Invasive species management can benefit from eDNA

(Sepulveda et al. in prep, TREE)

Gordon Luikart, Steve Amish, Dulaney Miller, Cody Youngbull, Shawn Devlin, Phil Matson.

FLBS & Montana Conservation Genomics Lab, University of Montana

Monitoring network for mussel & milfoil eDNA

Thank you collaborators!

FLATHEAD LAKERS

THE CONFEDERATED SALISH AND KOOTENAI TRIBES OF THE FLATHEAD RESERVATION
A People of Vision

Flathead Basin Commission

FOREST SERVICE US DEPARTMENT OF AGRICULTURE

USGS science for a changing world

Whitefish Lake INSTITUTE

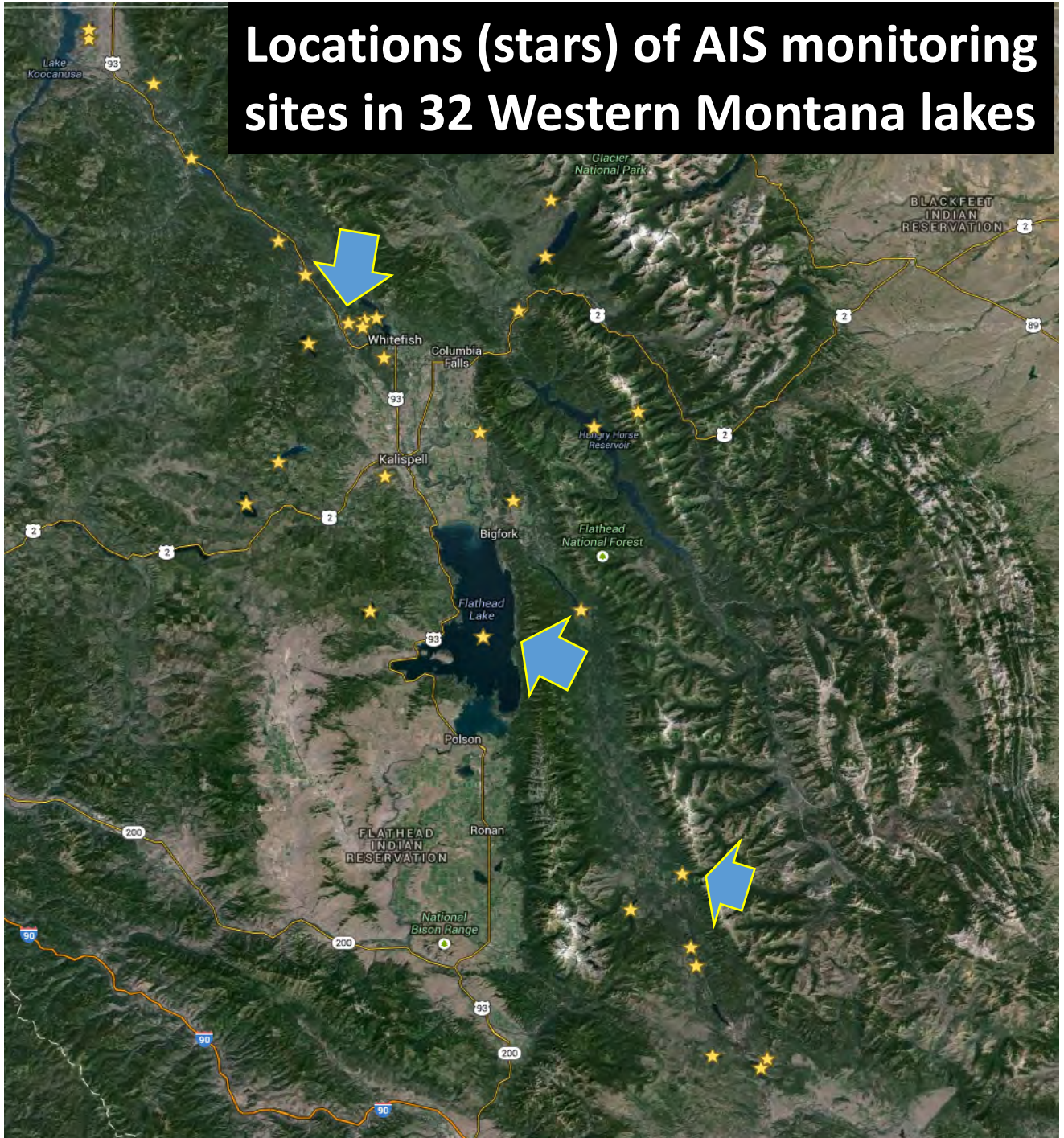
GLACIER NATIONAL PARK

Montana Fish, Wildlife & Parks

WEED DISTRICT MISSOULA COUNTY & EXTENSION

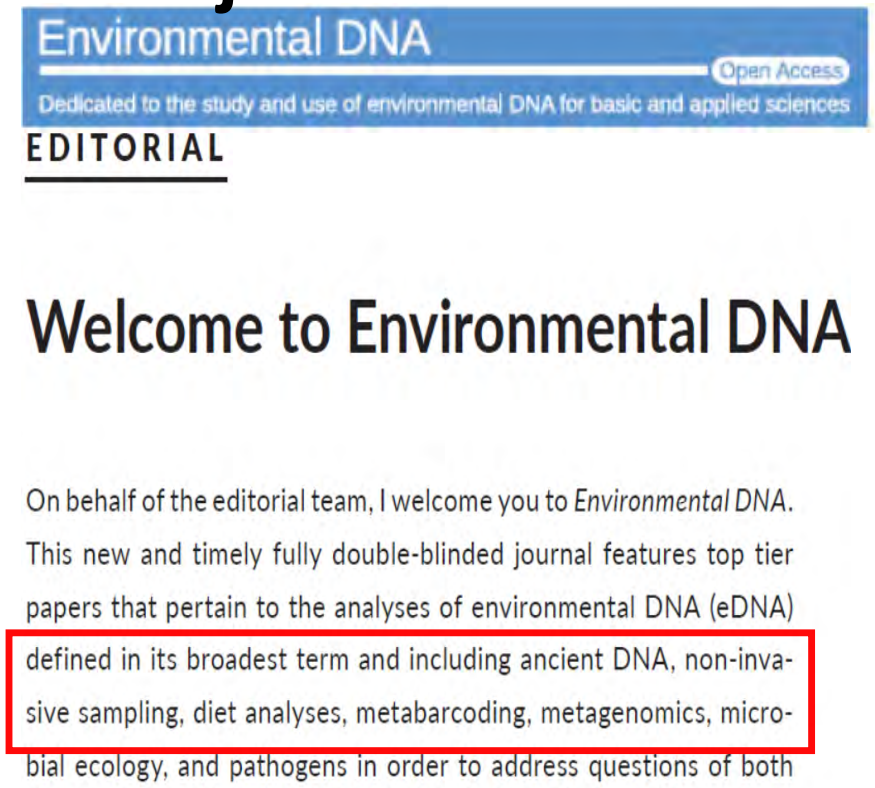
Clearwater Resource Council

Locations (stars) of AIS monitoring sites in 32 Western Montana lakes



eDNA is a mature science & reliable monitoring tool

New journal


Environmental DNA

Open Access

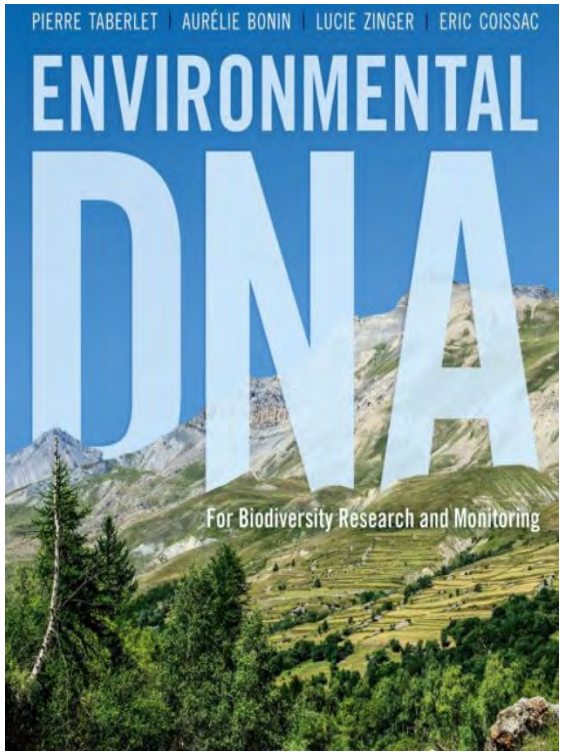
Dedicated to the study and use of environmental DNA for basic and applied sciences

EDITORIAL

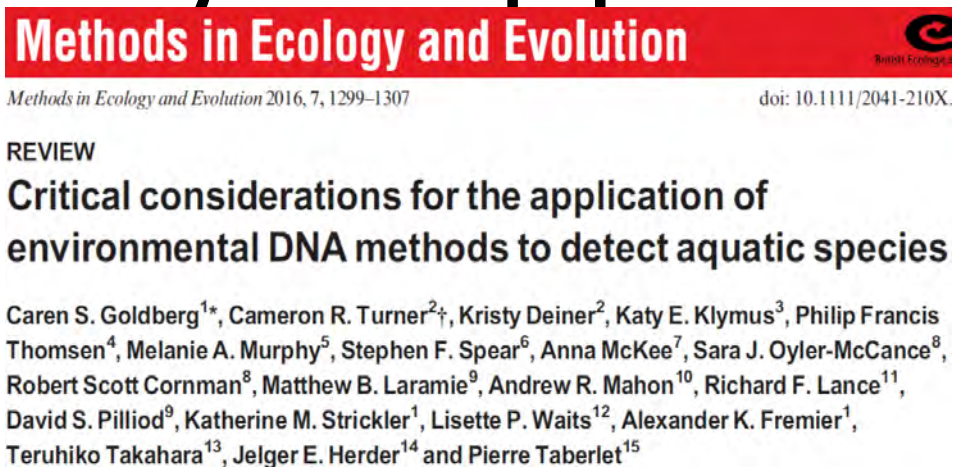
Welcome to Environmental DNA

On behalf of the editorial team, I welcome you to *Environmental DNA*. This new and timely fully double-blinded journal features top tier papers that pertain to the analyses of environmental DNA (eDNA) defined in its broadest term and including ancient DNA, non-invasive sampling, diet analyses, metabarcoding, metagenomics, microbial ecology, and pathogens in order to address questions of both

New book



Many review papers


Methods in Ecology and Evolution

Methods in Ecology and Evolution 2016, 7, 1299–1307 doi: 10.1111/2041-210X.

