ALBERTA INNOVATES

AI PROJECT FINAL REPORT

Project Title:	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
AI Project Number:	2332
Project Leader:	Patrick Hanington
Lead Institution:	University of Alberta
Project Partners:	ALMS, AHS, AEP, Freshwater Solutions, Biomeme Inc, Chai Biosystems, City of Edmonton
Project Start Date:	May 1, 2016
Project End Date:	February 28, 2020
Project Budget:	$312,045
AI Funding:	$297,045

Executive Summary:

Some of the most important hazards associated with ambient recreational water in Canada are biological in nature, and can have significant, negative impacts on human/animal health and local and Provincial economies. Because of the diversity of biological hazards present in Alberta's natural recreational waters, and the myriad factors that can influence their presence and abundance in a lake, stream or wetland, it can be incredibly difficult to accurately predict their occurrence, or identify their presence in a timely manner. Relying on existing approaches to monitoring, such as visual inspection of a water sample, microscopic cell count or species identification, cell culture analysis or laboratory tests which can have lengthy times between sample collection and sample processing, runs the risk of either being over-zealous in closing beaches to public use, or in missing an emerging problem thereby risking the health of recreational water users. While the above testing approaches all have certain advantages, it is becoming clear that molecular detection tests for water-based organisms surpass traditional tests in many ways. Most important are: significantly improved sensitivity (number of organisms required for a positive result) and specificity (providing a positive result only for the organisms of interest), more rapid results, and the ability to test for multiple organisms of interest in a single test. Historically, molecular tests based on the detection of a specific DNA target have been limited in their application because of financial and technical hurdles that have outweighed the benefits. This project is focused on developing and implementing innovative new technologies, developed in Alberta, to provide a means for near-real-time monitoring of biological hazards in ambient recreational water.

Introduction:

Natural freshwater resources in Alberta, and Canada, are incredibly valuable. The measurable contribution of water to the Canadian economy is estimated to be as high as $23 billion annually, which is comparable to agricultural production and other major components of the Canadian economy (1). A significant proportion of that value is associated with recreational uses. For example, in 2010 recreational fishers contributed a total of $8.3 billion to various local economies in Canada (2), and estimates of the total annual value of recreational water sites in Alberta range between $500,000 and $1 million per year, per site (3,4). In addition to the obvious economic value of recreational water in Canada, these resources are also of significant health, environmental, biological and social importance; serving as places for social gathering, outdoor activity, animal and plant habitat and often as a source for irrigation and drinking water.

Threats to Alberta's recreational water resources impact the extent to which any specific water body can be utilized, and thus the value it has. While there are numerous types of threat to the lakes, streams and wetlands of Alberta, this project will focus on those that have had a significant impact in the past decade (cyanobacteria and swimmer's itch), and those that pose immediate threats to future recreational water security (quagga and zebra mussels). Cyanobacteria and swimmer's itch represent health risks to recreational water users and led to the closure of over 25 beaches in Alberta in 2015, reflecting 1,616 lost days of recreational use (5). These closures often last until the end of the season and thus have significant impacts on local businesses, municipal and provincial events, tourism to Alberta parks and property values at summer

villages. Invasive species such as quagga and zebra mussels pose another enormous and growing threat to recreational waters in Alberta. Significant investment has been allotted to Provincial programs aimed at preventing these organisms from establishing in Alberta lakes, and recent reports indicate that Ontario spends ~$75-91 million annually dealing with their infestation, and over $7 billion is spent annually throughout Canada (6). Evidence suggests that only through early detection of these invasive species can infestation be prevented in a lake, emphasizing the need for sensitive tools capable of monitoring for them before they establish (7).

Existing Gaps in Knowledge and Technology:

The current approaches to monitoring recreational water in Alberta are reactionary, rather than proactive. Tests to monitor for priority organisms in recreational water are either completely lacking (swimmer's itch), or are slow to yield definitive results (cyanobacteria and invasive mussels). We currently lack an early warning system that is sensitive enough to measure these hazards at low environmental levels, information that could be used to identify potential issues before an invasive species or outbreak gains a foothold (7). Thus, the health of Alberta's natural recreational waters and the safety of those using them is not effectively protected. Moreover, communication to the public regarding these issues is ineffective, leaving the average recreational water user or summer village property owner frustrated, which ultimately leads to them risking their health and safety, or compromising the security of the recreational waters they utilize by disregarding warnings and spreading misinformation.

Parallel to these gaps in in our ability to monitor recreational water effectively, is a fundamental lack of biological understanding related to many organisms that are considered biological hazards/nuisances of recreational water. For example, our understanding of the ecological and environmental drivers of a swimmer's itch outbreak in Alberta is largely based on data that our research group collected during the past three years, and is thus limited to a narrow time frame and sample set. Similarly, the drivers of toxin-producing cyanobacterial blooms are poorly understood, and the relationship between toxin-producing and non-toxin producing cyanobacteria over a season is not well understood. Arising from these knowledge gaps is an inability to make accurate and meaningful predictions about how recreational water hazards will respond to changes in environmental or ecological factors from year to year, or even within a season. Having this ability would be a powerful tool for mitigating the impact of these recreational water hazards, and is a parallel aim of this project which will be addressed as we advance our monitoring tools.

From Bench to Beach – An Innovative Solution for Recreational Water Monitoring:

Meaningful advances in monitoring must deliver improvements over existing testing methodologies. This requires increased sensitivity and specificity of the tests, reduced time to results, simplification, expanded testing range/breadth, and movement towards real-time monitoring.

Innovative approaches to monitoring recreational water are required to advance our ability to protect both the natural water ecosystems of Alberta as well as recreational water users. This proposal targets the development and comprehensive validation of DNA and RNA-based molecular detection tests that deliver on all of the above requirements of an improved monitoring test.

Project Description:

The following objectives summarize the aims and goals of this project.

Primary objectives:

A) Develop, validate and molecular detection tests targeted at improving upon the sensitivity, specificity and speed in which swimmer's itch parasites, microcystin-producing cyanobacteria, and quagga and zebra mussels can be identified in natural recreational waters.

B) Adapt the above tests for use in an innovative quantitative polymerase chain reaction (qPCR) system that allows for the implementation of DNA-based qPCR testing at the point of sample collection.

Secondary objectives include:

A) Utilize data collected during this project to improve our understanding of the relationships between environmental, ecological and biological factors that influence recreational water hazards.

B) Integrate these molecular tests into the operational toolkit of relevant recreational water monitoring programs.

