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September 6, 2017

### MEMORANDUM

**TO:** Fish and Wildlife Committee members

**FROM:** Kendall Farley

**SUBJECT:** Developing a field-based environmental DNA sampling and detection system

### BACKGROUND:

**Presenter:** Dr. Austen Thomas  
Director of Molecular Species Detection Technologies at Smith-Root Inc.

**Summary:** Environmental DNA is an emerging tool that can be used for sensitive detection and quantification of rare or invasive species, identifying pathogenic bacteria and disease as well as determining habitat range of an individual species. In light of growing threats of invasive mussels and other invasive species in the Columbia Basin, there is a need for rapid detection of these species to facilitate quick decision-making and efficiency and efficacy for planning and mitigation efforts.

Smith-Root has been developing a hand-held device that can produce quantitative results on the relative abundance of a species' DNA detected right in the field in less than an hour, versus sending data to a genetics lab and waiting for results. This type of emerging technology could help Columbia Basin researchers and managers in recovery and restoration efforts across the basin. The presentation will show examples of studies and proof-of-concept in which this technology produced results similar to

those generated from a lab. It will offer ideas for differing applications and usage of this technology across the Columbia Basin and in Pacific Northwest watersheds.

**Relevance:** The 2014 Fish and Wildlife Program's list of emerging priorities includes priority #3: Preserve program effectiveness by supporting: (1) expanded management of predators; (2) mapping and determining hotspots for toxic contaminants; and (3) aggressively addressing non-native and invasive species.

**More Info:** Dr. Austen Thomas is a molecular ecologist and the Director of Molecular Species Detection Technologies at Smith-Root Inc. in Vancouver, Washington. Austen received his PhD from the University of British Columbia and his MSc from Western Washington University. His work has mainly focused on seal and sea lion interactions with fisheries, in addition to a recent focus on new applications of environmental DNA in fisheries management. He is currently working on novel methods for integrating environmental DNA survey techniques into the fisheries scientist's toolbox.

# Design of a field-based eDNA sampling and detection system

Austen Thomas, Jesse Howard, Mieke Sinnesael, Caren Goldberg



# Smith-Root

*Technology for fisheries conservation*



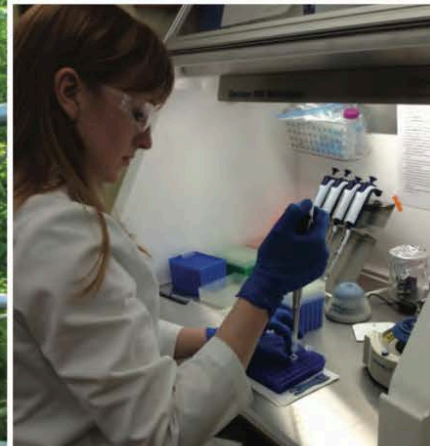
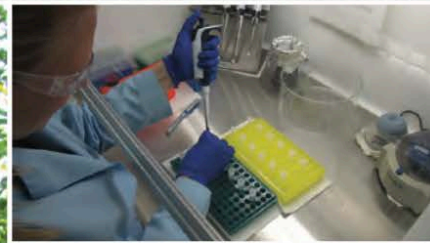
# Species detection with environmental DNA (eDNA)



Photo Credit: fishbio.com

Prepared in collaboration with the University of Minnesota

# Detection of Environmental DNA of Bigheaded Carps in Samples Collected from Selected Locations in the St. Croix River and in the Mississippi River





