

Using qPCR to track whirling disease in Alberta using DNA

Rapid, Multi-Parameter Assessment of Natural Recreational Waters in Alberta:
Detection of Health Risks, Invasive Species and Nuisance Organisms using
Point of Contact Molecular Tests

Patrick C. Hanington
University of Alberta

AI WIP Forum



Warning: Whirling Disease

Whirling disease is caused by a microscopic parasite that infects trout and mountain whitefish.

Prevent the spread! The disease can be spread through the movement of fish, water, sediments or equipment such as felt-soled waders or watercraft.

CLEAN: Rinse all mud and debris from equipment and aquatic gear with clean water.

DRAIN: Drain water before leaving the river or lake.

DRY: Dry boats and gear between trips.

**CLEAN • DRAIN • DRY
YOUR BOAT**

Alberta
Government

For additional information visit aep.alberta.ca



What is whirling disease?

- Debilitating and chronic disease of fish caused by the myxosporean parasite *Myxobolus cerebralis*
- Affects salmonid fish
- Juvenile fish are most susceptible because the parasite infects cartilage
- Causes nerve damage and skeletal deformities, characteristic black tail
- Affected fish have reduced mobility, inability to feed and increased mortality
- Currently no treatment is available



http://media.spokesman.com/photos/2009/07/20/WHIRLING_DISEASE_RAINBOW_TROUT_07-20-2009_PTGFJM.jpg

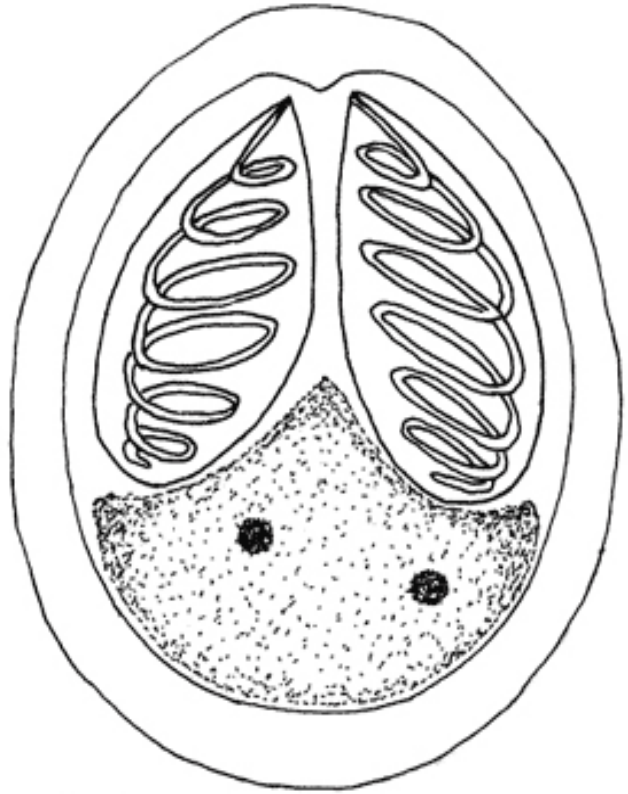


<https://fishpathogens.net>

Myxobolus cerebralis is most closely related to jellyfish



A nematocyst in a cnidocyte - Cnidarian



A polar capsule - Myxozoan

What is whirling disease?



Brown Trout with black, deformed tail.

Image credit: Barry Nehring

The history of whirling disease in North America and why we should care about it being in Alberta

- Discovered in 1893 in Germany, from non-native, imported fish
- First found in commercial fisheries in the USA in the late 1950s
- First confirmed in natural populations in the Rocky Mountains in the United States around 1990 (1987 Colorado)
- First found in Canada in August 2016 in Johnson Lake in Banff National Park, Alberta
- Concerns with reduced populations affecting the recreational fishing industry
 - In 2010, \$171 million CAD was spent in Alberta on fishing related expenses

