

Protecting our Lakes using multiple eDNA-based approaches

Gordon Luikart, Leif Howard, Phil Matson, Flathead Lake Biological Station-UM



UM WATER WARRIORS
DEFEND AGAINST
INVADING MUSSELS

Monitoring network for mussel & milfoil eDNA



Zebra/quagga mussels

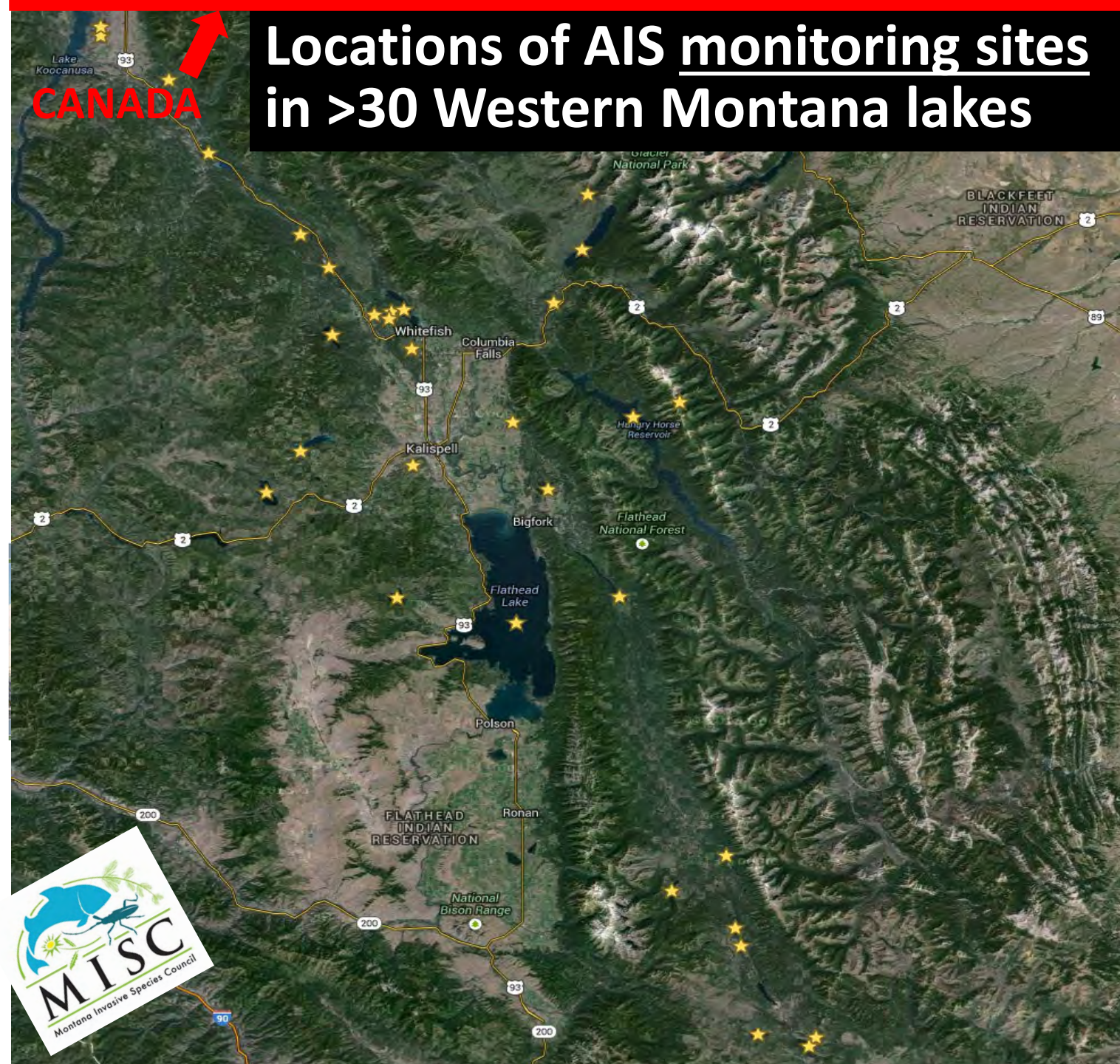


Eurasian milfoil

Thanks to collaborators and donors!



A People of Vision



Locations of AIS monitoring sites in >30 Western Montana lakes

Outline and Conclusions from 3 studies:

 1. Tow nets provide higher sensitivity than traditional filter samples

(Miller et al. In review)

2. Tow nets detect Dreissenids (eDNA) when microscopy does not (veligers)

(Dahlquist et al. In prep)

3. qPCR results from tow nets (& filters) are reproducible by different labs and sampling teams ($r^2 = 0.75$ to 0.95)