OPEN

## Validated methodology for quantifying infestation levels of dreissenid mussels in environmental DNA (eDNA) samples

Received: 12 February 2016

Accepted: 17 November 2016

Published: 14 December 2016



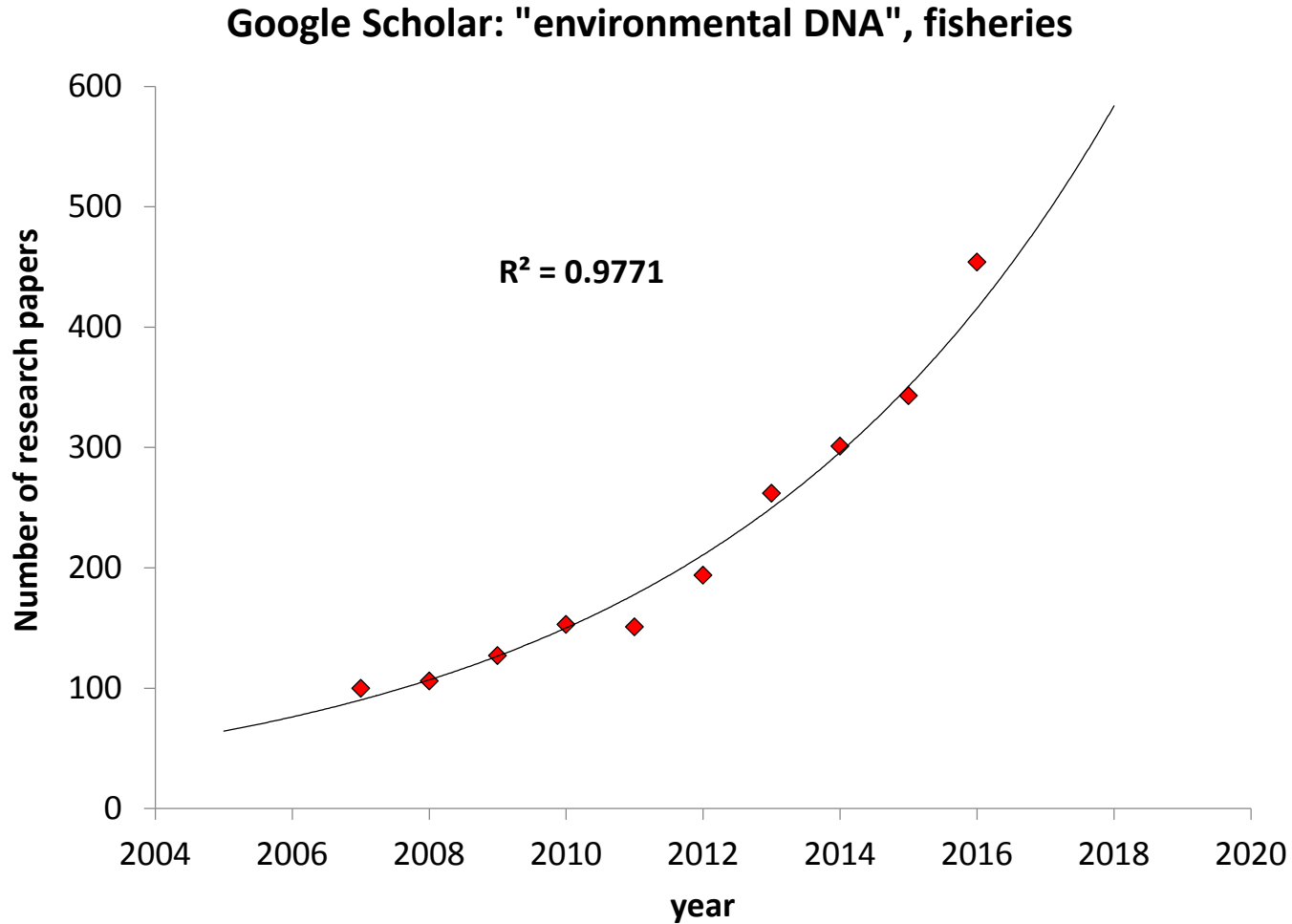
# Why do we care about eDNA?

Google Scholar: "environmental DNA", fisheries





# Why do we care about eDNA?



Laramie et al., 2015

Prepared in cooperation with Washington State University

# Environmental DNA Sampling Protocol—Filtering Water to Capture DNA from Aquatic Organisms

Carim et al., 2015



**Rocky Mountain Research Station**

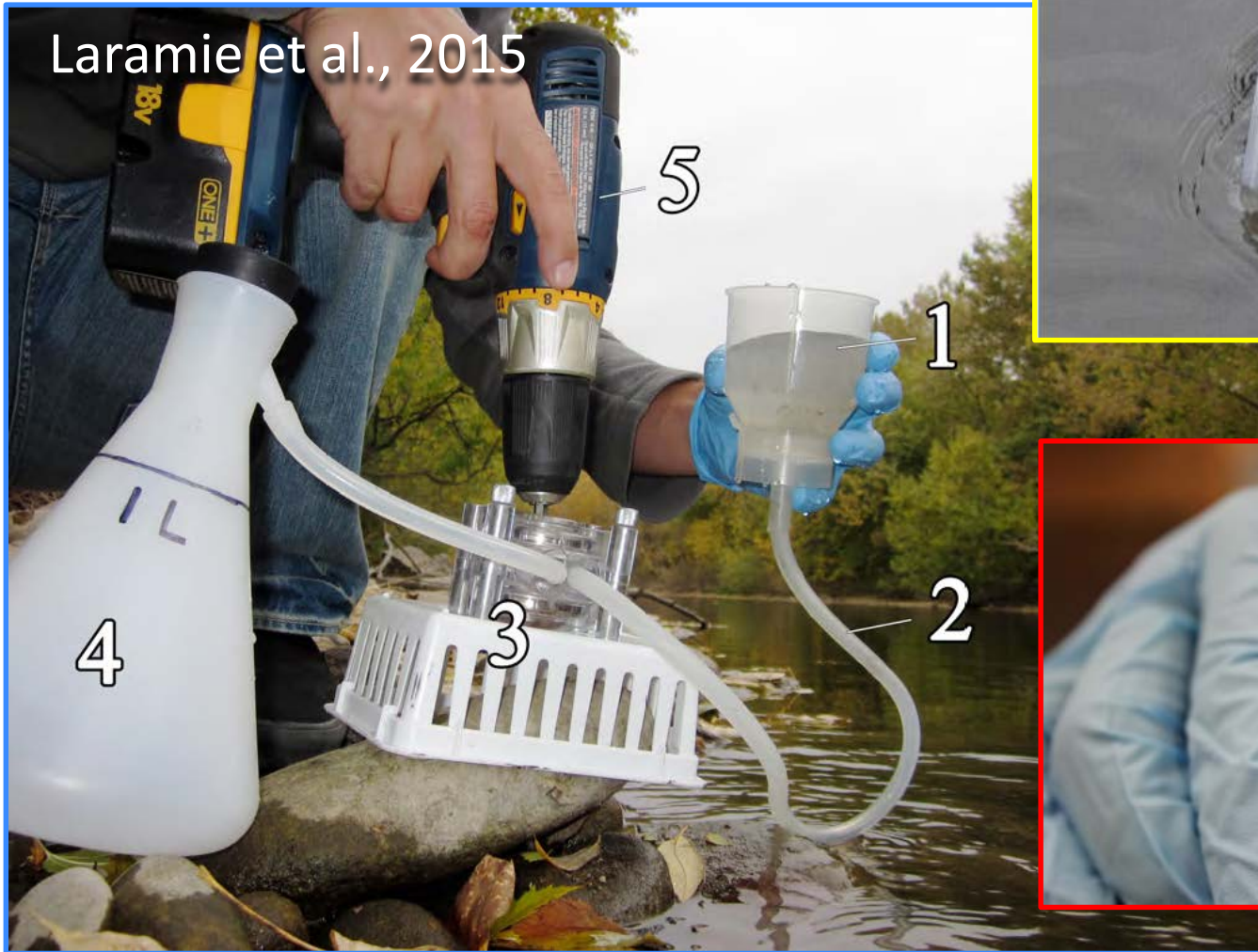
Protocol for collecting eDNA samples from streams

