Development and applications for environmental DNA (eDNA) with droplet digital PCR (ddPCR)

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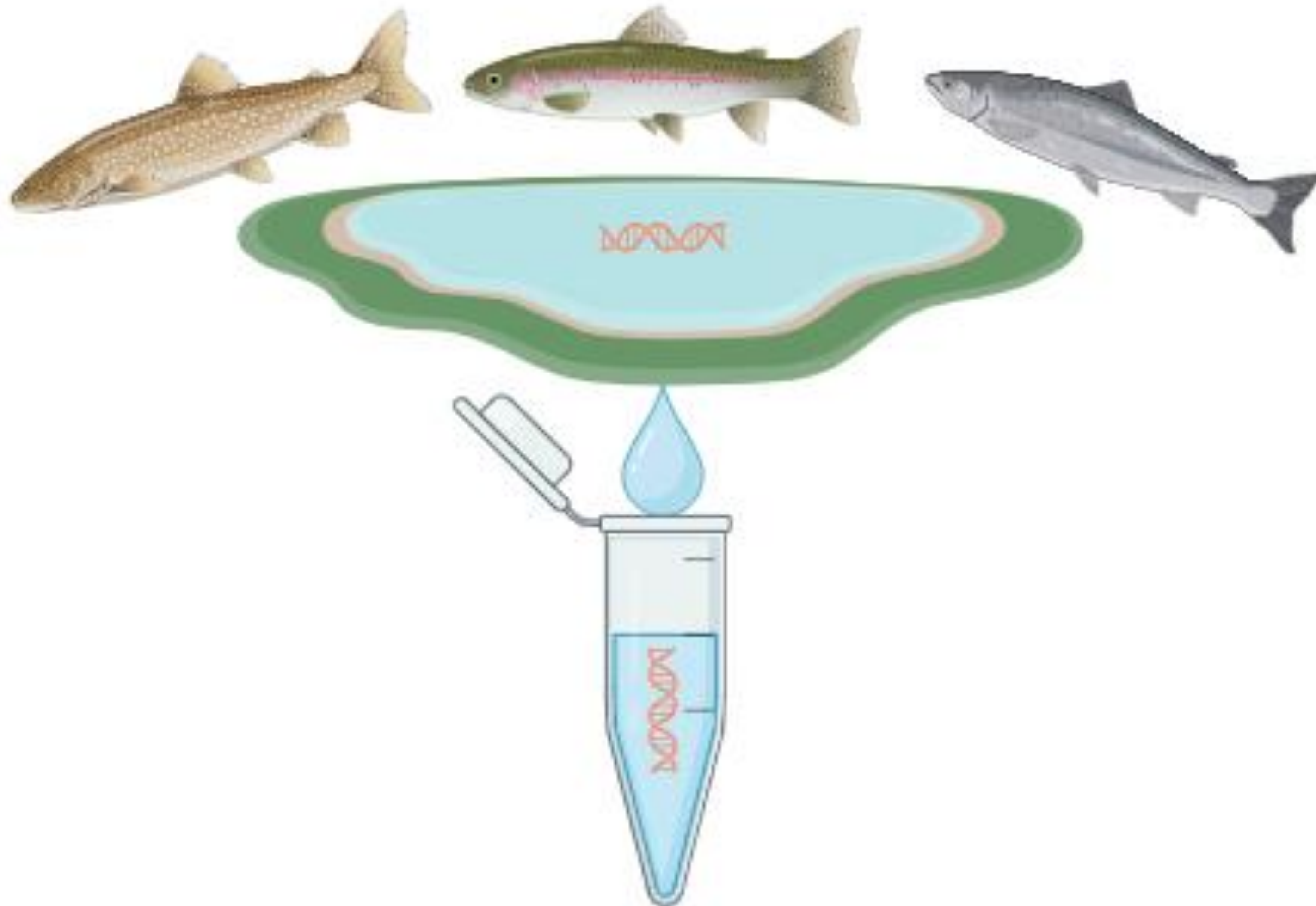
environmental Deoxyribonucleic Acid (eDNA)



- Genetic material shed into the environment
- Collected from a variety of matrices:
  - Sediment, water, air, spider webs, etc.

