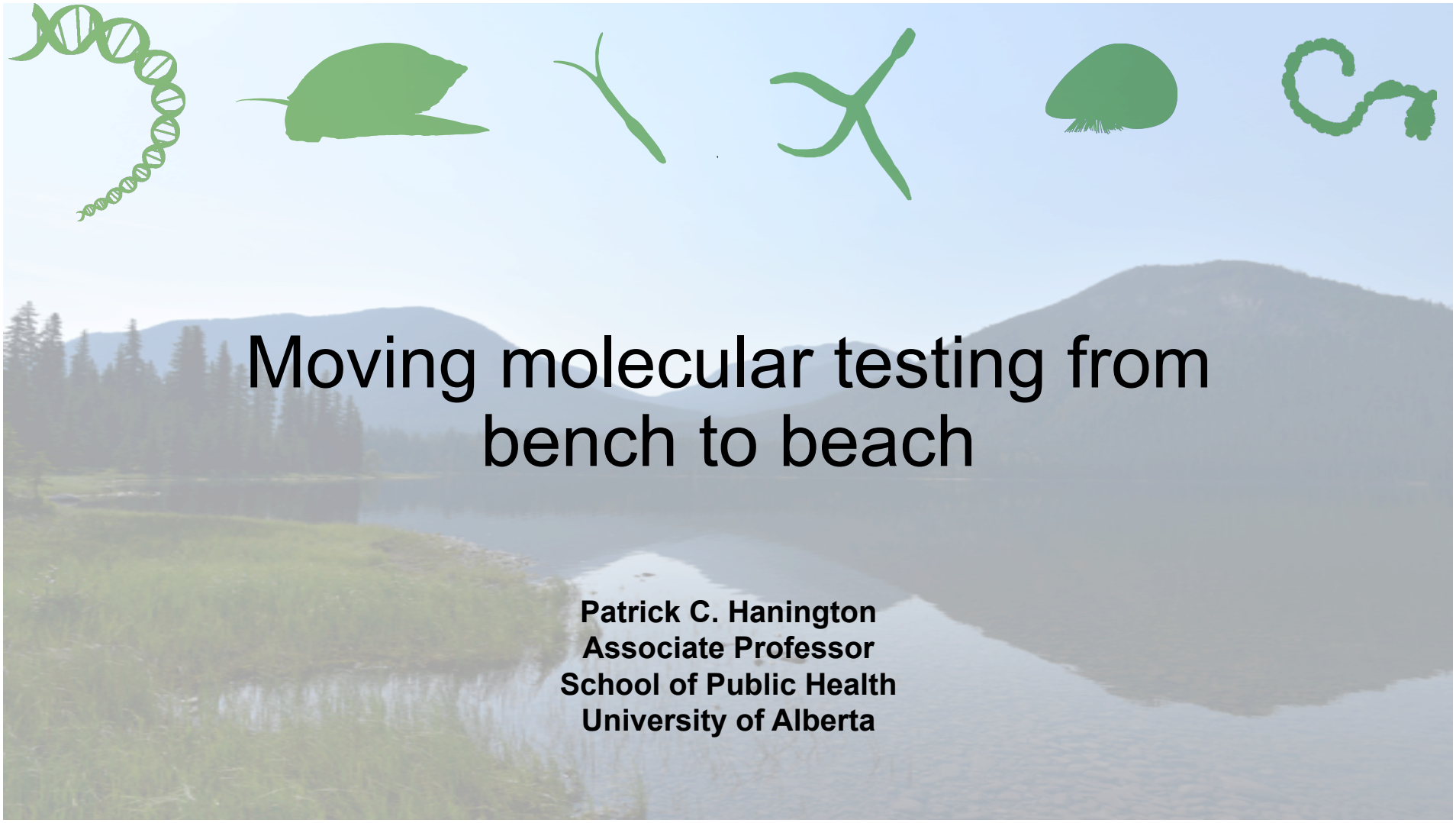


Moving molecular testing from bench to beach

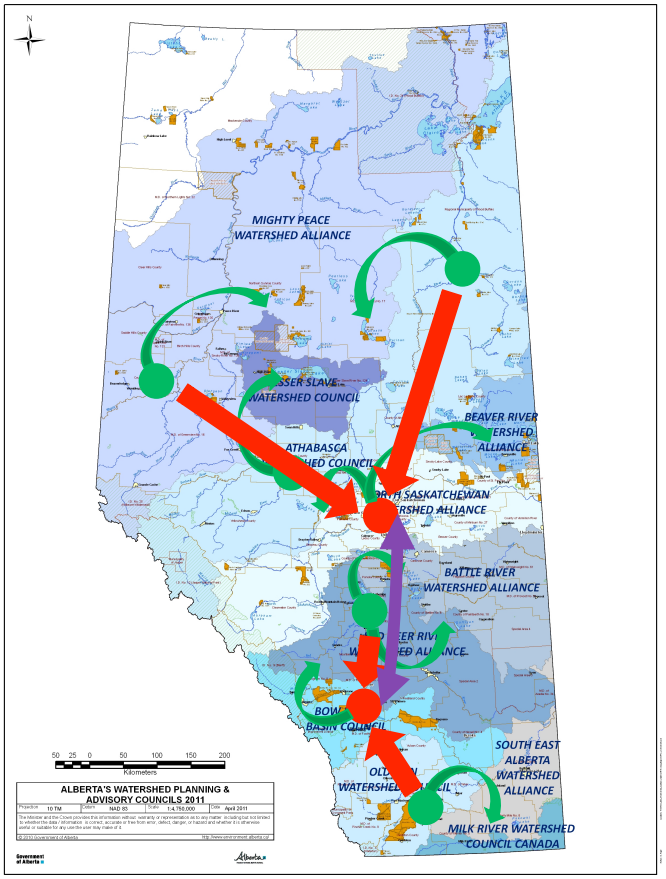
Patrick C. Hanington
Associate Professor
School of Public Health
University of Alberta

Humans are allergic to change. They love to say, 'We've always done it this way. – Grace Hopper, 1976

Monitoring natural water in Alberta: Health Example

What does this mean?

1. Testing is done by trained staff using well established tests
2. Transport conditions are important and could impact results.
3. There is up to 48 hours between sample collection and a result.
4. Different samples are collected for different types of test.
5. Results from a Monday sample are used to make decisions about the weekend.



In 2016 weekly monitoring at:
49 microbiology sites
32 cyanobacteria sites

AHS office
-samples taken weekly
-Monday or Tuesday

Transport back to Prov Lab must be within 24 hours of sample collection

Provincial Lab for Public Health

Most beach monitoring test results are reported within 24 hours of sample drop off

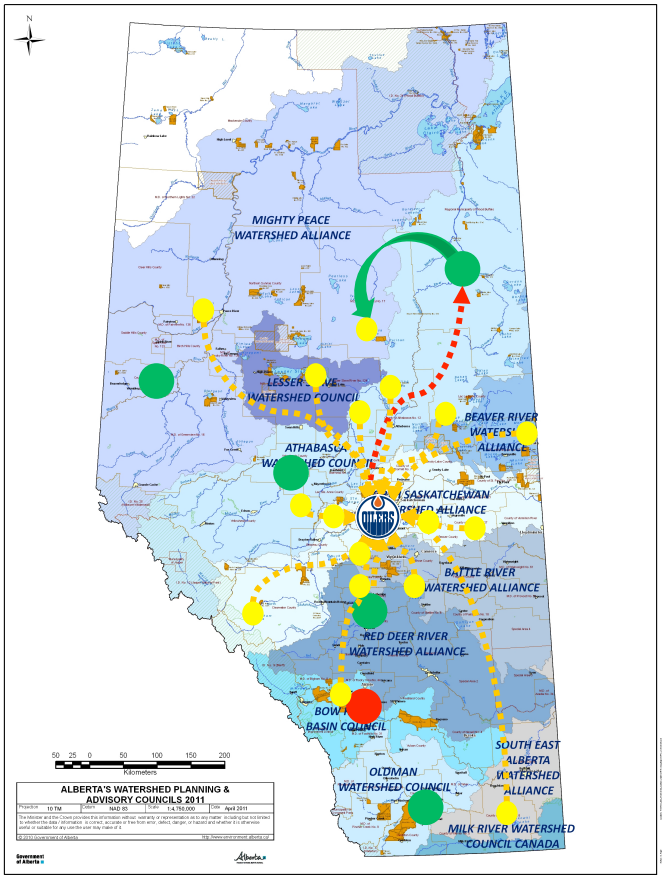
Provincial Lab for Public Health

Additional testing might take place elsewhere
eg. Cyanobacteria toxin testing

What we hope to do for monitoring methods

What does this mean?

1. Initial testing is done by a 'citizen scientist'
2. Regulatory level testing is still done by trained staff using well established tests
3. No initial transport of samples.
4. Near immediate results for preliminary test, and nearly no lag for regulatory test.
5. Many tests can be done from same sample
6. Testing could be undertaken more routinely or, close to high use times/days



Park managers, lake association volunteers, property owners, teachers/ classrooms.. anyone!