C) Incorporate environmental and target organism data collected on specific Alberta-lakes throughout this project to build frameworks for the assessment of factors that exacerbate risk.

D) Design and initiate tailored strategies for communicating to the public the importance of, and risks related to, threats to Alberta recreational water resources.

Updates to Project Objectives:

In early 2017, we amended our project to include development and validation of a qPCR-based test for *Myxobolus cerebralis*, the parasite that causes whirling disease that was recently found, for the first time, in Alberta. This amendment added $81,420.00 to our total project budget, and extended the project until February 28, 2020.

Project Plan

Task #	Task Description	Status	Comments on Tasks and Milestones
Task 1	Swimmer's itch qPCR test		
1.1	Test development and validation	complete	
1.2	Implementation and refinement	complete	We can now distinguish between the four primary species of parasite that can cause swimmer's itch in Alberta via qPCR
1.3	Continued monitoring	complete	We now make use of the general swimmer's itch and the new species-differentiating tests at sites throughout Alberta.
Task 2	Cyanobacteria mcyE qPCR test		
2.1	Test development and validation	complete	
2.2	Continued monitoring	complete	AHS, ALMS, City of Edmonton (Triathlon and Borden Park), and our own research group continue to make use of this test.
2.3	Development and validation of a qPCR test that reflects total cyanobacteria	complete	A 16s-based qPCR test has been developed and validated for providing estimates of total cyanobacteria. This test was utilized by ALMS in 2019 to supplement the Beach Monitoring program to provide data on cyanobacteria blooms across Alberta.
Task 3	Quagga and Zebra Mussel qPCR		
3.1	Test development and validation	complete	
3.2	Implementation and refinement	complete	AEP and Aquality continue to collect samples that we will test using our existing qPCR assay.
Task 4	Whirling disease qPCR		
4.1	Test development and validation	complete	We have selected a qPCR test for M. cerebralis that targets the 18s rDNA gene (which is in high copy number). This test has been used for detection of M. cerebralis from known positive samples of fish head homogenates and spiked sediment and water samples.
4.2	Sediment and water sampling procedure	complete	
4.3	Sample assessment	complete	With both a test and a sampling procedure in hand, we have undertaken our own sampling of various sites across Alberta to establish a baseline for where whirling disease is located. This has been developed in parallel with the AEP sampling strategy which was

Task #	Task Description	Status	Comments on Tasks and Milestones
			contracted out and included sites that we tested using our qPCR assay. In total, we samples over 700 sites throughout Alberta as part of this program.
Task 5	Transition to field qPCR		
5.1	Assessing reliability of field PCR compared to core facilities	complete	We have found that portable qPCR platforms tend to have a 1-log lower sensitivity than their core facility counterparts.
5.2	Assessing reliability of 'citizen scientists'	complete	From our 2017 and 2018 data we have a high degree of confidence in our citizen scientist training methods and the data that they generate using our tests.
5.3	Implement field qPCR tests	complete	Having run four field qPCR units in 2017 and 2018, we have now trialed units with AHS, AEP, Aquality, ALMS, Freshwater Solutions, The City of Edmonton, and at one school in Southern Alberta. Each of these partners continues to be interested in building this community-based monitoring program.
Task 6	Online public engagement	complete	We have an established site, www.swimmersitch.ca, that reliably connects with over 100,000 unique user IP addresses each spring/summer. It targets informing the public about recreational water hazards.
Task 7	Beach outreach	complete	Each spring/summer we place one MPH student with ALMS to assist with beach outreach. This student focuses on engaging with the public on rec water issues of a biological nature: invasive species, biological hazards, etc.

Methodology and Project Results:

Cyanobacteria qPCR assay

We have completed validation work for two different cyanobacteria qPCR assays. One targets the *mcyE* gene, which is responsible for producing the microcystin toxin that makes many cyanobacteria blooms in Alberta a health risk. The second test is designed to detect a region of the 18s gene of cyanobacteria, and it is meant to serve as a surrogate for the traditional cell count assay. The 18s test reflects total cyanobacteria in a sample, which the *mcyE* test reflects the fraction of cyanobacteria that are able to produce microcystin toxin.

The *mcyE* assay can detect 5 copies of the *mcyE* gene in a water sample, which means that we can detect as few as 5 cyanobacteria. Either of these assays can easily detect exceedance of either official recreational or drinking water thresholds for cyanobacteria (cell count or toxin). Moreover, we have found that our *mcyE* assay results match well with microcystin toxin levels measured in water, with a R^2 value of 0.84 comparing our assessment of *mcyE* gene abundance in a sample with microcystin toxin levels measured by ELISA.

To date, we have validated the qPCR assay for the *mcyE* gene using cataloged water samples from Pigeon lake and using samples collected from across Alberta. One of our more targeted collecting sites is Hawrelak Park lake, which has had microcystin producing cyanobacterial blooms for the past three seasons. These blooms have preceded the International Triathlon Union (ITU) triathlon event that is held in the lake each year. This lake is unique in that it is chlorinated each year prior to the ITU event, and this chlorination has been used to clear the lake of cyanobacteria for the past two years. This can be a risky endeavor however, as killing the cyanobacteria can release the microcystin toxin, exacerbating the risk associated with using the water for recreational activities. In our sampling this year, we sampled three locations at Hawrelak Lake as well as the area that is protected each year from the chlorination (natural area). Hawrelak Park lake was tested for total cyanobacteria signal as well as for the *mcyE* gene. All sample times points were negative for *mcyE*, but, a strong total cyanobacteria signal was detected, particularly in June. This emphasizes the point that blooms can be present but produce no microcystin toxin (Figure 1).