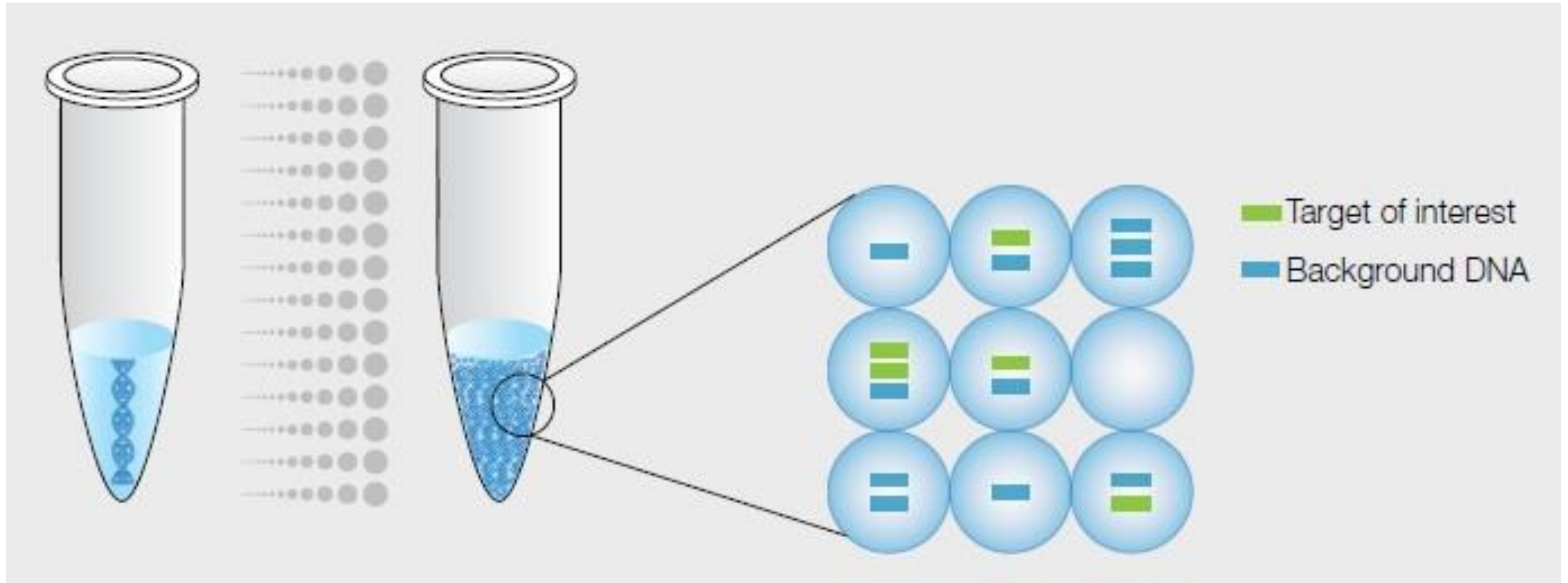
eDNA is one tool in the toolbox

- Widely applicable with a broad range of uses; BUT,
- Still just one tool in the toolbox for aquatic ecology

droplet digital Polymerase Chain Reaction (ddPCR)

- The process of amplifying a target sequence with repeated temperature cycles
- Digital PCR is a newer technology than RT-qPCR that is precise, but can be pricey

ddPCR provides ‘absolute quantification’