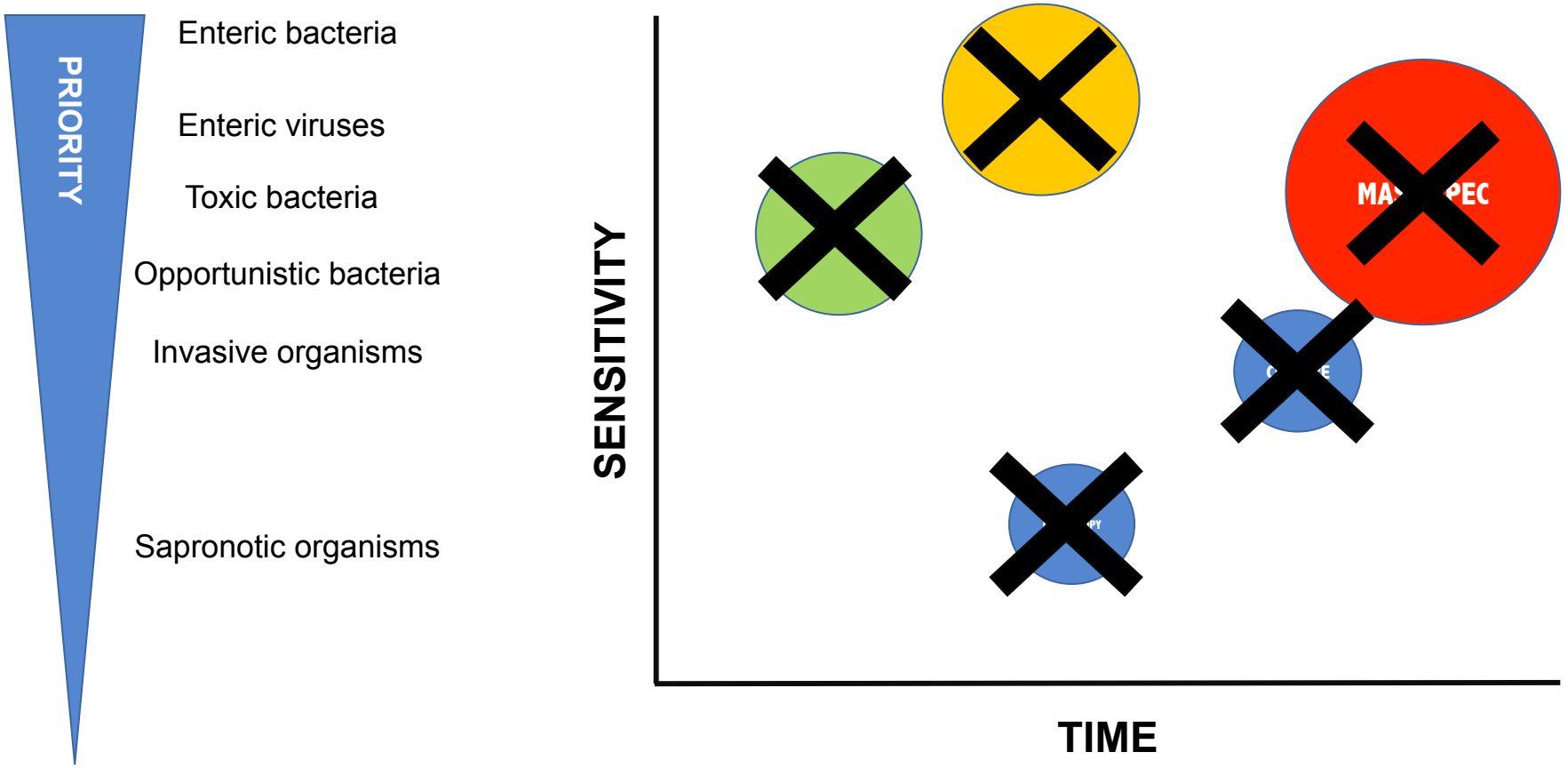
Data reported via the cloud to central labs

Locations with potential issue are then reported and sampled using traditional tests

