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Environmental DNA (eDNA) Detection of Aquatic Invasive Species in Manitoba Lakes, a comparative analysis of microscopy and DNA detection methods.

2026 Invasive Species Forum.

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Enviro-Responsible

DECEMBER 23, 2025

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Introduction

- Aquatic invasive species (AIS) threaten **ecosystems, fisheries, and infrastructure**
- Early detection is critical for **rapid response and cost control**
- Traditional microscopy:
  - Reliable for **established populations**
  - Poor sensitivity for **early-stage invasions**
- eDNA offers a **high-sensitivity alternative**

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Objective

This study assessed the presence of three Aquatic Invasive Species (AIS) *Dreissena polymorpha*, *Dreissena bugensis*, and *Bythotrephes spp.* in samples collected between 2022 and 2024 from several lakes of the Manitoba basin using both conventional microscopy and environmental DNA analysis.

- **Why these species?**
- High ecological and economic impact
- Known or emerging presence in Manitoba waters

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Study Sites

- **Lake Manitoba:** Established zebra mussel populations
- **Cedar Lake:** Spiny water flea hotspot; emerging dreissenid signals

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Study Objectives

- To compare **microscopy vs. eDNA (qPCR)** detection
- To assess **sensitivity and agreement** between methods
- To identify **early-stage invasions** missed by microscopy because of very low-density populations
- To better inform **integrated AIS monitoring strategies**

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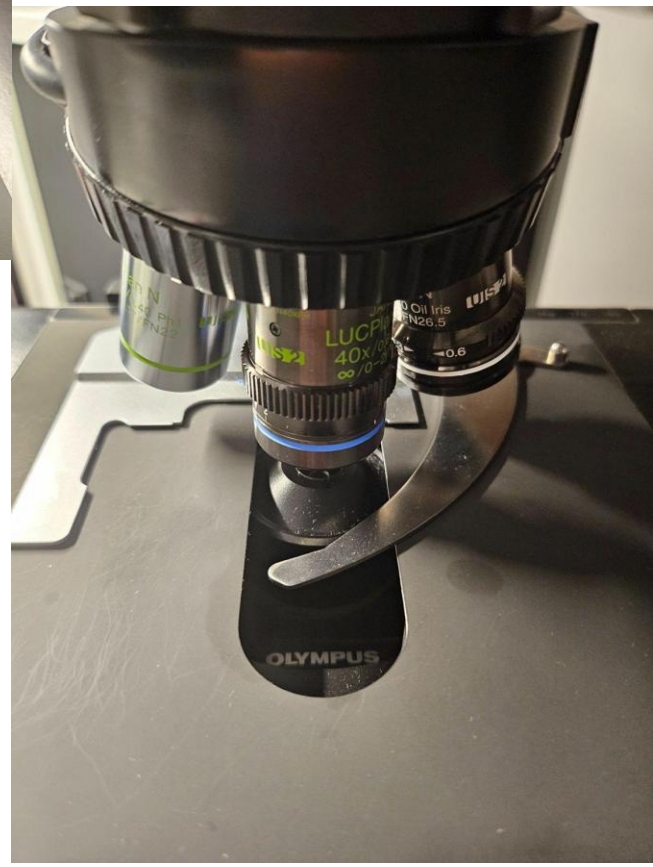
Sampling overview

- **29 water samples (70% ethanol-preserved)**
- Collected across **21 sites (2022–2024)**
- Parallel analysis using:
- Light microscopy and polarized light filter for veligers
- eDNA extraction + qPCR



