wildfire equipment and OHVs. On average, a Level 3 decontamination performed by a Decontamination Technician took approximately 3 hours to complete.

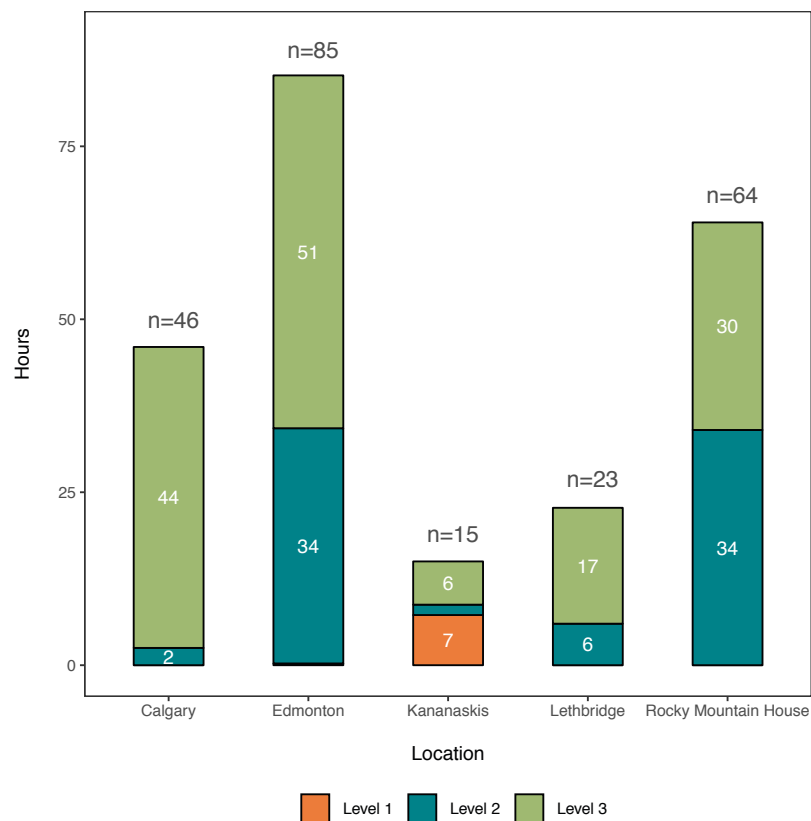


Figure 17: Total number of hours spent providing decontamination assistance to the Government of Alberta by the decontamination technicians in 2018. Data are broken down by the five locations where mobile decontamination units and technicians were stationed throughout the summer. Total number of hours for each location is displayed at the top of each bar.

Decontamination technicians also provided resources and assistance to external contractors completing Government of Alberta construction projects as well as other external clients that work closely with the Government of Alberta such as the Alberta Conservation Association, Environment Canada, the University of Alberta, the Alberta Biodiversity Monitoring Institute and Trout Unlimited Canada.

Decontamination technicians are available to provide decontamination services, training sessions and education on the protocol to Government of Alberta groups as well as information sessions to public or recreational interest groups. See the “For More Information” section (p.61) for contact information.

Mobile Decontamination Units

To support decontamination efforts throughout Alberta, the Whirling Disease Program purchased five mobile decontamination units in 2017. The decontamination units are equipped with a high-pressure wash system, which includes steam that can reach $> 90^{\circ}\text{C}$ and decontamination supplies to thoroughly clean, disinfect and dry equipment (Figure 18). The use of mobile decontamination units is currently limited to the Government of Alberta and contractors to assess capacity and resource use. However, decontamination requests can be sent to AEP.DECON@gov.ab.ca.



Figure 18: A mobile decontamination unit equipped with a high-pressure wash system, and decontamination supplies to clean, disinfect and dry equipment.

Permanent Decontamination Stations

The Whirling Disease Program is in the process of establishing two permanent stations which will provide decontamination services for Government of Alberta equipment used in or around water. The decontamination stations will be located in Edmonton and Lethbridge and will be operated by decontamination technicians. In 2019, decontamination services will be available out of the two permanent stations. In the first season of operation, the use of the permanent stations is limited to the Government of Alberta and contractors to assess capacity and use.

Permanent decontamination stations will be located at:

- Lethbridge: #3-109 Broxburn Boulevard: Opening date: June 01, 2019
- Edmonton: 3703 56 Avenue: Opening date: TBD

More information on these permanent stations will be posted on the Stop the Spread website as it becomes available (see For More Information for the website link, p.61).

Education

Education plays a key role in preventing the spread of whirling disease in Alberta. Initially, educational efforts focused on raising awareness of the presence of whirling disease in Alberta. In the last year, education and outreach has shifted to promote a deeper understanding of the impacts of whirling disease and the actions that help prevent the spread of the disease. The ultimate goal is that Albertans will not only undertake AEP's recommended actions to prevent the spread, but that they will also report observations of clinical signs which might be due to whirling disease, help to raise awareness of the issue and encourage others to take action.

The following education and outreach projects summarize key elements of the past year:

- Raising awareness of the "Clean, Drain, Dry Your Gear" and "Prevent the Spread of Whirling Disease" campaigns through various advertisements in fishing and outdoor magazines, newspapers, digital newsletters and online posts through AEP's social media channels.
- Various video clips and interviews aimed at improving the understanding of whirling disease were completed by AEP and in collaboration with Michael Short of "Let's Go Outdoors". These were made available on YouTube and promoted through social media.
- Nearly 8000 individuals were reached at 55 public events such as trade shows, community events, lake and parks events, school field trips, fishing events and at popular angling and water recreation launch points in key locations throughout the province. At these events, a whirling disease display and educational resources were available for the public to view and government personnel educated these target audiences through drop-in style interactive activities like trivia questions and a fish ID pond.
- Seventeen whirling disease and aquatic invasive species presentations were delivered upon request at conferences and to organizations such as fish and game clubs, lake groups, and boating clubs, reaching an audience of 1150 people.

- Various educational resources supported this outreach, including display banners, posters, pamphlet stands, a taxidermy mount of whirling disease infected fish, quick fact cards and behaviour prompt tools such as angling lures, trout stress balls and boot brushes.
- 1675 print copies of quick fact cards and posters were restocked upon request at angling retailers, tourism centres, Government of Alberta offices and external stakeholder groups, through the AEP information centre.
- Quarterly meetings were held with the whirling disease stakeholder committee, comprised of federal government representatives, environmental non-governmental organizations, research organizations and angling groups. The meetings provide an opportunity to update these groups on work of the Whirling Disease Program and gain insight and recommendations on the direction of future work.
- Encouraging the public to get involved with citizen science and the reporting of suspect whirling disease observations was enabled through the launch of the EDDMapS Alberta app (Early Detection and Distribution Mapping System), in collaboration with the Alberta Invasive Species Council. The app allows users to submit observations of invasive species through their smartphones and thus directly connect with the Whirling Disease Program.
- Training and education of government and contractor agencies in decontamination protocols was a focus of education and outreach. Efforts were made to support the public's efforts in decontamination and opportunities will continue to be explored to provide watercraft cleaning and equipment decontamination stations for the public. More information is provided below.
- Updated signage was ordered to replace emergency response signage installed in the fall of 2016, which had a warning statement that whirling disease may be present. The new signs clarify whether a waterbody has had a positive detection of the whirling disease parasite, as well as further details on how to "Clean, Drain, Dry Your Gear". More information is provided below.
- Through angler surveys conducted by the Whirling Disease Program and Alberta Conservation Association, data was collected on anglers' level of awareness and understanding of whirling disease in three different waterbodies in Alberta. This data is useful for evaluating the effectiveness of education efforts and for tracking behaviour change in the target audience over time. More information is provided below.



Education and outreach information booth

Education Support for the Decontamination Protocol for Watercraft & Equipment

The Decontamination Protocol implementation included providing education and outreach support to partner agencies. The main contribution in 2018 was the collaboration with Alberta Parks in Kananaskis where decontamination technicians provided a roving information booth throughout the park during the summer. More than 1,000 users of Alberta Parks visited the information booth at various boat launches and were provided with information on aquatic invasive species and whirling disease threats in the province and most importantly information on Stop the Spread using the Clean, Drain, Dry methods.