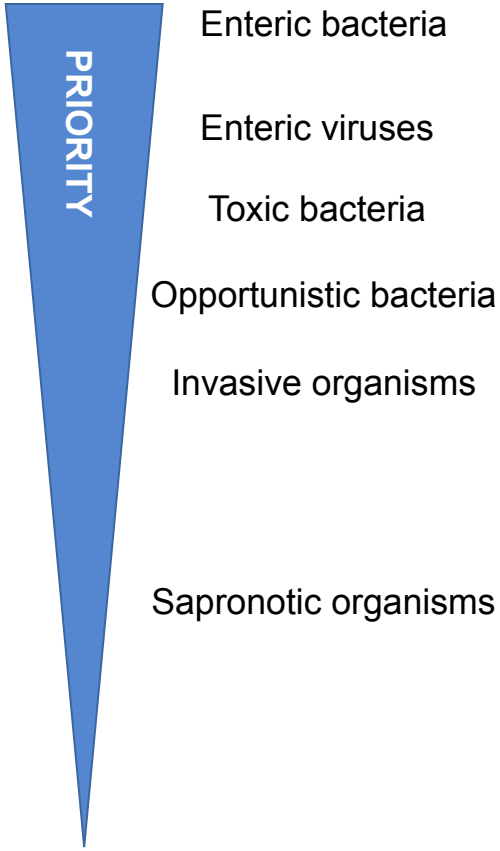
Provincial Lab for Public Health

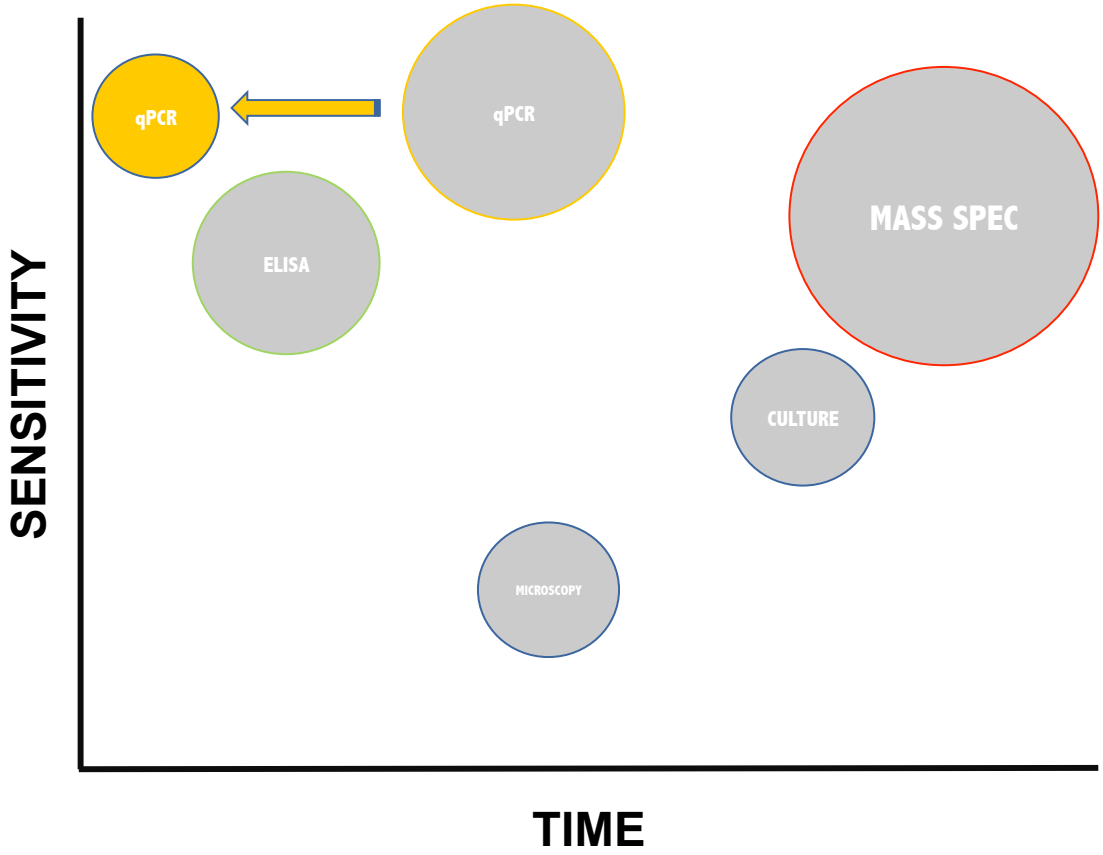
Provincial Lab for Public Health

Monitoring natural water in Alberta: Health Example

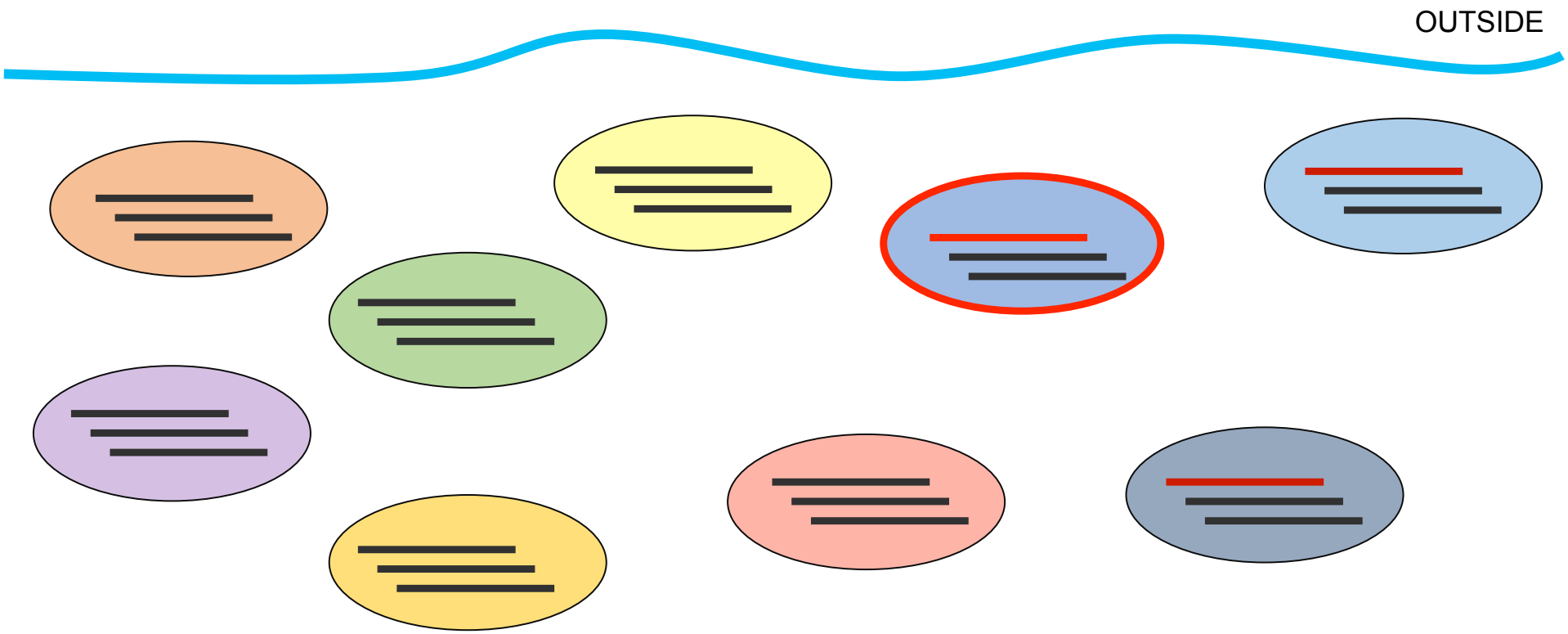


What we hope to do for testing methods



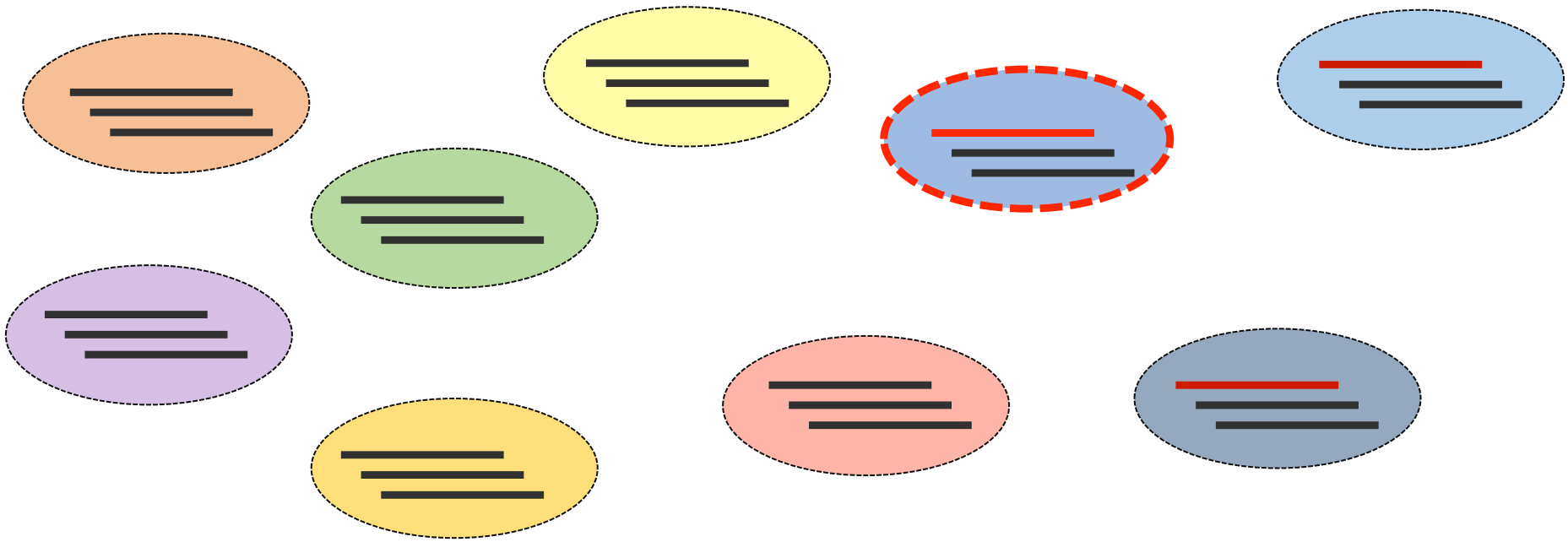


qPCR as a tool for water monitoring

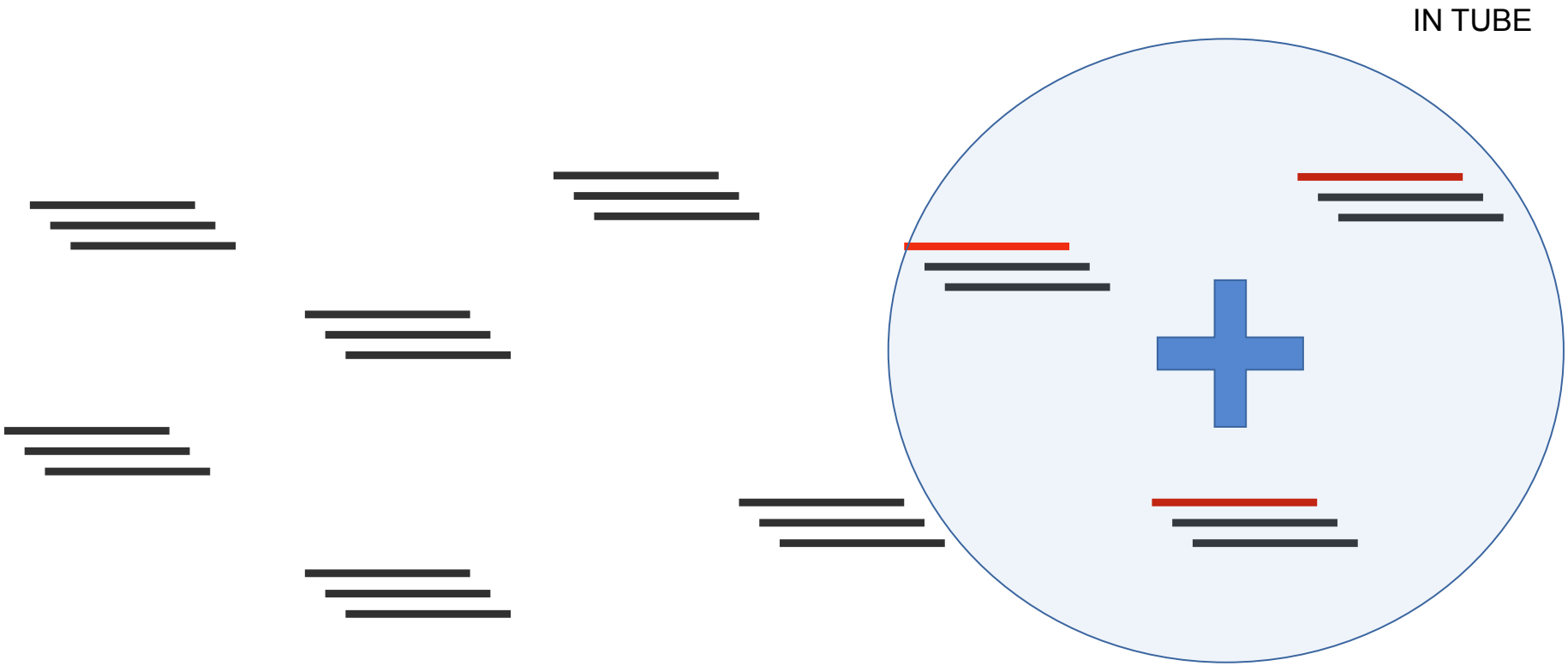


qPCR as a tool for water monitoring

IN TUBE



qPCR as a tool for water monitoring



qPCR as a tool for water monitoring

ZOOM IN TUBE

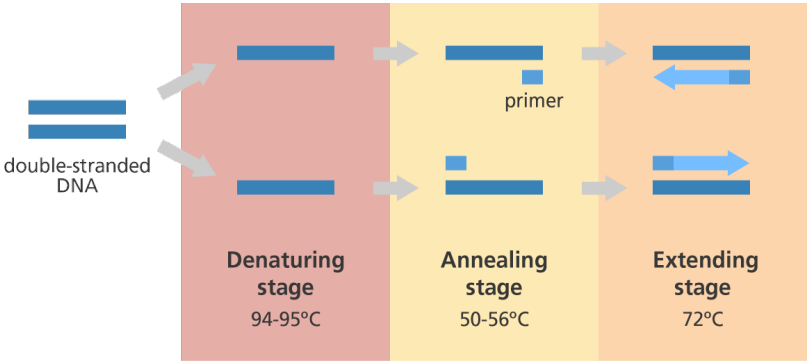
ACTTGAACGTTACGTACGATCAGTACAGTACCAA