Version 2.3- July 2015



# eDNA sampling methods

Laramie et al., 2015



# Current Tool Limitations

- Sampling gear is not purpose-built (highly cumbersome)
- Inefficient use of technician time

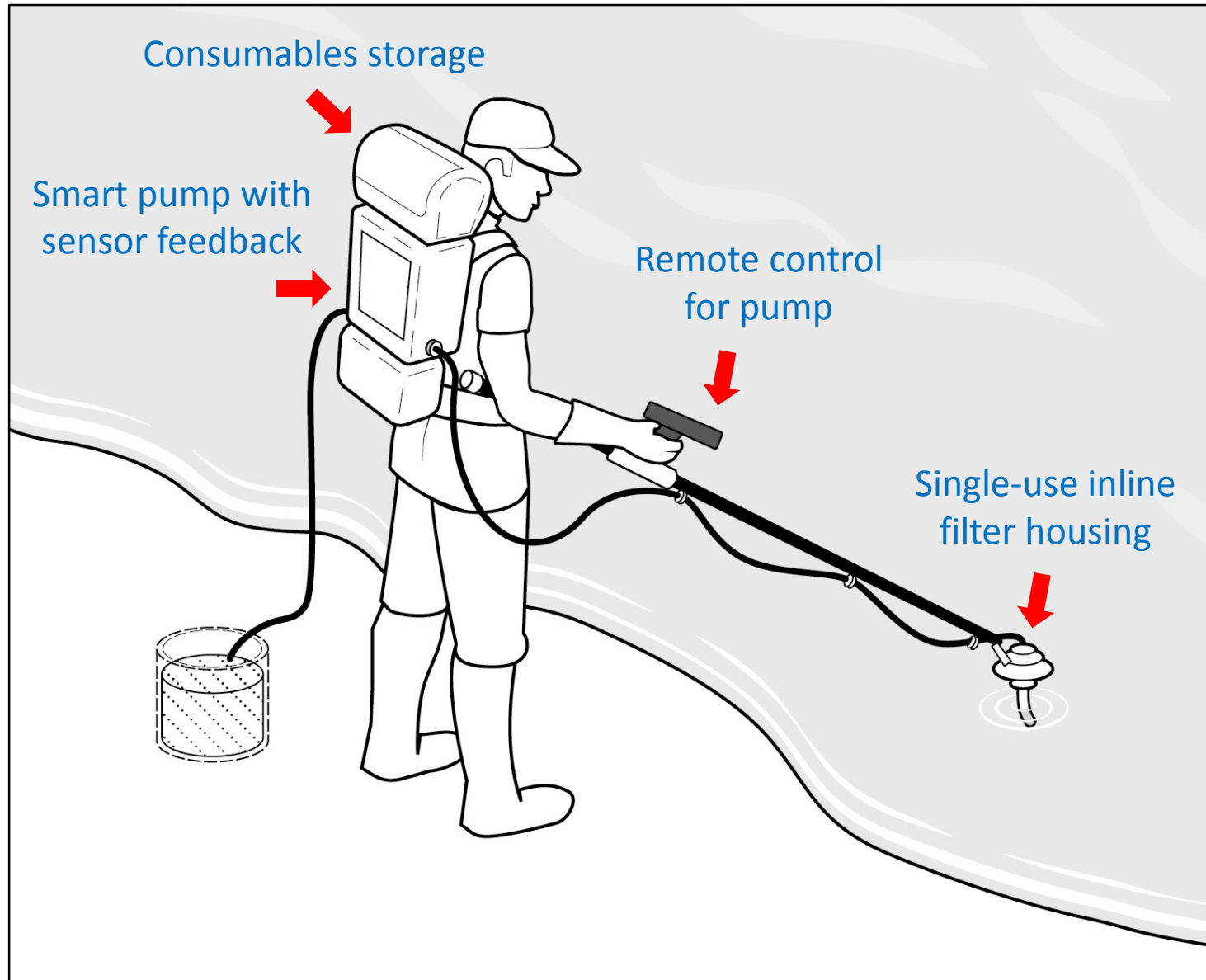


Carim et al., 2015



# The eDNA Sampling Backpack

(A fully integrated eDNA sampling system)