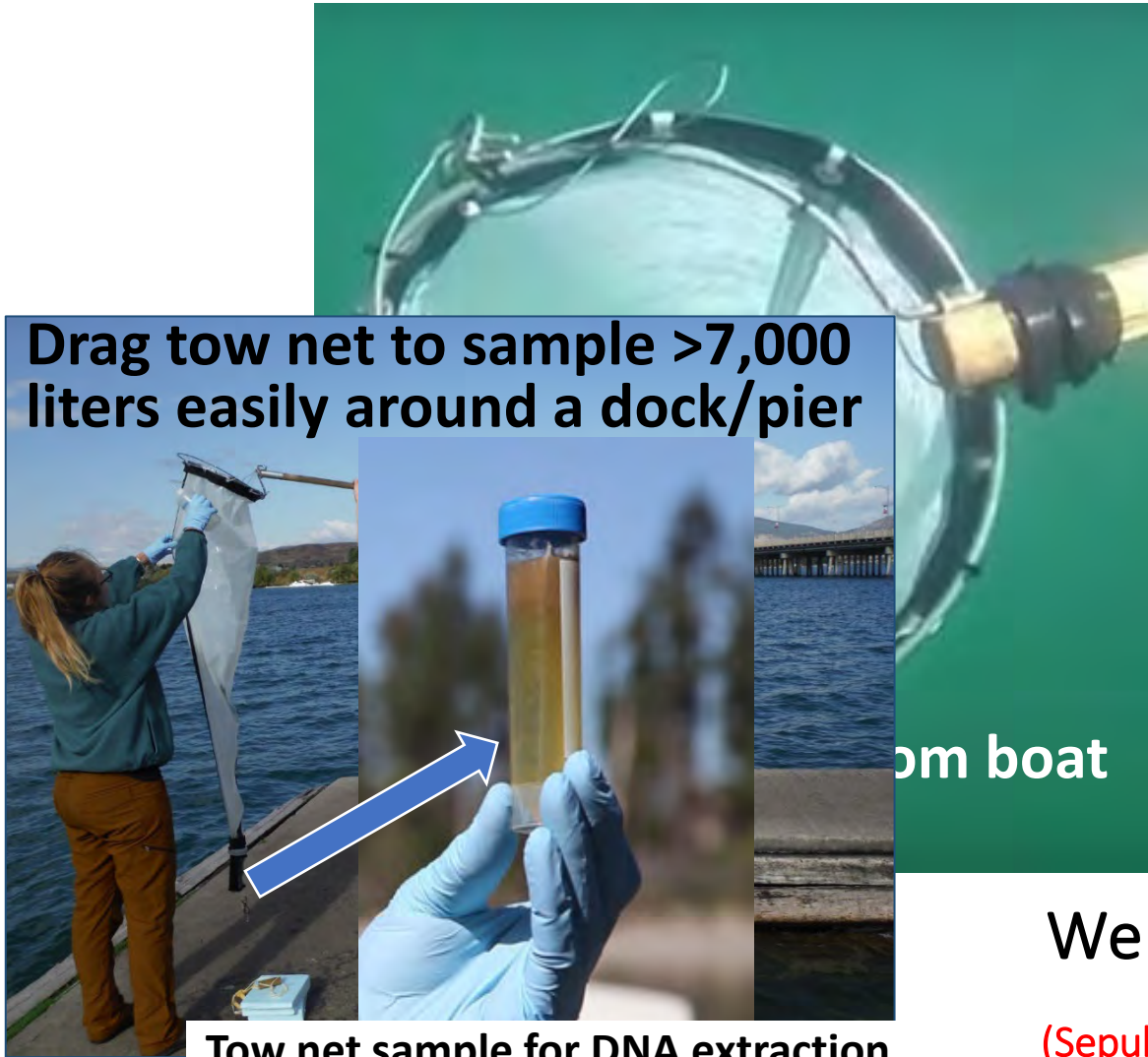
(Howard et al. In prep/MISC)

ZEBRA MUSSEL



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Do Large-volume plankton tow-nets (64 μm) provide better early detection of AIS than standard filters (1.5 μm)?



Drag tow net to sample >7,000 liters easily around a dock/pier

from boat

Tow net sample for DNA extraction
(Sampling is demonstrated in FLBS video)
<https://vimeo.com/97369920>



A filter clogs after only ~1 liter

The filter

We get MORE DNA from a tow net than a filter sample

(Sepulveda et al. 2019, Schabacker et al. 2020, Dahlquest et al. in prep.; Miller et al. in review.; Kirtane et al. in prep. = metabarcoding)