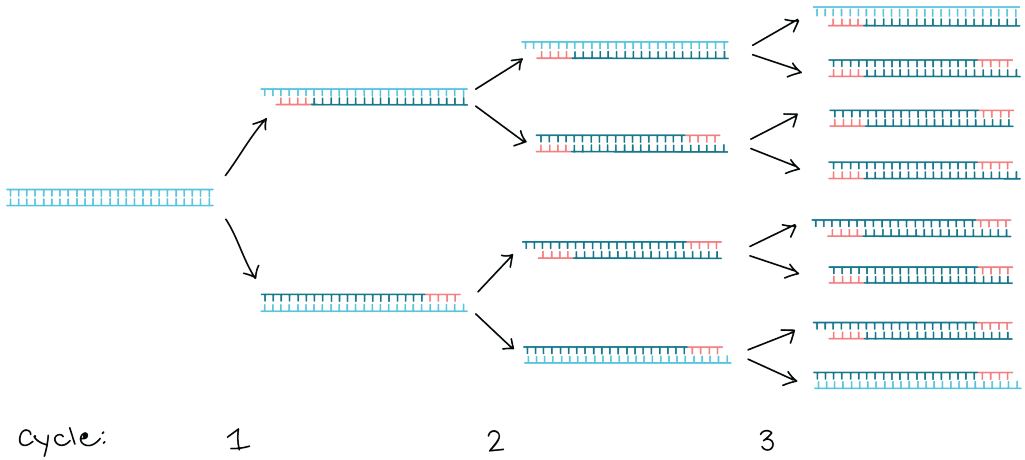
ACTTGAAC**ACA**ACGTACGATCAG**GGG**AGTACCAA

ACTTGAACGTTACGTACGATCAG**GGG**AGTACCAA

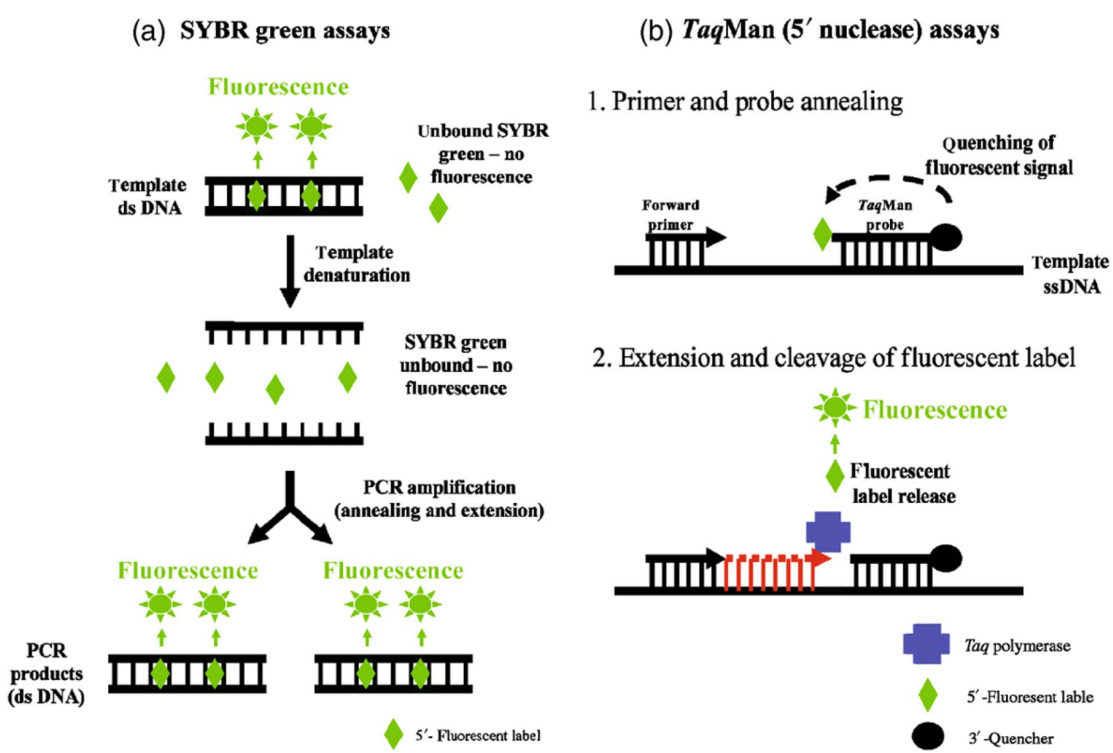
qPCR as a tool for water monitoring

- PCR emerged in 1980 as a tool for amplifying DNA in a tube (*In vitro*)
- In 1993 qPCR was developed as a way to quantify the DNA that was amplified
- qPCR has been taken up as a method for monitoring of numerous waterborne and water-based organisms





Quantitative PCR as a method for monitoring rec water



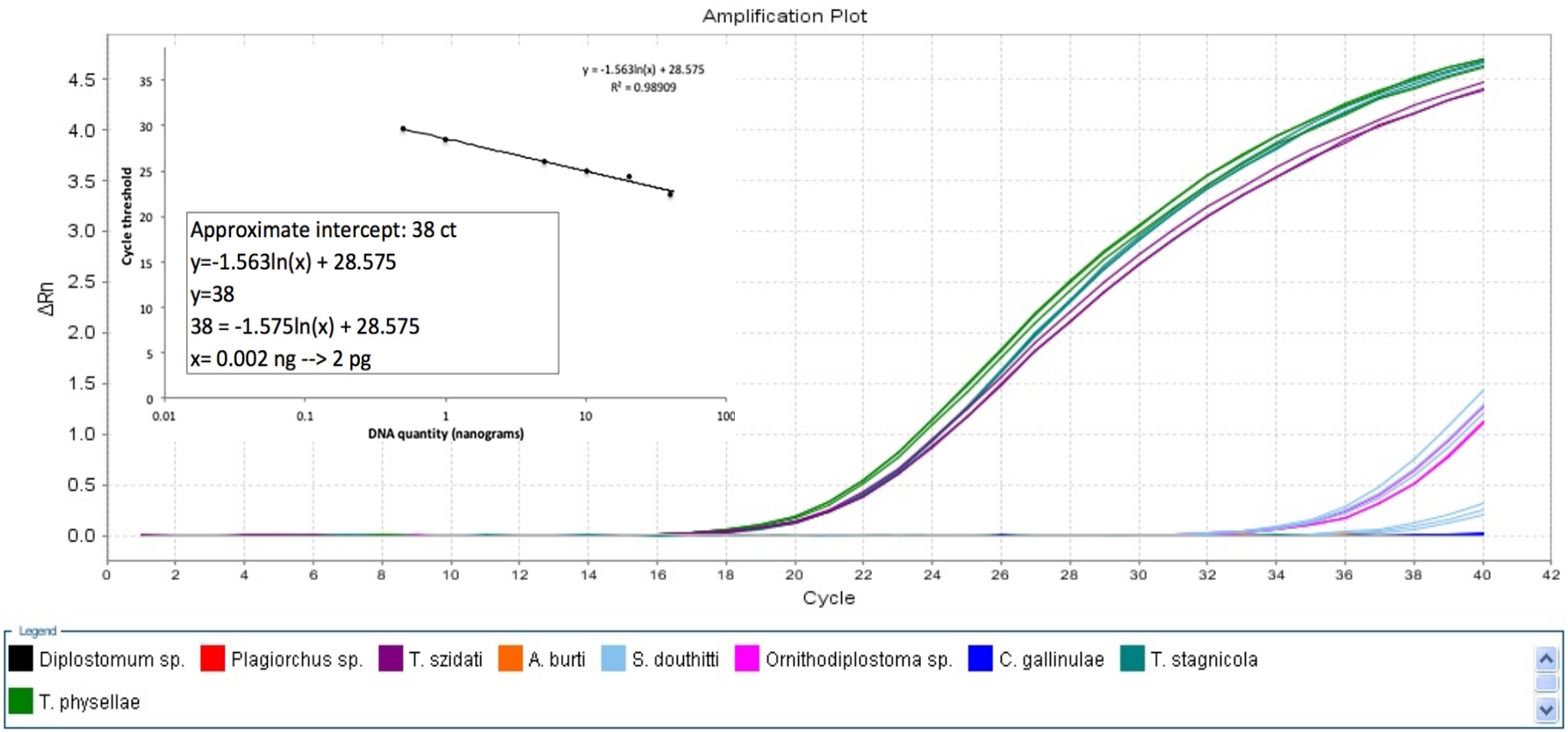
Advantages of qPCR as a monitoring method