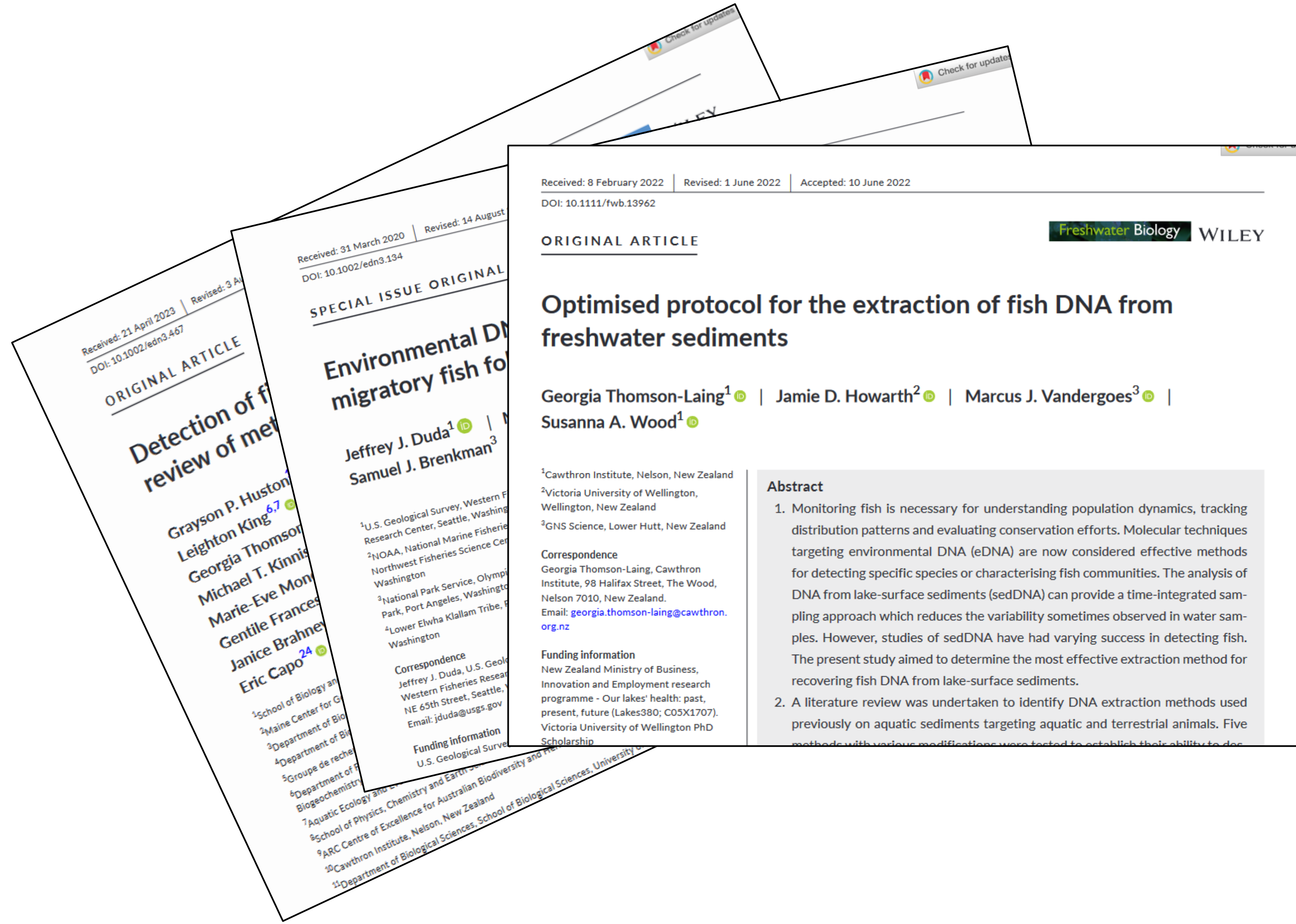
- Digital PCR forms 10-20,000 droplets, each may or may not contain the target, each is individually read as positive or negative

How do we design a ddPCR eDNA assay?

- Case Study: Game fish species in sediment
- Targets:
  - Coho salmon
  - Rainbow trout
  - Brook trout
  - Arctic grayling
- Matrix:
  - Sediment
- Location:
  - Boardman River watershed

Literature review

- We start in the literature for a validated primer and probe set
- Often RT-qPCR as it is older and more widely used



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ORIGINAL ARTICLE

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Optimised protocol for the extraction of fish DNA from freshwater sediments