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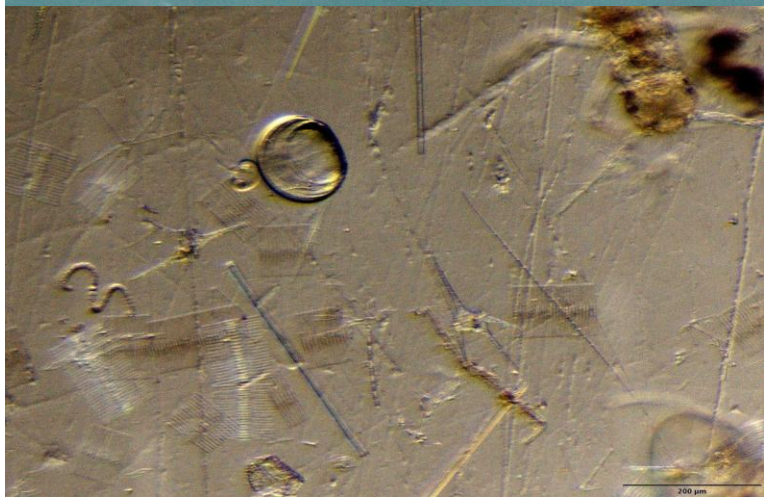
Microscopy Methodology

- Visual identification of:
  - Adults of SWF
  - Larvae (veligers)
  - Zooplankton stages
- Strengths:
  - Confirms established populations, **viable and dead organisms, as well as SWF embryos and resting eggs.**



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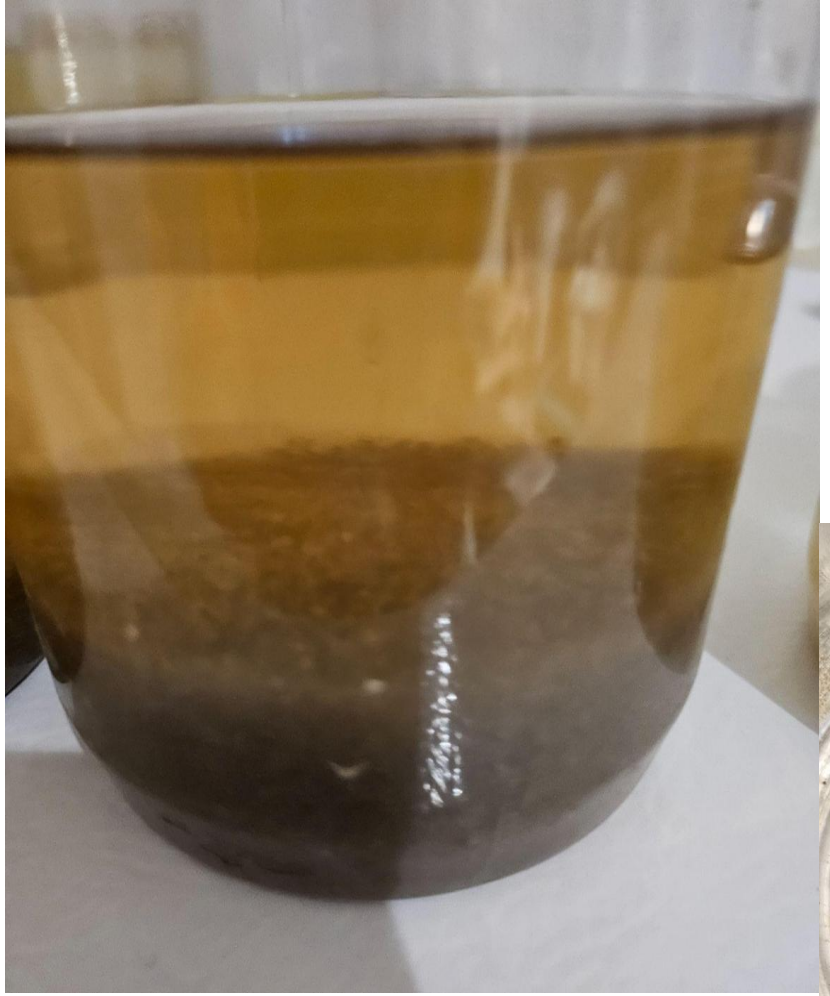
Microscopy Methodology

- Limitations:
  - Low sensitivity at very **low abundance of organisms or early invasion**
  - Time consuming for samples with extremely high density plankton, including algae, and/or sediment.