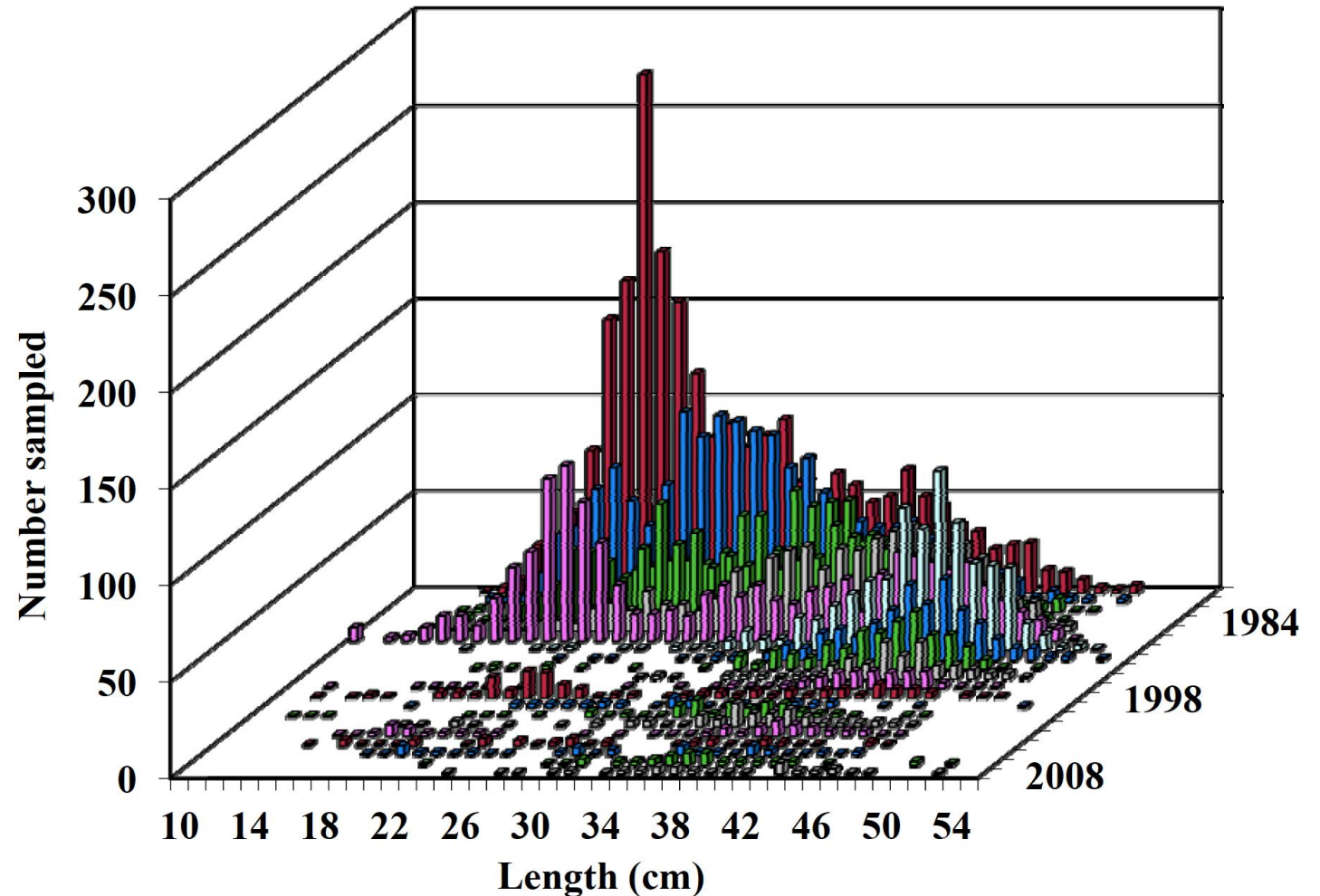


Figure 4.2. Upper Colorado River historic rainbow trout length-frequencies at Kemp-Breeze State Wildlife Area.

Whirling disease in Alberta

Whirling Disease Decontamination Zones

Decontamination Zones

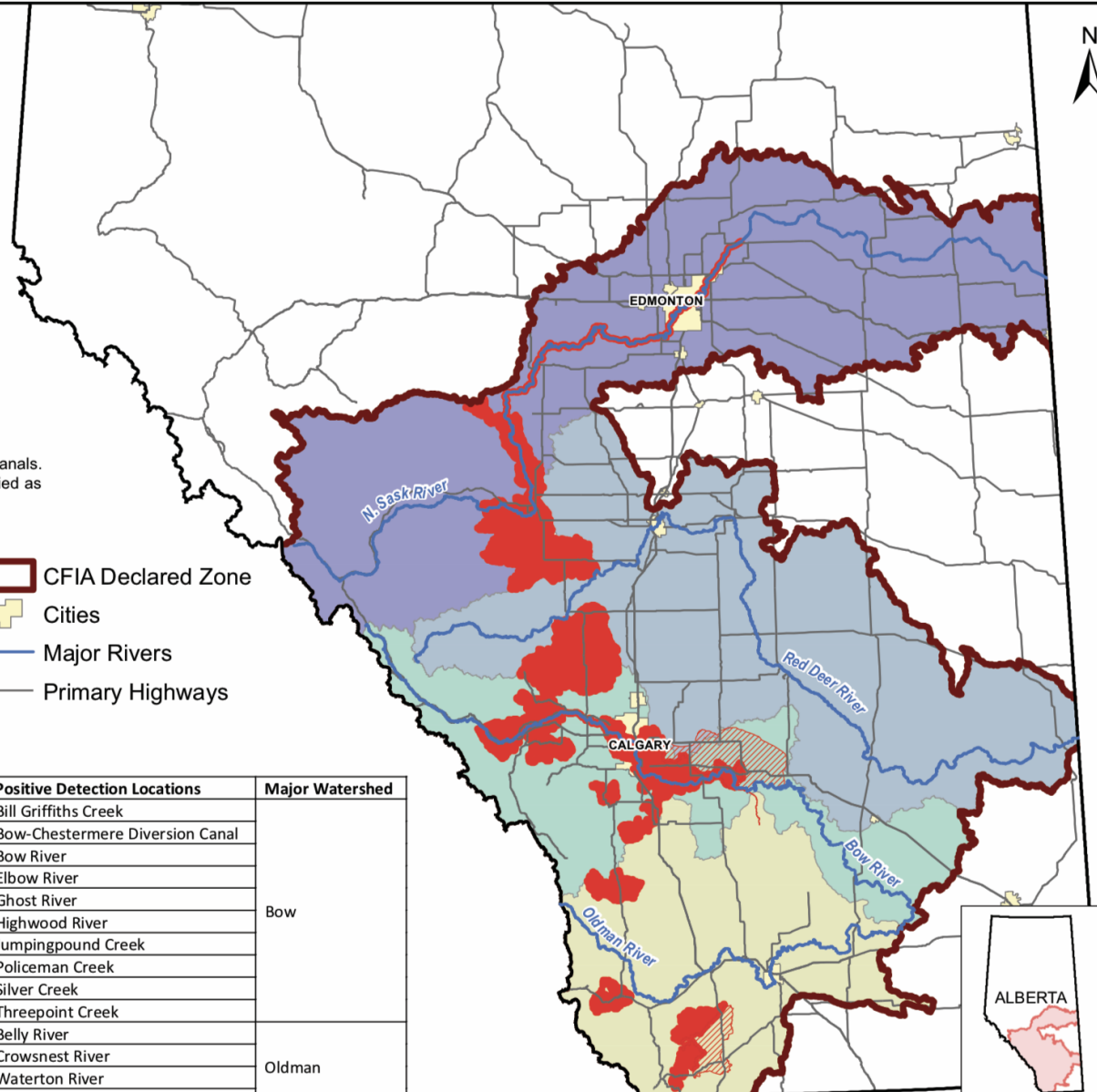
- Confirmed Red Zone
- Irrigation Red Zone