Other highlights included information booths set up at boat launches along the Bow River in conjunction with the whirling disease angler survey, attending multiple community events in collaboration with the AEP aquatic invasive species program including RiverFest in Edmonton, Neighbour's Day in Sundre, Calgary River Valleys Annual Redd Count, Sturgeon Lake First Nation Information Night and the Glenbow Ranch Park speaker series.

Whirling Disease Signage Updates

In 2016, when whirling disease was first detected in Alberta, signs were installed throughout the province near major waterbodies and publicly stocked ponds to inform the public that the disease had been found in the province. Additionally, the signs were used to promote AEP's "Clean, Drain, Dry" messaging. However, there was concern that the signs may be misinforming the public regarding the distribution of whirling disease, as signs were installed at locations where the parasite had been detected as well as locations where the parasite had not been detected.

In 2018, AEP began removing signs from waterbodies where the parasite was not detected. AEP replaced outdated signs (Figure 19a) with updated signs (Figure 19b) at popular access points for whirling disease positive locations. Updated signs have a specific whirling disease message indicating the presence of the parasite in the waterbody and are complemented by a “Clean, Drain, Dry Your Gear” sign (Figure 19b).



Figure 19: Original whirling disease signage (a) is being updated with new signs that indicate the presence of the parasite in specific waterbodies, in conjunction with a “Clean, Drain, Dry Your Gear” sign below (b) to encourage best practices by recreational users.

To date, 239 signs have been removed that were previously installed at waterbodies where the parasite had not been detected. Sixty-nine (69) original signs that were located in known positive locations were updated with the new whirling disease signs including “Clean, Drain, Dry Your Gear” campaign signs (Figure 19b). In 2019, the Whirling Disease Program will continue removing, replacing, and updating signage throughout the province to more accurately reflect the known distribution of *M. cerebralis*.

Angler Survey Education Questions

In 2018, three angler surveys were conducted in Alberta. The Whirling Disease Program conducted an angler survey on the Bow River, while the Alberta Conservation Association conducted angler surveys on the Wapiti and Upper Oldman Rivers. Education-based questions were asked to 3932 anglers on the Bow River, 40 anglers on the Wapiti River, and 958 anglers on

the Upper Oldman River. Three common whirling disease questions were asked throughout the angler surveys:

1. Have you heard of whirling disease?
2. Do you clean your gear after angling?
3. Do you wear felt-soled boots?

Additional whirling disease questions were asked as part of the Bow River angler survey to gauge the depth of whirling disease education amongst Bow River anglers. AEP's Environmental Education Framework was utilized as the basis for the Bow River angler survey, which outlines five levels of education on a literacy ladder (Figure 20). The literacy ladder illustrates that learning is a linear process, which builds on awareness and progresses into deeper knowledge, a change in attitude, the development of skills and finally results in taking action.

THE ENVIRONMENTAL LITERACY LADDER

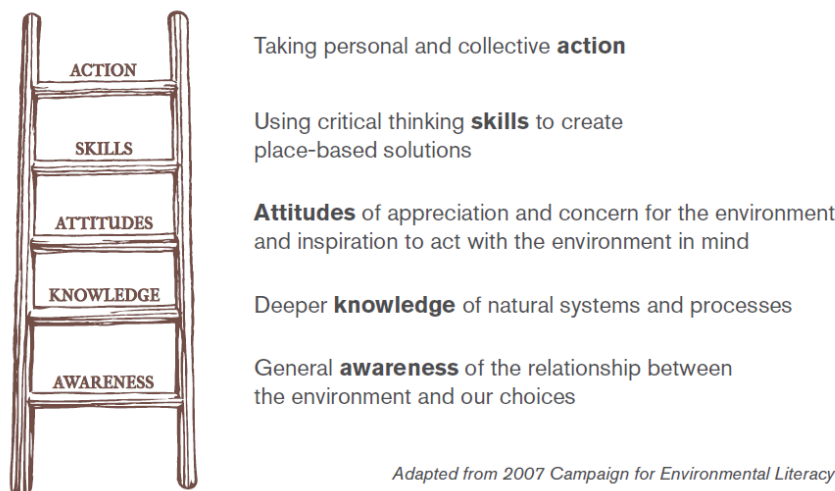


Figure 20: The Bow River Angler Survey used the Alberta Environment and Parks Environmental Education Framework, which outlines five levels on a literacy ladder as a basis for the survey. The ladder indicates that learning is a linear process, where learning begins with awareness and progresses to deeper knowledge, increased attitudes of appreciation and concern, using specific skills and finally taking action.

Building awareness of whirling disease is important; however, it is also important that Albertans develop deeper knowledge of the parasite's lifecycle so that they will understand the steps they can take to prevent the spread of the disease. The Bow River Angler Survey was an opportunity to reach thousands of recreational anglers to gauge awareness and knowledge of whirling

disease. Fisheries technicians provided targeted information to the anglers that were not aware of whirling disease and its potential impacts on salmonid populations.

The following sections discuss the results of the angler surveys broken up by the five levels on the literacy ladder.

Awareness

Awareness refers to the basic understanding that whirling disease exists in the province of Alberta, and where it has been found to date. Four questions were asked to determine general awareness of whirling disease, where it is found in Alberta and the province's Clean, Drain, Dry campaign. Awareness questions included:

- Have you heard of whirling disease? (Figure 21b)
- Are you aware that whirling disease is in the Bow River? (Figure 22)
- Do you know it is also found in the Oldman, Red Deer and North Saskatchewan watersheds? (Figure 22)
- Have you seen or heard about the "Clean, Drain, Dry" campaign? (Figure 22)

Results of the angler surveys indicate that 79% of Bow River anglers have heard of whirling disease, while 91% of anglers in the Upper Oldman River and 40% of anglers in the Wapiti River have heard about whirling disease (Figure 21b). Currently, there are positive detections for whirling disease in the Bow River and Upper Oldman River watersheds, but no detections of whirling disease in the Wapiti River watershed, which may explain the lower awareness of whirling disease from anglers in the Wapiti River.