- High specificity and sensitivity for target
- Rapid results: 1 – 5 hours
- Similar chemistries can often be run using the same thermocycling parameters
- Starting sample can be small
- Same sample can be used for assessment of multiple targets

Disadvantages of qPCR as a monitoring method

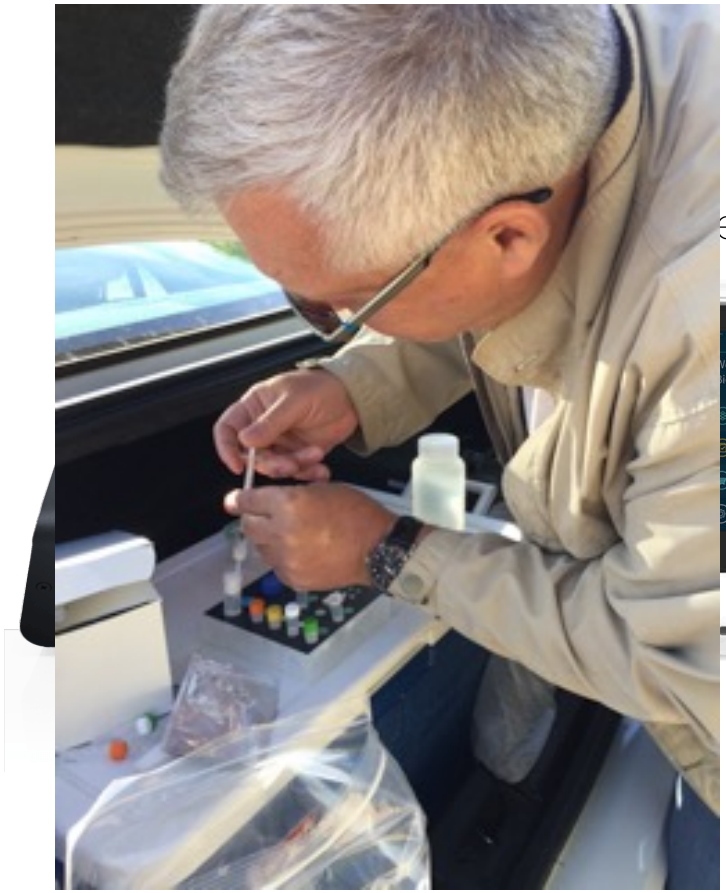
- Often requires expensive equipment
- Typically relies on core laboratory testing facilities
- Does not reflect viability or infectivity if only testing for DNA

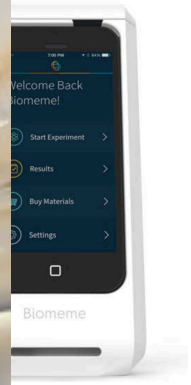
qPCR as a tool for water monitoring



Moving qPCR to the point of sample collection for near-real time assessment of hazard presence

$40k-75k
96-384 samples
Core facility





$3,700
3 samples (9 soon)
Battery powered

Challenges with field qPCR

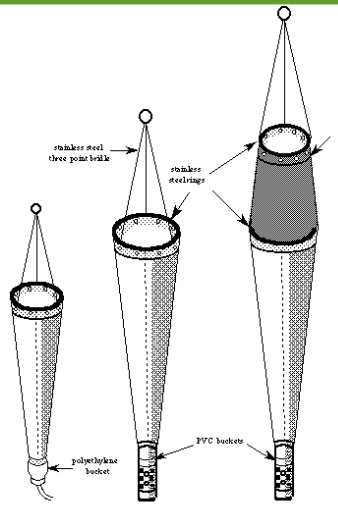
Sample collection

PCR inhibition

DNA extraction

Reaction preservation (coldchain)

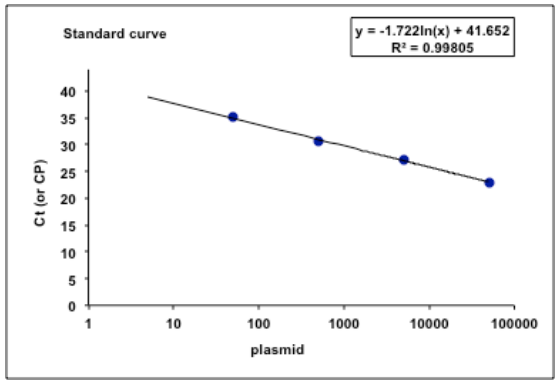
Power



Comparison of traditional core qPCR instrument and Chai platform – in lab

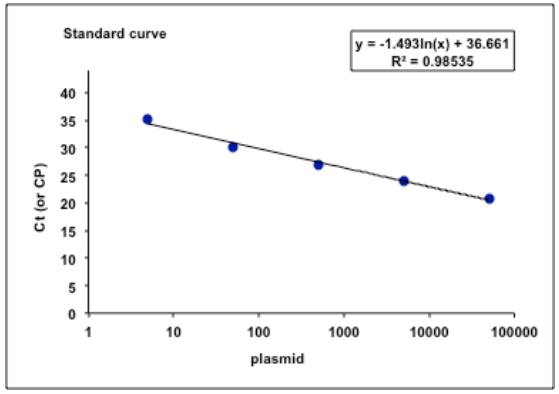
Chai

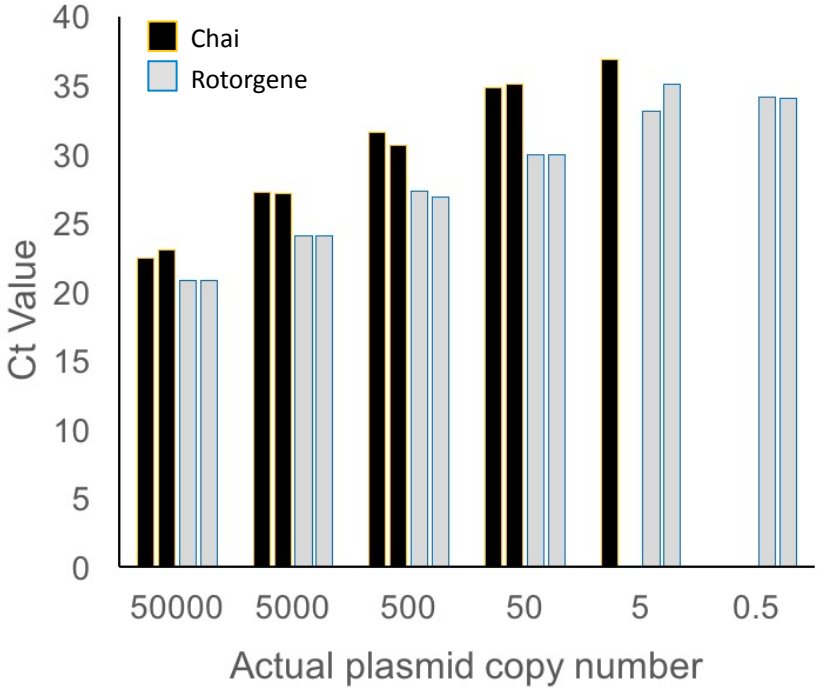
R2= 0.998
Eff= 1.6-1.7 (ideally 2)
LOD95: 50 copies