Note: Irrigation red zone only applies to irrigation canals. The remainder of the CFIA declared zone is classified as high to moderate risk (yellow).

Major Watersheds

- North Saskatchewan River
- Red Deer River
- Bow River
- Oldman River
- Cities
- Major Rivers
- Primary Highways
- CFIA Declared Zone

Positive Detection Locations	Major Watershed	Positive Detection Locations	Major Watershed
Alford Creek	North Saskatchewan	Bill Griffiths Creek	Bow
Clear Creek		Bow-Chestermere Diversion Canal	
Mud Creek		Bow River	
North Saskatchewan River		Elbow River	
Prairie Creek		Ghost River	
Swan Creek		Highwood River	
Beaver Creek	Red Deer	Jumpingpound Creek	Oldman
Dogpound Creek		Policeman Creek	
Fallentimber Creek		Silver Creek	
Grease Creek		Threepoint Creek	
Little Red Deer River		Belly River	
North Raven River		Crowsnest River	
Raven River		Waterton River	
		Willow Creek	



© 2018 Government of Alberta; Map Created: April 2018
 Created By: Whirling Disease Program, Fish and Wildlife Policy Branch, Alberta Environment and Parks
 Information depicted is subject to change, therefore, the Government of Alberta assumes no responsibilities for discrepancies at the time of use. Base map data provided by the Government of Alberta under the Alberta Open Government Licence.