REVIEW

Critical considerations for the application of environmental DNA methods to detect aquatic species

Caren S. Goldberg[1]*, Cameron R. Turner[2]†, Kristy Deiner[2], Katy E. Klymus[3], Philip Francis Thomsen[4], Melanie A. Murphy[5], Stephen F. Spear[6], Anna McKee[7], Sara J. Oyler-McCance[8], Robert Scott Cornman[8], Matthew B. Laramie[9], Andrew R. Mahon[10], Richard F. Lance[11], David S. Pilliod[9], Katherine M. Strickler[1], Lisette P. Waits[12], Alexander K. Fremier[1], Teruhiko Takahara[13], Jelger E. Herder[14] and Pierre Taberlet[15]

"The strength of evidence depends on the frequency & consistency of positive eDNA samples from a location"

Evidence is now extensive that eDNA detections can be reliable & often precede visual detections of invasive species establishment.

(Rees 2014; Bohmann et l. 2014; Goldberg et al. 2015; Gingera et al. 2017, Hosler 2017)

Do large volume plankton tow net samples provide better early detection of AIS than standard filter samples?

Drag tow net to sample easi
around a dock or pier

Tow net sample for DNA extraction
(Sampling demonstrated in our video)

A filter samples only 1 liter before filter clogs

50 µ human
6 µ filament

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

(Sepulveda et al.; Schabacker at al.; Amish et al. in prep/revision)

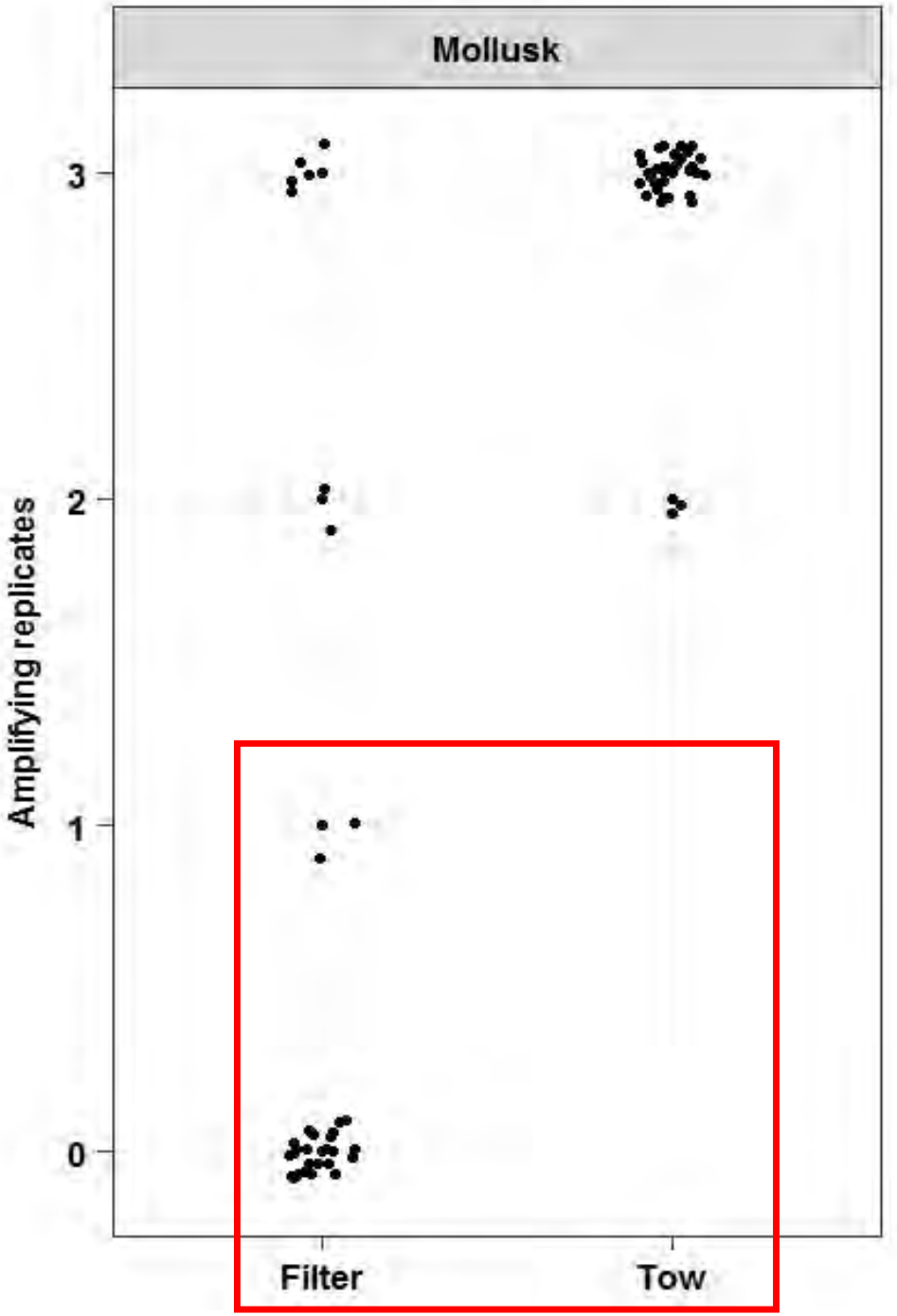
“Improved environmental DNA detection using a novel high volume water sampling method”

(Shabacker et al. In review)

- Tow net >3,000 liters (64 micometer pore size)
- Paired filter sample of 1 liter (0.45 micometer)
- 34 locations (Flathead, Holland, Beaver Lakes)
- 3 qPCR assay replicates per sample for:
  Northern milfoil (*Myriophyllum sibiricum*)
  and mollusks (including *Helisoma anceps*)