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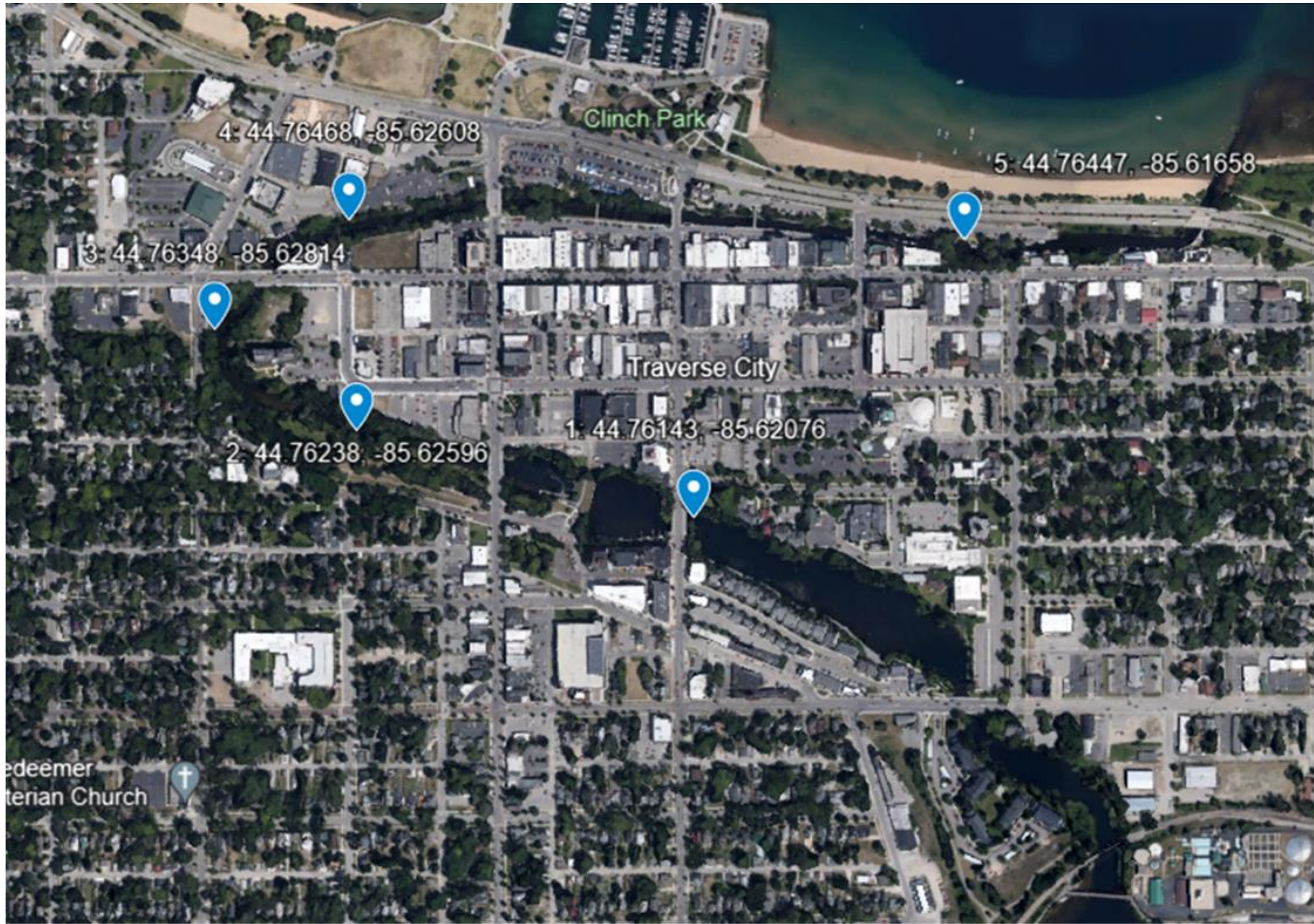
Abstract

1. Monitoring fish is necessary for understanding population dynamics, tracking distribution patterns and evaluating conservation efforts. Molecular techniques targeting environmental DNA (eDNA) are now considered effective methods for detecting specific species or characterising fish communities. The analysis of DNA from lake-surface sediments (sedDNA) can provide a time-integrated sampling approach which reduces the variability sometimes observed in water samples. However, studies of sedDNA have had varying success in detecting fish. The present study aimed to determine the most effective extraction method for recovering fish DNA from lake-surface sediments.
2. A literature review was undertaken to identify DNA extraction methods used previously on aquatic sediments targeting aquatic and terrestrial animals. Five

Sample Collection: Mitchell Creek sediment cores

Sample Collection: Lower Boardman River surface sediment



Sample Collection:
Grayling Fish Hatchery & Harrietta Hills Trout Farm

Charlie Buckmaster at the Grayling Fish Hatchery

Dan and Jim Vogler at Harrietta Hills

- Both raising rainbow trout
- Collected detritus from concrete raceways and surface sediment, respectively