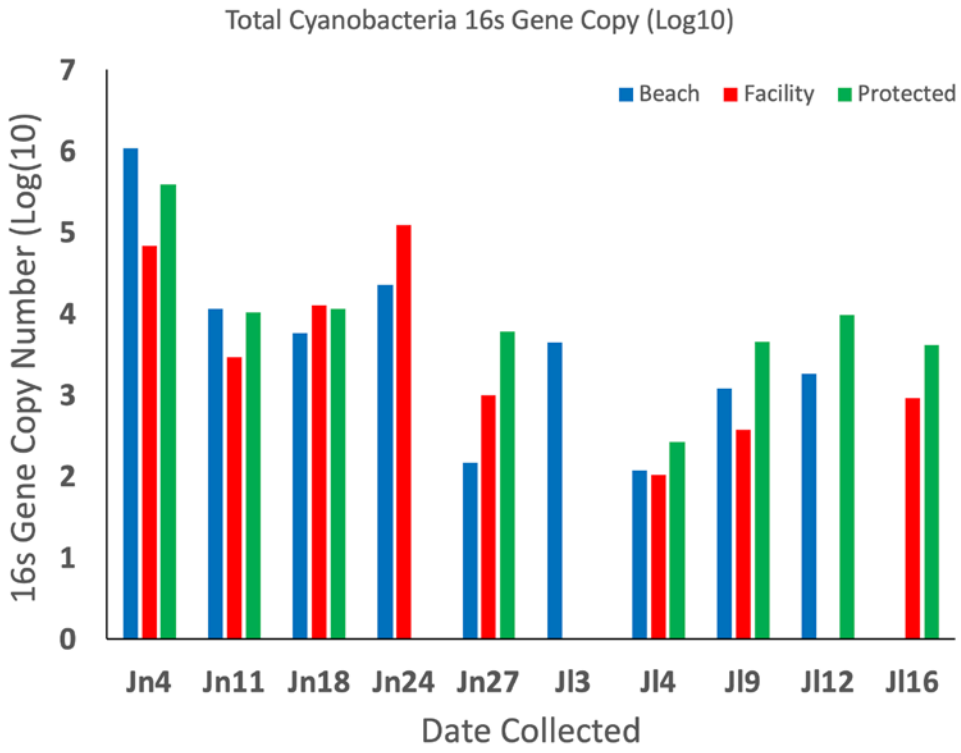

Figure 1. Total cyanobacteria 16s gene abundance in 50 mL water samples collected from Hawrelak Park Lake (beach, facility and protected natural area).

Swimmer's itch qPCR assay

One of the most important developments stemming from the swimmer's itch qPCR test has been the collecting method that was designed to isolate small, multicellular parasites from the water. This collecting approach has been utilized for collecting larval mussels and whirling-disease causing parasites. This technique involves passing a known quantity of water though a 20µM zooplankton collecting net, and then further filtering that sample using a 0.2µM filer which is then placed in 100% ethanol. The entire filer is then homogenized along with all the sample using microscopic silica beads. This technique ensures reasonable extraction of DNA from the sample, regardless of how 'dirty' the filtrate is.

Using the above approach and qPCR assay, we have followed our plan to assay water samples collected in both Alberta and Michigan for swimmer's itch causing parasites. To date, we have completed assessed over 300 water samples collected from Alberta lakes and over 1,500 samples collected from ten lakes in Northern Michigan, where swimmer's itch is the most prominent recreational water issue. In 2016 the Michigan State government allocated $750,000 over three years to control and monitor swimmer's itch. Our partner company Freshwater Solutions (FWS) has been contracted by a number of lake communities to attempt control efforts for swimmer's itch by relocating the primary avian host of the parasite there, the common merganser. Our assay has become central to confirming the efficacy of this control effort, providing FWS and these lake communities with data related to the abundance of parasites in

the water before and after control. While this work in Michigan consumes none of our AI funding, we receive significant benefit from this partnership. First, this is an ideal venue for us to implement our test. Second, the tests run in Michigan by FWS serve as a fantastic example of how to implement qPCR on site using our portable systems and methods. Currently, two portable qPCR units are running in Michigan for swimmer's itch testing. Finally, this work has allowed us to design new qPCR assays that are specific for individual species of swimmer's itch-causing parasite, and also to publish our work on swimmer's itch.

Our published manuscripts on swimmer's itch are attached to this final report. The main highlights of these manuscripts are detailed below.

The first manuscript published from our swimmer's itch work is entitled: Swimmer's itch in Canada: a look at the past and a survey of the present to plan for the future. This article was published in the journal Environment Health, and it focused on summarizing the data from our swimmersitch.ca website. It was published in collaboration with Dr. Tyler Cobb of the ABMI and the Alberta Museum. The main message of this manuscript was to highlight the importance of utilizing multiple data sources to assess swimmer's itch risk in Canada; self-reporting, host species surveys, and public engagement are all found to be important elements of a successful swimmer's itch campaign. We present the lakes across Canada from which we have had swimmer's itch reports, and also show that based on both reports and species surveys, that swimmer's itch risk in Canada, and in particular, Alberta, is highest in July and early August. We emphasize those species of migratory bird and snail in Alberta that are important for facilitating the swimmer's itch parasites life cycles and also show that these hosts have extensive ranges throughout all of Alberta, emphasizing that any lake in Alberta could be home to swimmer's itch.

The second published manuscript focuses more on our qPCR assay and its utilization to monitor for swimmer's itch parasites in Michigan. This manuscript is entitled: Use of qPCR-based cercariometry to assess swimmer's itch in recreational lakes. This study outlines the use of our qPCR test for assessing and quantifying swimmer's itch parasites in the waters of lakes throughout Northern Michigan in collaboration with FWS. Validation of the test and confirmation of its ability to quantify parasites is described as are a series of field experiments in which we use this test to show that these parasites are found in the upper water column, typically early in the morning and often are higher in density when concentrated by onshore winds. This study also forms the foundation for two other manuscripts currently in submission. First is a study in which we use the qPCR test to evaluate whether targeted chemical treatment of a lakeshore area can be effective at reducing swimmer's itch risk. This study is summarized in a paper currently under review entitled: Evaluation of targeted copper sulfate ($CuSO_4$) application for controlling swimmer's itch at a freshwater recreation site in Michigan. The results of this study indicate that while targeted $CuSO_4$ application is able to eliminate significant numbers of snails from a site, which is what it is intended to accomplish, it does not actually significantly impact swimmer's itch parasites in the water. The second submitted manuscript reports on an update to the qPCR test. It utilizes the data we've gathered over the past 6 years, sampling swimmer's itch-causing parasites throughout North America. These specimens have allowed us to design species-specific qPCR tests that allow us to identify specific species of parasites causing swimmer's itch in an area. This manuscript is entitled: Parasite source tracking: four species-specific Trichobilharzia qPCR assays deployed in recreational water yield insights into host-parasite dynamics. Within, we

detail the validation of these four new tests and use them to break down those species of parasite that have been causing swimmer's itch in Michigan and Alberta.

Finally, our last published manuscript is entitled: A fine-scale phylogenetic assessment of digenean trematodes in central Alberta reveals we have yet to uncover their total diversity. This manuscript represents a culmination of all of our past AI support. It summarizes the phylogenetic work we've done over the past 6 years to identify as many of the species of parasitic flatworm in Alberta as we can. This manuscript identifies a number of new species from our sampling and suggests that we have over 79 different species of parasitic flatworm in Alberta. This work is now being leveraged in a new study to determine whether we can now exploit our understanding of these parasites to more easily assess the biodiversity of their hosts at reclaimed wetlands in Northern Alberta.