Tow nets detected mollusk & milfoil DNA more often than filter samples

Planorbid snails

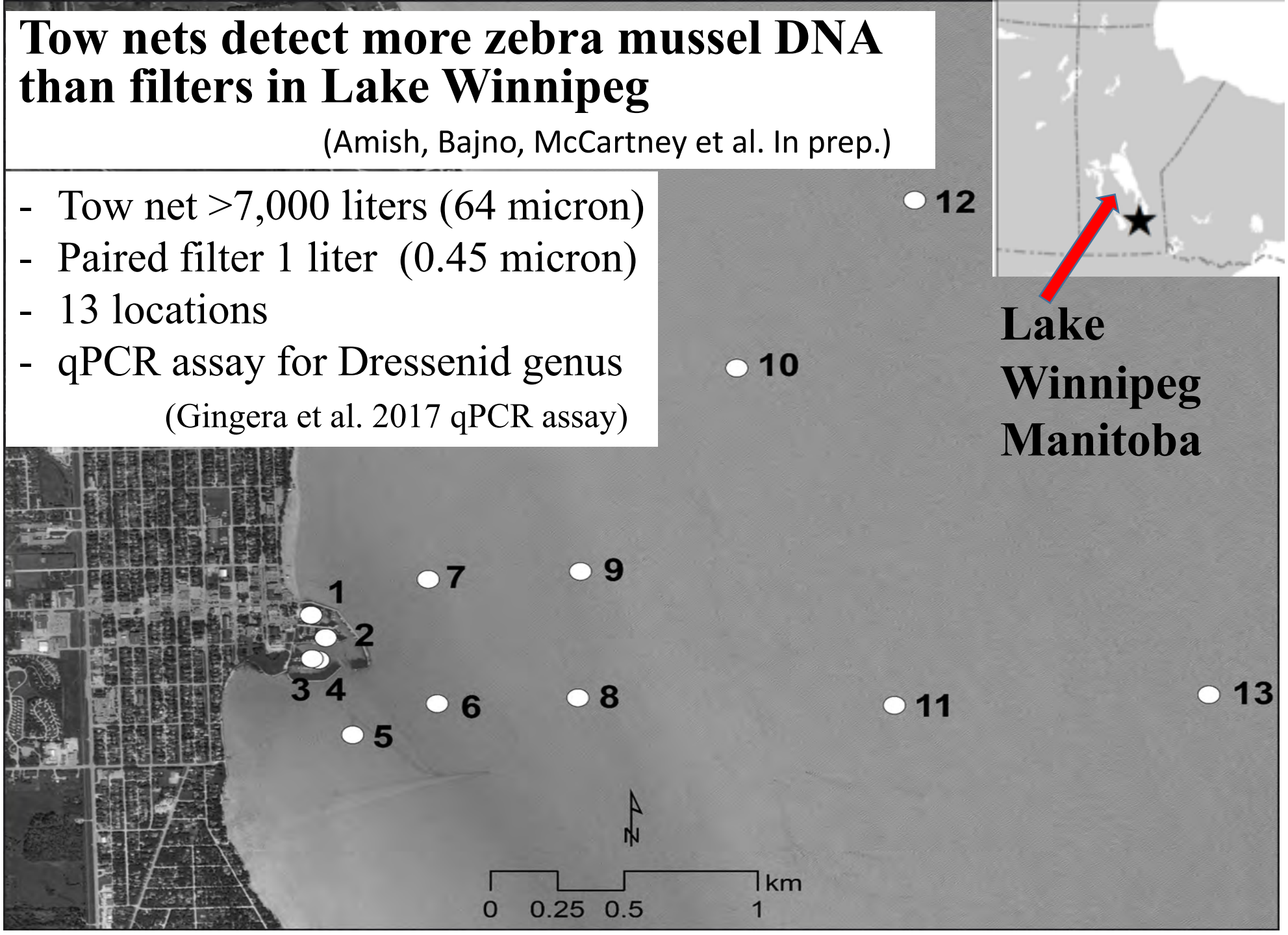


Tow nets detect more zebra mussel DNA than filters in Lake Winnipeg

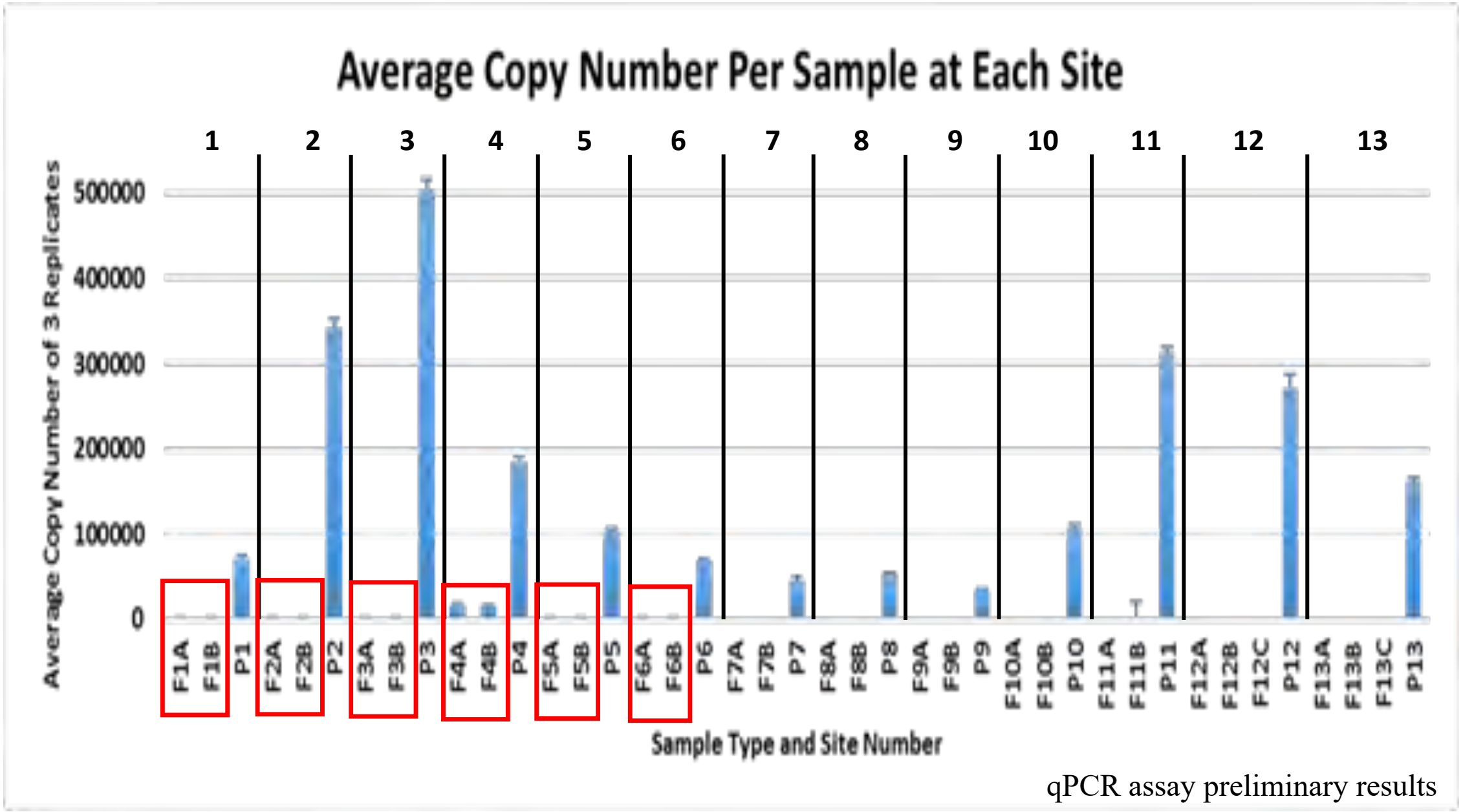
(Amish, Bajno, McCartney et al. In prep.)

- Tow net >7,000 liters (64 micron)
- Paired filter 1 liter (0.45 micron)
- 13 locations
- qPCR assay for Dressenid genus

(Gingera et al. 2017 qPCR assay)



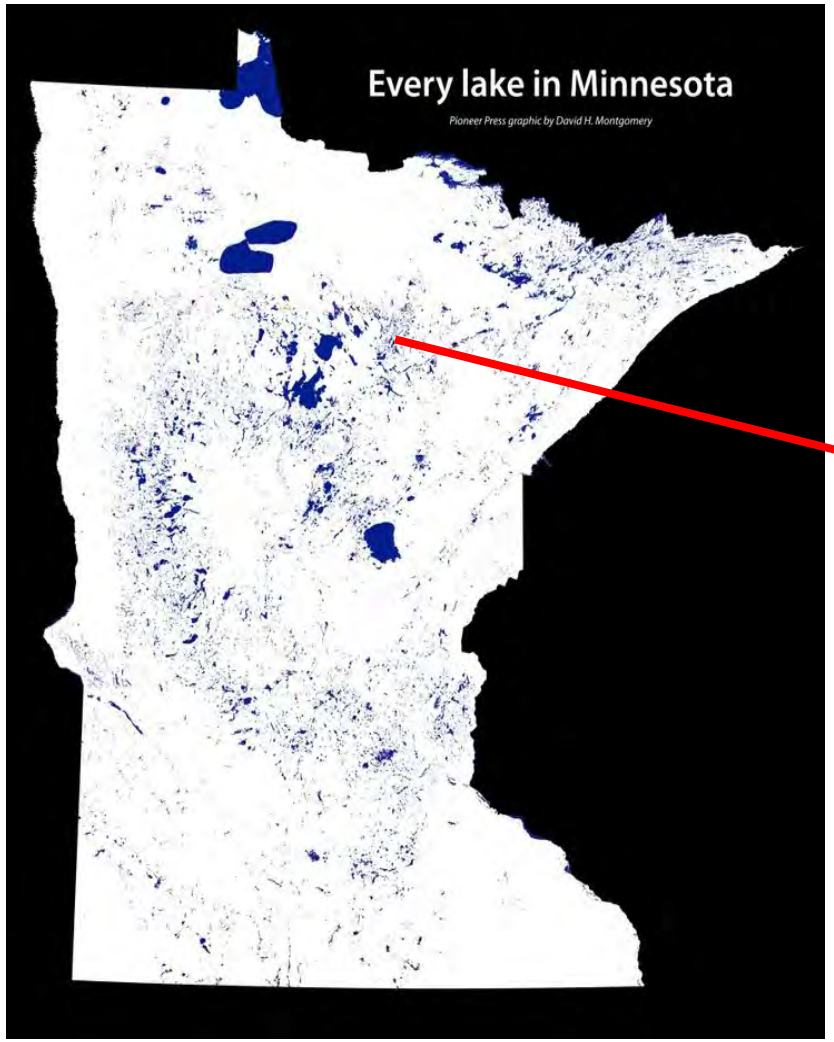
Tow nets detect more DNA than filters (F) in Lake Winnipeg

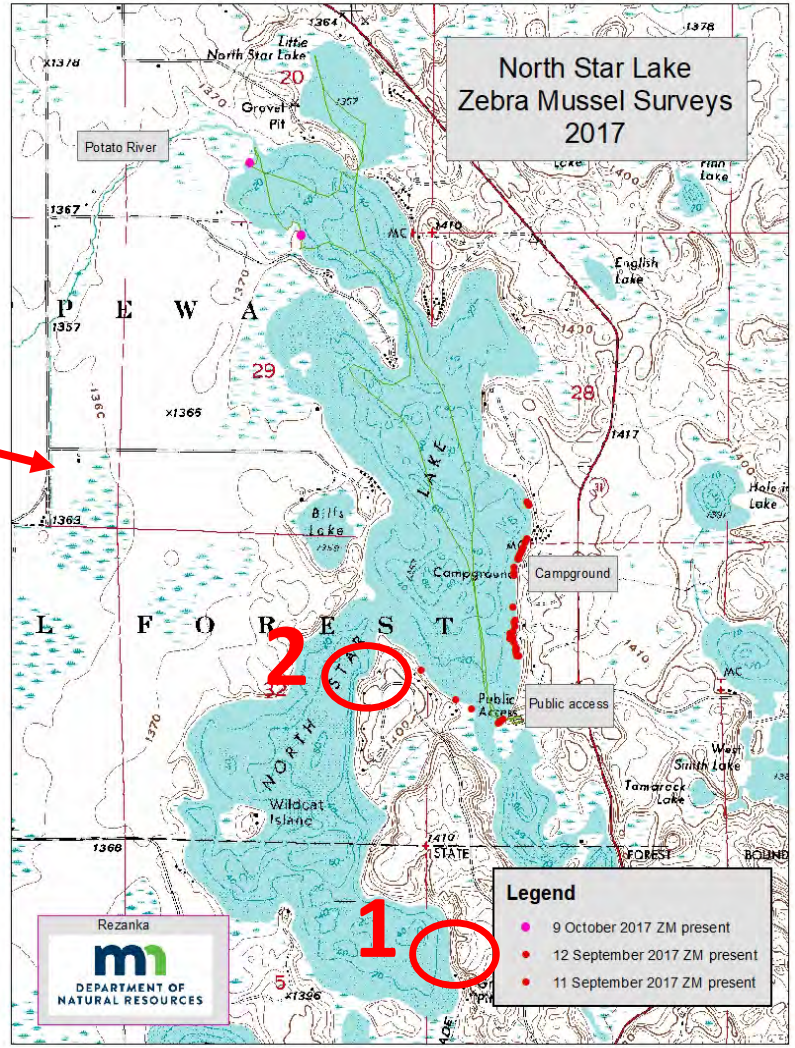


qPCR assay preliminary results

Tow nets detect more zebra mussel DNA than filters in North Star Lake, MN

(Amish, Bajno, McCartney, et al. In Prep)





DNA copy number

Tow net	Filter	
51	00	1
433	04	
475	00	
109	06	
449	01	
10,809	16	2
4,862	00	
4,673	01	
6,869	14	
4,839	22	

Gingera et al. 2017 qPCR assay results

Flathead eDNA testing via tow nets since 2012

12 locations, **2** times per year 2012-2015

30 locations **3** times per year since 2016 Tibor detections

No positives for ZM or QM. Positives for native taxa.