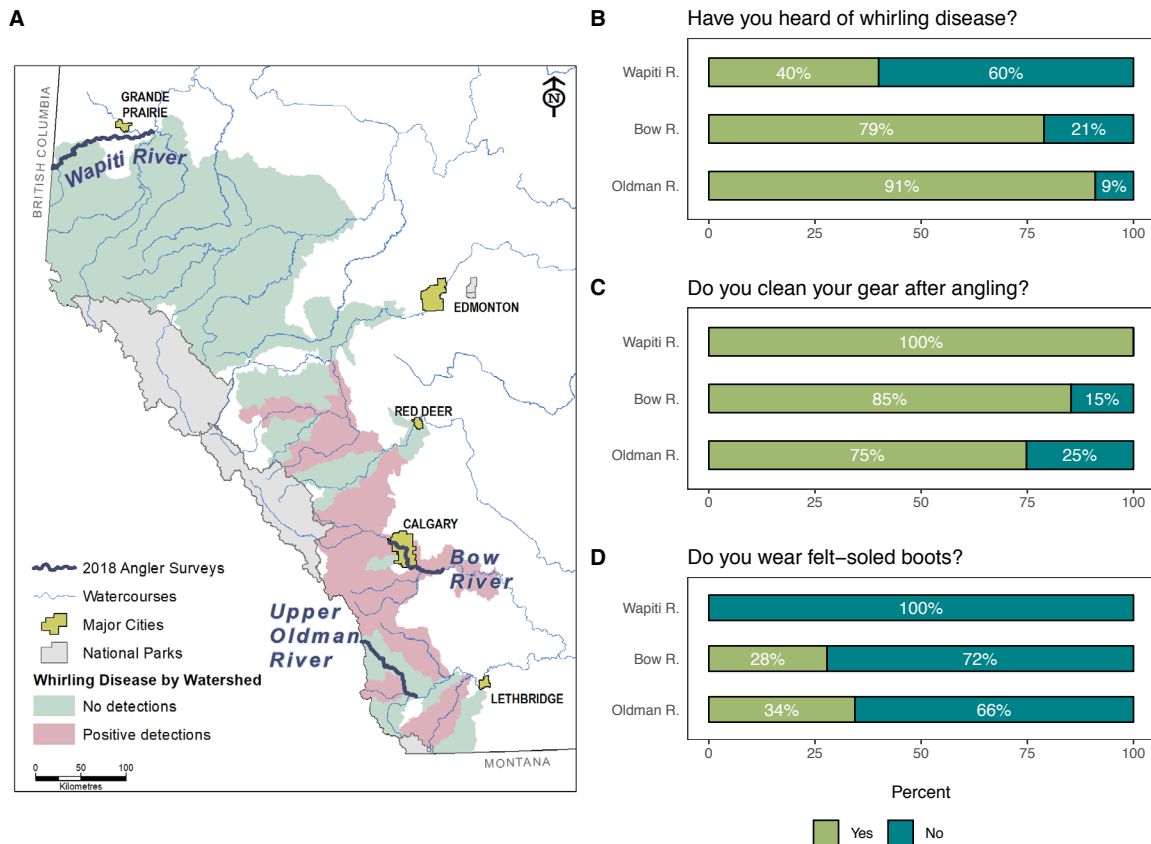


Figure 21: Map illustrating locations of three concurrent angler surveys conducted in Alberta in 2018 on the Wapiti River (Alberta Conservation Association survey), Bow River (Whirling Disease Program survey), and Upper Oldman River (Alberta Conservation Association survey) (A). Three whirling disease questions were asked to anglers at each of these locations. Answers to the following questions are illustrated here: “Have you heard of whirling disease” (B), “Do you clean your gear after angling?” (C), and “Do you wear felt-soled boots?” (D). Responses were restricted to Yes (green) and No (blue).

87% of Bow River anglers surveyed knew that whirling disease was present in the Bow River and its tributaries (Figure 22). 53% of anglers did not know that other watersheds (Oldman, Red Deer, and North Saskatchewan) had tested positive for the disease but 69% were aware of the provincial “Clean, Drain, Dry” campaign. The results indicate that education and awareness may need to focus on watersheds where the disease has not yet been detected.

Awareness

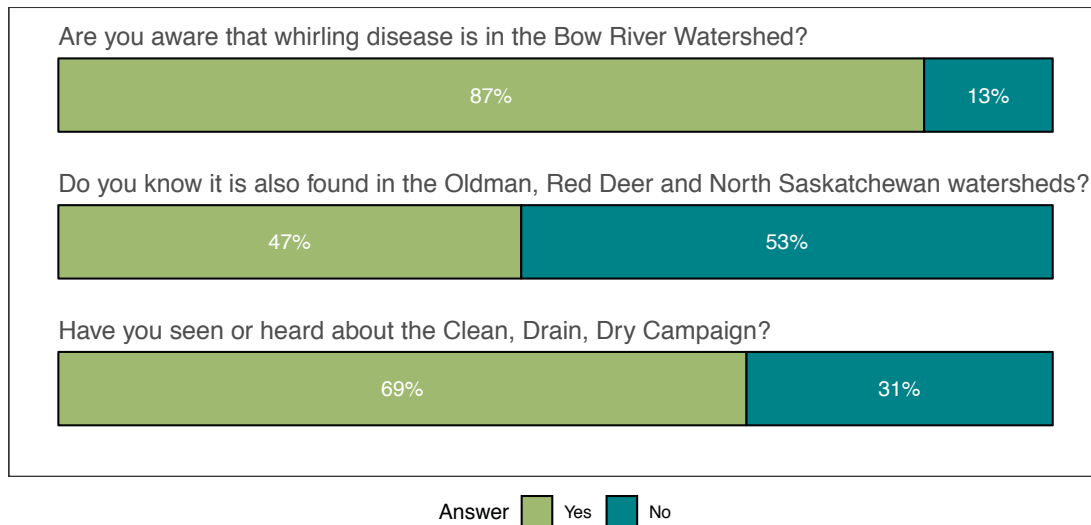


Figure 22: Answers to awareness-related questions from the Bow River Angler Survey, 2018.

Knowledge

Knowledge questions were designed to gauge anglers' understanding of Alberta's natural systems and how human actions and interventions affect the movement of whirling disease of the landscape. Knowledge questions included:

- Are you aware that whirling disease can spread through human actions? (Figure 23)
- Do you know that whirling disease can kill fish? (Figure 23)

In the Bow River angler survey, 88% of respondents indicated they were aware whirling disease can be spread through human actions and 91% indicated they knew the disease could result in fish death (Figure 23).

Knowledge

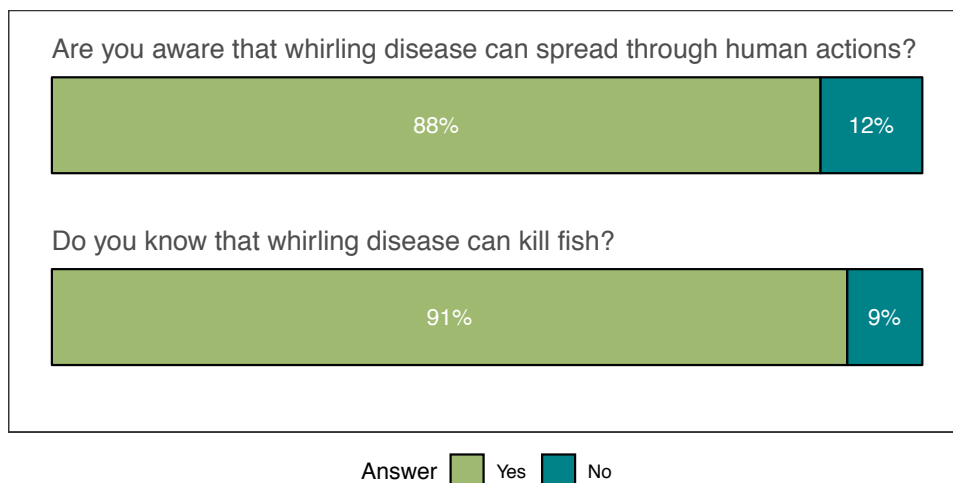


Figure 23: Answers to knowledge-related questions on the Bow River Angler Survey, 2018.

Attitude

Questions on angler's attitudes were used to identify if anglers were concerned about whirling disease and whether they were willing to take action to help prevent the spread of the disease. Developing an attitude of concern or appreciation for the disease should generate motivation amongst the public to foster environmental stewardship. Attitude questions included:

- Are you concerned about whirling disease in Alberta? (Figure 24)
- Are you willing to take action to help prevent the spread of whirling disease? (Figure 24)

87% of the respondents indicated they were concerned about the effects of whirling disease on fish populations, and 98% agreed they would take action to help stop the spread of the disease (Figure 24).

Attitude

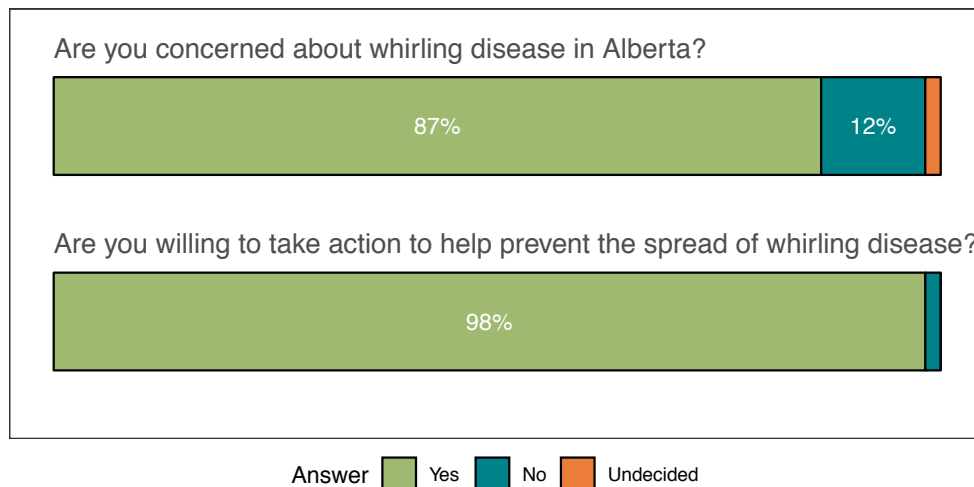


Figure 24: Answers to attitude related questions on the Bow River Angler Survey, 2018

Skills

One skill-based question, which refers to the application of newly acquired knowledge, was asked on the survey. The skills question was:

- Do you know what actions you can take against the spread of whirling disease? (Figure 25)

93% of Bow River anglers indicated that they are aware of the actions they can take against the spread of whirling disease (Figure 25).