Plankton

Sediment in plankton sample

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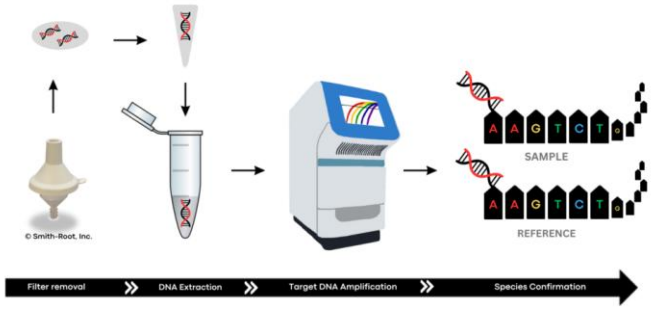
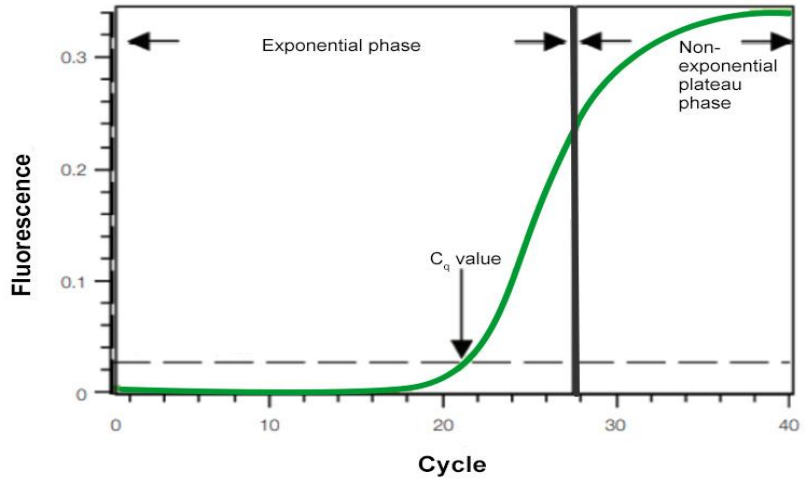
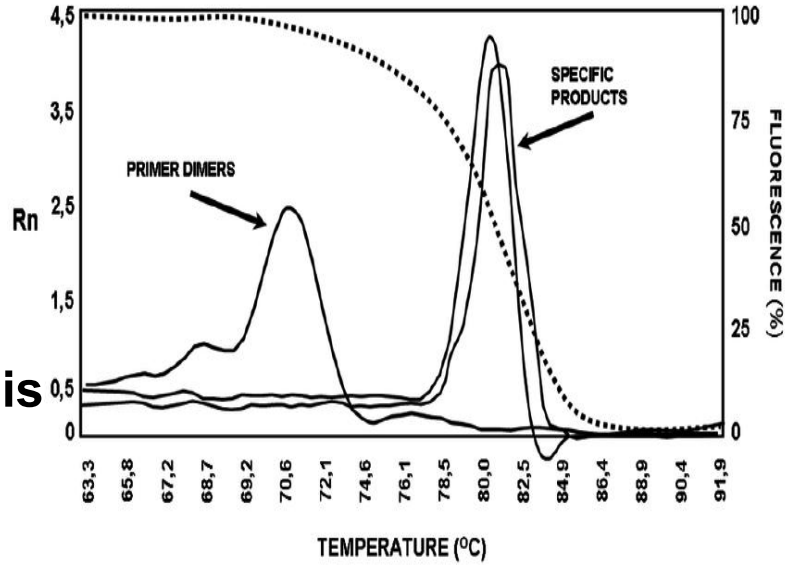
eDNA & qPCR Workflow





- Filtration (26 mm pore-size filter)
- DNA extraction (PowerWater kit)
- qPCR (SYBR Green):
- COI (species-specific)
- 18S (internal control)
- Positive = **Ct ≤ 40**
- Verification by **melt curve + gel electrophoresis**