Water

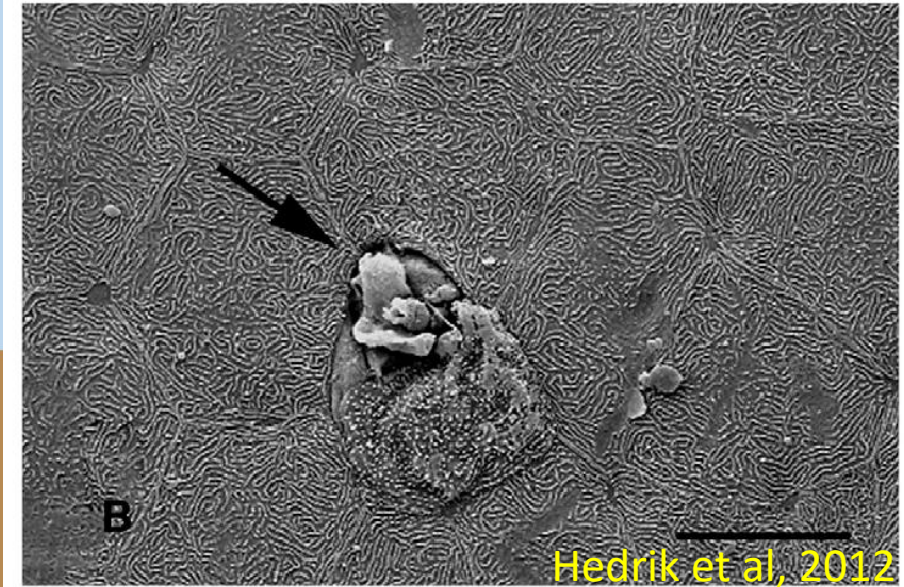
TAM (triacinomyxon)
stage of



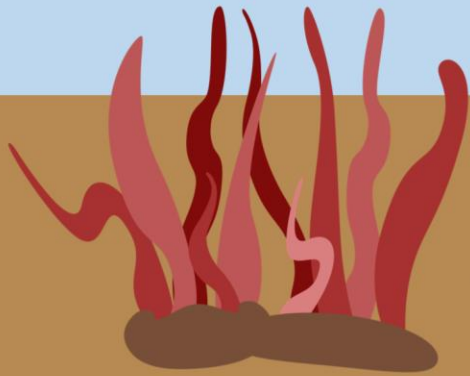
Infected salmonid fish



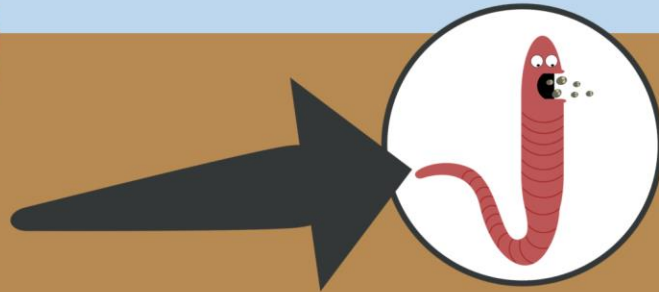
Lifecycle of
*Myxobolous
cerebralis*



Hedrik et al, 2012



Infected Tubifex worms



Sediment

Testing for whirling disease (*M. cerebralis*): it's all in the head

Electrofishing – catch
~150 fish per site

Pool fish heads in
groups of 5 and
digest with enzymes

Look for the
myxospore stage in
head digest

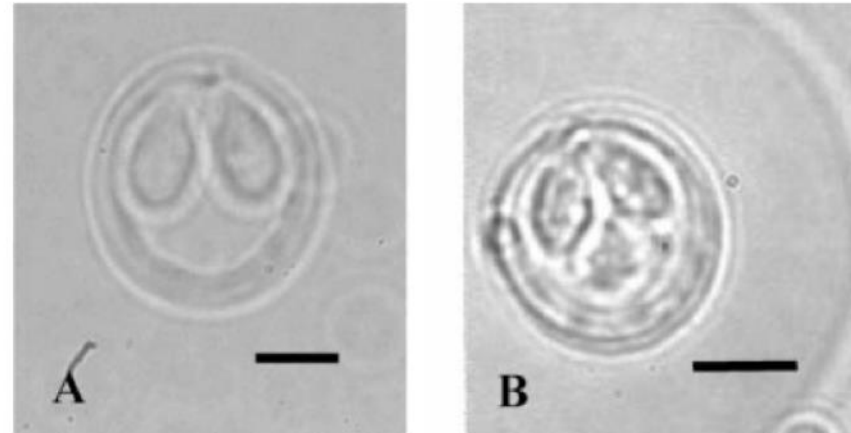


FIGURE 3.—Pepsin–trypsin digests of the spores of (A) neurotropic *Myxobolus* sp. and (B) *M. cerebralis* showing no obvious morphological differences; bars = 4 μm .

How we detect *M. cerebralis* DNA

Quantitative (q) Polymerase Chain Reaction (PCR)

- Method of looking for a specific gene target (or specific DNA sequence) in a DNA sample
- q= quantitative
 - The gene target is replicated (or amplified) using primers that are found at each end
 - As each gene target is replicated, a fluorescent dye is released from a probe and detected by a camera in the qPCR machine
 - This allows calculation of the number of gene copies in the original sample and detection down to a single copy of a gene

Target
DNA
Sequence

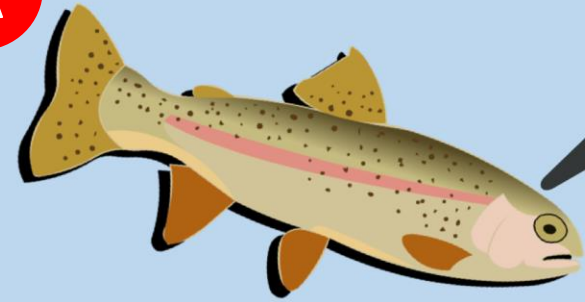


Water

TAM (triacinomyxon)
stage of
M. cerebralis



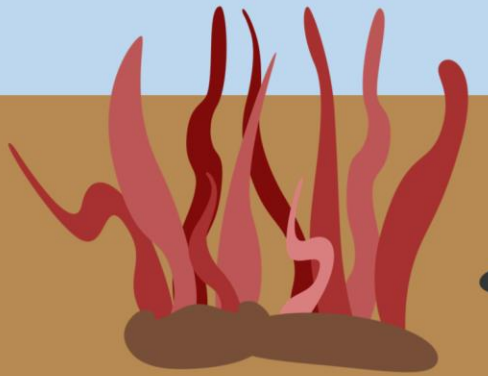
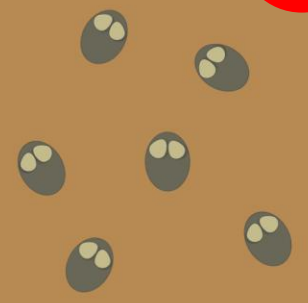
Infected salmonid fish