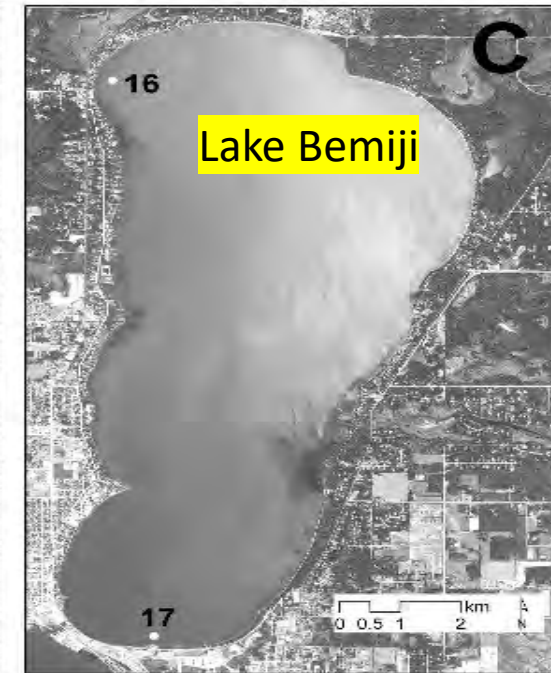
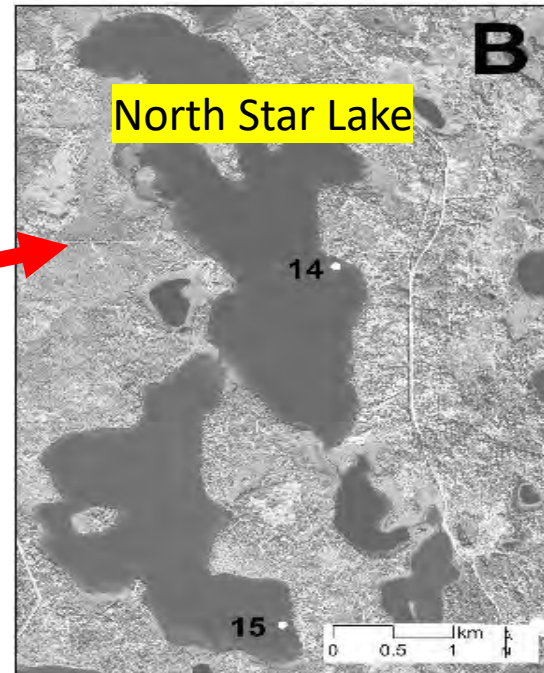
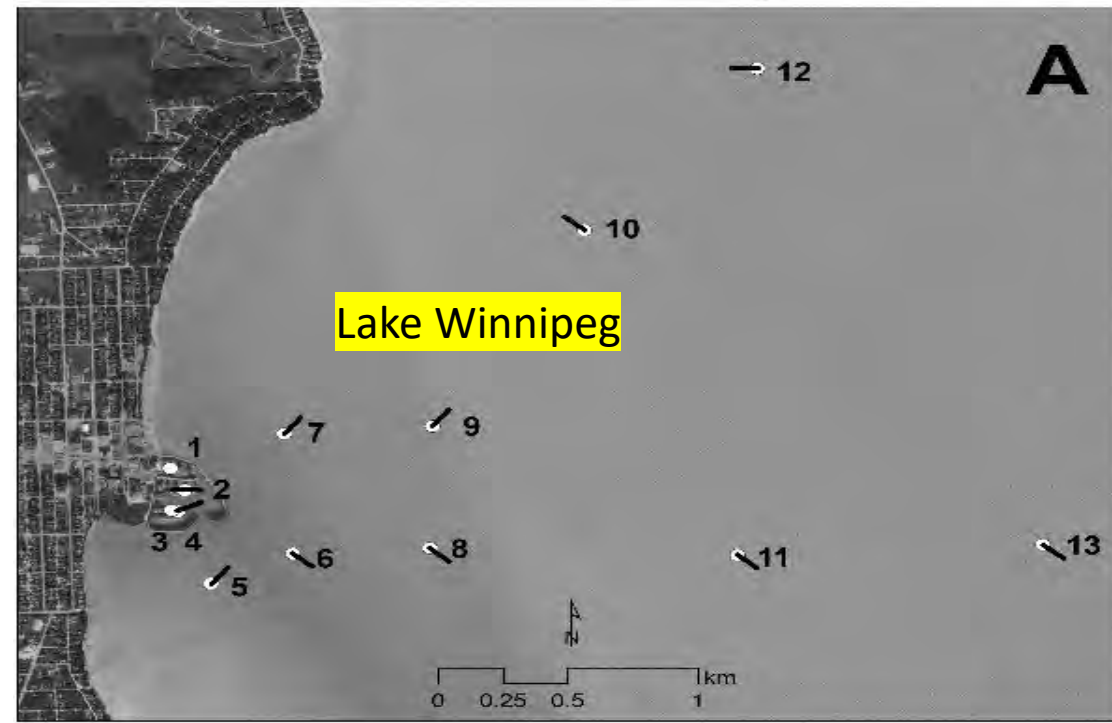
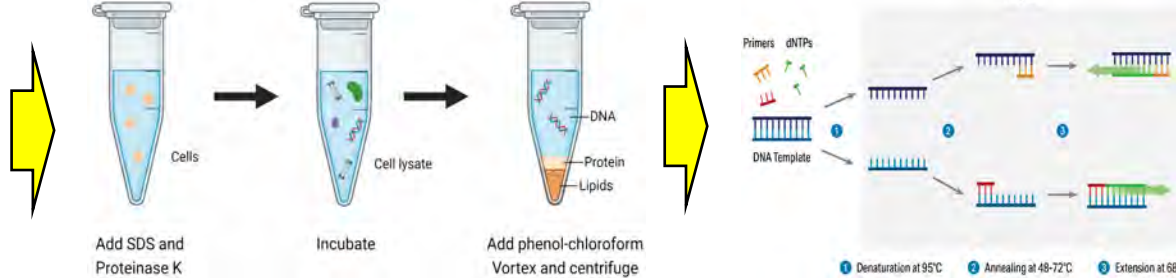
“Improved eDNA detection using high-volume sampling for invasive zebra mussels”

(Miller et al. In review)

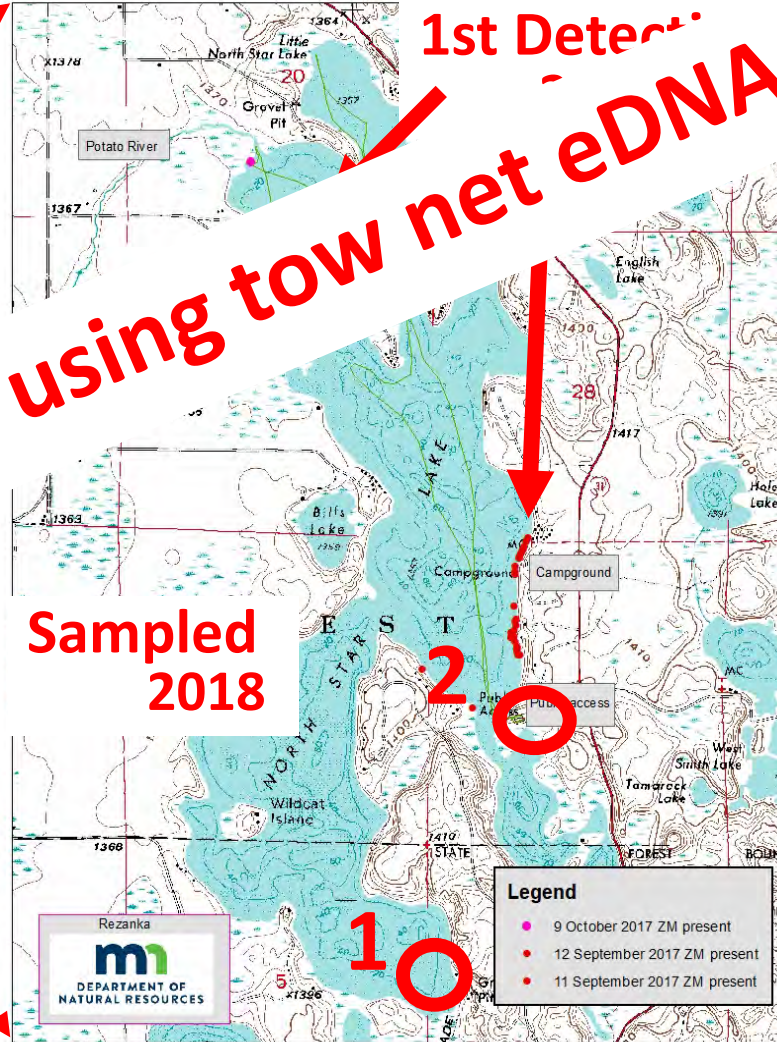
Methods:

Sampled: 5 tow nets & 5 filters, 17 sites, 3 Lakes

qPCR-tested: 3 replicates per sample extract



Tow nets vs filters for detecting zebra mussel eDNA in North Star Lake, MN



DNA copy number
Tow Filter

	00	1
433	00	
475	00	
109	00	
449	00	
10,809	16	2
4,862	00	
4,673	00	
6,869	14	
4,839	22	


Gingera qPCR assay results

Similar/same results in other lakes

Outline and Conclusions from 3 lines of AIS research:

1. Tow nets provide higher sensitivity than traditional filter samples

(Miller et al. In review)

 2. Tow nets detect Dreissenids (eDNA) when microscopy does not (veligers)

(Dahlquist et al. In prep)

3. qPCR results from tow nets (& filters) are reproducible by different labs and sampling teams ($r^2 = 0.75$ to 0.95)

(Howard et al. In prep/MISC)

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“Integrating eDNA testing with microscopy improves early detection of zebra mussels”

(Dahlquist et al. in prep.)

Methods:

Collected 85 tow net samples from 3 different lakes in Minnesota

Compared veliger detection (microscopy) to eDNA detection via qPCR

Site (Minnesota)	# of Samples [^]	# of Samples with Veligers detected	# of Samples with eDNA detected*
Lake Superior	16		
Mille Lacs	15		
Winnibigoshish	54		
Total	85		

[^]Collected by M. McCartney

*Gingera et al. (2017) qPCR assay

“Integrating eDNA testing with microscopy improves early detection of zebra mussels”

(Gingera et al. in prep.)

Methods:

Collected 85 tow net samples from

Compared veliger detection

Site (Minnesota)	Total Samples	Veliger Detected	Samples with eDNA detected*
La			15 (93.8%)
Mi		3 (20%)	11 (73.3%)
Win	54	20 (37%)	19 (35.2%)
Total	85	23	45

CR

eDNA testing complements microscopy.
eDNA-testing useful year-round & pre spawning.
We could eDNA-test the 1000s of tows collected annually for only microscopy.

^Collected by M. McCartney

*Gingera et al. (2017) qPCR assay

Outline and Conclusions from 3 lines of AIS research:

1. Tow nets provide higher sensitivity than traditional filter samples

(Sepulveda et al. 2019; Schabacker et al. 2020; Miller et al. In review)

2. Tow nets detect Dreissenids (eDNA) when microscopy does not (veligers)

(Dahlquist et al. In review)

➔ 3. qPCR results from tow nets (& filters) are **reproducible** by different labs and sampling teams ($r^2 = 0.75$ to 0.95)

(Howard et al. In prep/MISC)