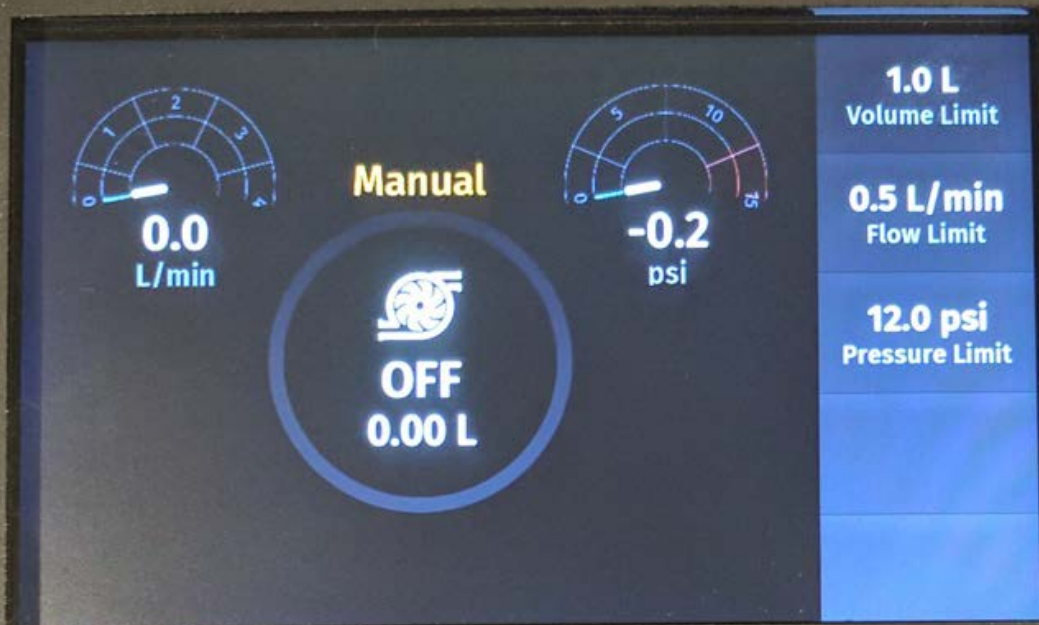


# ANDe™

THE eDNA SAMPLING BACKPACK





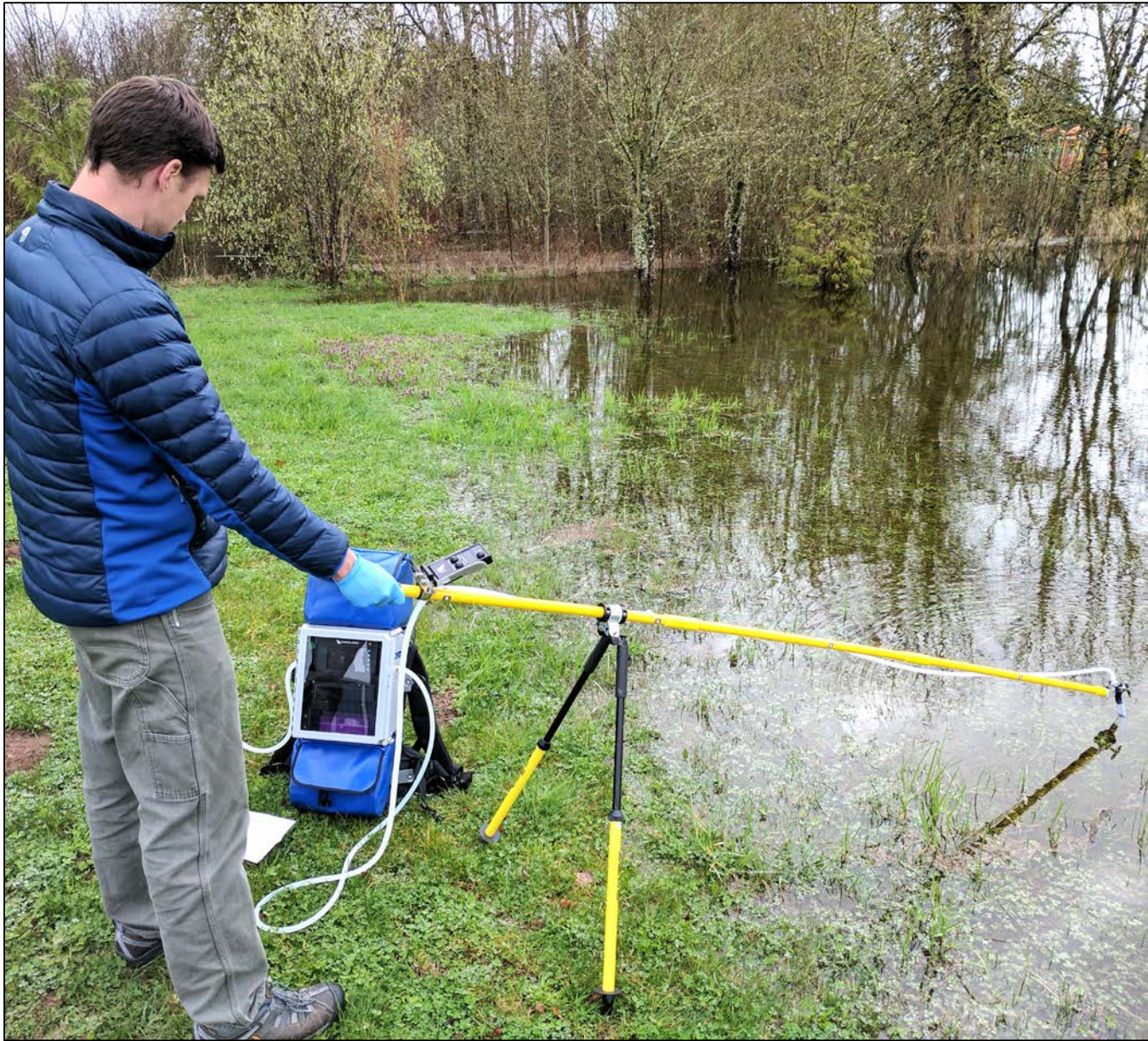






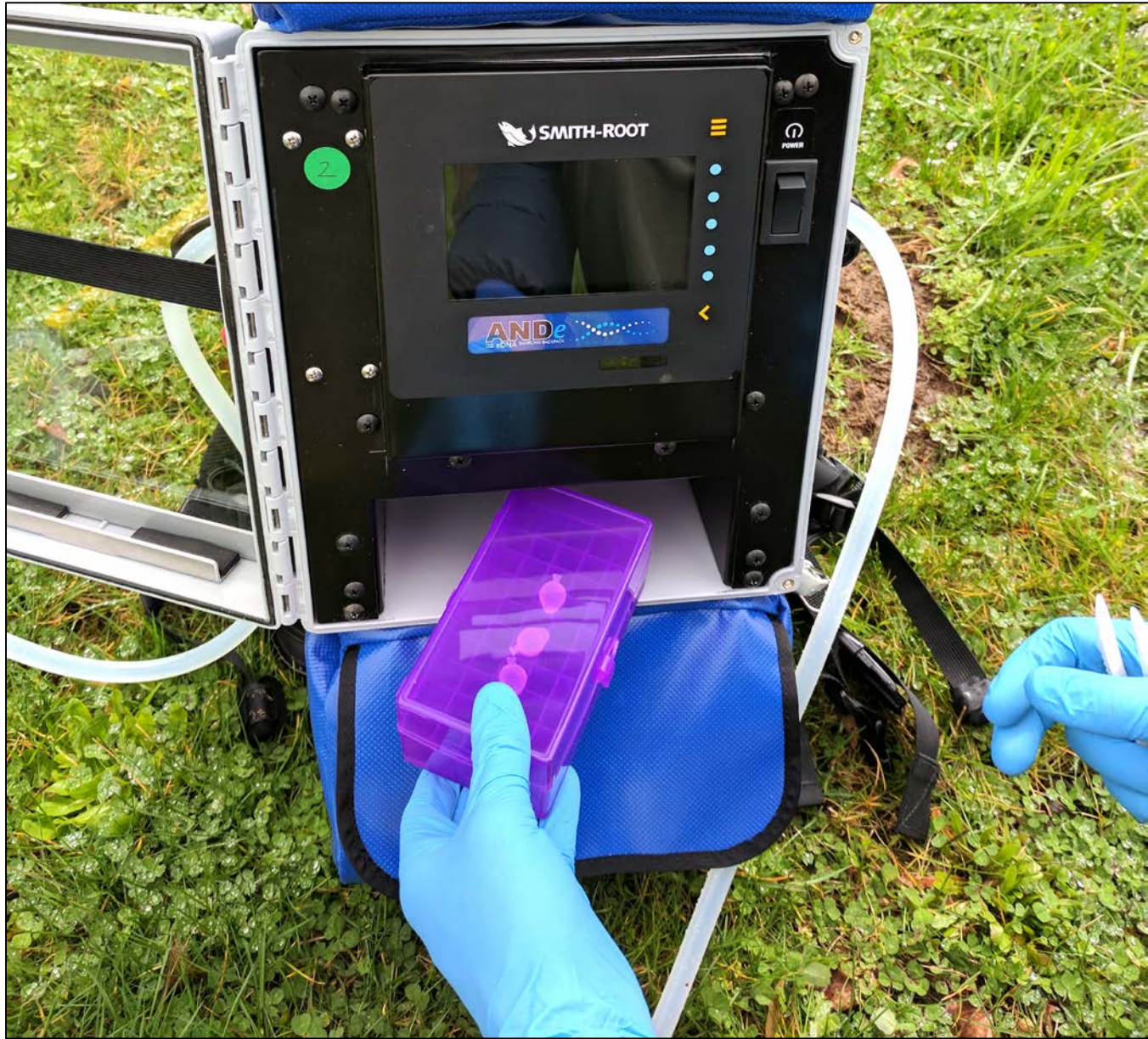








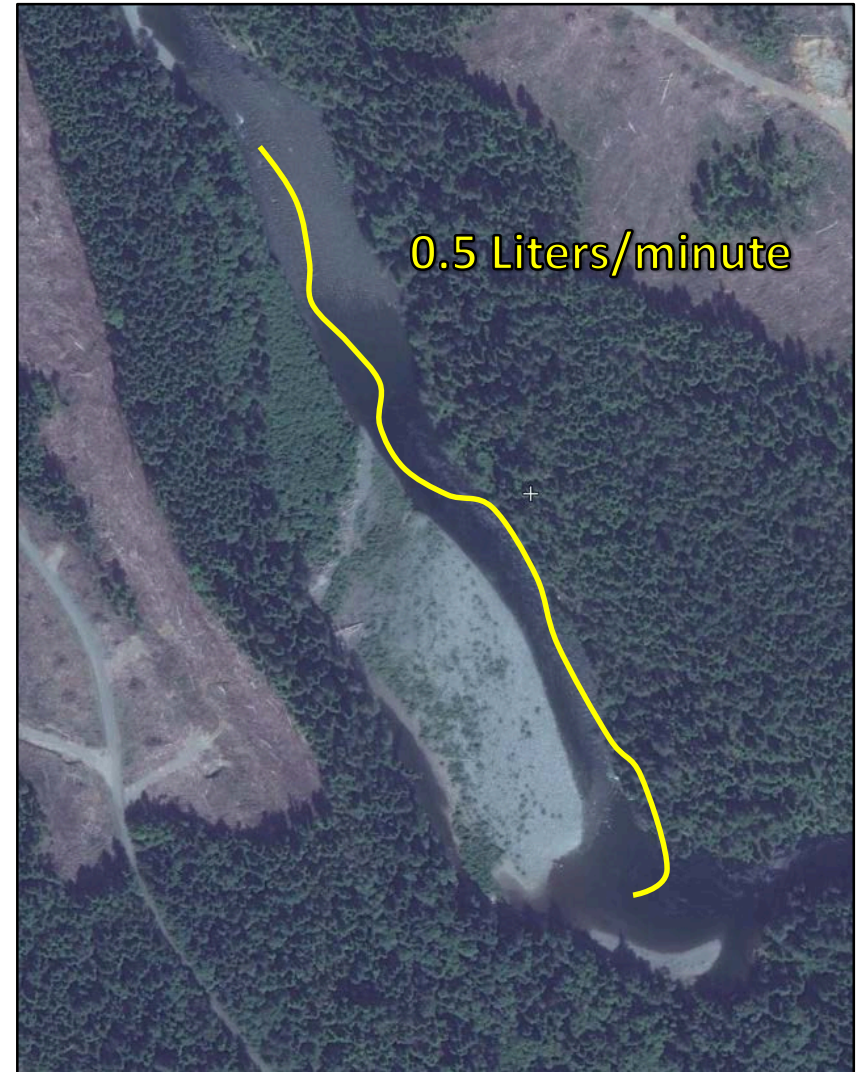




# Continuous transect sampling for eDNA



## Sampling Standardization





# Movie 1





## eDNA sampling

- **Sterility**
- **Optimization**