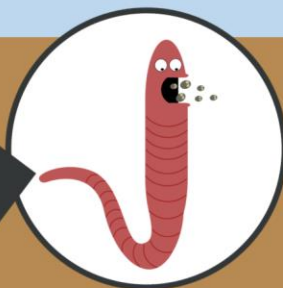
Lifecycle of
Myxobolous
cerebralis



Myxospore
stage of
M. cerebralis

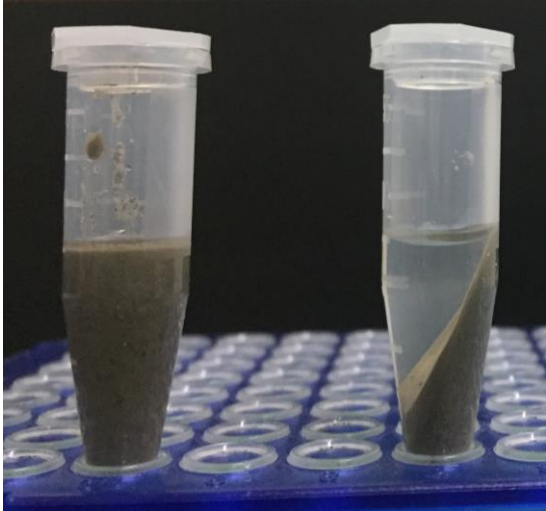
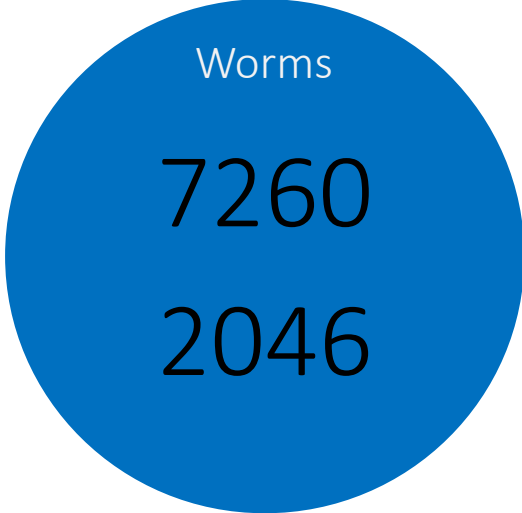


Infected Tubifex worms

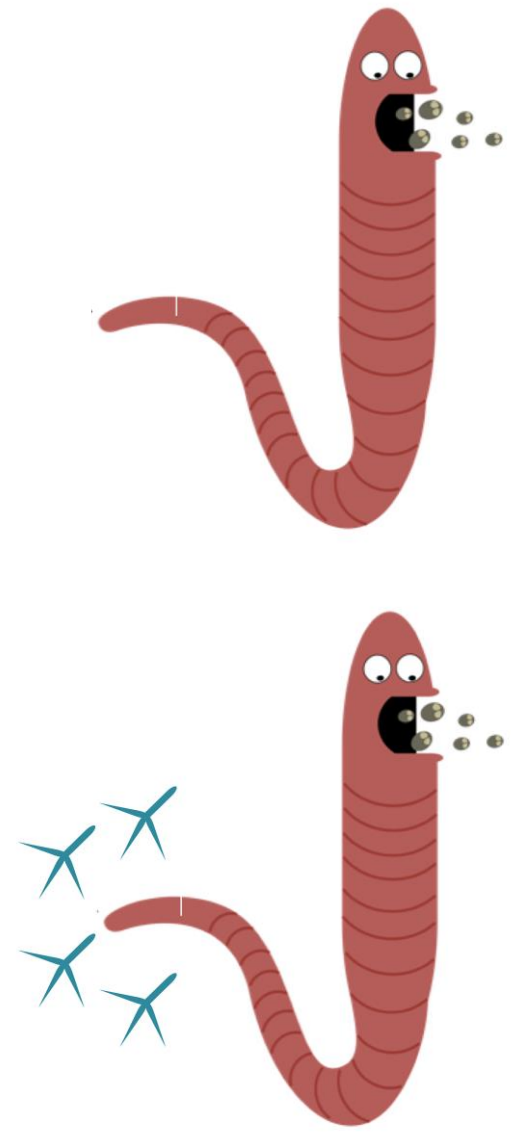
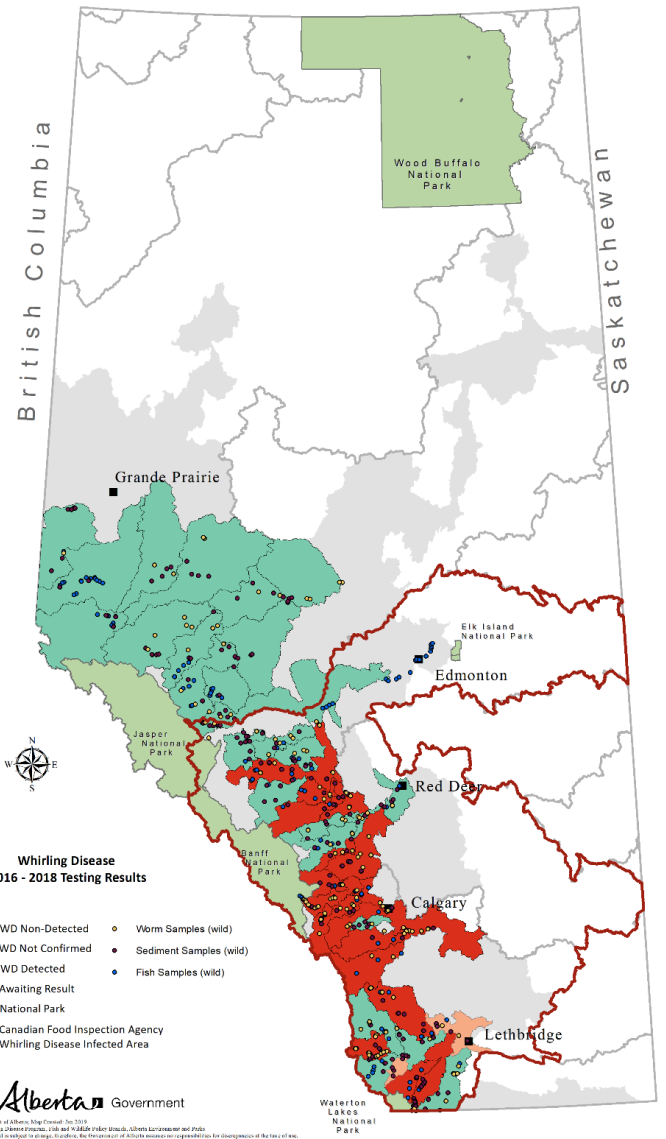