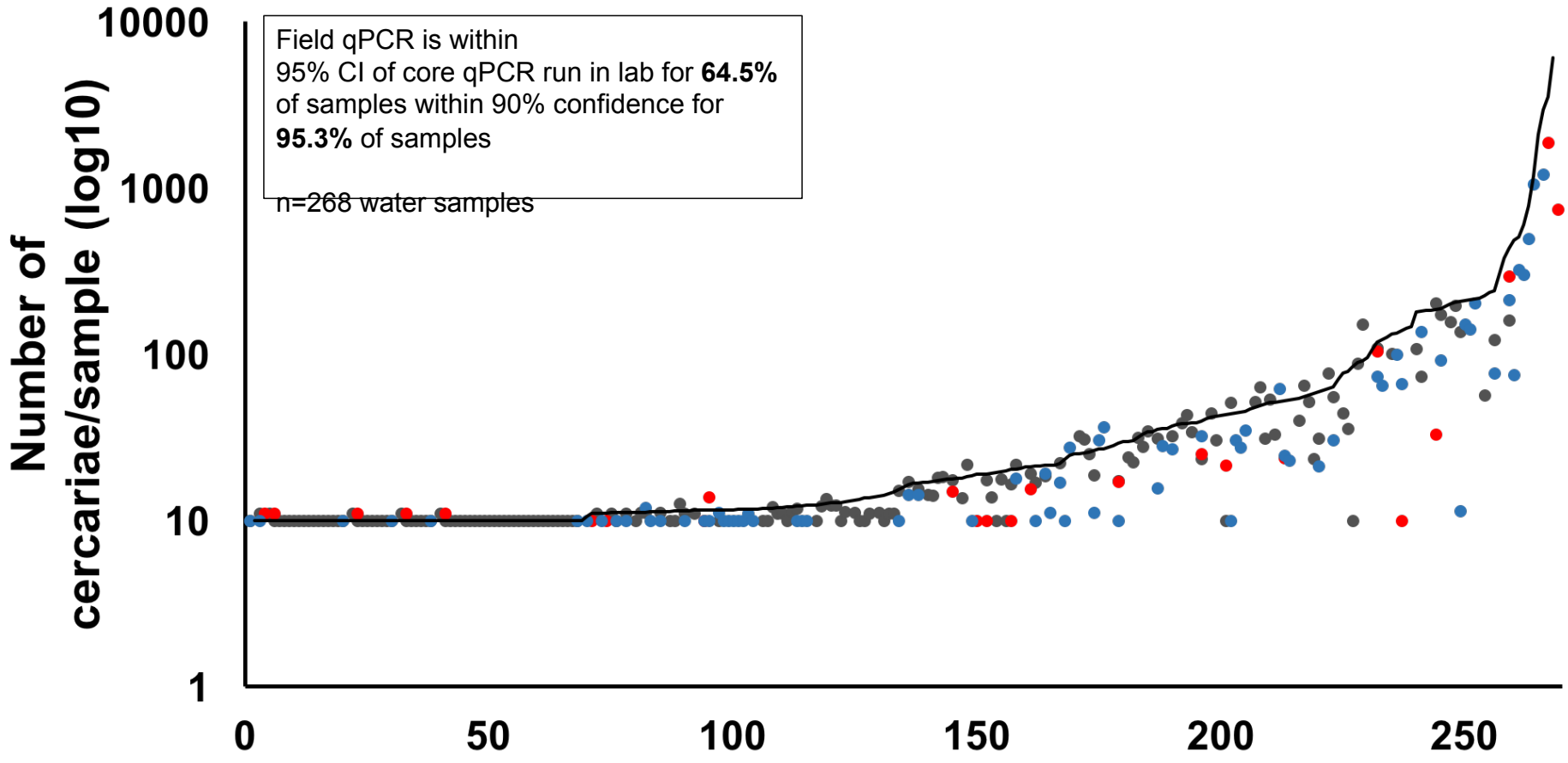
Rotorgene

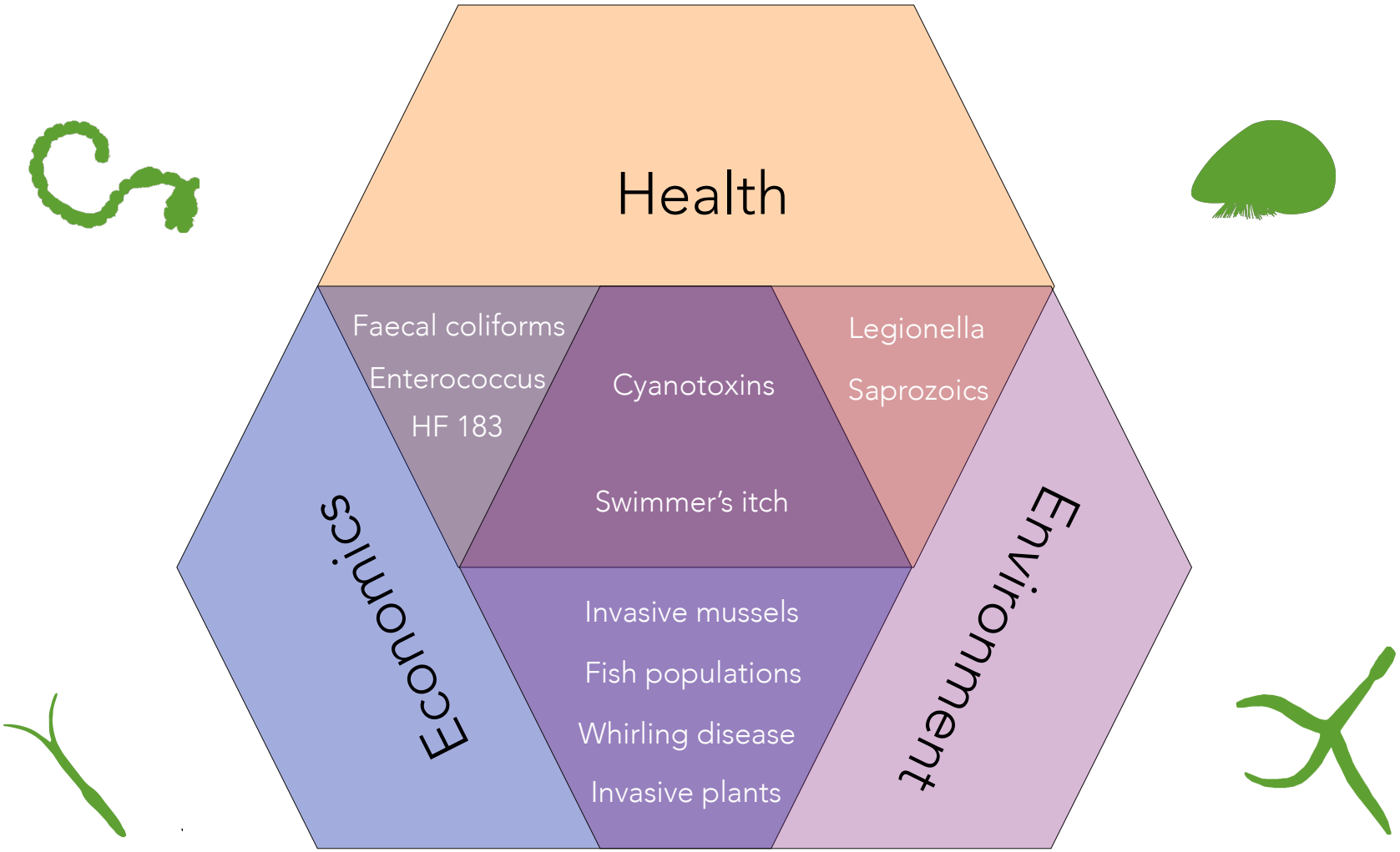
R2= 0.985
Eff= 1.9 (ideally 2)
LOD95: 2 copies