The move towards being able to accurately identify a specific species of swimmer's itch-causing parasite from a single water sample is emphasized by data that was collected in the last year of this project. This longitudinal and multi-site assessment of three species of swimmer's itch causing parasite on three lakes gives us unprecedented resolution of where and when swimmer's itch is a risk (Figure 2).

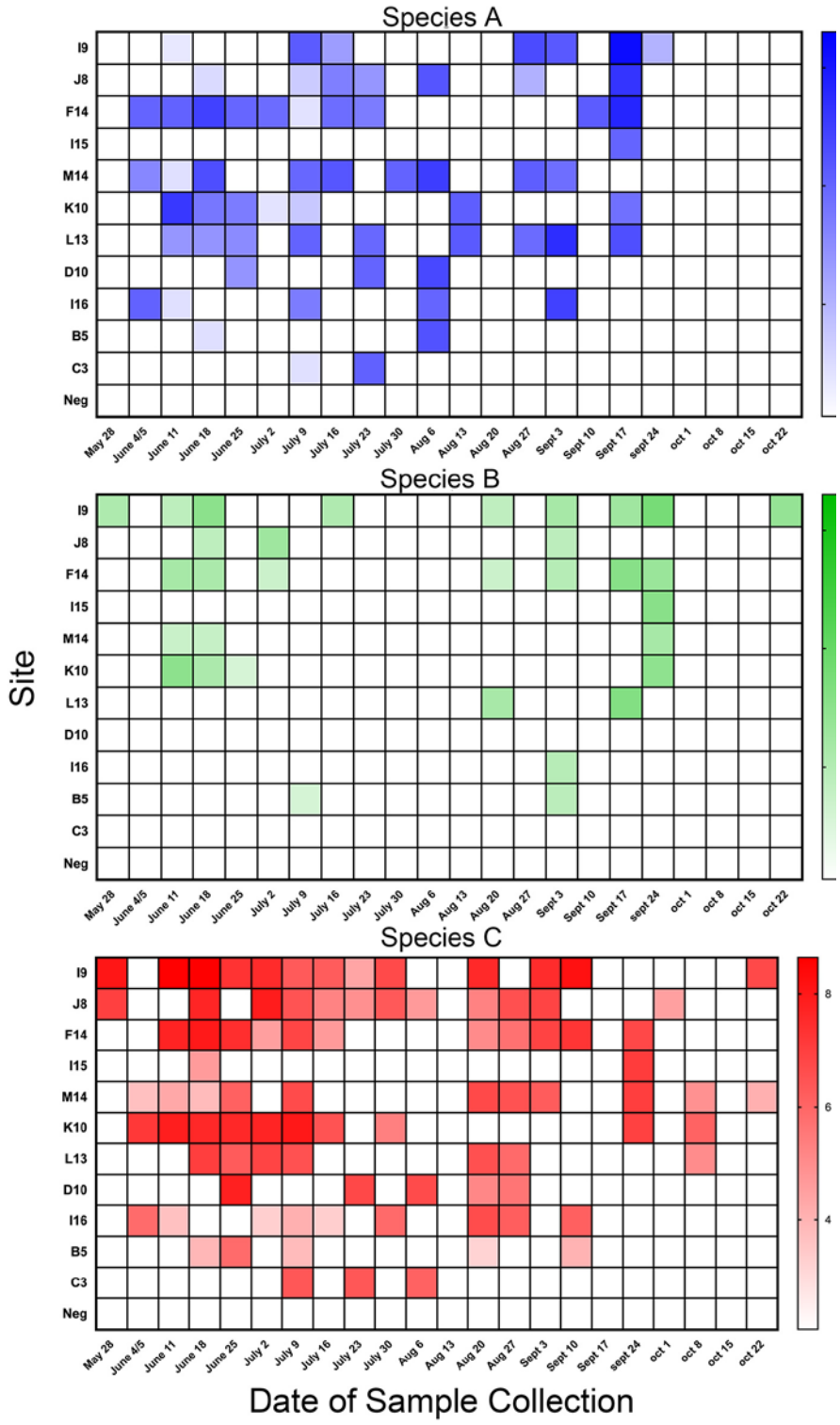

Figure 2: Spatio-temporal assessment of three species of swimmer's itch causing parasites. Species A (*Trichobilharzia stagnicolae*), species B (*T. physellae*) and species C (Schistosomatidae sp. C) were assessed using qPCR from DNA extracted from water samples taken Tuesday morning at 10am on each date indicated. Heatmap colour reflects species assessed and colour intensity reflects the relative abundance of the parasite at the site.

Larval mussel qPCR assay

We completed extensive validation of our qPCR test for larval zebra mussels. Initially, we intended to design a test that was able to detect both zebra and quagga mussels, however, this proved challenging without additional genetic information on each species. We were able to design a zebra mussel-specific test, and a quagga mussel-specific test. Of the two, the zebra mussel test passed all validation trials, while the quagga test requires some improvement.

To validate the zebra mussel test, we performed four trials. The first was to confirm that it amplified zebra mussels, this was accomplished using mussels collected by AEP as part of the boat monitoring program. Second, this test was used in an internal and blinded trial in which tap water samples were run alongside water samples from Michigan, which are known to be positive for zebra mussels. The third validation step was to confirm that the zebra mussel test displays no cross reactivity with any known species of bivalve in Alberta. Finally, with confidence in the test, we ran water samples collected as part of the AEP southern Alberta irrigation reservoir sampling project, to determine if any locations in Alberta resulted in a positive assessment using this test.

Our assessment of samples collected from Southern Alberta irrigation reservoirs in 2017 yielded some interesting results. We detected larval mussels at five sites. These results were relayed to Dr. Ron Zurawell, and initiated a more thorough investigation into these sites and our qPCR test. While we continued to detect zebra mussels in the initial positive samples, we have not detected any mussels since. To date, we have not found any native Alberta bivalves to cross react with our test, so we have no reason to doubt our results. Although we are relieved that there does not seem to be evidence to support the establishment of zebra mussels in Alberta as of yet.