Sediment

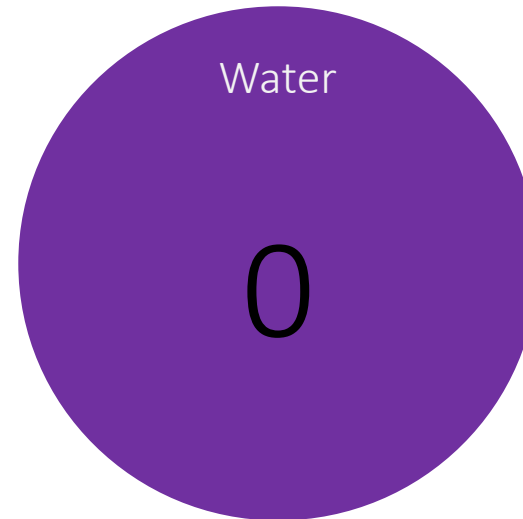
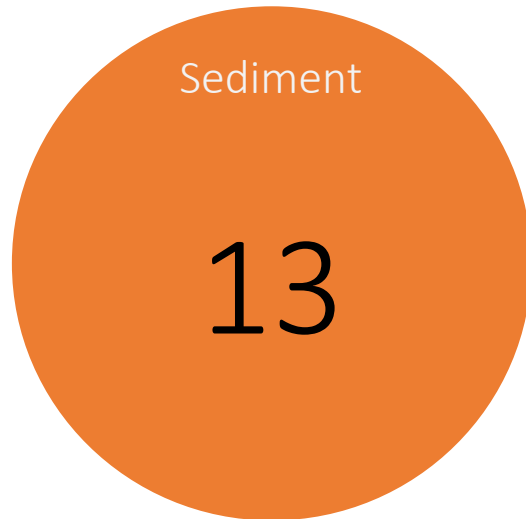
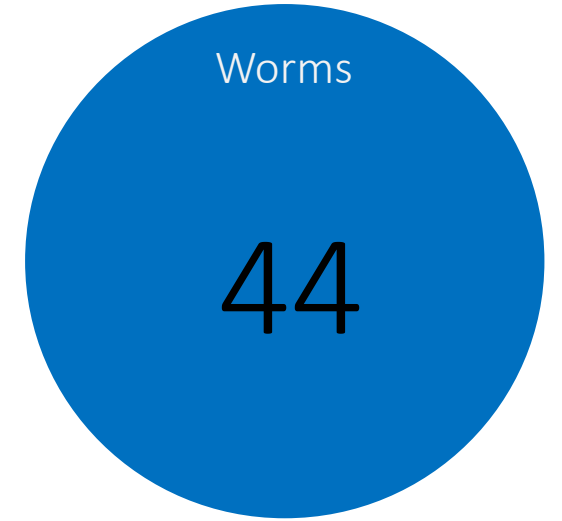
Environmental monitoring program for whirling disease