Skills

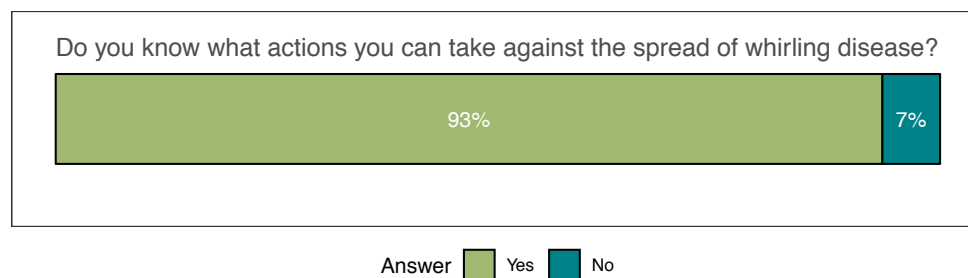


Figure 25: Answers to a skills related question on the Bow River Angler Survey, 2018

Action

The ultimate goal of the Whirling Disease Program's environmental education and outreach component is to develop the capacity for action and participation from all stakeholders. This requires a combination of the lessons learned on each step of the literacy ladder. At the action stage, anglers have an opportunity to take action on what they have learned for the benefit of the environment and healthy fish populations. Action questions included:

- Do you clean your gear after angling? (Figure 21c)
- Do you wear felt-soled boots? (Figure 21d)
- Have you spoken to family or friends about whirling disease? (Figure 26)
- If you saw a fish you suspected had whirling disease, what would you do? (Figure 26)

85% of the respondents of the Bow River survey indicated that they clean their gear after angling, compared to 75% of anglers in the Upper Oldman watershed, and 100% of respondents in the Wapiti watershed (Figure 21). These results are a positive indication that anglers are taking appropriate actions to help prevent the spread of whirling disease and other aquatic invasive species.

However, 28% of anglers in the Bow River indicated that they wear felt-soled waders (Figure 21). This may indicate that anglers are not aware that felt-soled waders are a vector of transport for the disease. The Whirling Disease Program is encouraging anglers not to wear felt-soled waders. The Alberta Conservation Association found that 66% of anglers in the Upper Oldman River, and 100% of the anglers interviewed in the Wapiti River do not wear felt-soled waders (Figure 21).

Ongoing messaging on the ways anglers can spread the disease would be beneficial, as the use of felt-soled waders can result in the spread of whirling disease to uninfected waters.

Anglers that communicate or discuss whirling disease to either friends or family demonstrate mitigation through personal experience and influence. 63% of respondents from the Bow River indicated they speak to family about the disease (Figure 26).

There was a wide breadth of responses of what to do if an angler saw a fish they suspected had whirling disease. The answers to this open-ended question were grouped by either desired or undesired behaviours. Desired responses included: “do not kill the fish” and/or “report findings” to either Fisheries Management or the Whirling Disease Program. Undesired behaviours included “not reporting the suspect fish” and/or a combination of simply “releasing the fish”, or “kill the fish”. 50% of respondents indicated a desired behaviour, 20% suggested undesired behaviours, and 30% were unsure what to do (Figure 26). Additional messaging is necessary to make certain anglers are aware of what to do if they encounter a potentially infected fish.

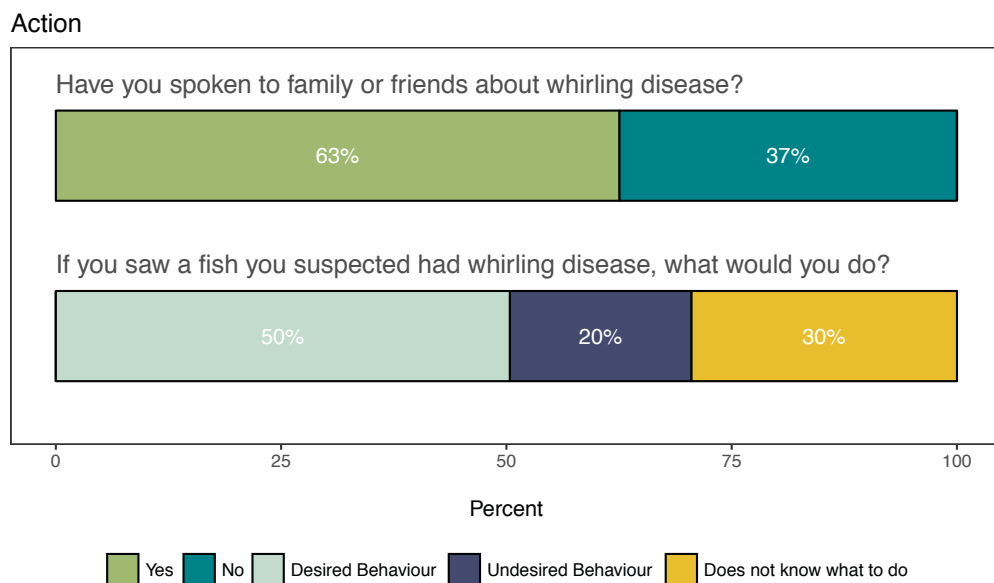


Figure 26: Answers to action related questions from the Bow River Angler Survey, 2018

Recommended actions if you suspect a case of whirling disease

If Albertans suspect a case of whirling disease in the wild or in a stocked pond, they should call the Aquatic Invasive Species Hotline 1(855) 336-BOAT (2628) or email AEP.Whirl@gov.ab.ca to report the observation. Always follow the fishing regulations and do not kill the fish; clinical signs of whirling disease can be caused by other conditions (e.g., other viruses, bacteria, aquaculture conditions, electrofishing) and whirling disease is impossible to diagnose in the wild based on observation alone.

Please record the following information if reporting a case of whirling disease:

1. Date and time of observation
2. Location (river or lake name, GPS coordinates, if possible)
3. Approximate count of suspect fish, size and species
4. Description of how the fish looked or acted
5. A photograph, if possible

The success of the Whirling Disease Program's education efforts is measured by a change in Albertan's knowledge, attitudes, skills or practices. The results of the angler surveys indicate that there is a wide range of whirling disease knowledge amongst anglers. Overall, education and awareness through social media and face-to-face interaction has been relatively successful in watersheds where the parasite has been detected; however, more emphasis may be required in areas where the parasite has not yet been detected to prevent the spread into new watersheds. Continued education to Alberta's recreational user-groups will allow users to take appropriate action to help stop the spread of whirling disease.