Whirling Disease

Beginning in 2017, we included the development and validation of a qPCR test for the parasite *Myxobolus cerebralis*, which is the causative agent of whirling disease in salmonid fish. Our intention was to utilize this test to detect *M. cerebralis* from sediment, water and within the Tubificid worm host to complement existing testing for the parasite using fish head homogenates. Test development has been successful and our preliminary data demonstrating that this test performs well when investigating environmental samples made it appealing enough for AEP to contract to us to run environmental samples for them until the end of 2018. Inclusion of these AEP environmental samples has vastly expanded the scope of our initial Alberta Innovates project amendment (Figure 3). To date we have analyzed 424 sediment samples (13 were positive), 2046 worm samples (44 were positive) and 35 water samples (0 were positive). The AEP molecular biology team began running this test as their primary means of detecting M. cerebralis in spring/summer of 2019.

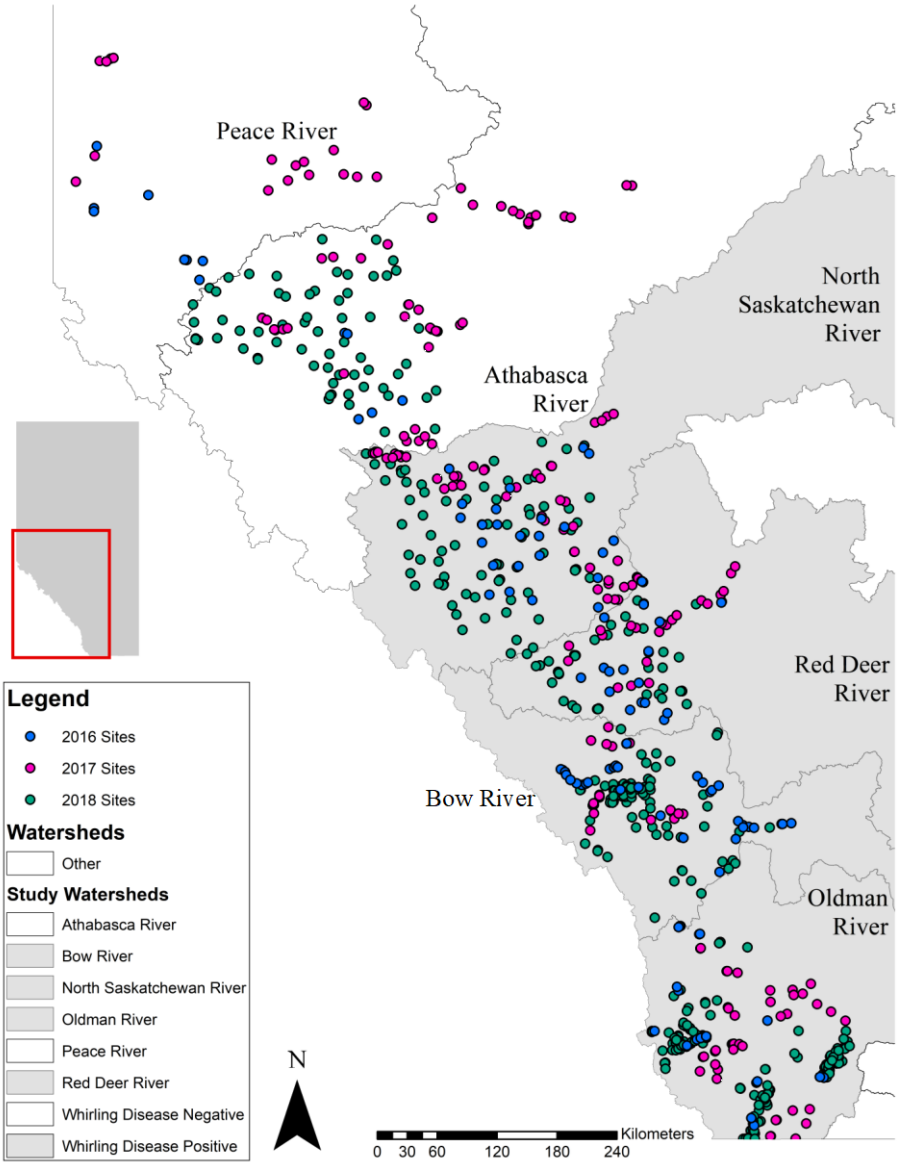

Figure 3: Lotic sample sites from 2016-2018, covering six watersheds. Watersheds declared positive for whirling disease (*Myxobolus cerebralis*) by the Canadian Food Inspection Agency (CFIA) are shaded in.

In addition to now having a comprehensive understanding of where *M. cerebralis* is found in Alberta, we also undertook a large-scale phylogenetic assessment of Tubificid worms in Alberta. This part of our Alberta Innovates project amendment was intended to facilitate a better understanding of where *M. cerebralis* transmission might be able to occur in Alberta, by determining where suitable worm host ranges are found. To date, we've used DNA barcoding of the CO1 gene to identify a total of 31 unique species of oligochaete from the sediment of Alberta rivers and streams. For each of these worms, we also have assessed their infectivity with *M. cerebrais*, and thus, we know that *Tubifex tubifex* is the primary host in Alberta (as expected) and

that it is found throughout the Province. We also discovered that another species of worm, *Limnodrilus hofmeisteri*, is also a potential host here in Alberta. It is less common than *T. tubifex* but is also found ubiquitously in Alberta (Figure 4).

Our work on developing and implementing this qPCR-based test for whirling disease is currently being written up along with AEP partners for publication in two manuscripts.

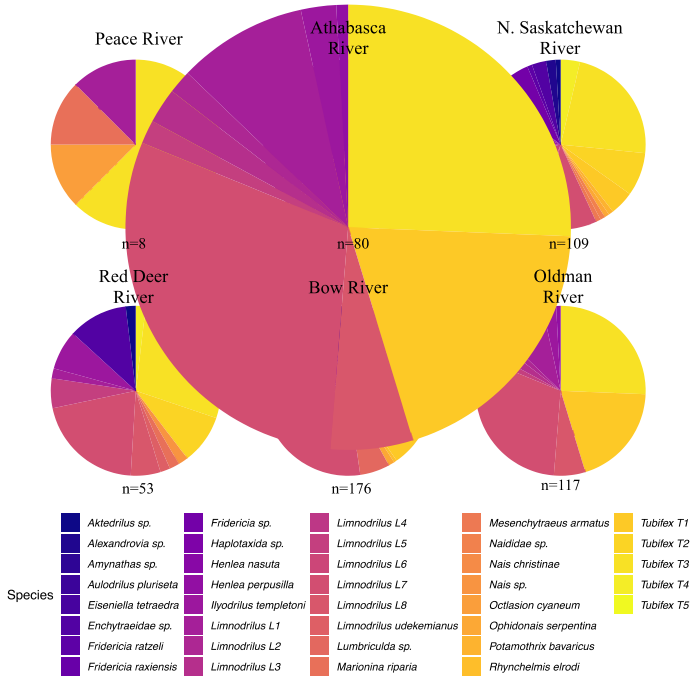

Figure 4: Oligochaete biodiversity in Alberta based on watershed.