ZEBRA MUSSEL



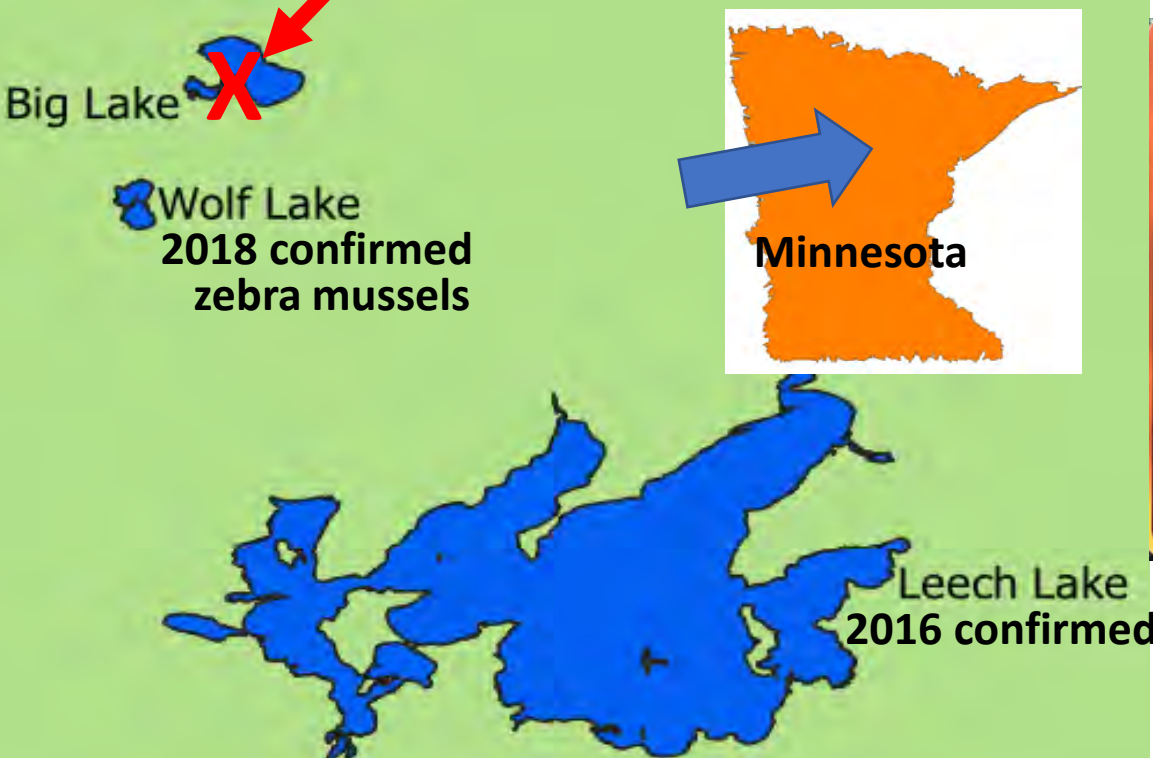
Flathead Lake Bio Station

Methods

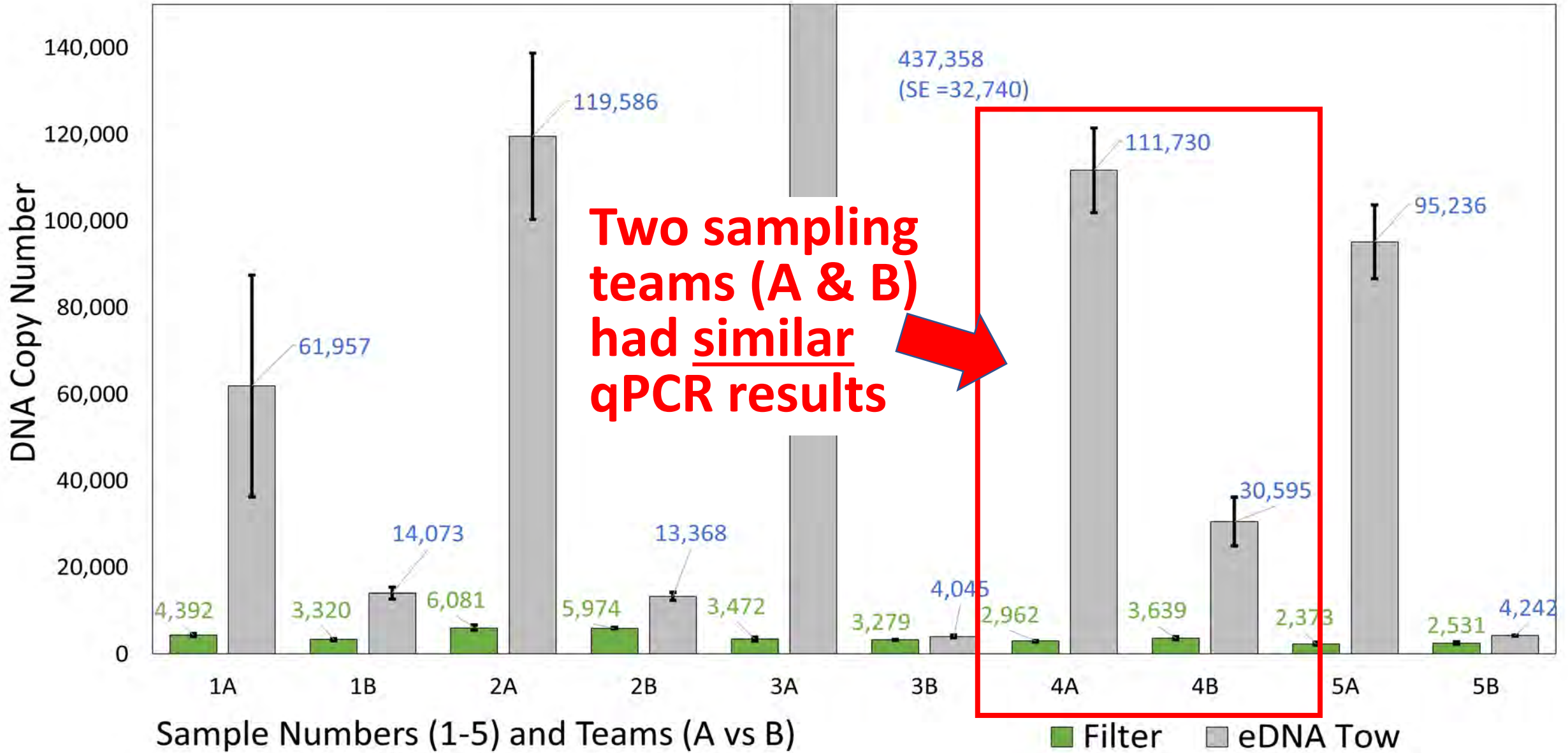
Tow net & qPCR validation:

- 5 Tow nets & 5 filter samples collected by each of 2 teams (UM & MN-DNR)
- 2 labs extracted & qPCR-tested (USGS, MCGL)
- Assessed reproducibility of results

No Detections by either lab (1 adult 2020, 2022!)