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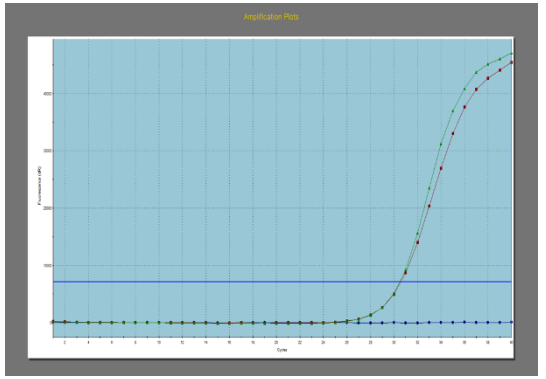
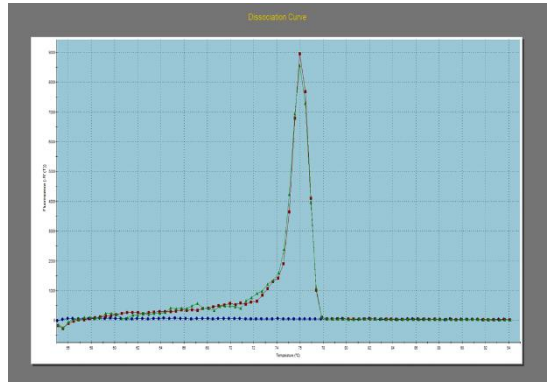
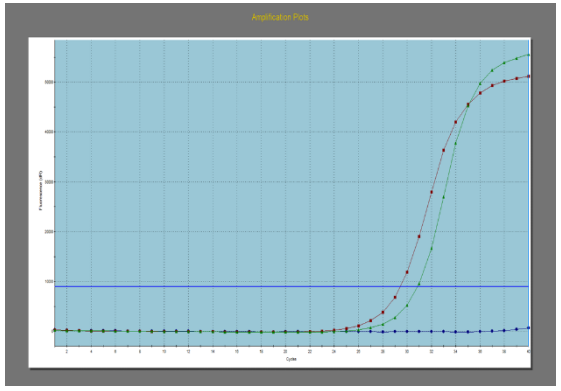
eDNA targets amplification

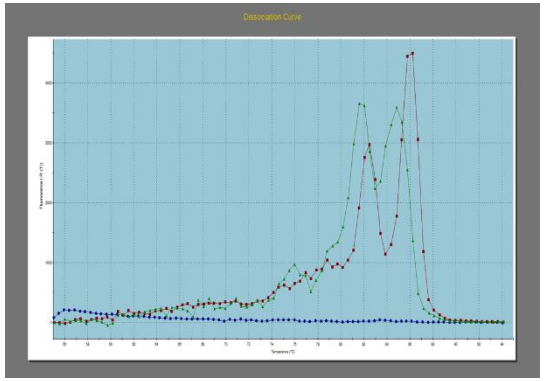
- Clear amplification:
  - **Ct range:** 28–36
  - **Single melt peaks**

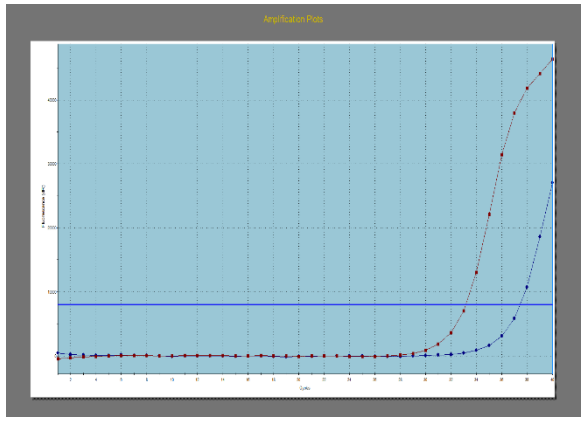
Primer ID	Primer sequence	Reference
Bytho_F	GCAGGAACTGGCTGAACA	Walsh et.al., (2019)
Bytho-R	AATAATAAAAGGAGGGCTGTAATACC	
COI-DP -F3	TAATGGGGGGATTCGGAA	Williams et al. 2017
COI-DP -B3	GCTCCCCCAATATGAAGAG	
COI-DB-F3	ATTTGGTGGGGGTTGAAC	Williams et al. 2017
COI-DB--B3	GGCTAAAACAGGTATTGCTAA	
18S -F3	GTTAGCCCAGACCAACGC	Williams et al. 2017
18S -B3	CTTCCTTGGATGTGGTAGCC	