Enteric Bacteria and Borden Park

With the validation of our qPCR tests being complete, we wanted to show real-world application of site-specific qPCR. We were engaged by Borden Park, which is a Natural Water Recreation area that is run by the City of Edmonton. Borden Park pool is unique in Canada as being the only designed swimming area that is not treated. The water flows through a series of ponds and gravity filters before re-entering the swimming pool area. As a pilot project, the Borden Park project was interested in gathering as much data on pool performance as they could this past year. The pool opened to the public in July and was immediately a success, reaching maximum capacity of 900 bathers per day numerous times. We placed a portable qPCR unit at Boden Park and trained the staff on how to test for enteric bacteria and pseudomonas prior to opening. Then, the staff ran the qPCR unit every two day until the pool closed in September. We served as quality control for their data, and worked with the City of Edmonton staff to determine how to best proceed into the future. Currently, it appears as though Borden Park will continue to monitor the pool with the portable qPCR unit, moving to a daily sampling schedule for three targets.

Online engagement

Our website, www.swimmersitch.ca, continues to serve as both an information disseminating and gathering resource. 2016 was the first year in which AHS agreed to utilize reporting data from this website as a way to assess swimmer's itch risk at Alberta lakes, and agreed to post warning signs at lakes that exceeded 5 reports over 2 days. The website continues to gain a user base, exceeding over 100,000 unique IP address visits in 2018 and 2019.

Key Learnings:

The primary and secondary objectives of this project were met. In addressing these objectives, we have learned a great deal about the advantages of DNA-based water monitoring, particularly when envisioned in a decentralized network that allows for sample collection and partial analysis to take place at the site of sample collection. Data gathered using our portable qPCR approach has allowed us to learn a great deal about many of the organisms that we chose to monitor for as part of this project. Finally, we have concluded that community partnership is a reliable and logical way forward for DNA-based water monitoring efforts, particularly when community partners are integral to planning the project. This approach built reliable and long-term partnerships that can generate far more data related to biological targets in water than could an equally funded centralized water monitoring system. Moreover, engaging public stakeholders builds trust and ensure water literacy amongst the stakeholder group most impacted by recreational waters.

Project Outcomes and Impacts:

- We designed and validated qPCR tests for cyanobactera, cyanobacteria toxin *mcyE,* avian schistosomes (causative agent of swimmer's itch), five different species of swimmer's itch causing parasite, zebra mussels and the whirling disease causing parasite *Myxobolus cerebralis.*
- Each of the above tests was implemented in a context-specific way to advance our understanding of the target organism in Alberta and recreational water in general.
- The results of this project have emphasized the utility and reliability of site-specific qPCR assessment of water and have garnered interest from a number of partners; AH, AHS, AEP.
- This is first example of qPCR monitoring in the field for specific organisms of interest from a recreational water health and environment perspective.

Outcomes and Impacts:

Training of HQP

Name	Institution	Level	Period	Thesis Title/Project	Status
Sydney Rudko	University of Alberta	PhD	5 years	Development and validation of portable qPCR tests for detection of recreational water hazards	Complete
Michelle Gordy	University of Alberta	PhD	6 years	Examination of the diversity and assembly processes of digenean trematode communities in Alberta, and the implications of spatio-temporal community dynamics on swimmer's itch transmission in recreational lakes	Complete
Danielle Barry	University of Alberta	MSc	2 year	Detection of whirling disease from environmental samples	In progress
Cerina Lee	University of Alberta	MPH	2 years	Public engagement and beach outreach with ALMS	Complete
Emily Buss	University of Alberta	MPH	1 year	Rec water economic value assessment	Complete
Alyssa Turnbull	University of Alberta	MSc	1.5 year	Identification of Tubifex worm species in Alberta	In progress
Leah Brummelhuis	University of Alberta	Technician	1.5 year	Optimization of sediment DNA extraction	Complete
Lily Han	University of Alberta	Technician	1 year	qPCR analysis of environmental samples for M. cerebralis	Complete
Arnika Oddy-van Oploo	University of Alberta	Technician	1 year	Non-lethal sampling for whirling disease using sediment	Complete

Benefits:

Economic benefits

Ambient recreational water resources in Canada are incredibly valuable. For example, in 2010 recreational fishers contributed a total of $8.3 billion to various local economies in Canada, and estimates of the total annual value of recreational water sites in Alberta are range between $500,000 and $1 million per year, per site. Biological hazards associated with these waters are the most frequent reasons that they are made inaccessible to the public, and therefore, these hazards represent the potential for significant economic loss. Cyanobacteria and swimmer's itch led to the posting of over 25 lakes in Alberta in 2015 and 33 lakes were posted in 2016, reflecting 1,616 lost days of recreational use in 2015, and over 2,320 lost days of recreational use in 2016. These postings often last until the end of the season and thus have significant impacts on local businesses, municipal and provincial events, tourism to Alberta parks and property values at summer villages. Our analysis of the economic value of recreational lakes in Alberta suggests that many Provincial parks and summer village-associated beach access areas are worth in excess of $500,000 annually, making lake recreation areas an important economic factor to local and Provincial economies. From an economic standpoint, a number of benefits will arise from accomplishing this project.

1. Reduced cost of water sample testing. The exact costs associated with existing monitoring efforts for recreational water are difficult to accurately determine. However, what is certain is that for each water sample, multiple tests are carried out for assessment for each specific hazard. This fragmentation carries with it increased costs and time to result, and requires a prioritization of certain hazards over others. Most of our tests are designed to run in a dehydrated matrix with or without DNA extraction, which eliminates significant costs, to the point where we have budgeted for as low as $2.50 per test. Ultimately, this means that one could assess a water sample for up to 16 targets (minus necessary controls), retaining the high specificity and sensitivity of qPCR-based testing, without adding significant costs or requiring specific expertise for different analyses. We expect that adoption of these tests, and this testing approach will lead to a reduction in costs associated with monitoring to a large extent.