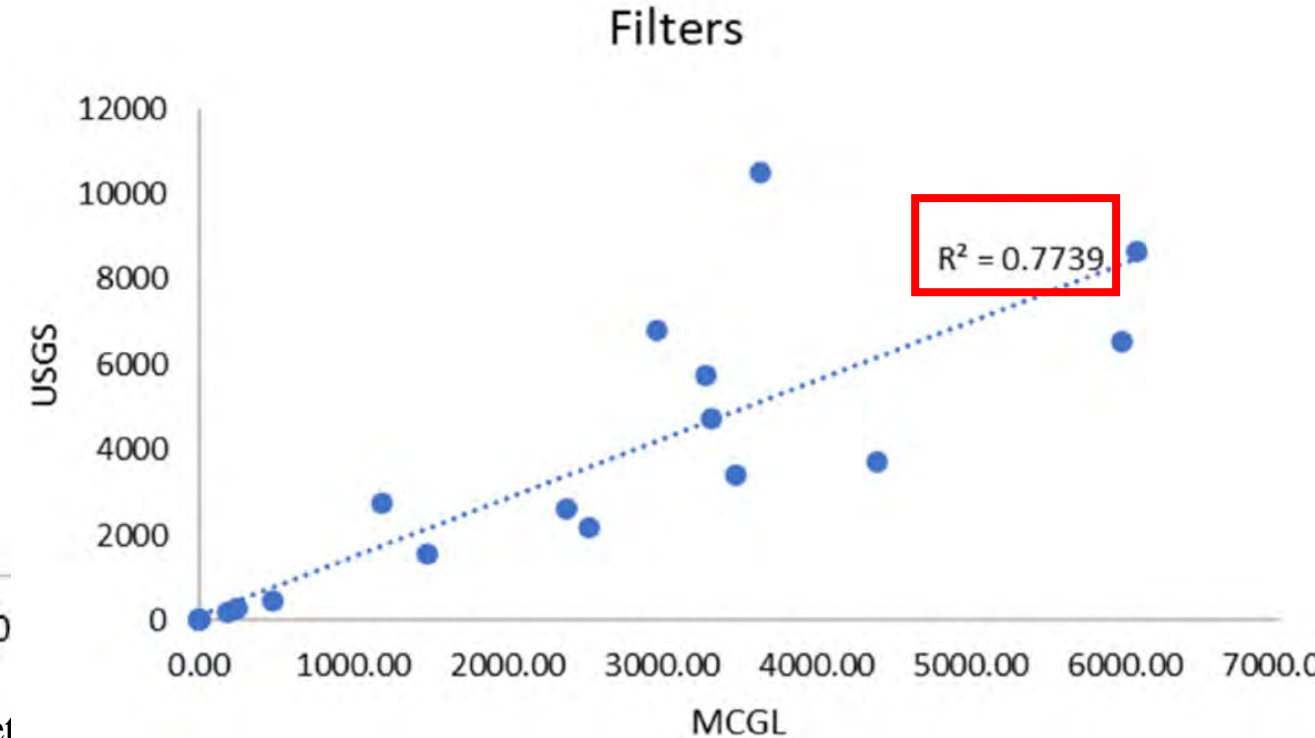
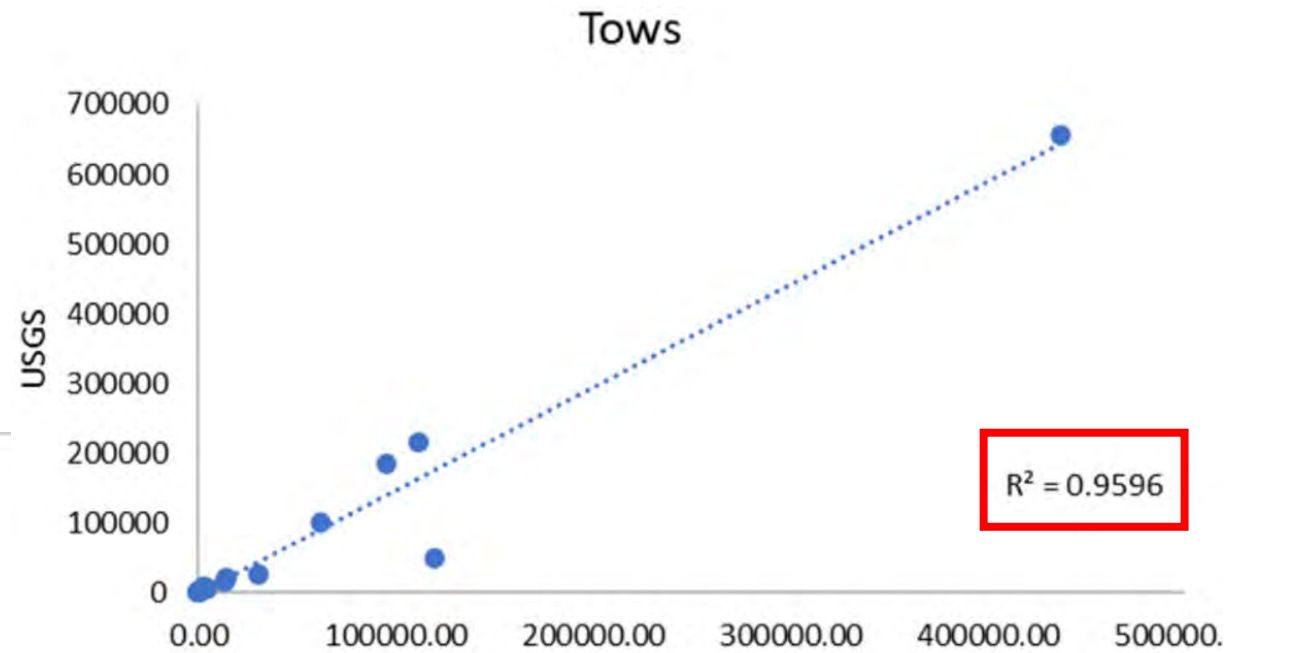
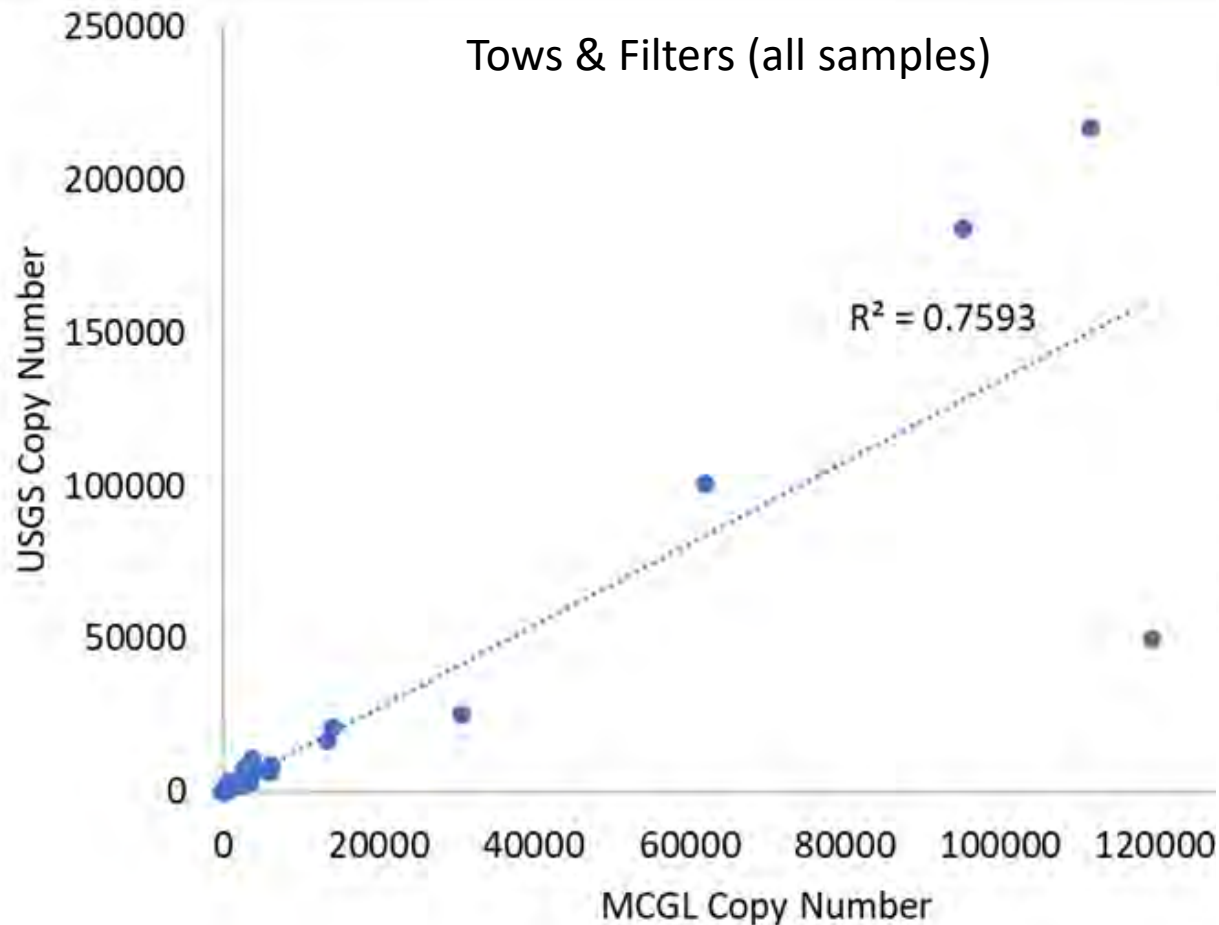