- **Standardization**
- **High-throughput**

# Current model of eDNA detection

Field sample preservation



Benchtop DNA extraction/detection



# Current model of eDNA detection

Field sample preservation



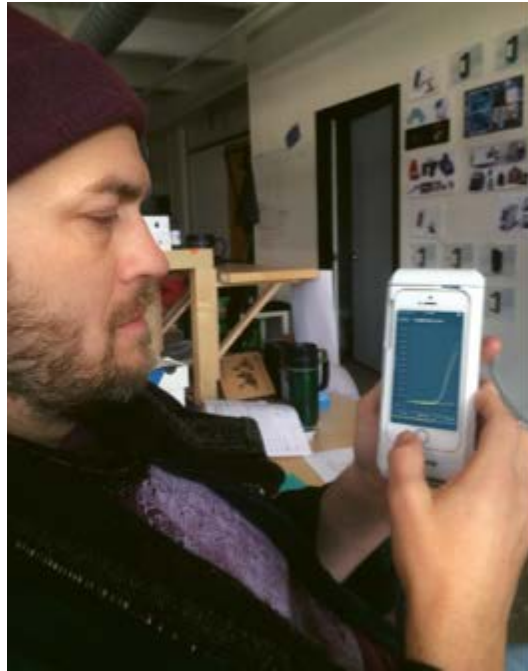
Benchtop DNA extraction/detection



## Model Limitations

- Not all users have access to a lab
- Results can take weeks to get back
- Prevents adaptive monitoring

# A revolution in diagnostics portability (Bringing the lab to the sample)



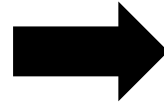
## PCR heads into the field

NATURE METHODS | VOL.12 NO.5 | MAY 2015 | 393

Vivien Marx

Analyzing samples with PCR is routine in the lab. New approaches let researchers do this assay wherever they need.