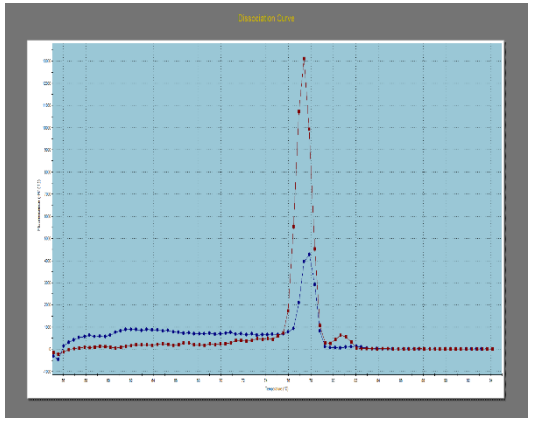


Fig 2 Amplification plots and dissociation profiles for the COI and 18s DNA segments using the primer pairs developed by William et. al, 2021. A) Amplification of *D. polymorpha* COI in eDNA samples V46 and V47 and NTC, B) Dissociation profile for the COI target in samples V46 and V47 and NTC; C) Amplification of the bivalve small subunit ribosomal RNA (18s) in eDNA samples V46 and V47 and NTC; D) Dissociation profile for the 18s target in samples V46 and V47 and NTC; E) Amplification of *Bytrothrephes longimanus* in samples V4 and V5 from Cedar Lake F) Dissociation profile for the *Bytrothrephes longimanus* target in samples V4 and V5 and NTC.

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qPCR Validation Results

- Agarose gels:
  - Single bands (~200 bp)
  - No amplification in NTCs
- Confirms **specificity and reliability**