eDNA Detections by the two Teams A & B (for filters vs tows)



Correlated copy numbers of eDNA detected at sites (dots) between labs: USGS & MCGL (Montana Cons. Genet. Lab)

Each dot is the mean copy number from 3 independent qPCR tests per sample



Summary and Conclusions

1. Tow nets provide higher sensitivity than traditional filter samples

(Sepulveda et al. 2019; Schabacker et al. 2020; Miller et al. In review; Howard et al. In prep.)

2. Tow nets detect Dressenids (eDNA) when microscopy does not (veligers)

(Dahlquist et al. In prep)

3. Tow nets, filters, and qPCR results are reproducible by different labs and sampling teams ($r^2 = 0.75$ to 0.95)

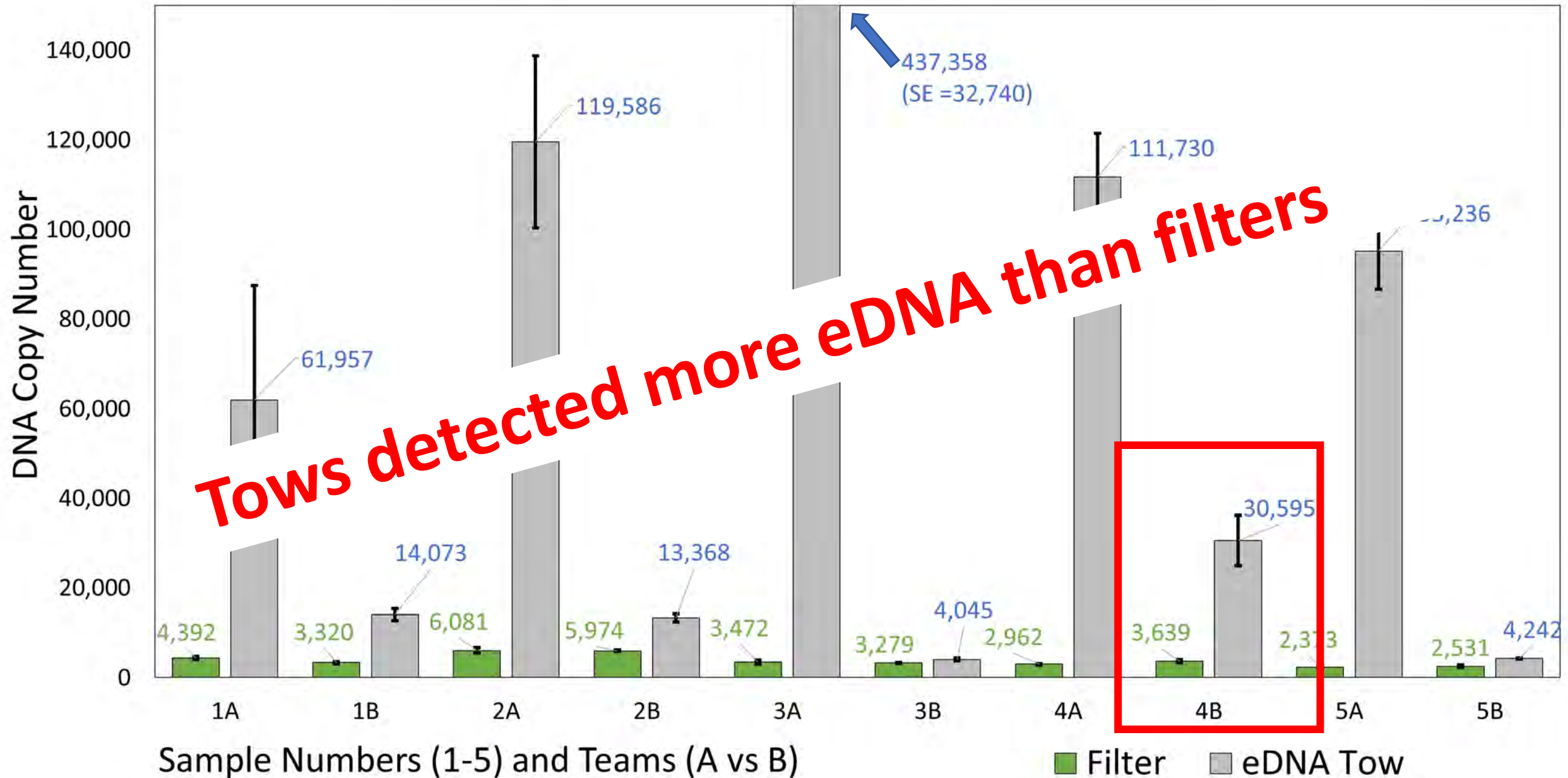
(Howard et al. In prep)