# A revolution in diagnostics portability (Bringing the lab to the sample)



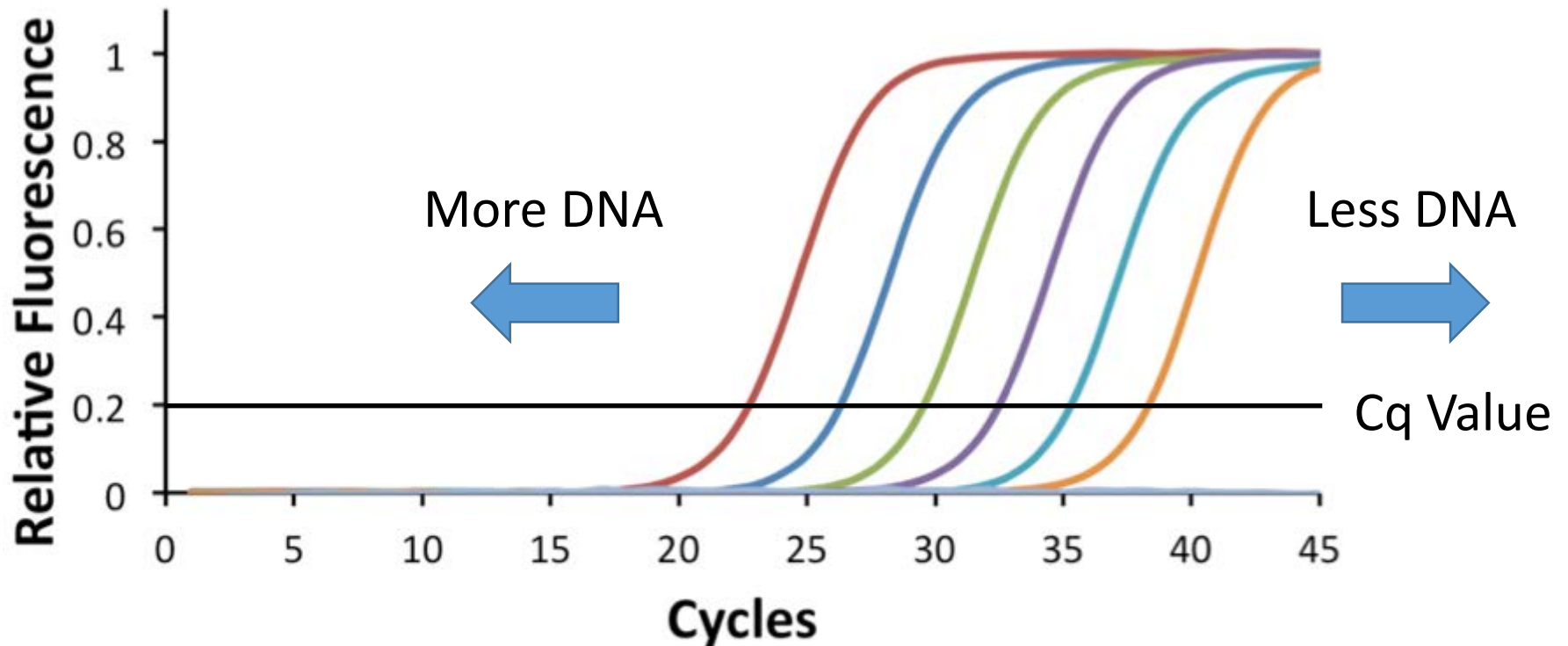
## PCR heads into the field

NATURE METHODS | VOL.12 NO.5 | MAY 2015 | 393

Vivien Marx

Analyzing samples with PCR is routine in the lab. New approaches let researchers do this assay wherever they need.

# What is quantitative PCR (qPCR)?



# Biomeme field-portable qPCR system



1. Field DNA sample prep



2. Species-specific test packets



3. Portable qPCR device



4. Data portal services



# Movie 2





# DNA detection using species-specific packets



Customizable App displays +/- or DNA quantity



Data Portal > Folder Hub > View Basic Data

## View Basic Data

### Experiments

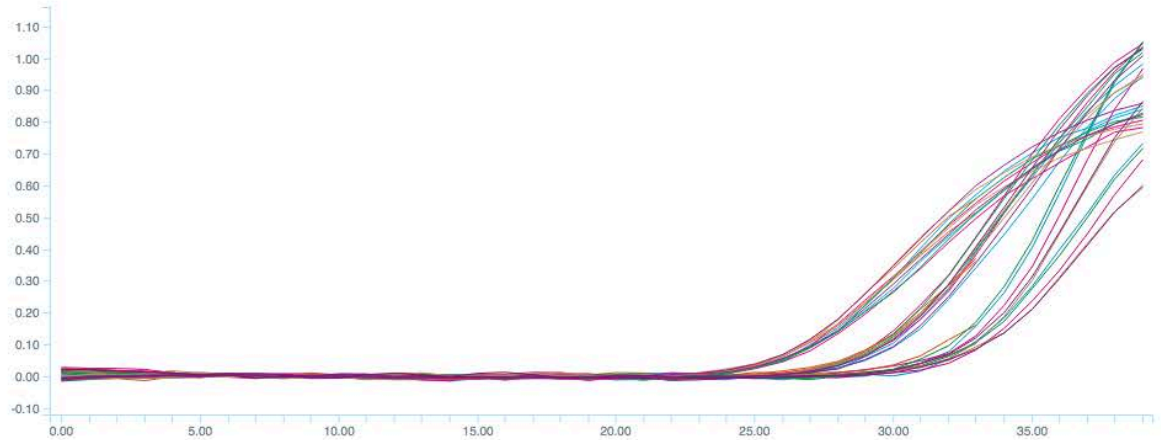
	1	2	3
XMS 003: HW 104 run 3	Unk	Unk	Unk
XMS 003: HW 104 run 4	Unk	Unk	Unk
XMS 003: HW 104 run 5b	Unk	Unk	Unk
XMS 003: HW 104 run 2	Unk	Unk	Unk
XMS 003: HW 104 run 6b	Unk	Unk	Unk
XMS 003: HW 104 run1	Unk	Unk	Unk

SHOW THRESHOLDS

SHOW RAW DATA 

### Targets

E3	E2	E1
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### 1

<b>FAM</b>	Cq: 27.80	Cq: 27.68	Cq: 27.66
<b>JUN</b>	Cq: 28.03	Cq: 28.90	Cq: 26.63

<b>FAM</b>	Cq: 28.85	Cq: 26.77	Cq: 26.68
<b>JUN</b>	Cq: 28.72	Cq: 27.18	Cq: 27.86

### 2

<b>FAM</b>	Cq: 29.39	Cq: 30.36	Cq: 28.75
<b>JUN</b>	Cq: 31.72	Cq: 30.74	Cq: 28.88

<b>FAM</b>	Cq: 30.12	Cq: 30.51	Cq: 30.35
<b>JUN</b>	Cq: 30.49	Cq: 30.97	Cq: 31.55

### 3

<b>FAM</b>	Cq: 32.58	Cq: 33.44	Cq: 31.49
<b>JUN</b>	Cq: 33.11	Cq: 33.88	Cq: 32.59

<b>FAM</b>	Cq: 32.13	Cq: 33.06	Cq: 33.68
<b>JUN</b>	Cq: 33.47	Cq: 33.92	Cq: 33.54