Early detection offers hope of eradication, suppression, and **<u>containment</u>**.

THE CONFEDERATED SALISH AND KOOTENAI TRIBES OF THE FLATHEAD RESERVATION

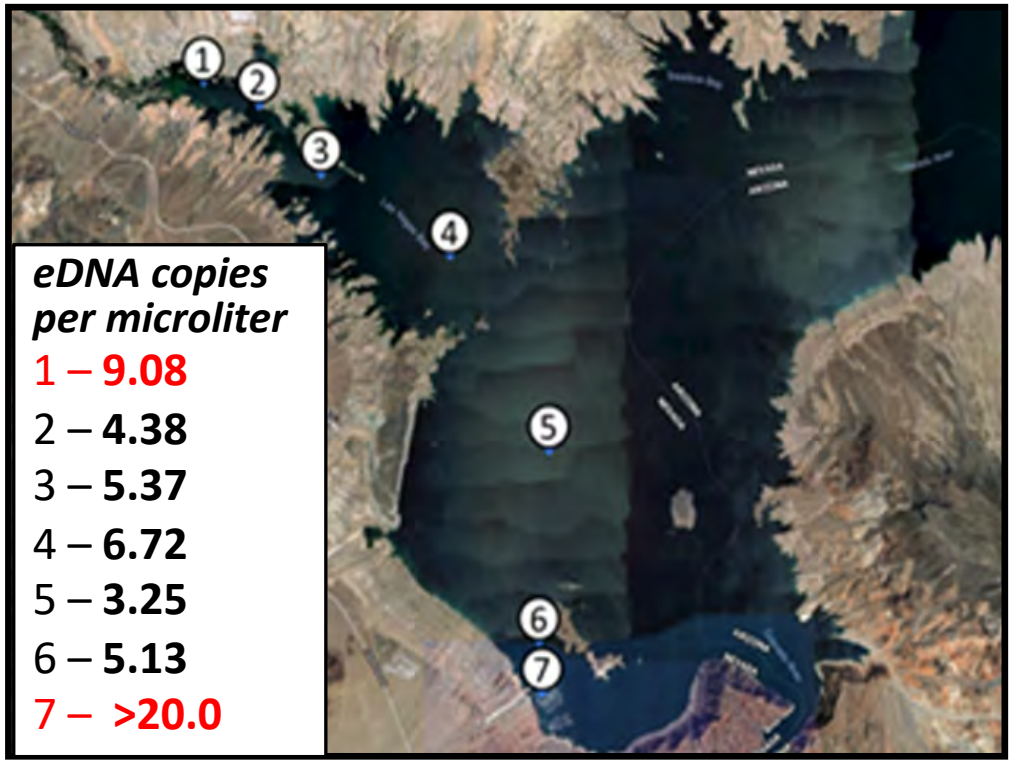
A People of Vision

World's only continuous-flow quantitative PCR machine allows source tracking and quantification while in transit

The DNA-Tracker operating by the side of a lake to continuously monitor for eDNA.
(Youngbull et al., in commercialization)

Sites 1 to 7 are points where water samples were collected from a speed boat.

DNA concentration of invasive zebra mussels increased as the boat approached colonies (sites #1 & #7) in Lake Mead. See data table in legend for DNA copy number detected per site.



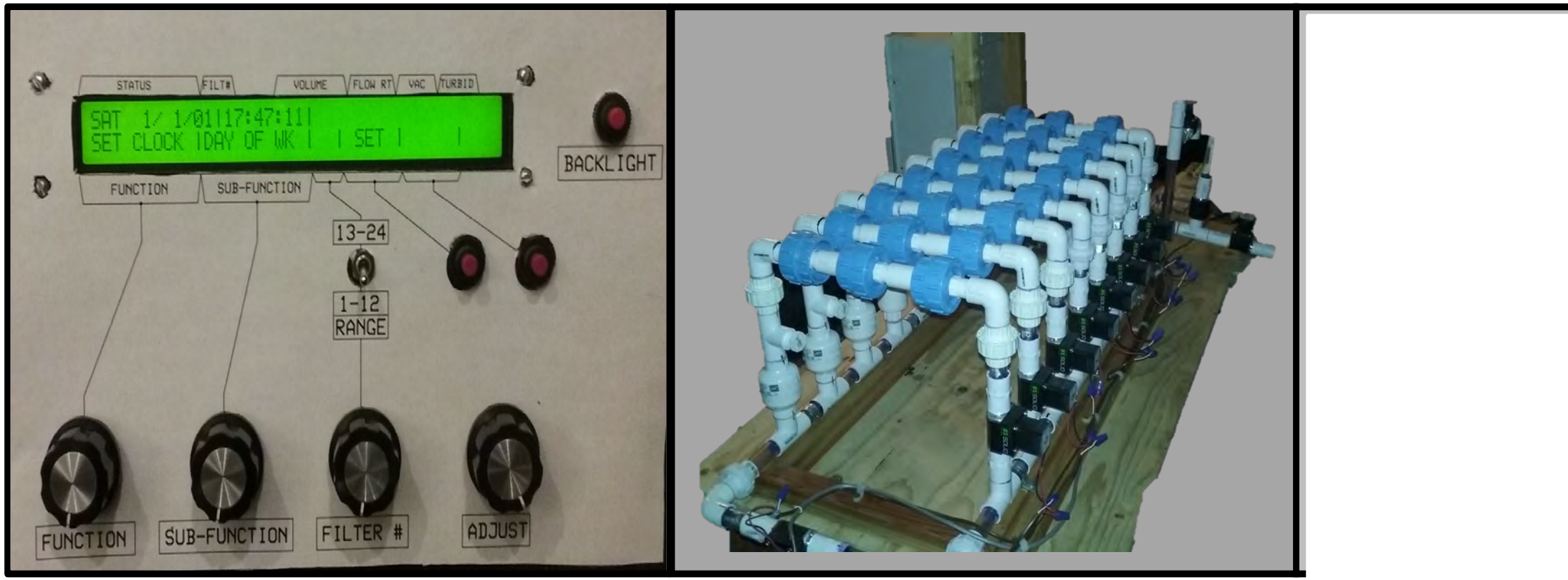
Photos from C. Youngbull, Flathead Lake Biological Station, Univ. of Montana.

Autonomous eDNA sampling instrument

Sampling time is programmable. Collection can be triggered by readings from sensors for temperature, pH, turbidity, or flow.

Control panel for setting water-collection parameters for each sample (date, etc.)

Rows of independent **filtering units**, each collecting eDNA samples.



In development by Amish et al. (Photos from Flathead Lake Biological Station. University of Montana)

NASA project: "Predicting the Spread of Aquatic Invasive Species Using Remote Sensing, Genetics, and Climate Modeling"

Goal: Provide managers with tools for AIS management

1. Evaluate & improve **cell phone apps** for AIS early detection & management

(Leif Howard et al. in prep)

2. Improve & 'crowdsource' **databases** for AIS early detection & management
   - Provide computer programs for managers to upload **& visualize data on AIS detections** (states, provinces, tribes, & federal agencies)
   - Add eDNA data to USGS's NAS web site and other databases

3. Build predictive models to identify hotspots of future AIS spread
   - Dressenids, brook trout, rainbow trout, bass

Conclusions:

1. Plankton tow nets **often detect more DNA than traditional filter methods** for diverse taxa (milfoil, mollusks, zebra mussels; & bass - see Sepulveda et al. In Press.)

2. Researchers & managers should **consider using large volume tow net samples** to improve sensitivity and early detection

3. Real time DNA tracking, autonomous sampling, cell phone aps, and predictive models (with heat-maps of invasion hotspots) can improve AIS early detection and management.