Research

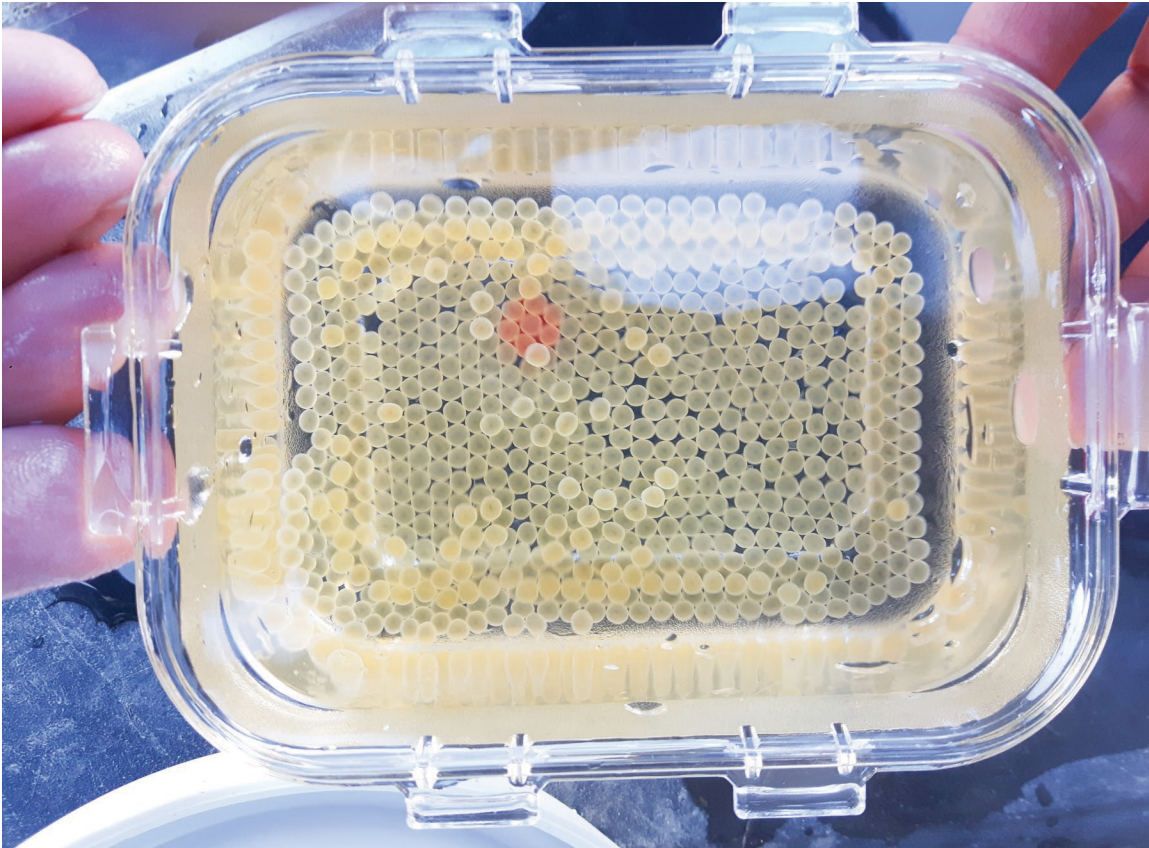
AEP has initiated a number of research projects on whirling disease in Alberta to increase general parasite knowledge, improve diagnostic methods, assess impacts to Alberta's trout populations, understand parasite host resistance and susceptibility for Alberta species, evaluate parasite vectors, and predict where the parasite is likely to spread in Alberta. The following sections provide a brief overview of the research initiatives undertaken by the Whirling Disease Program.

Bayesian Risk Assessment Modeling

Whirling disease presence in Alberta adds an additional threat to the sustainability of Alberta's trout and whitefish populations. Although removal and elimination of the parasite, *M. cerebralis*, is not practical, it may be possible to mitigate the risk of parasite transfer to populations in areas where the disease has not yet established. This requires a decision support tool to aid managers in estimating the likelihood of salmonid exposure to *M. cerebralis* in watersheds throughout Alberta as well as understanding the spread of the parasite within a stream system following infection.

In 2017, the Whirling Disease Program established a collaborative research project with Dr. Mark Lewis and Dr. Russ Greiner at the University of Alberta to develop a Bayesian-belief network for risk assessment modeling for whirling disease in Alberta. The research project will inform AEP management efforts by quantitatively assessing the potential risk of *M. cerebralis* transfer from known infected locations to unknown and potentially uninfected locations. With this approach, AEP can estimate the effects of various management actions on the spread of whirling disease. These assessments will inform AEP's management efforts to reduce, minimize or eliminate whirling disease vectors and the subsequent spread of the parasite.

In 2018, a conceptual pathway model for whirling disease spread was developed with the assistance of hydrologists, fish disease specialists, subject matter experts on whirling disease in Canada and the United States, as well as researchers from the University of Alberta. The project is currently in the data gathering and model development stage, using the Oldman River watershed as a pilot study for developing and testing the model.



Fertilized Brook Trout eggs collected by Hemmera Environmental Consultants from Jumpingpound Creek

Susceptibility Study of Alberta's Salmonids

In 2018, AEP, in conjunction with the University of Alberta, Nautilus Environmental, Hemmera Environmental Consultants and with support from Colorado Parks and Wildlife, initiated a laboratory-based susceptibility study to provide information on the relative risk of whirling disease to Alberta's salmonid species. Salmonid species will be tested for susceptibility to *M. cerebralis* through controlled laboratory exposures.

This project consists of three stages:

1. Collection of salmonid eggs and milt from natural systems of interest;
2. Fertilization, disinfection and incubation of collected eggs in a quarantined facility until the eyed-egg developmental stage; and
3. Hatching eyed-eggs in, or introducing hatchery reared fish to a bio-secure laboratory then exposing individuals to measured doses of the actinospore stage (TAM) of *M. cerebralis* and evaluating the development of infection and disease.

In fall of 2018, gametes were collected from three fall-spawning species, Mountain Whitefish, Brook Trout and Brown Trout, and transferred to a laboratory for fertilization and a separate facility at the University of Alberta for challenge tests. In spring of 2019, gametes will be collected from the following spring-spawning species: Westslope Cutthroat Trout, Athabasca Rainbow Trout, naturalized Rainbow Trout, and Arctic Grayling. In the fall of 2019, gametes will be collected from wild Bull Trout populations as well as a select group of hatchery strains of salmonids to be challenged with infection.

Genetic Mechanisms of Susceptibility in Alberta's Salmonids

In 2018, AEP approved a research grant submitted by Dr. Patrick Hanington at the University of Alberta to determine the genetic mechanisms of susceptibility or resistance to whirling disease in Alberta's salmonids. This project builds upon information gathered through the laboratory susceptibility study to determine the genetic mechanisms driving susceptibility or resistance in a population. The objective of this study is to develop a rapid response method to determine resistance in a population using non-lethal fin clips. Determining the genetic resistance of a population to the whirling disease parasite would allow biologists to assess the risk of whirling disease-related population declines before infection occurs, using fewer resources and also identify naturally resistant populations of wild trout. Additionally, it would allow biologists to monitor changes in population-level genetic resistance over time. In the 2017 and 2018 field seasons, AEP collected fin clips from approximately 5000 susceptible salmonids to be used in this project.

WhIRL Grant Program

In 2018, the Whirling Disease Program launched a grant program titled "Whirling Disease Innovative Research and Learning (WhIRL) Grant Program". The WhIRL Grant Program was created to fulfill AEP's obligation of dedicated funding for research to help AEP maintain populations of wild and stocked salmonids in the presence of the whirling disease parasite. A total of \$1 million has been allocated to fund research proposals that align with AEP's priorities. In 2018/19, six research proposals were selected and approved for funding. These research projects include a wide variety of whirling disease research topics that are outlined in Table 5.

Applicant	Organization	Project
Dr. Julie Alexander, Dr. Patrick Hanington	Oregon State University University of Alberta	Tubificid host susceptibility, community competence and whirling disease risk
Dr. Daniel Barreda	University of Alberta	Contributions of dynamic thermoregulation to mitigate whirling disease: climate change challenges and novel strategies for harnessing fish natural capacity to combat parasite infection
Dr. Patrick Hanington	University of Alberta	Characterizing the genetic and transcriptomic foundations of oligochaete susceptibility to the whirling disease-causing parasite <i>Myxobolus cerebralis</i>
Dr. Mark Poesch, Dr. Mark Lewis	University of Alberta University of Alberta	Assessing the movement and behavior of anglers and the risk of spreading whirling disease
Dr. Mark Poesch Sean Simmons	University of Alberta Anglers Atlas	Using citizen science and mobile phone technology to engage anglers and quantify their real-time behavior and movement across Alberta waterbodies and their potential to spread whirling disease
Dr. Rolf Vinebrooke Dr. Patrick Hanington Dr. Mark Poesch	University of Alberta University of Alberta University of Alberta	Tracking spatio-temporal dynamics of whirling disease in Alberta using paleo-eDNA

Table 5: Successful recipients of WhIRL Grant Funding for the 2018/2019 fiscal year.

WhIRL Grant Program Proposal Summaries

Tubificid host susceptibility, community competence and whirling disease risk

Dr. Alexander and Dr. Hanington propose a series of manipulative experiments to examine worm host (*T. tubifex*) susceptibility to *M. cerebralis* and determine how invertebrate community structure (density and diversity) affects parasite transmission. *T. tubifex* will be collected from whirling disease positive watersheds in southern Alberta to establish laboratory colonies and quantify parasite transmission in native worms. Experiments manipulating worm community structure (parasite compatible and non-compatible hosts) will determine if non-compatible worm hosts (e.g. *Limnodrilus hoffmeisteri*) can act as filters for the parasite and reduce parasite transmission. Experimental results will be used to inform a risk model for Alberta's salmonid bearing watersheds. Fish disease risk is directly related to spore release from infected worms. Knowledge of the susceptibility of *T. tubifex* will enhance risk assessments and inform whirling disease management in Alberta.