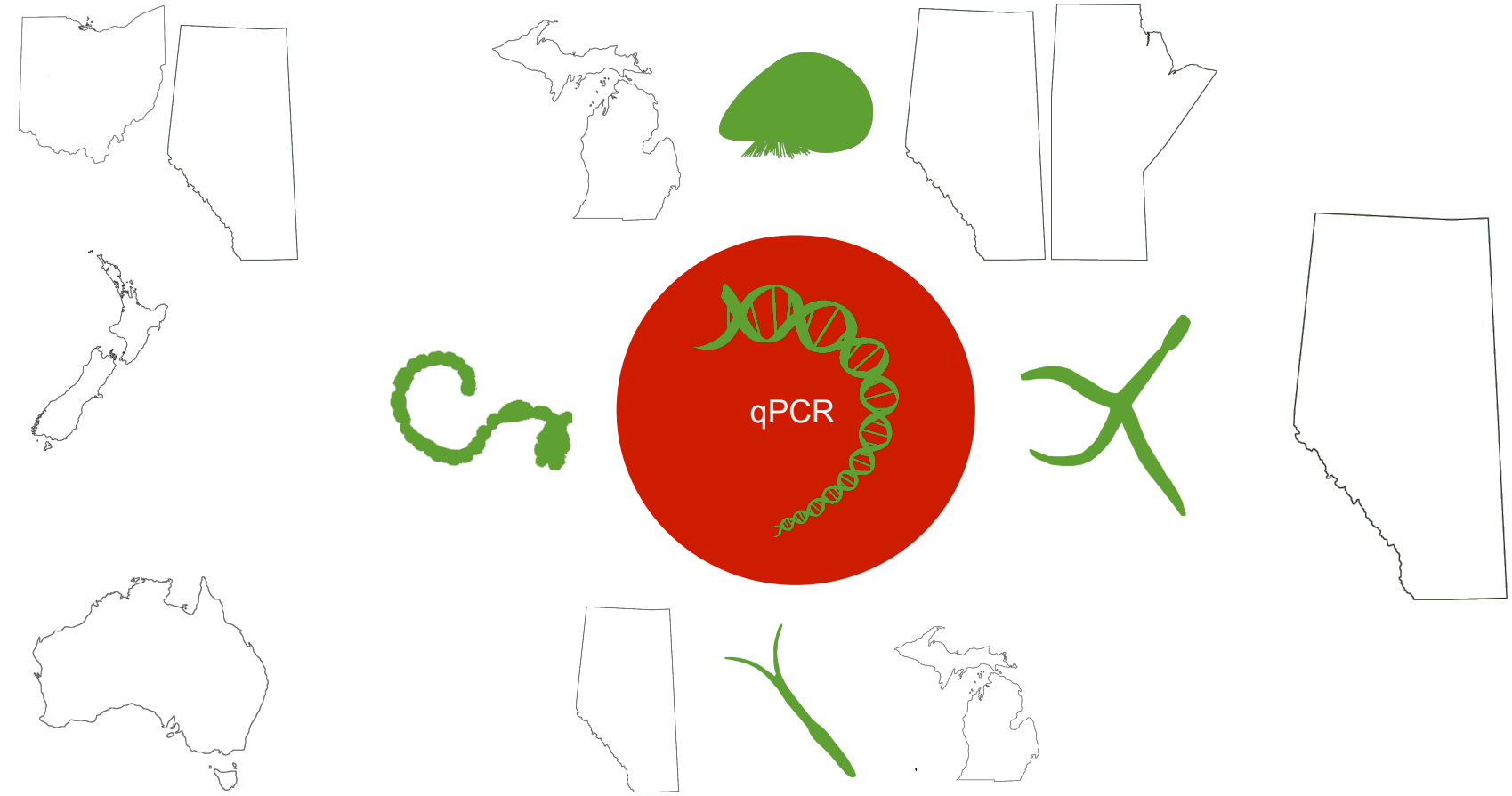


How often does field qPCR match what we get in the lab?



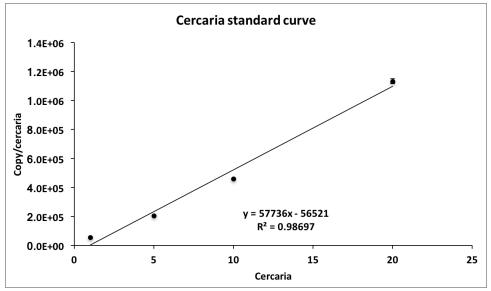

Health
Faecal coliforms
Enterococcus
HF 183
Cyanotoxins
Legionella
Saprozoics
Swimmer's itch
Economics
Environment
Invasive mussels
Fish populations
Whirling disease
Invasive plants

Project summary

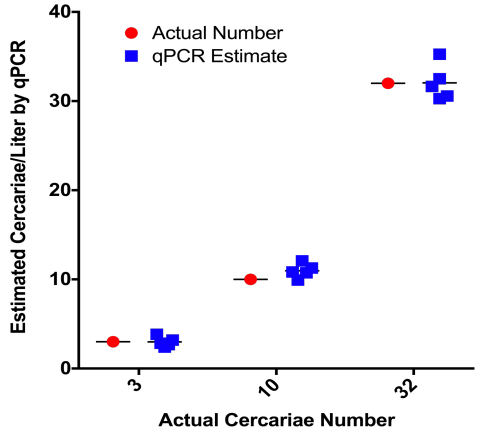


Swimmer's itch qPCR: 18s rDNA

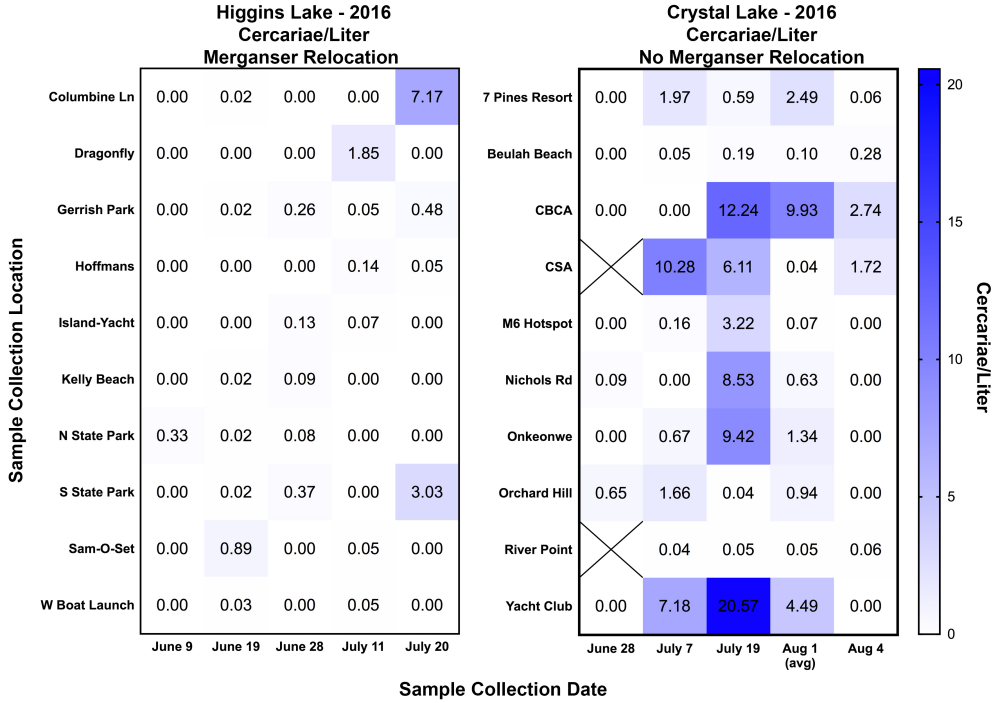
Confirm 18s gene copy number per cercariae



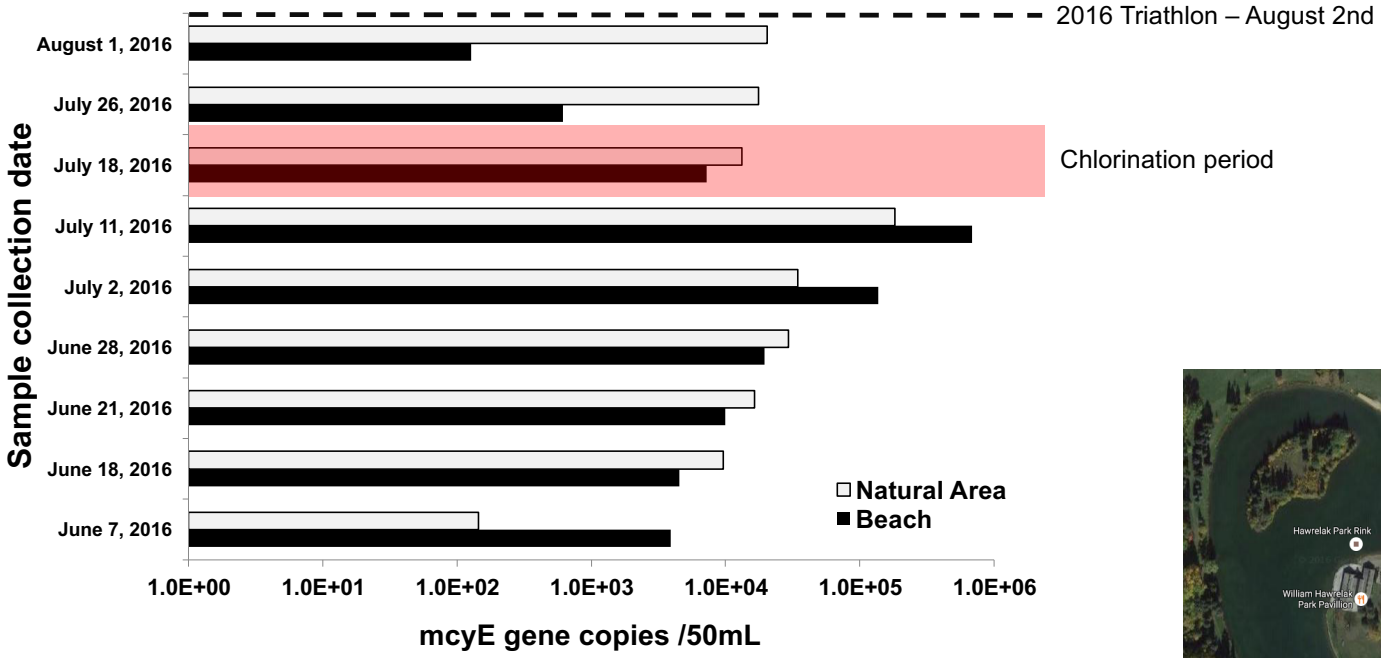
Blind study to confirm assay performance

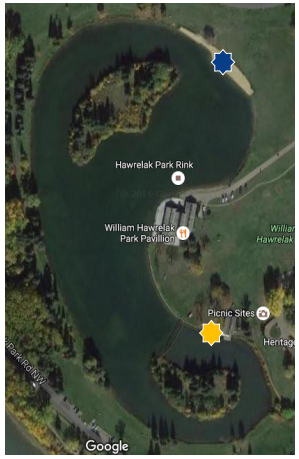


Example data corrected for cercariae per litre of water from a Swimmer's itch control initiative we are part of in Michigan



Example of field qPCR data: Hawrelak Park, Edmonton





Zebra and Quagga (Dreissenid) mussels

18s rDNA qPCR test

- Two different tests were developed for Quagga and Zebra mussels
- Samples from Manitoba and Michigan have served as known positives