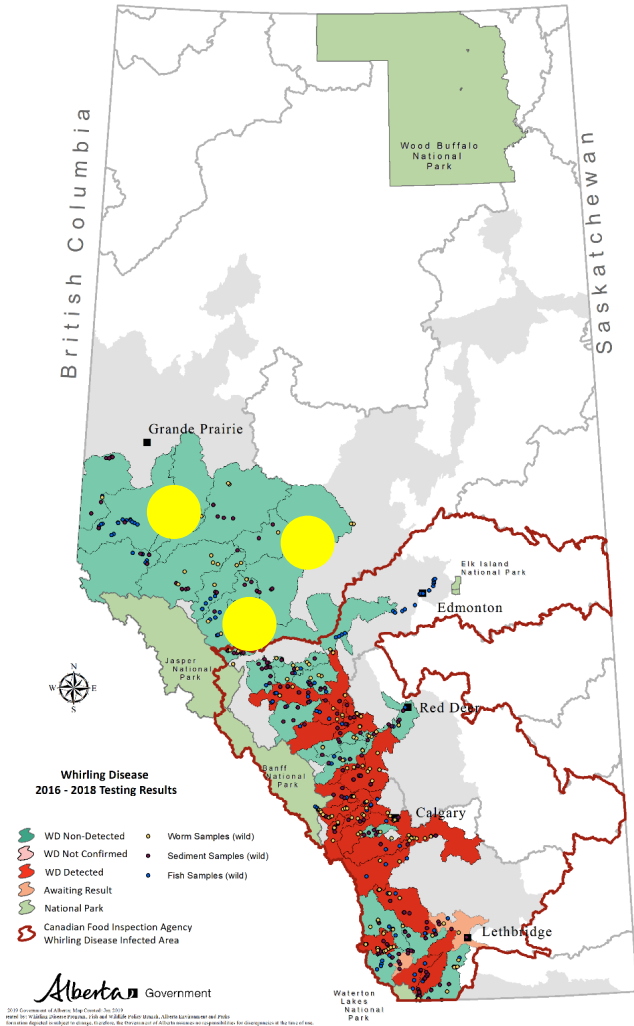
Wild site data summary: 688 locations sampled



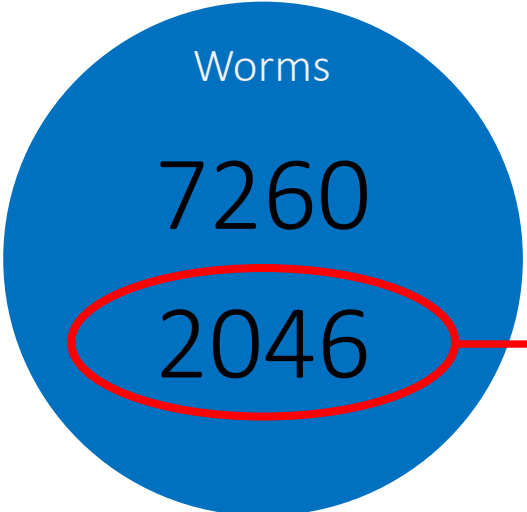
Summary of our three-year surveillance



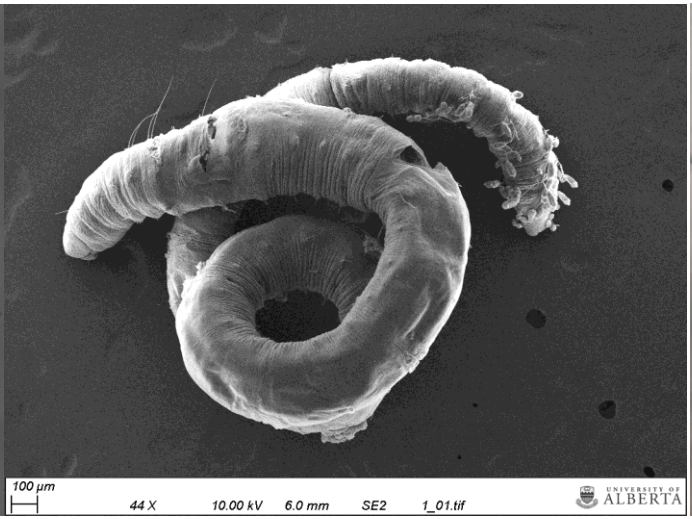
Environmental surveillance allows us to target investigations and resources for assessment and control