Future research and monitoring:

Develop decision tree with managers for interpreting and reporting on eDNA detections (Sepulveda et al. in prep., TREE)

Test aliquots from existing veliger tow net surveys for DNA from invasive mussels

Compare sensitivity of tow nets, filters, & DNA tracker near colonies of mussels (in Minnesota?) and invasive fish

Extend USGS round robin to include “optimized” qPCR assays and tow net samples

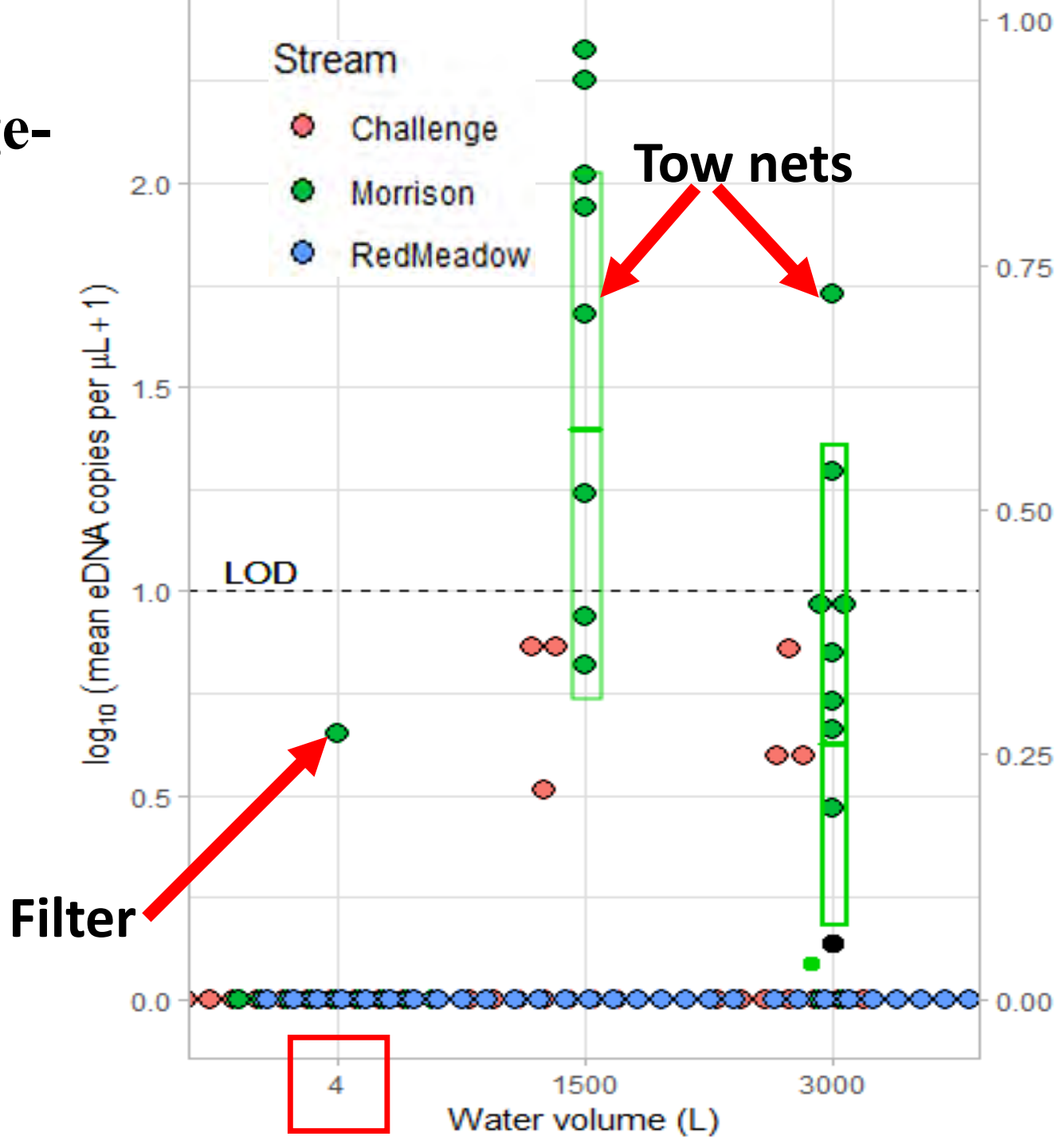
Thank you!

“Improved detection of endangered species via large-volume sampling of eDNA”

(Sepulveda et al. In Press)

Tow nets detected more bull trout DNA than filters in Montana streams

However, sampling higher water volumes increased the PCR inhibition so the DNA extraction protocol was modified



Environmental DNA (eDNA) is a reliable monitoring tool

Review Trends in Ecology & Evolution, June 2014, CellPress

Environmental DNA for wildlife biology

This body of work takes eDNA detection from a technical breakthrough to an established, reliable method

Biological Conservation 183 (2015)

ELSEVIER

journal homepage: www.elsevier.com/locate/biocon

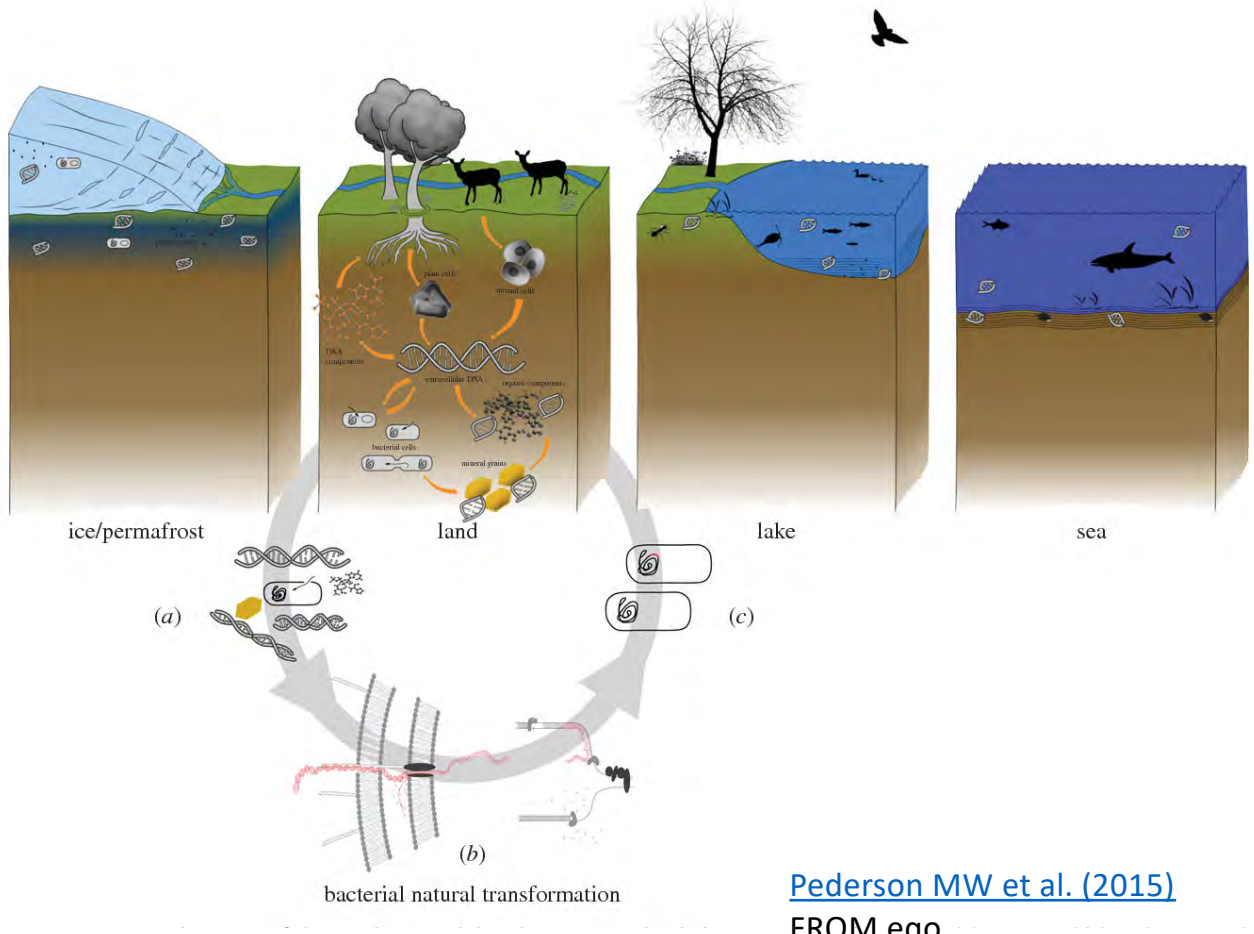
Special Issue Article: Environmental DNA

Moving environmental DNA methods from concept to practice for monitoring aquatic macroorganisms

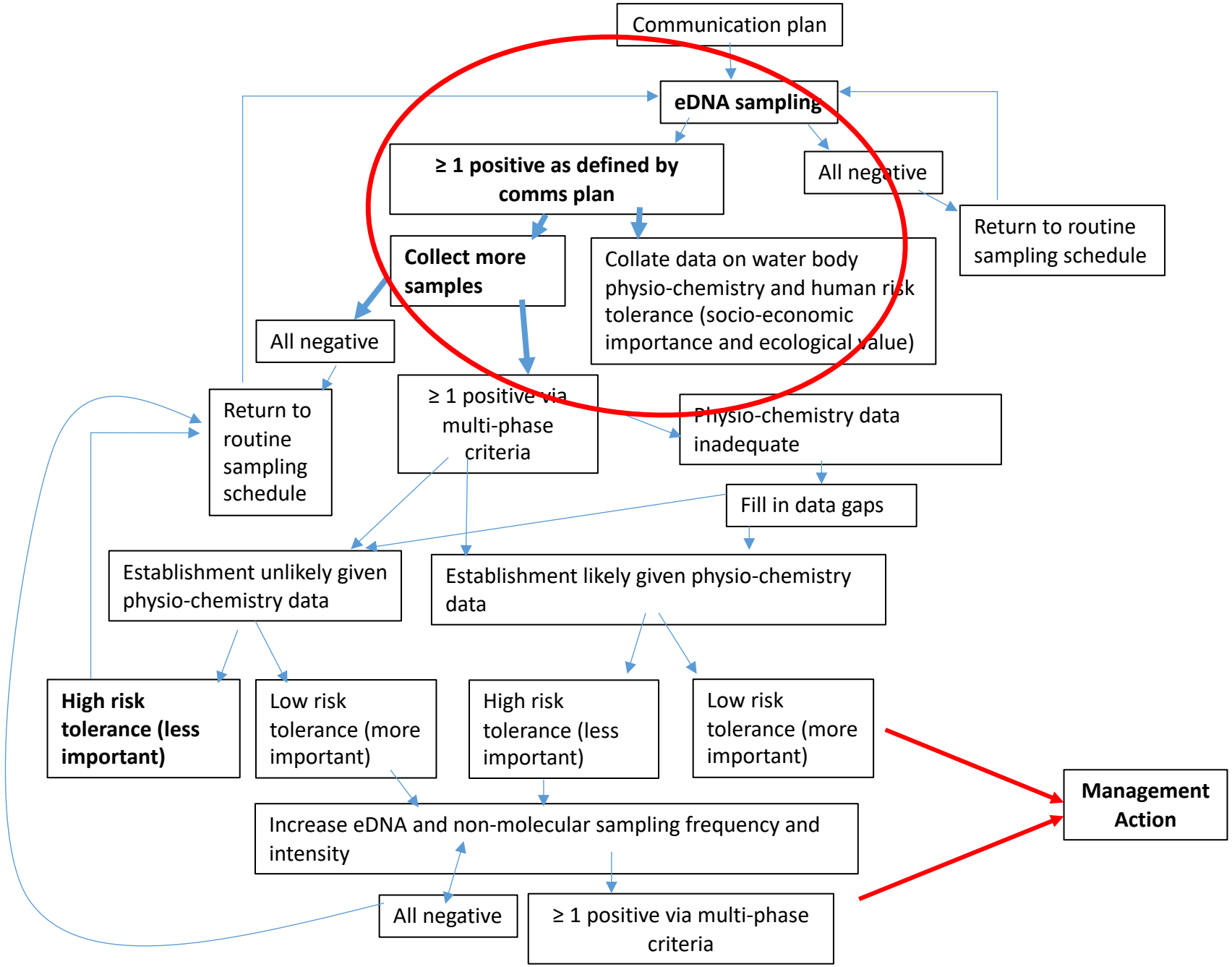
Caren S. Goldberg [a,*], Katherine M. Strickler [a], David S. Pilliod [b]