- ~28 species of bivalve characterized in Alberta
- Confirm that none of the native Alberta bivalves yield a positive qPCR result using either test
- Known number required for tests to be quantitative

Whirling disease (*Myxobolus cerebralis*)

Banff lake may be drained to stop spread of deadly whirling disease in fish

Parks Canada considers extreme measures to halt spread of deadly parasite

CBC News Posted: Nov 08, 2016 6:51 PM MT | Last Updated: Nov 11, 2016 5:44 AM MT

All the fish in this Banff lake are to be removed and killed to protect other lakes from whirling disease

Parks Canada trying to protect westslope cutthroat trout in nearby Two Jack Lake and Lake Minnewanka

Entire Bow River watershed infected with whirling disease, CFIA says

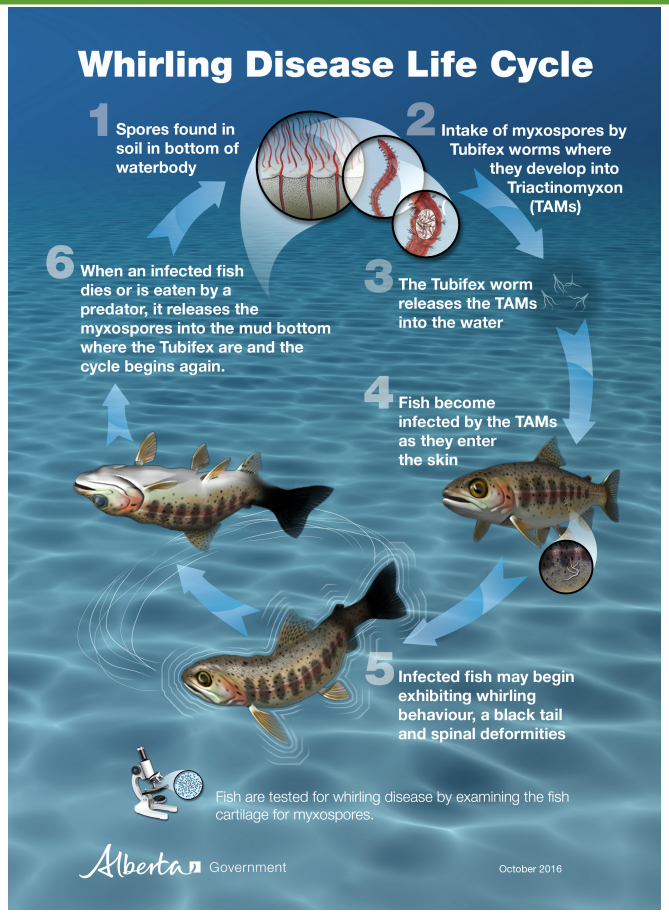
Agency declares province-wide 'buffer zone'

CBC News Posted: Feb 10, 2017 2:13 PM MT | Last Updated: Feb 10, 2017 2:13 PM MT

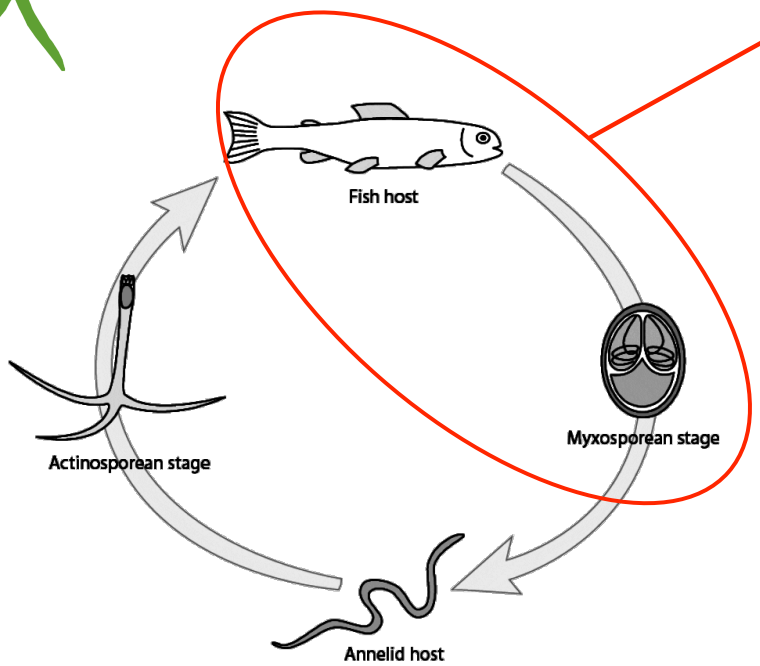
Whirling disease now infects entire Oldman River basin, including Waterton Lakes National Park

Deadly fish disease has spread southward after infecting Bow River basin, says Canadian Food Inspection Agency

CBC News Posted: May 01, 2017 1:25 PM MT | Last Updated: May 01, 2017 1:25 PM MT



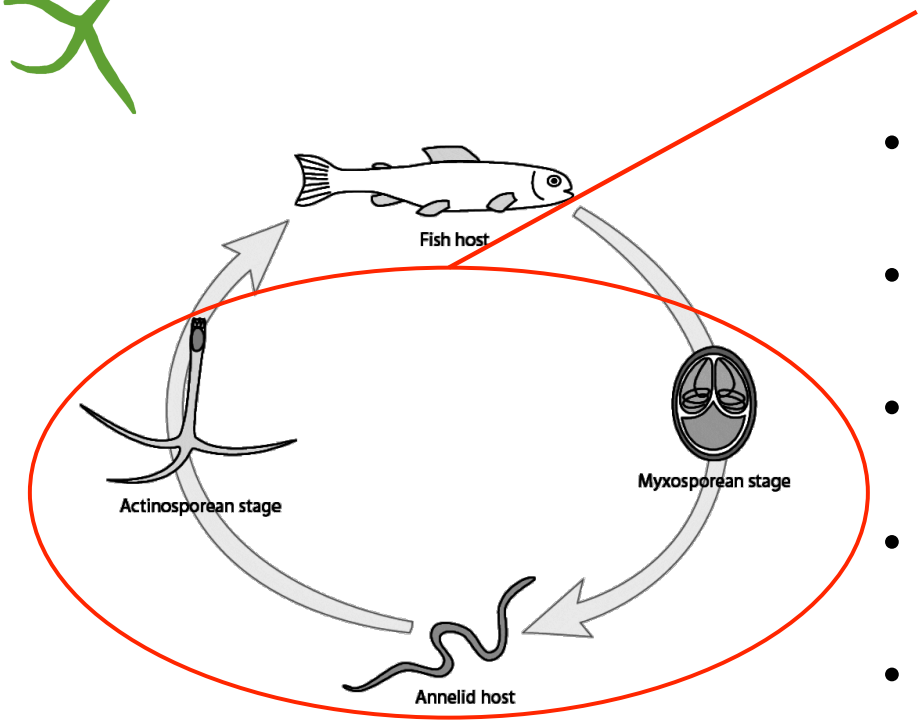
Whirling disease (*Myxobolus cerebralis*)



Current monitoring/detection

- Fish head/cartilage homogenization
- Visual identification of myxospore in homogenate
- Time consuming
- Requires expertise
- Requires handling of material that can spread infections if not decontaminated properly

Whirling disease (*Myxobolus cerebralis*)



Our qPCR strategy

- Test for environmental spore stages in concentrated river/lake water
- Analyze sediment samples for myxospores
- Identify worms and categorize infection status
- DNA can be extracted at sample collection point
- Minimize risk of contamination

Our program for the next two years

Cyanobacteria

- 4 Chai platforms operating in Alberta and US monitoring for cyanobacteria (16s and mcyE)
- 1 Chai platform operating in Sydney, Australia monitoring for cyanobacteria (16s, mcyE, anatoxin and saxitoxin)
- 1 Chai platform operating in New Zealand monitoring for cyanobacteria (16s, mcyE, anatoxin and saxitoxin)

Swimmer's itch

- We monitor three lakes in Alberta and partner with Freshwater Solutions in Michigan to evaluate SI control programs using qPCR

Quagga and Zebra mussels

- Retroactively testing water samples from Alberta, Manitoba and Michigan in 2017, field testing in 2018

Whirling disease

- Validation and sampling began this summer with AEP.
- Testing water, sediment, worm and fish samples collected this year.
- Repeated testing of specific sites, and finding the edges of the parasite in Alberta in 2017 and 2018.

Acknowledgments

Michelle Gordy | Sydney Rudko | Danielle Barry

Emmanuel Pila | Jacob Hambrook| Dr. Hongyu Li | Dr. Nick Ashbolt | Dr. Norm Neumann | Dr. Lilly Pang | Ron Reimink | Marie Veillard | Bev Larson | Clayton James | Peter Giamberardino | Dr. Mike Pauldon | Dr. Simon Otto | Bradley Peter | Arin MacFarlane Dyer | Dr. Jennifer Graydon

Mahmoud Tarrabain Lisa Kish Valerie Phillips Cerina Lee Emily Buss