Contributions of dynamic thermoregulation to mitigate whirling disease: climate change challenges and novel strategies for harnessing fish natural capacity to combat parasite infection

Dr. Barreda proposes a series of three experiments to determine if natural thermoregulation (regulation of body temperature) in Rainbow Trout (*Oncorhynchus mykiss*) and Brown Trout (*Salmo trutta*) can reduce or eliminate *M. cerebralis* infections. In the first experiment, infected trout will be placed in a tank with a water temperature gradient from 8°C to 18°C. Fish movement will be unrestricted for the 24 days of exposure allowing for natural thermoregulation. Following exposure, analysis of behavior, immune response indicators (inflammation, immune cell migration) and parasite load will determine whether fish are able to clear or reduce infection. Subsequent experiments would establish a dynamic thermoregulation based protocol that facilities could use to manage infected fish and assess the risk of *M. cerebralis* infection in wild salmonid populations associated with climate change related shifts in stream temperature. To date there is no cure or vaccine for *M. cerebralis* infection. If it is determined that fish are able to reduce or clear infections naturally, aquaculture facilities could use this information to manage outbreaks of *M. cerebralis*.

Characterizing the genetic and transcriptomic foundations of oligochaete susceptibility to the whirling disease-causing parasite *Myxobolus cerebralis*

Dr. Hanington proposes a series of experiments to advance the understanding of the genetics of tubificid worms and the susceptibility of different species and lineages. The proposal included the establishment of 4 worm colonies (2 strains of *T. tubifex*, *Limnodrilus hoffmeisteri* and *Limnodrilus claparedeanus*). The susceptibility of each colony to *M. cerebralis* infection would be determined by challenge studies conducted at a range of water temperatures. Worms resistant to infection would be isolated to determine if they produce resistant progeny. The genomes of each worm species will be sequenced and included in genomic databases. The complete transcriptome of resistant and susceptible worms will be sequenced to provide greater understanding of genetic basis for parasite resistance in the worm host.

Assessing the movement and behavior of anglers and the risk of spreading whirling disease

Dr. Poesch and Dr. Lewis propose using traditional angler surveys to characterize anglers in Alberta and identify behaviours that increase the risk of spreading whirling disease. This information will be used to develop a gravity model to aid in the development of a spatial risk assessment. The angler surveys will collect information related to angler demographic, movement and behaviour including high risk behaviours such as not cleaning equipment between trips. Gravity models incorporate human movement and environmental factors and will support the development of a spatial risk assessment (Bayesian Belief Network) currently in development in collaboration with AEP and Dr. Mark Lewis.

Using citizen science and mobile phone technology to engage anglers and quantify their real-time behavior and movement across Alberta waterbodies and their potential to spread whirling disease

Dr. Poesch and Sean Simmons propose to use data collected via the mobile app MyCatch to understand angler behaviour and movement to assess the risk of spread of whirling disease. Education and outreach campaigns would be developed and the current MyCatch app would be updated to track angler behaviour and movement in real time. Traditional angler surveys would

be conducted to validate information collected by the MyCatch app. A mobile app is an efficient low-cost way for managers and biologists to collect data on angler movement and behaviour. This research will be conducted in tandem with Dr. Poesch and Dr. Lewis' proposal above.

Tracking spatio-temporal dynamics of whirling disease in Alberta using paleo-eDNA

Dr. Vinebrooke, Dr. Poesch, and Dr. Hanington propose a study to determine the chronology (historical distribution) of whirling disease in Alberta. This research would identify if *M. cerebralis* was newly introduced to Alberta when it was detected in 2016 or if the parasite has been present for longer. Sediment cores would be taken from Johnson Lake (the site of initial whirling disease detection) and three additional reservoirs in Alberta. Each layer of sediment would be dated using radio isotopes and the presence or absence of *M. cerebralis* determined using DNA analysis. These methods would be validated by testing sediment cores from a reservoir with a known history of whirling disease in Colorado, USA. Knowledge of how long Alberta's salmonid populations have been exposed to *M. cerebralis* is invaluable and will inform risk assessments and provide context for interpreting the results of the whirling disease surveillance program.

Future Direction

The Whirling Disease Program will continue to focus on the three core elements of the action plan: Distribution/Monitoring, Education, and Mitigation. Aquaculture, surveillance and laboratory diagnostic components will continue to define the distribution and impacts of whirling disease in wild and stocked fish populations in Alberta. Decontamination and education efforts will continue to disseminate information to Albertans to help mitigate the spread of the parasite to uninfected regions. As research initiatives progress, they will help fill knowledge gaps in our understanding of whirling disease in Alberta and provide a scientific basis for management decisions and actions.