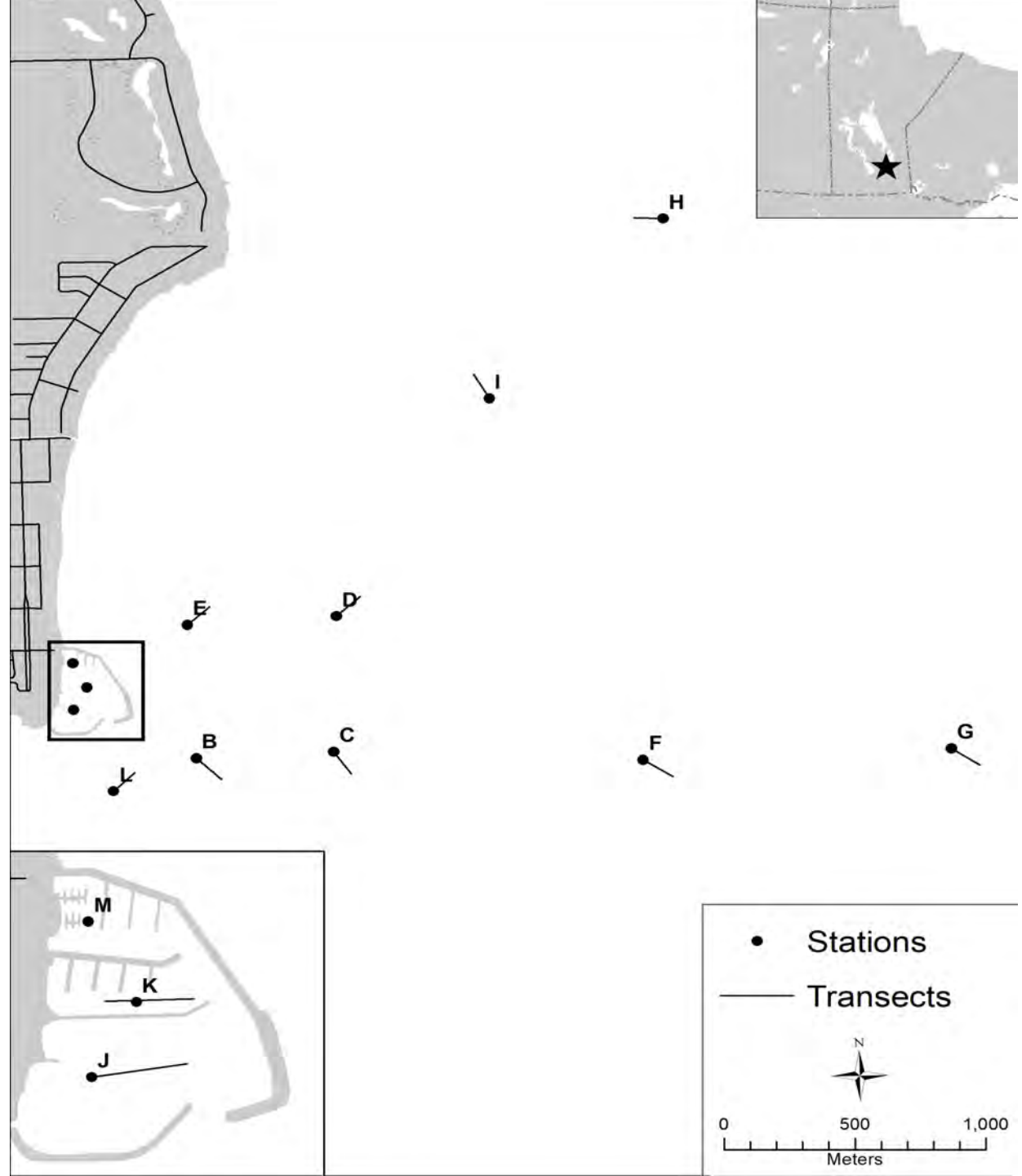
Utilizing eDNA - Limitations

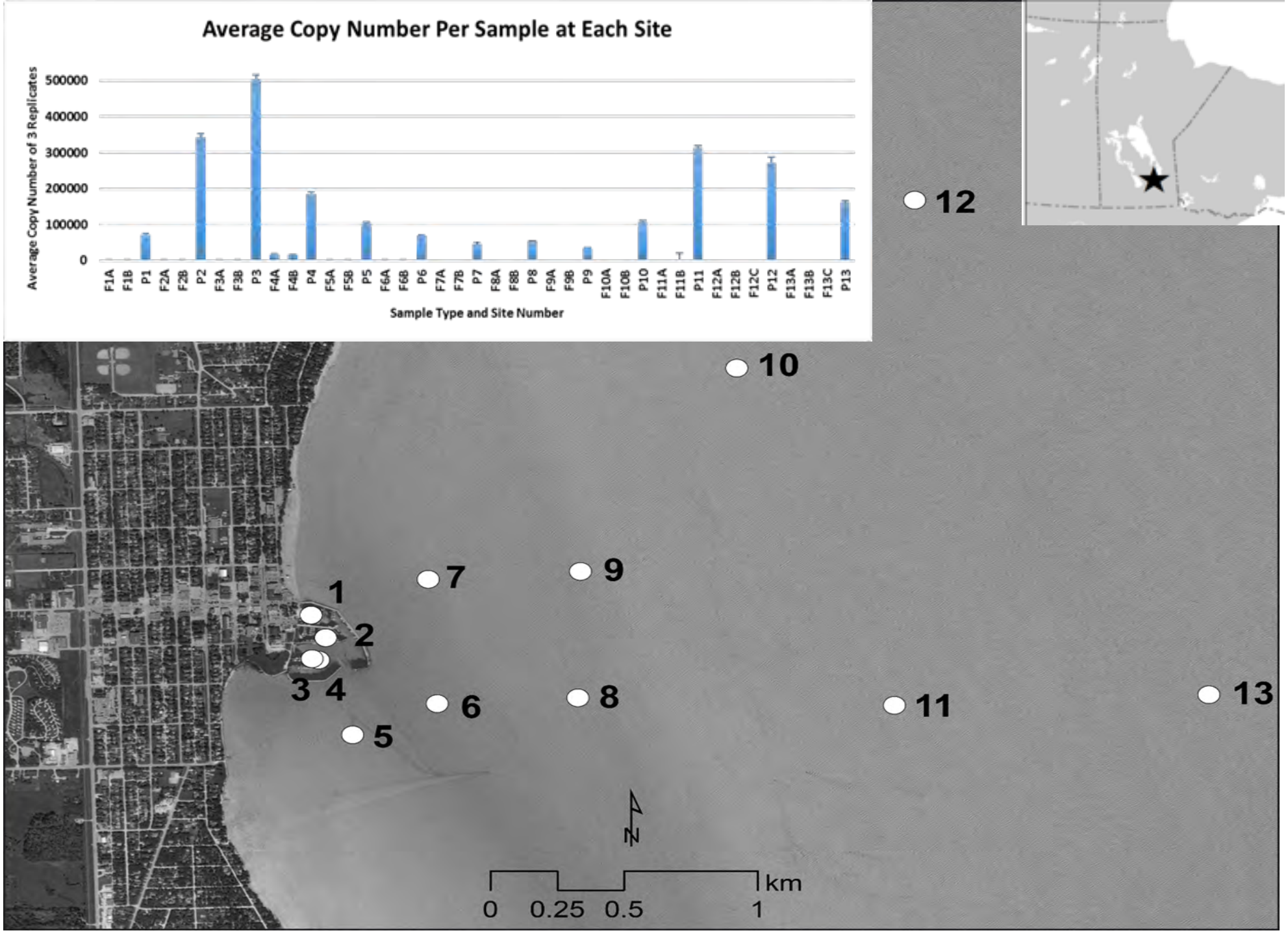
- Detects DNA, not necessarily organisms
- No differentiation between live and dead
- Source of DNA (primary, secondary, etc.) not determinable.
- Persistence of DNA in the environment is dependent on several factors that vary from water body to water body.