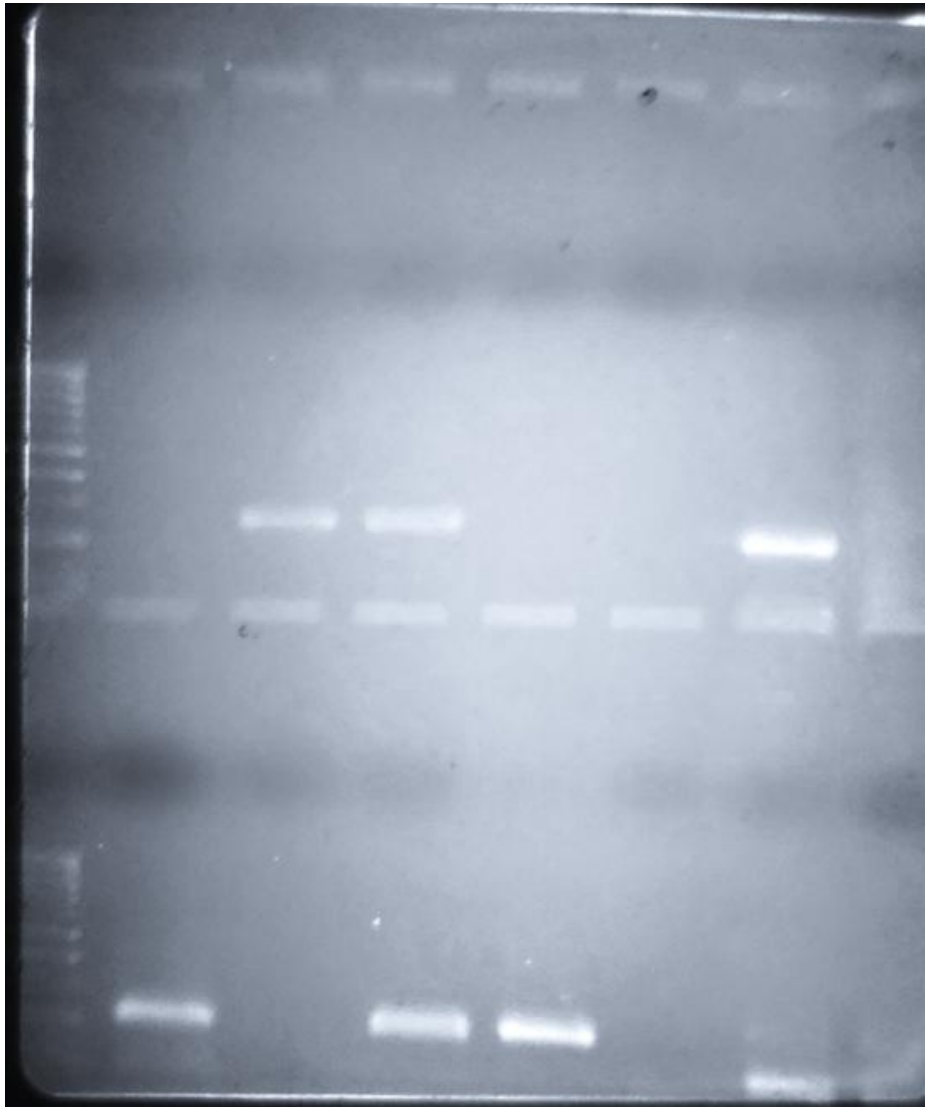

Fig.3 Electrophoresis of qPCR amplification products in 1.5% agarose. Single bands were observed for the target species D *polymorpha* COI and *Bythotrophes sp.* (COI) with sizes around 200 bp with no amplifications in the non-template control samples (NTC).

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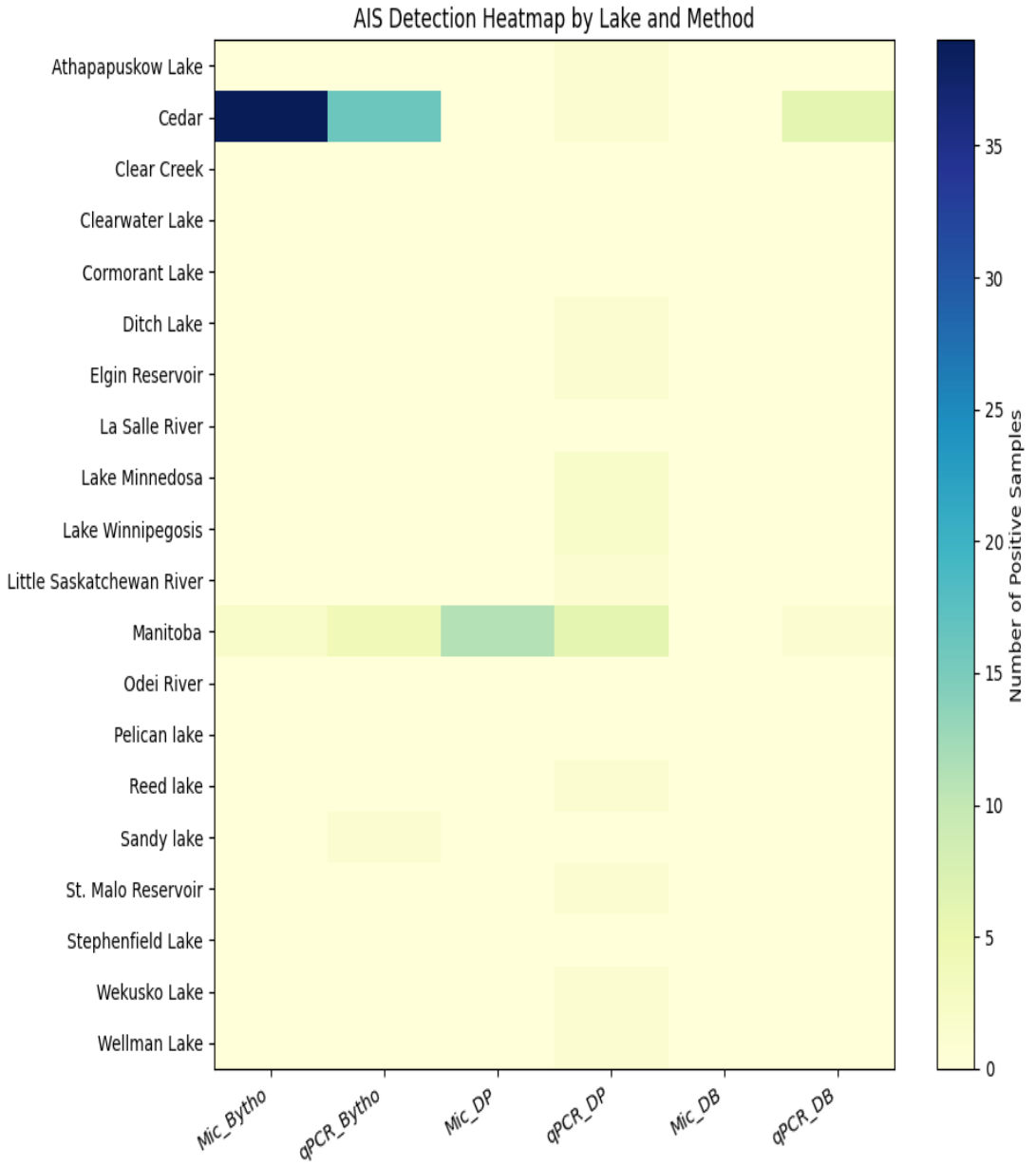
Key Results: Detection Patterns