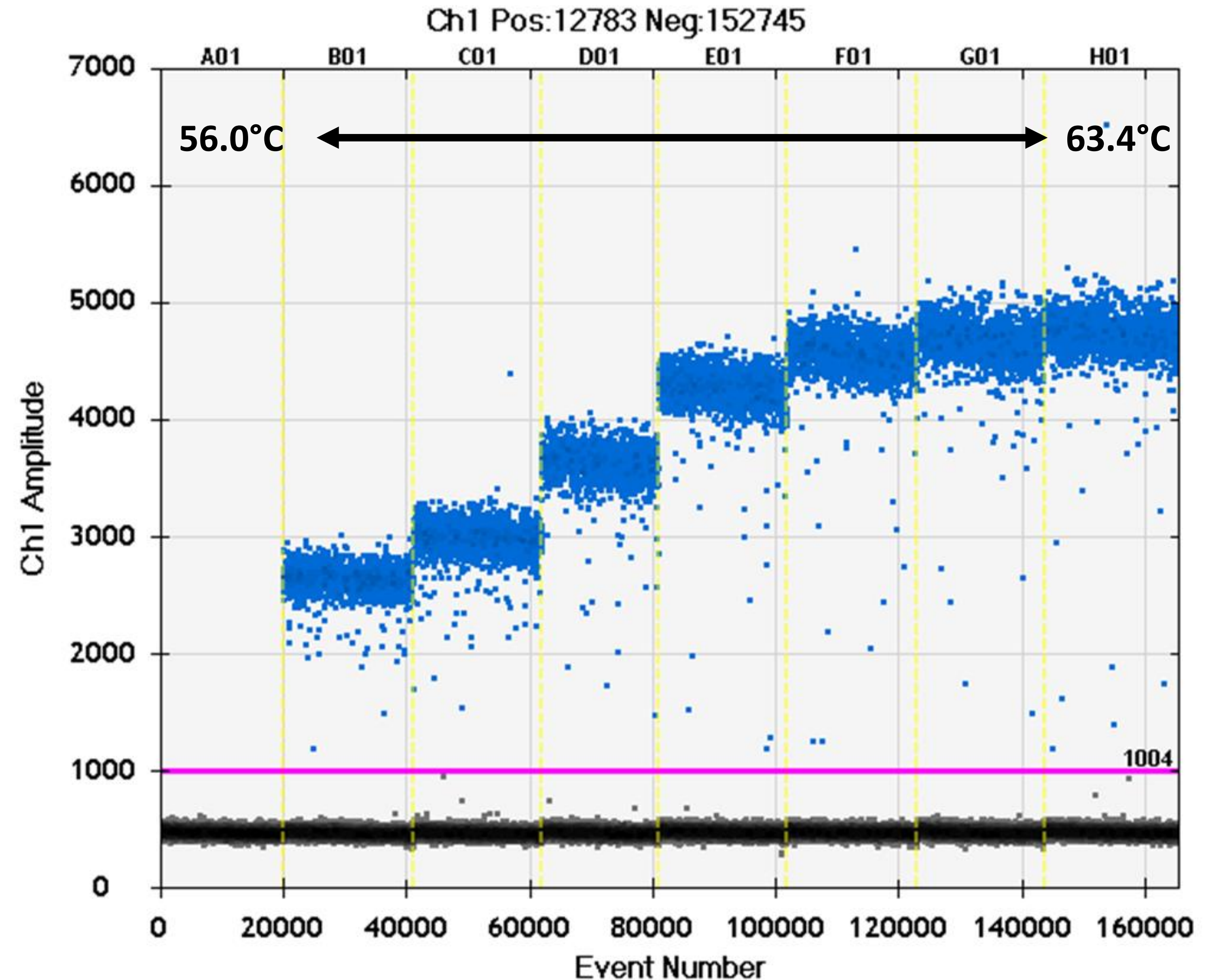
Positive Controls

AACCCAATTCCTATTCTGGGCCTTGGTGGCGGATATACTTATCTTAACCTGAATCGGGGGCATGCCCGTGGAACACCCATTCATCATT
ATCGGCCAAGTTGCCTCTGTGATCTACTTCGCCATCTTCCTAGTTCTTTCACCCCTAGCCGGCTGGCACCCTCTCTCACACTCCTCA
GTCTCTCCCTGTATATCGTCATGACATCTTCAGCTTTCCTCACATTAAAAACCAACAACTCTTTAACCATCAATACTCTCGCAACTTC
ATGAACTAAATCCCCAACCCTTGCCGCATTAACATTCAACTACAAGAACCTAATGGCCAACCTCCGAAAAACCCACCCACTCCTAA
AAATTGCTAATGACGCACTAGTCGACCTCCCTGCCCCCTCTAATATCTTGTAGATTTAACTATTTTCTCCCTTCACTTAGCTGGTATTT
CTTCTATTTTAGGGGCAATTAATTTTATTACAACCATTATCAACATGAAACCCCCAGCGATTTCTCAATACCAAACCCCTCTTTTTGTG
TGGGCTGTTCTGATTACCGCCGTCCTCCTACTTCT

- Designed a synthetic oligo which contains the targets of all four
- Collected fin clips and/or tissue for each species for future cross reaction