Using DNA to identify species of worm susceptible to *M. cerebralis* in Alberta




































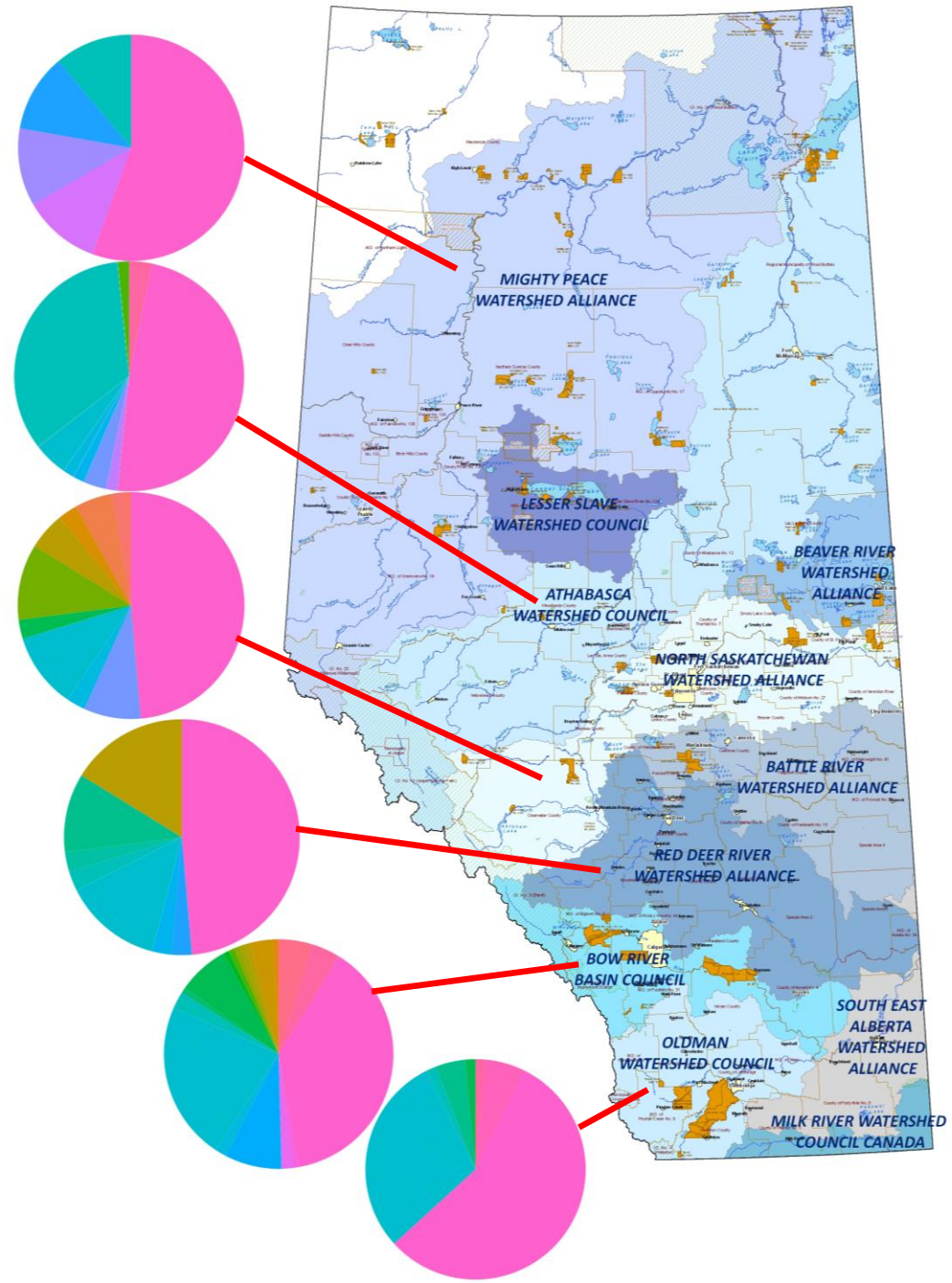
DNA Barcoding
- Compare to a huge online database of genes from identifies species



All watersheds in Alberta are suitable for whirling disease transmission

Species

	Aktedrilus arcticus			Limnodrilus hoffmeisteri
	Alexandrovina onegensis			Limnodrilus profundicola
	Aulodrilus plurisetus			Limnodrilus udekemianus
	Chamaedrilus cognetti			Lumbriculda sp.
	Eiseniella tetraedra			Marionina riparia
	Enchytraeidae sp.			Naididae sp.
	Fredricia semisetosa			Nais bretscheri
	Fridericia raxiensis			Nais christinae
	Fridericia sylvatica			Octolasion cyaneum
	Haplotaxida sp.			Ophidonais serpentina
	Henlea nasuta			Rhynchelmis elrodi
	Henlea perpusilla			T. tubifex
	Henlea ventriculosa			Tubifex sp.
	Ilyodrilus templetoni			Tubificinae sp.
	Lamprodrilus ammophagus			Undetermined
	Limnodrilus claparedeanus			



Summary: How DNA-based monitoring improves our ability to track whirling disease in Alberta

- DNA-based testing is incredibly sensitive, it allows us to detect single parasite spores in fish, water, sediment or worms
 - This is critical when an invasive species is establishing and our opportunity to curb or prevent invasion is highest
- The same test can be used to test each type of sample, this unifies testing methodologies and allows for direct comparison between sites and sample types
- Using DNA to ID species that are susceptible can highlight sites of high potential for transmission
- Integrating environmental sampling reduces pressure for lethal fish sampling and allows for routine monitoring at sites of interest
 - Especially important for fish species at risk

Acknowledgments

Danielle Barry | Sydney Rudko | Alyssa Turnbull

Jacob Hambrook | Hongyu Li | Abdullah Gharamah | Brooke McPhail | Norm Neumann | Heather Proctor | Simon Otto | Bradley Peter | Kathryn Wagner | Andre Morson | Jay White | Leah Brummelhuis | Arnika Oddy-van Oploo | Clayton James | Marie Veillard | Trish Kelly | Peter Giamberardino | Emmanuel Pila | Julie Alexander