2. Reduce the time that beaches are closed. Currently, cyanobacterial blooms will typically lead to the closure of a lake to recreational activities. Often the entire lake is closed because blooms are mobile, and can be very transitory, appearing and disappearing from one part of a lake, only to arise elsewhere. For certain lakes in Alberta, this can mean that a lake is closed for almost the entire peak recreational season. Lake Isle for example was closed in 2015 from June 16th until the end of the season, and Pigeon Lake was closed on July 8th. For traditionally busy recreational lakes, with active summer villages, such as Pigeon Lake, these closures represent significant loses to the local economy and the Provincial economy due to lost park fees, low recreational traffic and a reduction in non-water based recreational activities. We propose that this approach to the protection of the health of recreational water users is not ideal, and that it can be improved by implementing a more active monitoring plan. Near-real time monitoring of recreational water hazards would allow for the closure of specific sites that represent a health hazard, and would facilitate the re-

opening of sites that were once positive, and then test negative for a particular hazard. While this approach relies on a more active monitoring approach, our tests greatly reduces time investment for testing and monitoring, offsetting the increased time spent sampling. Ultimately, we expect that implementing this type of monitoring program better protects health, and greatly reduces beach closure time.

3. Improved understanding of the economic and health value of ambient recreational waters in Alberta through the undertaking of a comprehensive economic assessment.

Environmental benefits

Significant events like cyanobacterial blooms, or the introduction of invasive animals to an ecosystem can have measurable, negative impacts on a recreational water area. Cyanobacterial blooms that result in the establishment of toxin-producing species of cyanobacteria can alter entire freshwater animal communities by creating inhospitable environments, and altering the selective pressures on resident plants and animals. Invasive species also impact upon local ecosystems by altering habitat, outcompeting local species for resources and, in some cases, infecting and killing local wildlife. The parasite that causes whirling disease is Canada's most recent invasive parasite, having been identified in Johnson Lake (Banff) in late 2016. In past locations where this parasite has been introduced, it has had a devastating impact on local salmonid fish, impacting recreational fishing and also fish populations, including those at risk. Moreover, there is mounting evidence suggesting that parasitic flatworms can serve as sentinels for environmental change or water toxicity. Thus, possessing a comprehensive dataset regarding these organisms will be useful for monitoring long-term environmental changes. Accomplishing the goals of this project will yield a detection test for these organisms that will enhance the gathering of detailed information on emergence and establishment of toxin-producing cyanobacteria as well as invasive species of mussels. The information gathered as part of completing the aims of this project as well as by implementing these tests as part of ongoing monitoring will lead to a number of environmental benefits.

1. The ability to monitor for invasive mussels using a sensitive and specific test. This will allow for identification of water bodies that may have become infested, before any adult mussels can even been seen by eye, thereby greatly improving the likelihood that interventions, like molluscicide, will be successful and prevent establishment of these invasive organisms.
2. Detect and track whirling disease in Alberta to best protect watersheds that are still not infested by this parasite, and implement best practices to minimize movement of the parasite throughout Alberta.
3. Generate data related to the environmental factors that influence the development of toxin-producing cyanobacterial blooms.
4. Identify how environmental features at a recreational water site influence the presence of swimmer's itch causing parasites by comparing snail-shedding parasite data to measurements of larval parasites in the water.

5. We now have a sensitive and specific test that can be used throughout Alberta for monitoring the progression of Whirling Disease. This test is currently being used by AEP as part of their Provincial monitoring effort.

Social benefits

1. Health protection and promotion: The primary objective of this proposal is the development of improved monitoring tests for recreational water. These tests are able to deliver more accurate and sensitive assessments of recreational water safety/quality in a shorter time frame than currently employed approaches, thereby ensuring that unsafe conditions are recognized quickly so that the health of recreational water users can be protected effectively.

   Also within the scope of this project is an aspect of public engagement, education and risk communication. The detection of a recreational water hazard only protects the health of a recreational water user if the presence of the hazard is communicated effectively, to the correct audience, and to an audience that is informed. Thus, it is essential that we ensure that recreational water users understand hazards like swimmer's itch and cyanobacteria, and are informed as to how and why they pose a risk to health.

2. Improving the value of recreational water resources: Beyond the economic value of recreational water sites in Alberta, there is also a societal value associated with water. In a survey of North Americans (primarily in the US) water-based activities such as swimming, boating and fishing, were the three most frequently mentioned outdoor recreational activities. Moreover, respondents almost unanimously indicated that undertaking other types of outdoor recreational activities such as hiking or camping was more pleasurable if it took place near water. Outdoor recreational activities are known to positively influence both individual and community wellbeing, with clearly demonstrable benefits to enjoyment, fitness and health, social interaction and stress relief, stemming from such activities. Thus, negative impacts on recreational waters and the ability of individuals and communities to utilize them is expected to negatively impact wellbeing and health. It is therefore advantageous to ensure that the approaches to assessing the health and safety of recreational waters are optimized to ensure the safety of recreational water users, and the ability to access these resources. This approach is the foundation of our monitoring tests and it drives our interest in developing and seeing implementation of better tests for measuring recreational water hazards.

3. Strengthening stakeholder involvement: There are numerous stakeholders with ties to recreational water use, safety, risk assessment and monitoring. Certain groups of these stakeholders, such as AHS/AH and AEP have established recreational water monitoring and safety as priority areas, others, have historically been less engaged. For example, public users and local communities range in their level of interest and engagement on issues pertaining to recreational water. Community associations such as the PLWA are highly engaged, whereas the average beachgoer is often unaware of the types of recreational water risks that exist. An important aspect of this project is to encourage and diversify the approach to engaging the public on recreational water issues. Our past experience in this area has led us towards a two-sided approach to involving the public, the first is increased online presence and accessibility

via websites and apps focused at knowledge translation and data dissemination/collection. The second approach is via direct engagement at beaches, an approach that has been working incredibly well for ALMS in past years. This type of beach outreach specifically targets beach users with the intention of providing them with relevant information on recreational water issues, and providing them information on how they can become better engaged. Both approaches are central to our knowledge translation objectives in the project.

Recommendations and Next Steps:

The successes of this project demonstrated that qPCR can be implemented at the site of sample collection, and that a single environmental sample can serve as the template for numerous tests. Samples collected as part of a cyanobacteria monitoring effort, or an invasive species monitoring effort can be tested for other targets; this is one of the primary strengths of qPCR-based environmental monitoring. This means that we can learn far more about biological targets in our environment if samples are collected and processed with molecular testing in mind. It also suggests that we can rely on a wide range of partnerships with industry, government and the public to collect and process samples for later DNA-based testing.