Optimization Experiments: PCR conditions

- Temperature gradient, ddPCR reagent selection, primer and probe concentration



Optimization Experiments: Extraction efficiency

- Used Boardman River sediment
- Spiked 0.25 g sediment with 10 µL of gBlock positive at 716 GC/µL
- Extracted with Qiagen PowerSoil Pro column kits

- Efficiency of 18-29%
  - Area for improvement
  - Future work: compare alternative extraction methods to
  - Goal to minimize FALSE NEGATIVES

Optimization Experiments: Amplification control

- Used Mitchell Creek core sediment samples
- Spiked wells with 716 gene copies per well of positive gBlock
- Ran with standard PCR conditions

- Recovery of 80-91%
  - Low inhibition
  - One benefit of ddPCR

Sediment core DNA quantification

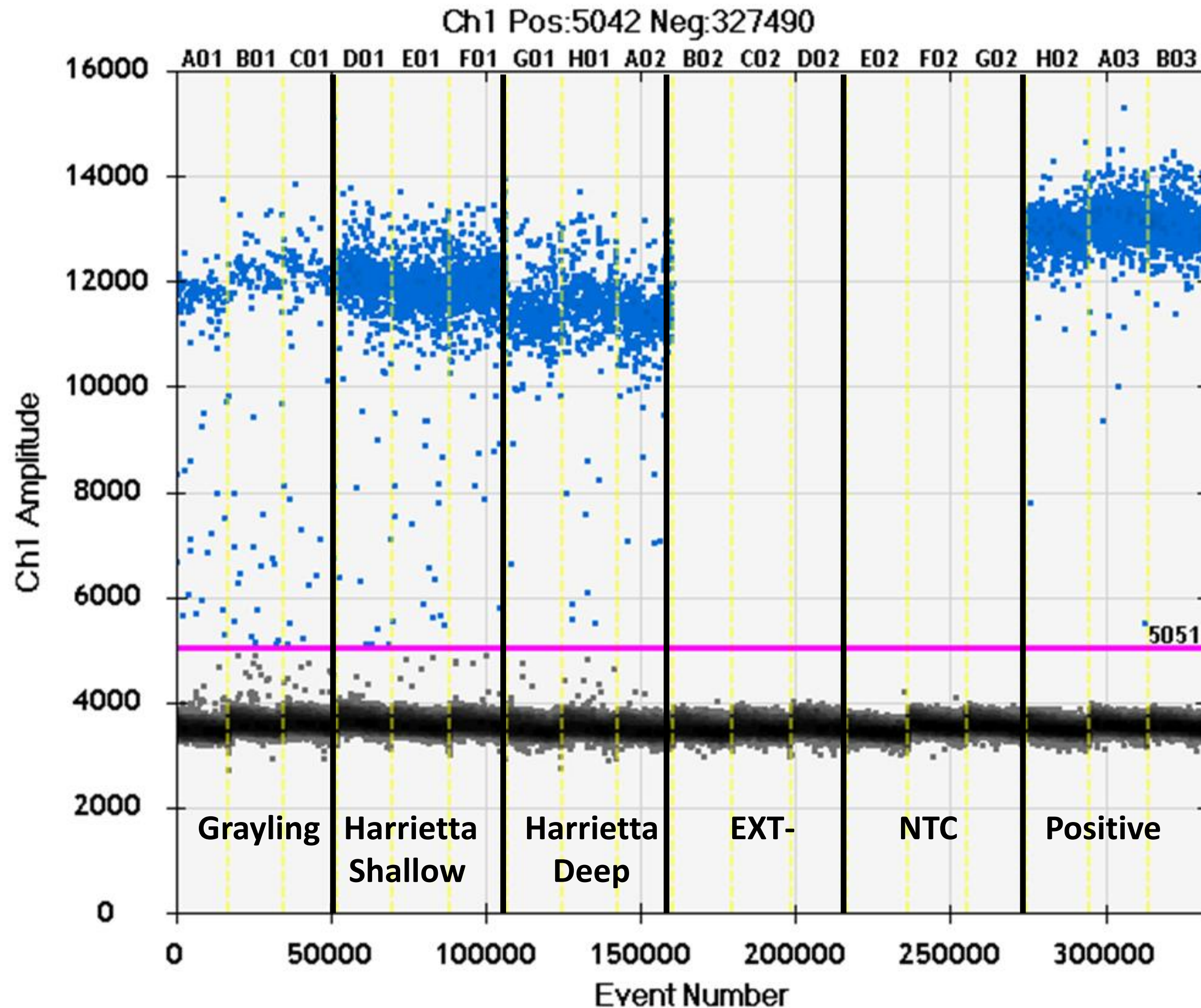
Core	Section	Depth (cm)	Extracted DNA Quantity (ng/µL)
1	Top	10-15	10.6
	Middle	90-95	16.6
	Bottom	133-138	0.0516
2	Top	5-10	17.2
	Middle	20-25	36.5*
	Bottom	150-155	0.504
3	Top	5-10	90.4*
	Middle	50-55	40.8*
	Bottom	110-115	3.84
Negative Controls			
	Coring Equipment		ND (< 0.02 ng/mL)
	Coring Ambient		0.0644
	Processing Equipment		ND (< 0.02 ng/mL)
	Processing Ambient		ND (< 0.02 ng/mL)
	Extraction		ND (< 0.02 ng/mL)