Overview Maps

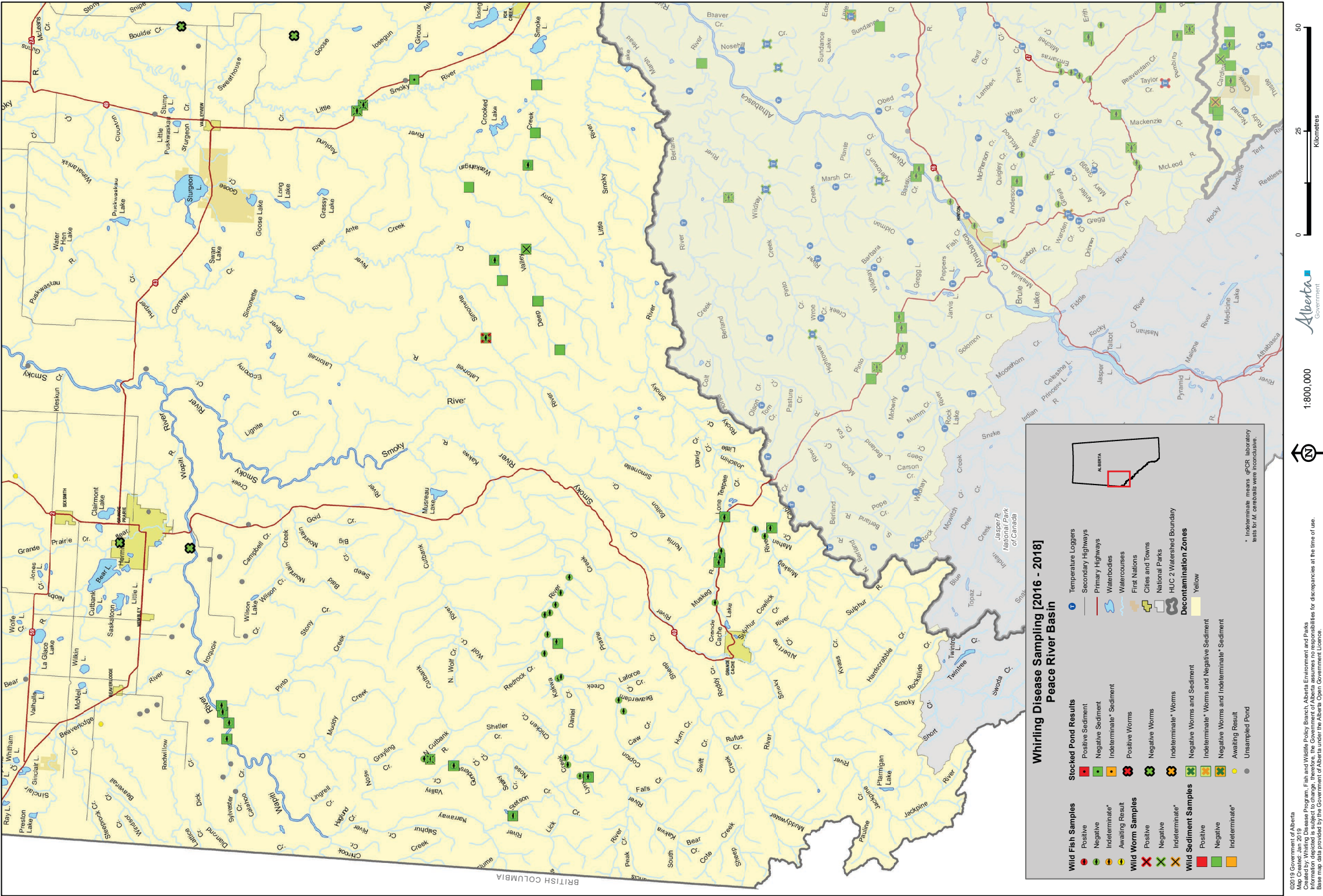
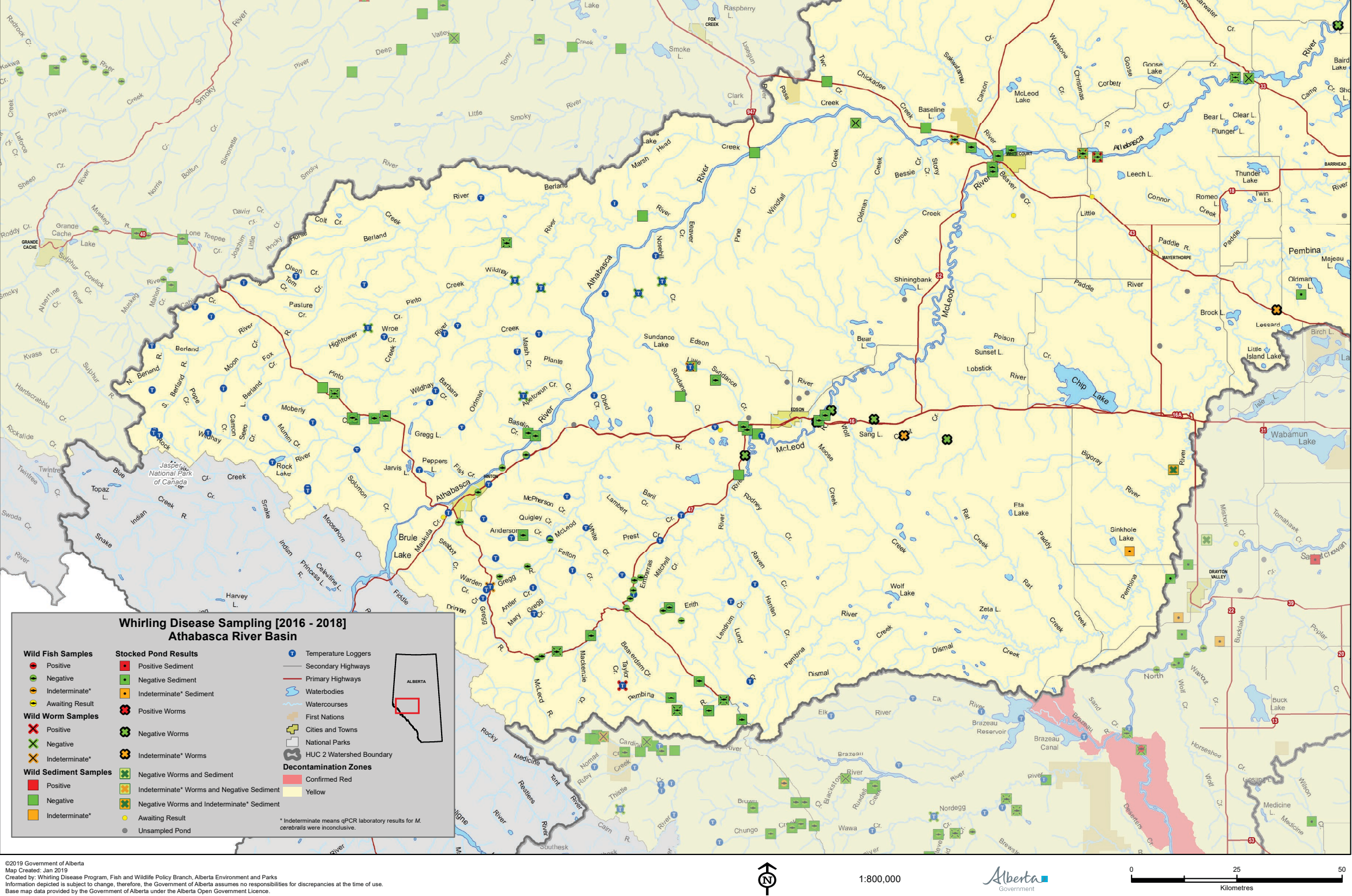