Important extensions of this project include ensuring that partnerships made with GoA, industry and the public continue to work towards the goal of improved environmental monitoring. Tests developed during this project are available for others to use and have already garnered interest by commercial testing labs for use in invasive species monitoring programs. Moreover, these tests can be used by GoA monitoring efforts to build upon existing programs. It will allow existing programs to unify around qPCR as a method for testing for biological targets in water. This aligns with transitions that AHS have already made to integrate qPCR-based testing into their recreational water monitoring programs.

Knowledge Dissemination:

Scientific Publications:

In preparation:

- Two manuscripts focused on whirling disease work
- Two additional manuscript focused on bacterial targets (cyanobacteria and enteric bacteria)

In Preparation: HQP associated with project are underlined

Barry, D.E., James, C., Veillard, M., Brummelhuis, L., Turnbull, A., Oddy-van Oploo, A., Han, X., and Hanington P.C. Comprehensive qPCR-based monitoring for the whirling disease-causing parasite *Myxobolus cerebralis* in Alberta, Canada.

Barry, D.E., James, C., Veillard, M., Brummelhuis, L., Turnbull, A., Oddy-van Oploo, A., and Hanington P.C. Molecular assessment of the invertebrate host, *Tubifex sp.*, for the causative agent of whirling disease, *Myxobolus cerebralis,* in a non-endemic area.

McPhail, B.A., Rudko, S.P., Turnbull, A., Gordy, M.A., Reimink, R.L., Froelich, K., Brant, S.V. and Hanington, P.C. Evidence of a novel species of avian schistosome infecting *Planorbella* snails.

Rudko, S.P., McPhail, B.A., Clyde, D., Turnbull, A., Reimink, R.L., and Hanington, P.C. Weekly assessment of five avian schistosome species within the context of host relocation efforts indicates that minimal host contribution is required to sustain schistosome populations.

Submitted: HQP associated with project are underlined

Rudko, S.P., Reimink, R.R., Peter, B., White, J. and Hanington, P.C., Democratizing water monitoring: evaluation of a community-based qPCR monitoring program for recreational water hazards. Under review with PLoS One.

Gordy, M.A., Koprivnikar, J., and Hanington, P.C. Environmental and ecological factors driving trematode parasite community assembly in central Alberta lakes. Submitted to IJP: Parasites and Wildlife.

Published: HQP associated with project are underlined

Froelich, K.L., Reimink, R.L., Rudko, S.P., VanKempen, A.P., and Hanington, P.C. Evaluating the efficacy of molluscicide copper sulfate ($CuSO_4$) at reducing cercariae concentrations at a recreation site in Michigan. Parasitology Research. In press. Journal impact factor: 2.098.

Gordy, M.A., and Hanington, P.C. A fine-scale phylogenetic assessment of digenean trematodes in central Alberta reveals we have yet to uncover their total diversity. Ecology and Evolution. 9(6), 3153-3238. Journal impact factor: 2.537.

Gordy, M.A., Cobb, T.P and Hanington, P.C. Swimmer's itch in Canada: a look at the past and a survey of the present to plan for the future. Environmental Health. 17(1), 73. Journal impact factor: 3.453.

Rudko, S.P., Gordy, M.A., Reimink, R.L., and Hanington, P.C. qPCR cercariometry as a method to quantify larval avian schistosome abundance and assess environmental and biological drivers of their concentration in recreational waters. EcoHealth. 15(4), 827-839.

Gordy, M.A., Locke, S.A., Rawlings, T.A., Lapierre, A.R., and Hanington, P.C. 2017. Molecular and morphological evidence for nine species in North American Australapatemon (Sudarikov, 1959): a phylogeny expansion with description of the zygocercous Australapatemon mclaughlini n. sp. Parasitology Research. 116 (8): 2181-2198. Journal impact factor: 2.098.

Gordy, M.A., Kish, L., Tarrabain, M., and Hanington, P.C. 2016. A comprehensive survey of larval digenean trematodes and their snail hosts in central Alberta, Canada. Parasitology Research. 115: 3867-3880. Journal impact factor: 2.098.

Conclusions:

The primary goal of this project was to develop novel DNA-based tests for biological agents that were of relevant to the natural ambient waters of Alberta. These tests were to be implemented in Province-wide monitoring programs that emphasized the use of DNA-based detection methods at the point of water/sediment sample collection. Tests developed as part of this project were combined with existing DNA-based tests for waterborne and water-based organisms of interest to create a comprehensive study that focused on evaluating the utility of point-of-sample DNA-based detection, and the development of methods that allowed for reliable sample collection and processing to isolate DNA on site. The primary conclusion of this 4-year project was that on site DNA-detection can be accomplished and relied upon and that via community partnerships, a comprehensive water surveillance network could be established using DNA-based detection of biological agents in water as a unifying method. A method was developed for sampling water for microscopic, yet multicellular, organisms. Eight novel qPCR tests were designed, validated, implemented and integrated into Provincial monitoring programs to facilitate research and detection of invasive zebra and quagga mussels, the parasite that causes whirling disease (*Myxobolus cerebralis*), and five species of parasitic flatworm that can be used as indicators of organismal biodiversity. These tests were paired with traditional qPCR tests for enteric bacteria, pseudomonas and cyanobacteria to facilitate tailored research and surveillance for geographically relevant biological targets throughout Alberta. On site qPCR testing was used to monitoring remote sites for whirling disease and invasive mussels. Satellite labs monitored over 50 locations for toxin-producing cyanobacteria blooms. And, a satellite qPCR system was integrated into a near-real time monitoring program at Borden Park recreational pool to monitor water quality on a daily basis.

Ultimately, all of the primary and secondary objectives of this project were accomplished and many exceeded initial expectations. Strong partnerships were formed by the research team and numerous agencies in Government of Alberta, Non-Governmental Organizations and communities throughout Alberta. Integrating sensitive and specific DNA-based tests into water surveillance programs led to the development of incredible datasets that have advanced our understanding of the targeted organisms in Alberta freshwater environments. This approach to research and monitoring has led to the publication of six peer-reviewed publications with two additional publications in submission and three in preparation with plans for submission in 2020.

Assessment of how to implement qPCR-based testing in a field context led to the finding that, with proper training and controls, a community partner can generate reliable results that compare well to expert results. Demonstrating that community partners can yield trustworthy data using DNA-based water monitoring methods provides a way forward for implementing satellite qPCR testing across Alberta as a companion to traditional testing programs. Partnerships made during this project have opened the way to implement such a surveillance network for aquatic invasive species and health related targets associated with ambient recreational waters.