Mitchell Creek results

Core	Section	Depth (cm)	Coho	Rainbow	Brook	Grayling
3	Top	5-10	125		624	
	Middle	50-55			125	
	Bottom	110-115				

- Only detected eDNA from fish targets in core 3, at the mouth of the river
- Found coho salmon and brook trout in the top
- Found just brook trout in the middle
- Nothing detected in the deepest section
- No Arctic grayling (expected) or rainbow trout (surprising)

Rainbow trout hatchery results



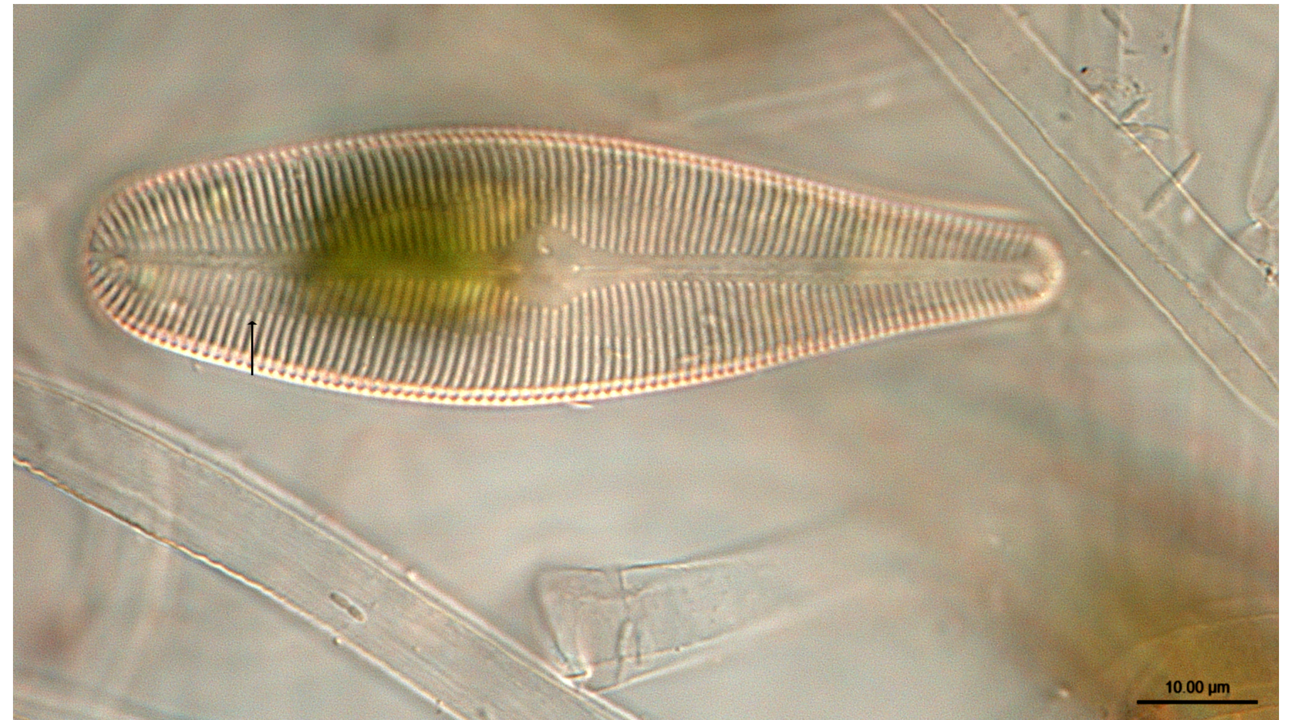
- Concerned about rainbow trout FALSE NEGATIVES and SENSITIVITY
- Collected sediment from known field sources of rainbow trout
- Very strong signal from all three rainbow trout sources

Next steps

- Compare extraction methods for best extraction efficiencies
- Extract DNA from fish tissue samples
  - Cross reactivity to determine SPECIFICITY to address FALSE POSITIVES
- Field validation with known occurrence data
- Surface water

Other and future projects

- *Didymosphenia geminata*
- ‘Rock snot’



Other and future projects

- New Zealand Mud Snail
- Boardman River as reference sediment for GLEC

Thank you!