4. Managers should consider adding eDNA-testing to monitoring programs – including ongoing tow-net microscopy for veligers

Questions?



Tow Net vs Filters: eDNA detections (Leech Lake)



Extended Glossary to help managers and non-eDNA experts

eDNA Glossary with Extended Definitions to Inform Managers

1. **Absence:**

Absolute demonstration that an organism is not present in an environment (Abbott et al. 2021). MCGL prefers for using the term “non-detection” instead of absence as it is generally impossible to demonstrate with absolute certainty that an organism is not present in an environment. Non-detection results only pertain to the absence of DNA in the SAMPLES. It is important to understand that these non-detection results for SAMPLES do not definitively establish that AIS are absent from a given water body. Instead, non-detection of DNA within samples increases our confidence that a water body is indeed free of AIS at the time of sampling. More sampling, with multiple methods including eDNA, would further increase our confidence in that conclusion.

2. **Amplification (PCR/qPCR):**

The enzymatic synthesis of specific DNA sequences, using two oligonucleotide primers that hybridize to opposite strands and flank the region of interest in the target DNA (Erich, H.A.1989). The binding of a primer to a target DNA sequence which subsequently leads to the probe to fluorescence. PCR amplifies DNA exponentially, doubling the number of target molecules with each amplification cycle. Amplification does NOT always indicate presence of target DNA, which requires amplification above the LOD/LOQ with low Ct Delay and corroborating amplifications in qPCR replicates

Question

Do metabarcoding & qPCR methods have similar Dressenid-detection rates from tow-net samples?

“eDNA detection and biodiversity assessments are influenced by the sampling (filter pore size) and analysis method (PCR vs metabarcoding)” (Kirtane et al. in prep.)

Methods: Sampled in MN, Florida, and Switzerland (using tow nets and filters)

Metabarcoding: ITS for plants, COI for metazoans (as in Deiner et al. 2017)

qPCR: Genus-specific assay from Gingera et al. (2017)

Results:

qPCR detected Dressenids in 40 of 50 tow samples (10 of 20 from Switzerland; all 30 from Minnesota)

Metabarcoding detected Dressenids in 30 of 50 samples (5 of 20 from Switz; 25 from MN)

Conclusions:

- 1. qPCR was more sensitive than metabarcoding in two labs and countries**
- 2. Use qPCR (or both lab methods) for early detection of rare spp.**

key phases within the eDNA workflow

- 1. *The pre-sampling stage*, where discussions occur between researchers and managers and during which decisions are made on the **criteria for what constitutes a detection** in the context of qPCR results, requires consistent interpretations of terminology and communication of thresholds for detections.
- 2. *The results interpretation phase* is where qPCR results are reviewed and translated into information for managers. The result file exported from a PCR machine contains a lot of information which must be formatted and analyzed before being translated into detection/nondetection results for managers (**determines if there was target DNA detected within the sample based on the number of positive amplifications (among the 3 technical replicates) and the LOD**)
- 3. *The response stage*, where a **decision needs to be made to invoke an action vs inaction**, or what to do and who is supposed to respond, should be already laid out within an agreed-upon framework. Here we **use 3 existing frameworks** (Figure 5) to decide what the response to our Big Lake results should be.

Benefits

- • Sampling methodology is simple, non-invasive, rapid and amenable to collection by non-specialists.
- • As a non-invasive technique no impacts occur to individuals or their habitats compared to some traditional approaches.
- • Can efficiently detect a wide range of taxa, including rare and elusive species often not detected by conventional methods (if reference database is available).
- • Rapid degradation of eDNA in freshwater samples (days to weeks) and marine samples (hours to days) means that a positive detection is likely to be associated with current presence of the species⁶.
- • Easy to store samples for long periods for future analysis.
- • Archived samples allow auditors to commission their own surveys using the exact same methods, eliminating any observer bias.
- • Cost effective before-and-after assessment of aquatic habitats and species to track project impacts⁷.

Challenges

- incomplete genetic reference databases,
- limited differentiation of recently evolved species clusters
- movement of eDNA within the environment
- eDNA can persist for decades in soil or sediments

carefully scoped out with specialists to ensure the approach can meet monitoring objectives,
integrate ecological and environmental expertise relevant to the specific project site

When is eDNA most likely to be useful?

- freshwater species and habitats, or
 - • where it is important to understand impacts on rare or elusive species, or difficult to access habitats, which are difficult to survey through other means, or
 - • where assessment of broad measures of ecosystem health and function is important, or
 - • where traditional survey methods are impracticable (e.g, for logistical, cost or health and safety reasons).
-
- As with any scientific data collection method, the value of eDNA depends on clear and precise framing of the survey objectives, careful planning of sampling, and appropriate analysis and interpretation of the data.

- eDNA can identify multiple taxa and species in a single sample, composite indicators can be selected as proxies for habitat quality or condition. These can be species-based (e.g., fish species diversity) or function-based (e.g., relative abundance of predatory vs herbivorous species). This can enable cost-effective monitoring of positive and negative changes in habitat quality over time, whilst expanding the scope of monitoring to ecosystem functioning.