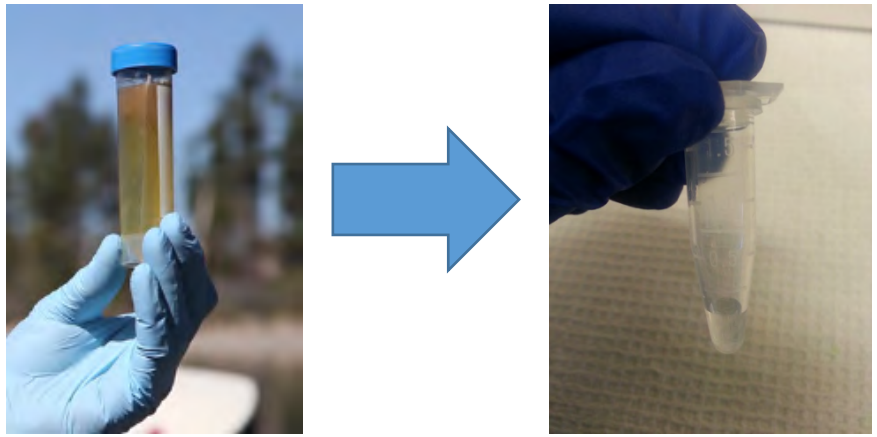
Pederson MW et al. (2015)
FROM eqo


Communication plan
eDNA sampling
≥ 1 positive as defined by comms plan
All negative
Return to routine sampling schedule
Collect more samples
Collate data on water body physio-chemistry and human risk tolerance (socio-economic importance and ecological value)
All negative
Return to routine sampling schedule
≥ 1 positive via multi-phase criteria
Physio-chemistry data inadequate
Fill in data gaps
Establishment unlikely given physio-chemistry data
Establishment likely given physio-chemistry data
High risk tolerance (less important)
Low risk tolerance (more important)
High risk tolerance (less important)
Low risk tolerance (more important)
Management Action
Increase eDNA and non-molecular sampling frequency and intensity
All negative
≥ 1 positive via multi-phase criteria


H
I
E
D
B
C
F
G
L
M
K
J
Stations
Transects
N
0
500
1,000
Meters

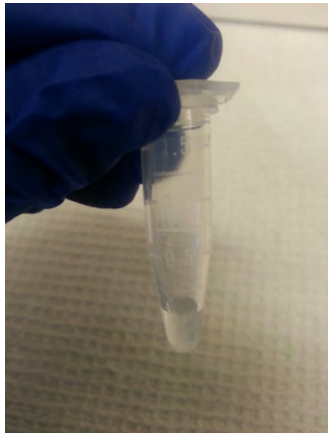

Average Copy Number Per Sample at Each Site
Average Copy Number of 3 Replicates
500000
400000
300000
200000
100000
0
F1A F1B P1 F2A F2B P2 F3A F3B P3 F4A F4B P4 F5A F5B P5 F6A F6B P6 F7A F7B P7 F8A F8B P8 F9A F9B P9 F10A F10B P10 F11A F11B P11 F12A F12B F12C P12 F13A F13B F13C P13
Sample Type and Site Number
12
10
7
9
1
2
3
4
6
8
11
13
5
N
0
0.25
0.5
1
km

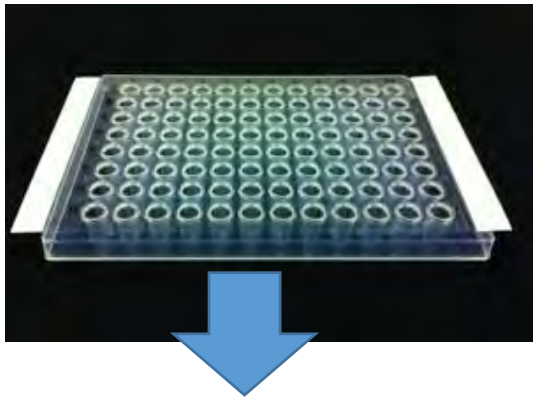
At the lab:



DNA extraction

At the lab:



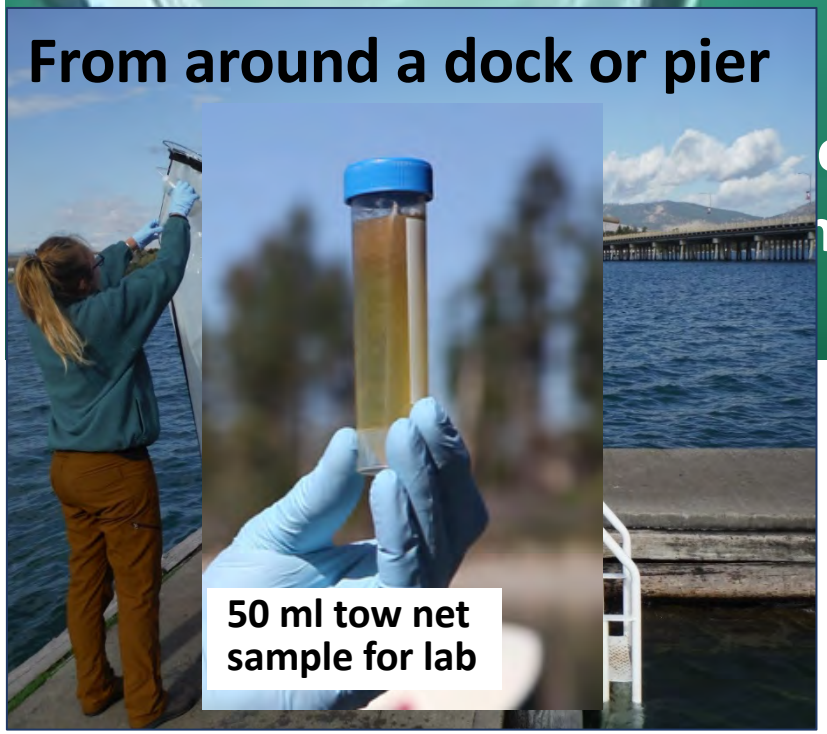


DNA extraction

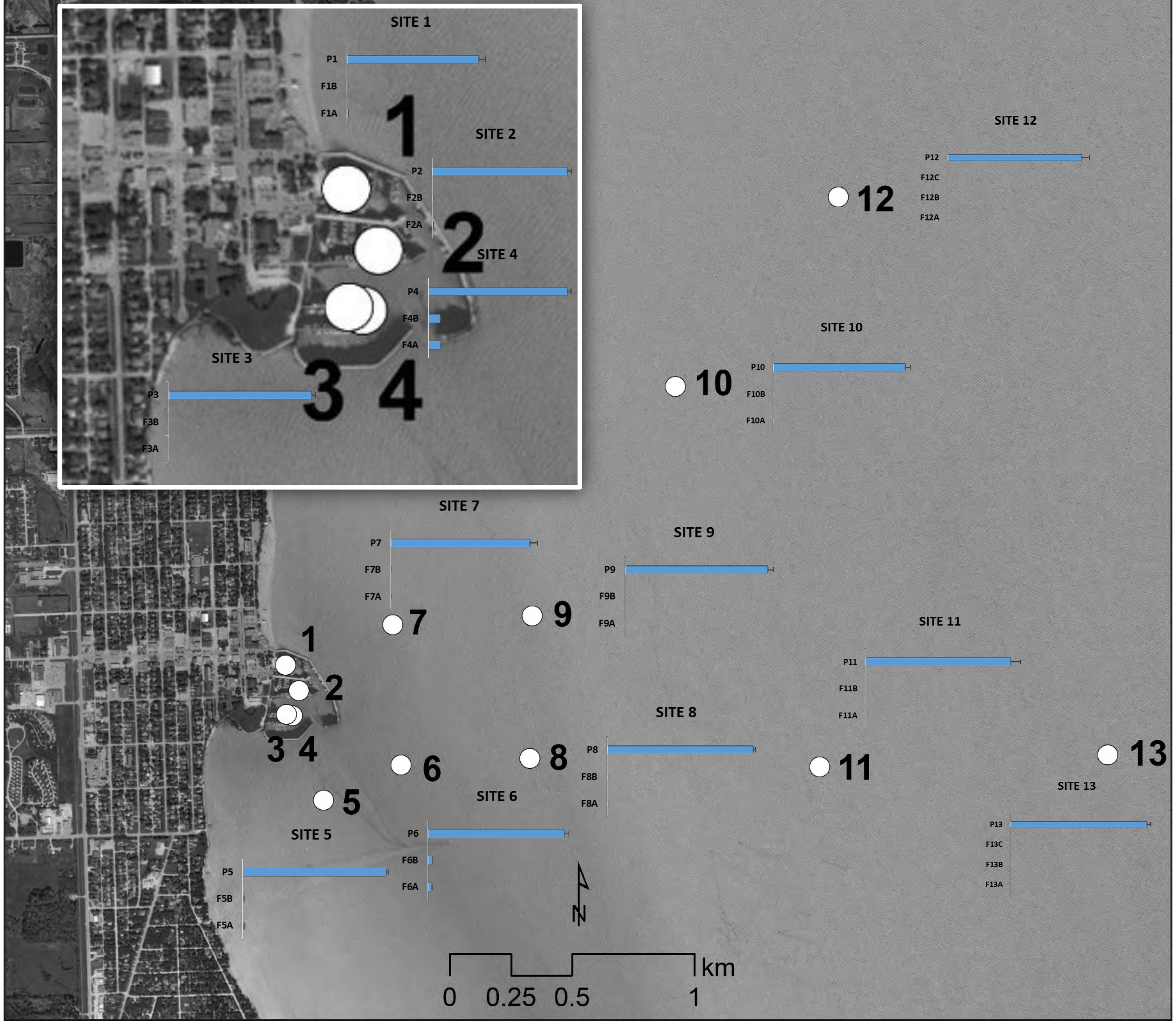
Real-time *q*PCR detection assay

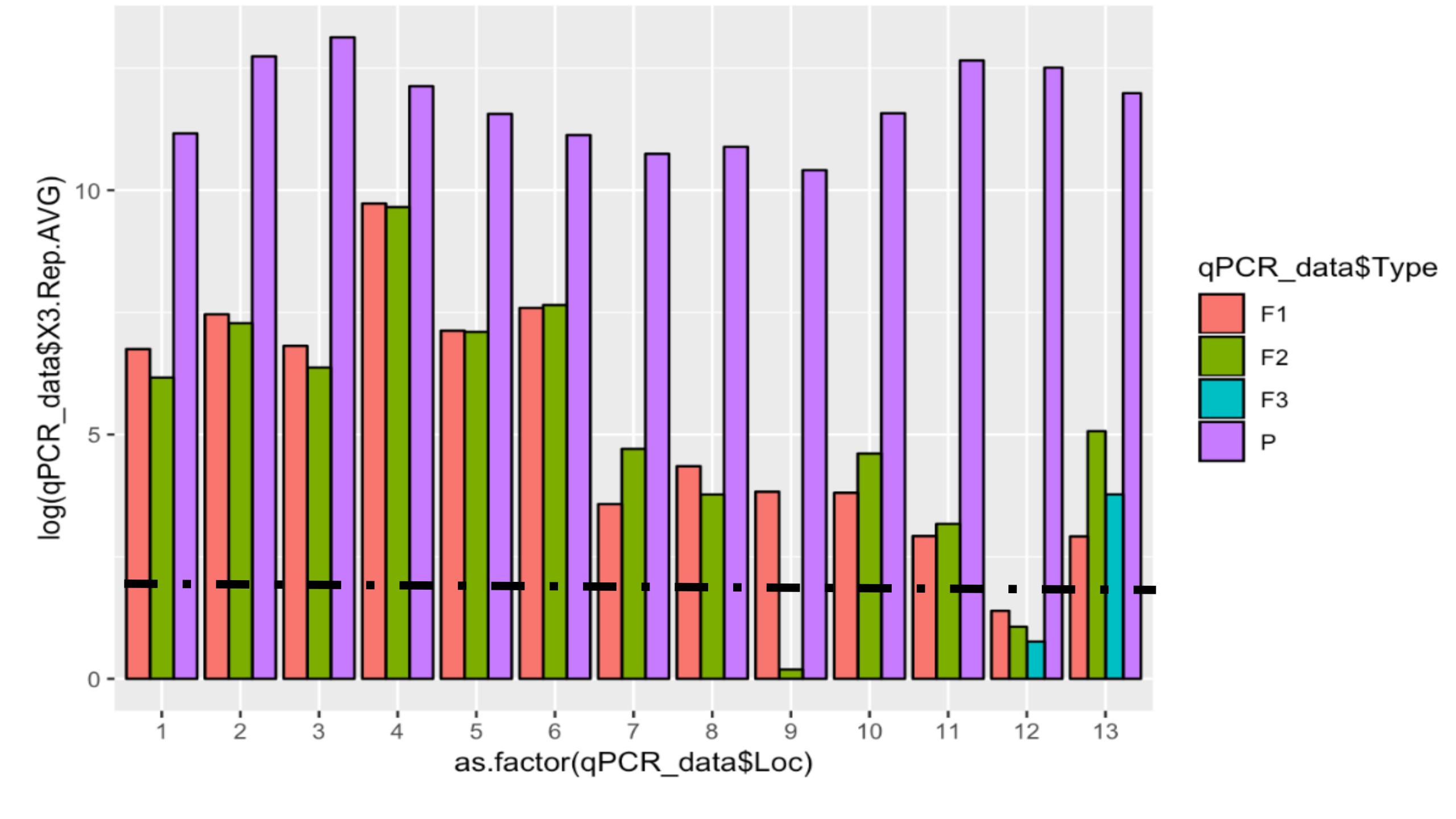
qPCR is most sensitive.
Allows multi-species ID

Plankton tow net (64 micron mesh size)

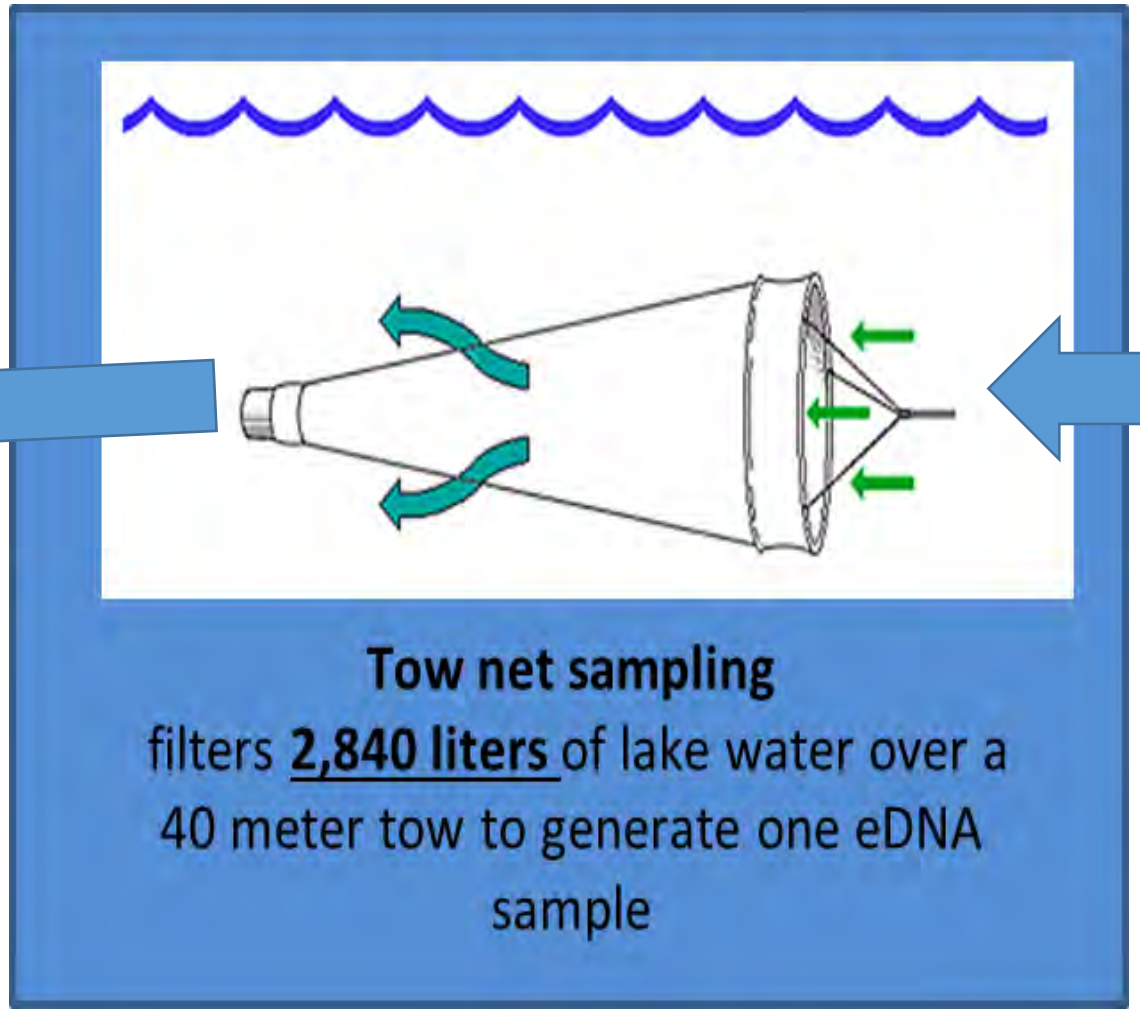


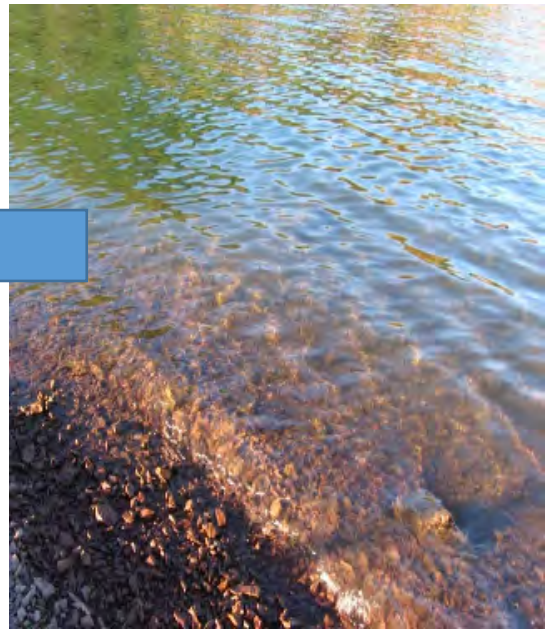
MORE total DNA from tow nets than filters


SITE 1
P1
F1B
F1A
SITE 2
P2
F2B
F2A
SITE 4
P4
F4B
F4A
SITE 3
P3
F3B
F3A
SITE 12
P12
F12C
F12B
F12A
SITE 10
P10
F10B
F10A
SITE 7
P7
F7B
F7A
SITE 9
P9
F9B
F9A
SITE 11
P11
F11B
F11A
SITE 8
P8
F8B
F8A
SITE 6
P6
F6B
F6A
SITE 5
P5
F5B
F5A
SITE 13
P13
F13C
F13B
F13A
N
0 0.25 0.5 1 km


log(qPCR_data$X3.Rep.AVG)
10
5
0
1
2
3
4
5
6
7
8
9
10
11
12
13
as.factor(qPCR_data$Loc)
qPCR_data$Type
F1
F2
F3
P

Plankton tow nets process 1000s of liters and detect more DNA than standard filter approaches





Sepulveda et al. in revision; Schabacker et al. in review; Miller et al. in prep

My immediate response would be (1) communication and (2) gather more information. I would do this regardless if it was just 1 faint signal or a consistent, strong signal from many samples. I would first communicate with FWP, who is the lead agency for AIS. They have a response plan. I would also do some “internal” communication. Perhaps surprisingly, I would not alert the public. That is a careful step that FWP wants to lead. Then I would attempt to size up the problem with more information. This would include veliger tows, more eDNA and possibly SCUBA divers. If the problem turns out to be substantial, my agency would then seriously consider actions such as manual removal, copper treatment and quarantine on the lake.