- **Microscopy**
  - *Bythotrephes:* 39 detections, **Cedar Lake only**
  - *Zebra mussel:* 13 detections, **Lake Manitoba only**
- **eDNA (qPCR)**
  - *Bythotrephes:* 29 detections across **8 lakes**
  - *Zebra mussel:* 21 detections across **12 lakes**
  - *Quagga mussel:* Rare or absent
- ➡️ eDNA reveals **broader, low-density distribution**



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Method Agreement (Cohen's κ)

Species	TP	FN	FP	TN	Sensitivity	Specificity	κ (Kappa)
Bythotrephes sp.	15	26	7	55	**0.37**	**0.89**	**0.27**
Dreissena polymorpha	3	8	18	74	**0.27**	**0.80**	**0.06**
Dreissena bugensis	0	0	7	96	NA	**0.93**	**0.00**

- **TP (True Positive)**: detected by both microscopy and qPCR
- **FN (False Negative)**: detected by microscopy, missed by qPCR
- **FP (False Positive)**: missed by microscopy, detected by qPCR
- **TN (True Negative)**: not detected by either method

Agreement between microscopy and qPCR was low to fair across taxa (κ = 0.00–0.27). Sensitivity of qPCR relative to microscopy ranged from 0.27 to 0.37, while specificity remained high (0.80–0.93). For *Dreissena bugensis*, no microscopy detections were recorded, resulting in zero agreement despite seven qPCR positives.

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Why Methods Disagree

- DNA degradation in stored samples
- PCR inhibition
- Patchy organism distribution
- Microscopy detects **presence and density of established populations**
- eDNA detects **genetic signal**, not viability
- ➡️ Differences are **expected and informative**

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Lake-Specific Insights

- **Lake Manitoba**
  - Zebra mussels eDNA dominant and persistent
  - Quagga mussels eDNA signal rare
  - Patchy spiny water flea signals
- **Cedar Lake**
  - Strong eDNA *Bythotrephes* establishment
  - Quagga mussel eDNA signal expansion suggested
  - Zebra mussel eDNA confirmed in **veligers (2025)**

*The language used in this slide has been changed from the original presented at the FORUM to avoid confusion.

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Management Implications

Aspect	Microscopy	qPCR
Sensitivity	Low–moderate	**High**
Early detection	❌ Poor	✅ Strong
Confirms live organisms	✅ Yes	❌ DNA only
Best use case	Established populations	**Early invasion surveillance**

Conclusions

eDNA analysis outperforms microscopy for **early detection**

Microscopy remains **critical** for confirmation of established populations.

EQUALS

Combined approach = Best practice

Supports proactive AIS management in Manitoba and in Canada

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Acknowledgements

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- A big thank you to the Invasive Species Centre for the opportunity to participate and share our research in this FORUM, and for organizing this and many other events.

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Thank you everyone!
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