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Please feel free to reach out:

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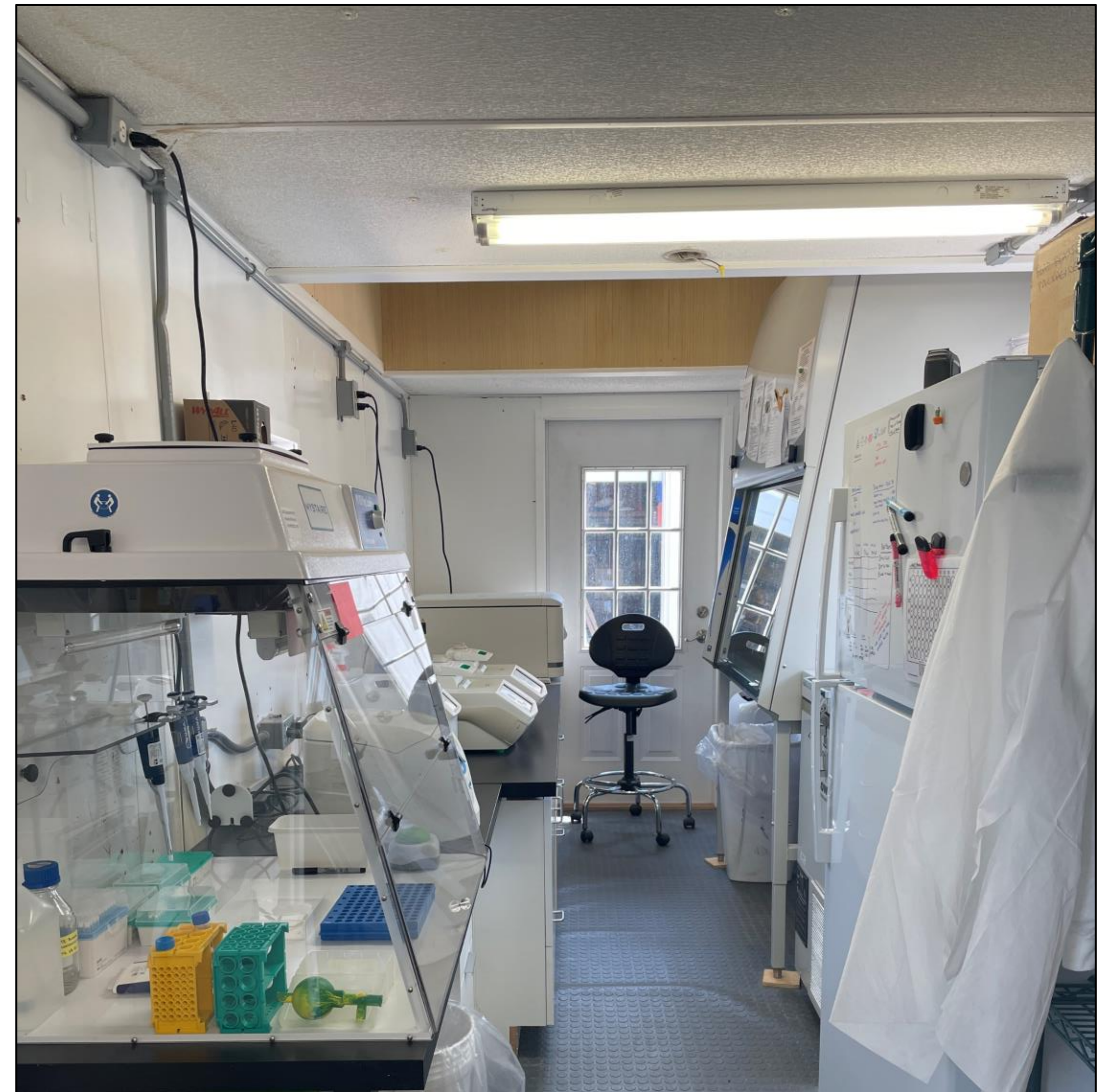

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