Figure 27: Sampling results in the Peace River Watershed, 2016-2018



28: Sampling results in the Athabasca River Watershed, 2016-2018

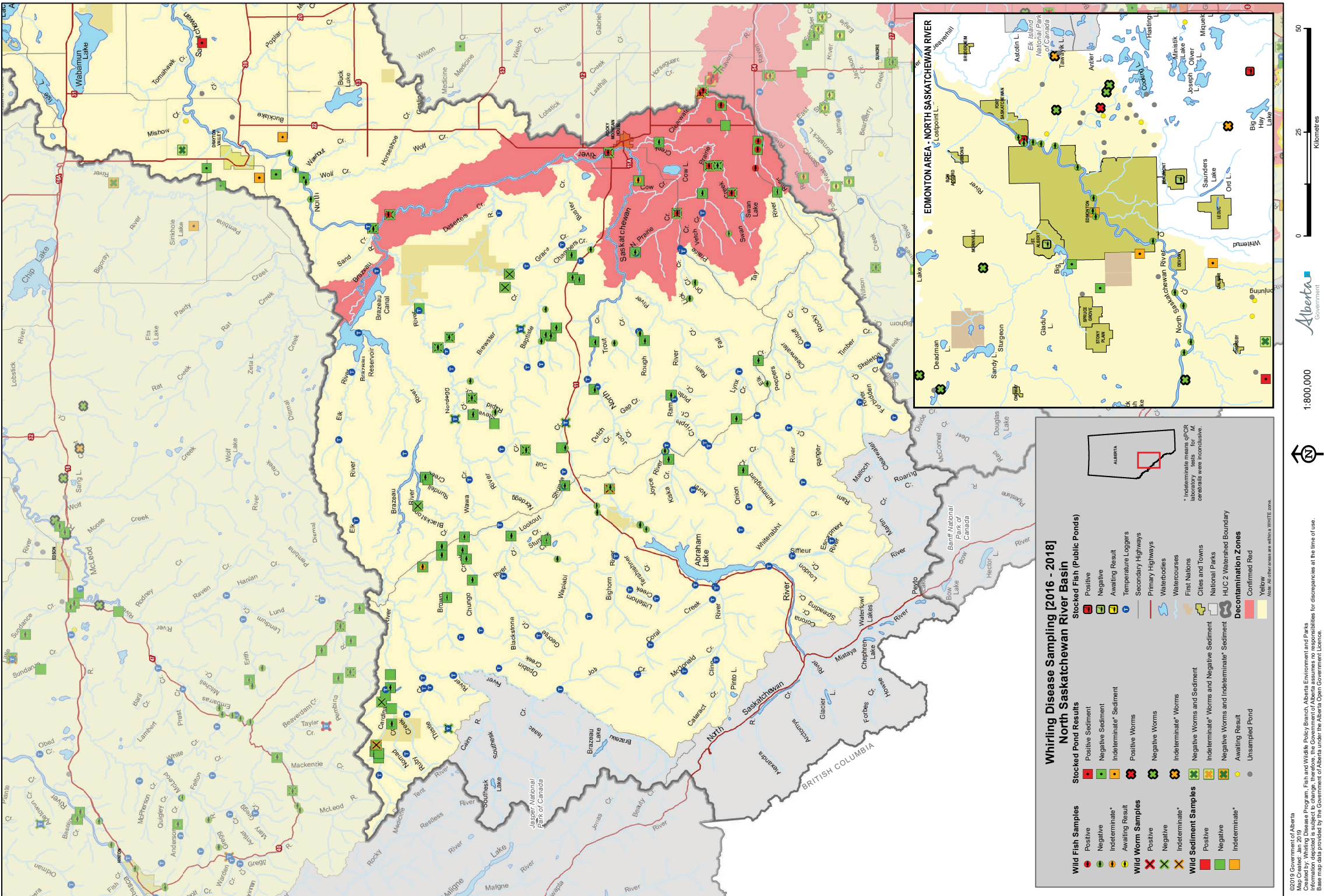


Figure 29: Sampling results in the North Saskatchewan River Watershed, 2016-2018

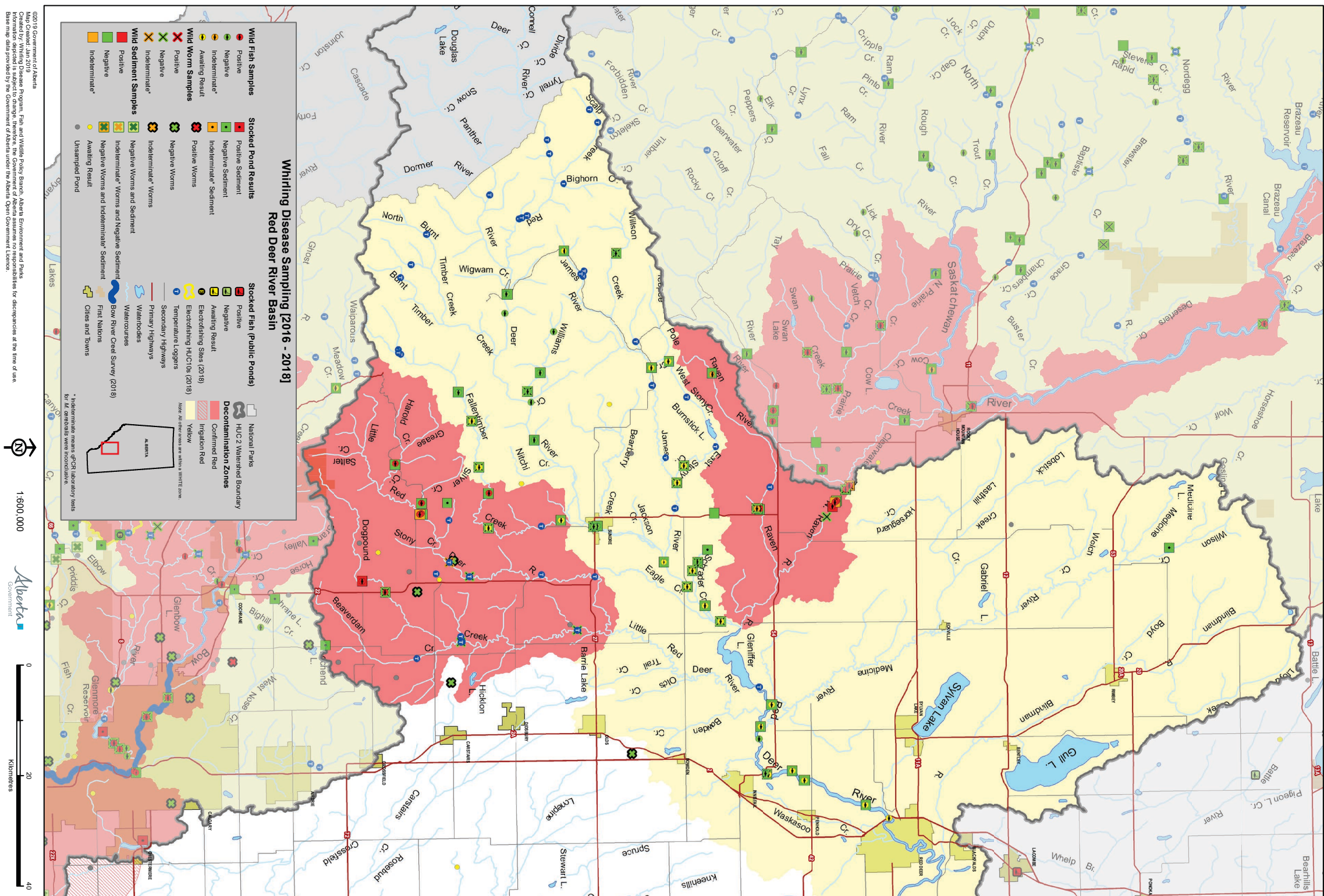


Figure 30: Sampling results in the Red Deer River Watershed, 2016-2018

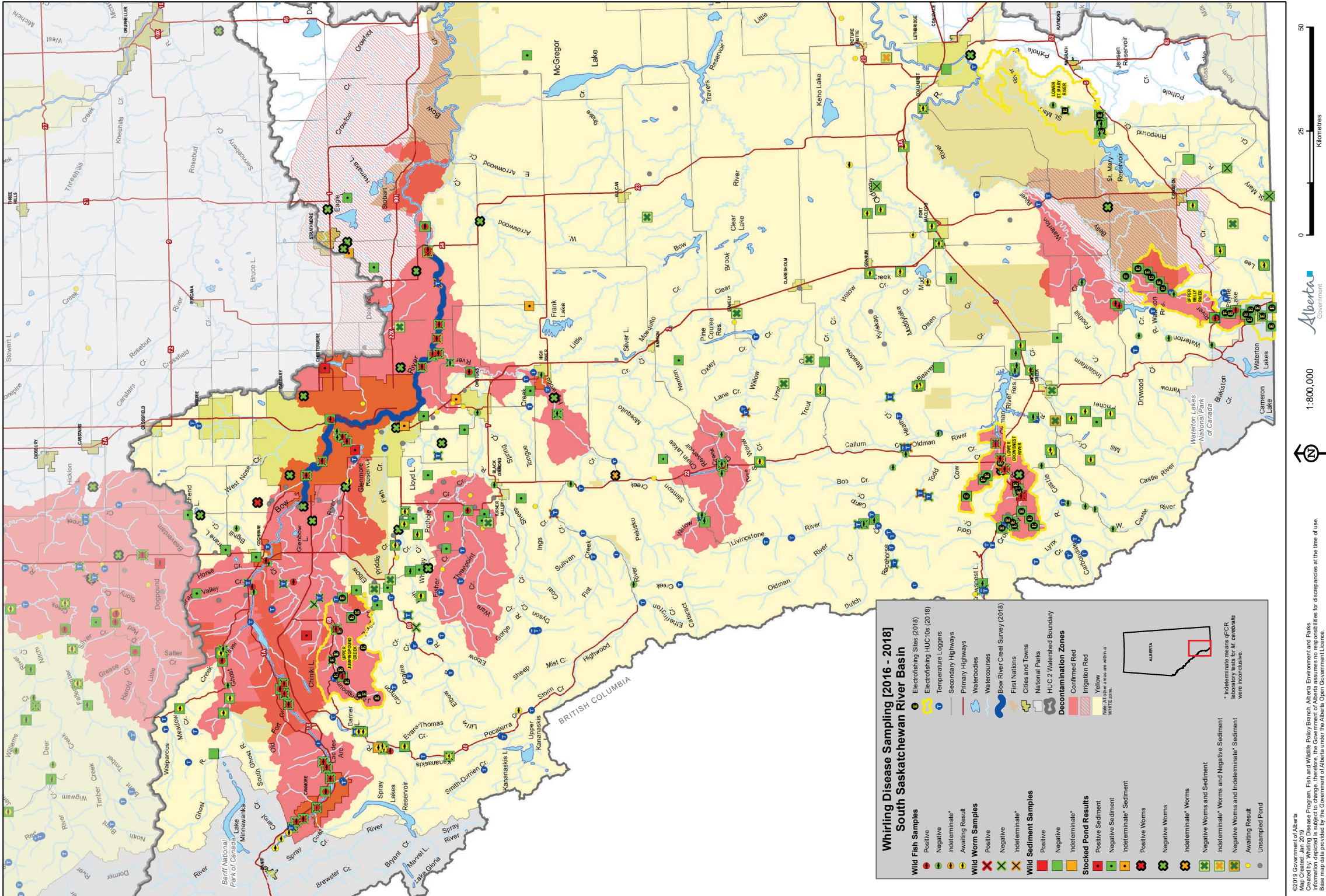


Figure 31: Sampling results in the South Saskatchewan River Watershed, 2016-2018

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Spatial Data Reference

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Decontamination Services: AEP.DECON@gov.ab.ca

AEP Whirling Disease Website: <https://www.alberta.ca/whirling-disease.aspx>

CFIA Whirling Disease Website: <http://www.inspection.gc.ca/animals/aquatic-animals/diseases/reportable/whirling-disease/eng/1336685663723/1336685826959>

Stop the Spread Website: <https://www.alberta.ca/stop-whirling-disease.aspx>

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