XMS 003: HW 104 run 3

XMS 003: HW 104 run 4

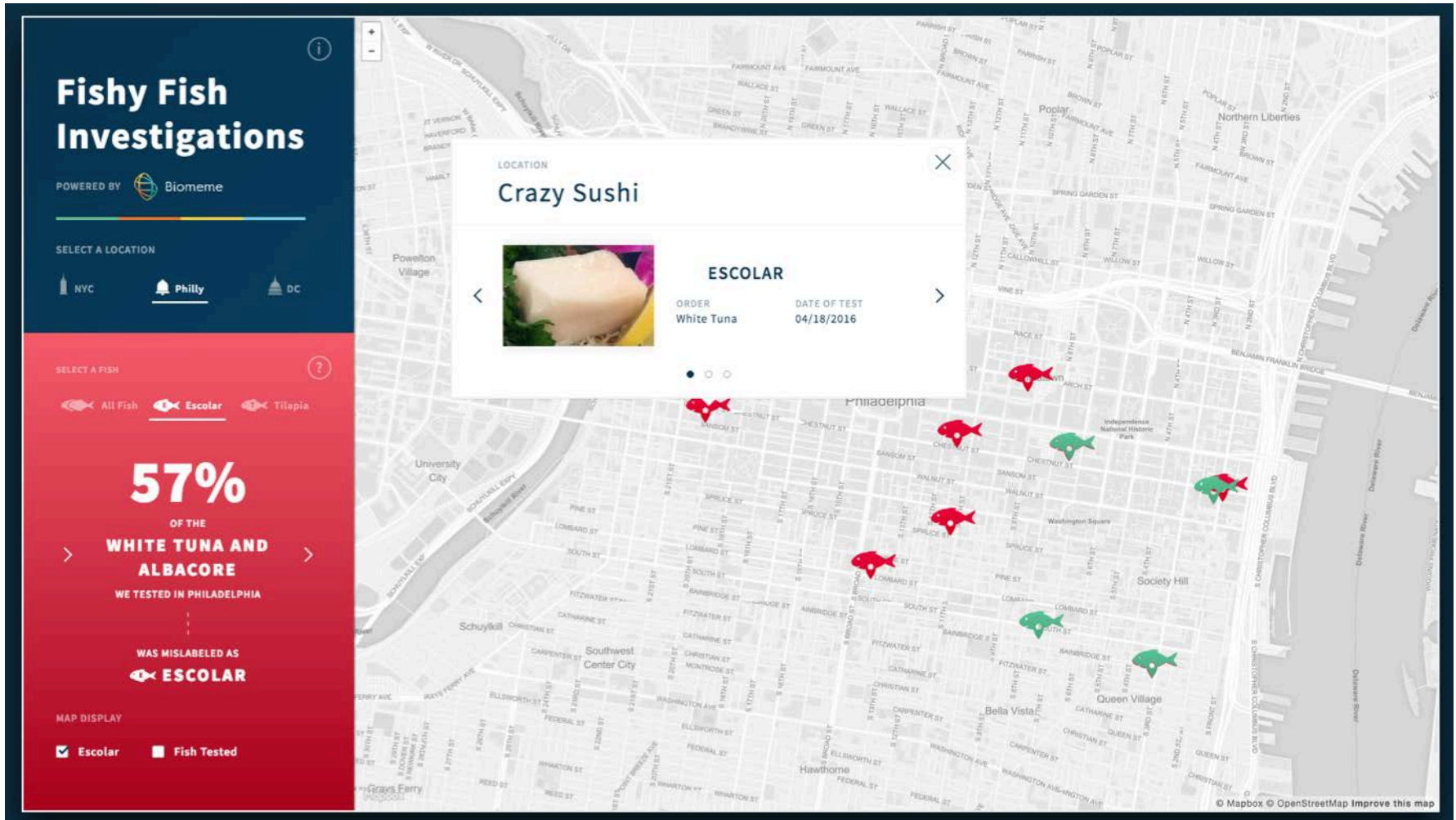
XMS 003: HW 104 run 5b

XMS 003: HW 104 run 2

XMS 003: HW 104 run 6b

XMS 003: HW 104 run1

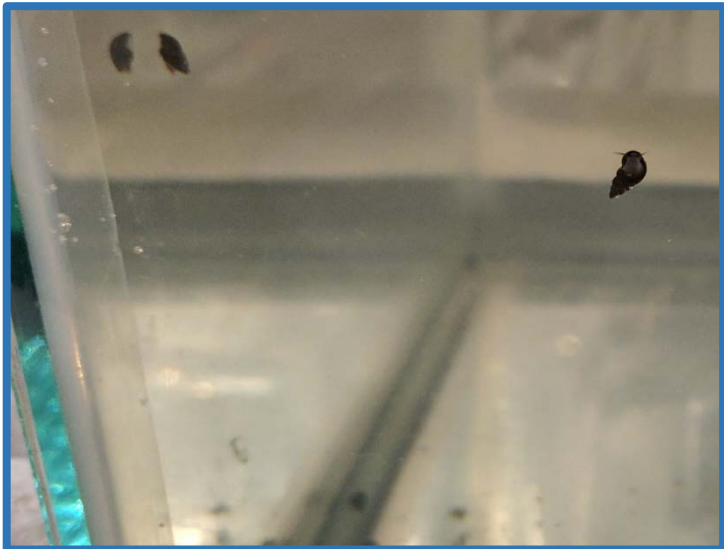
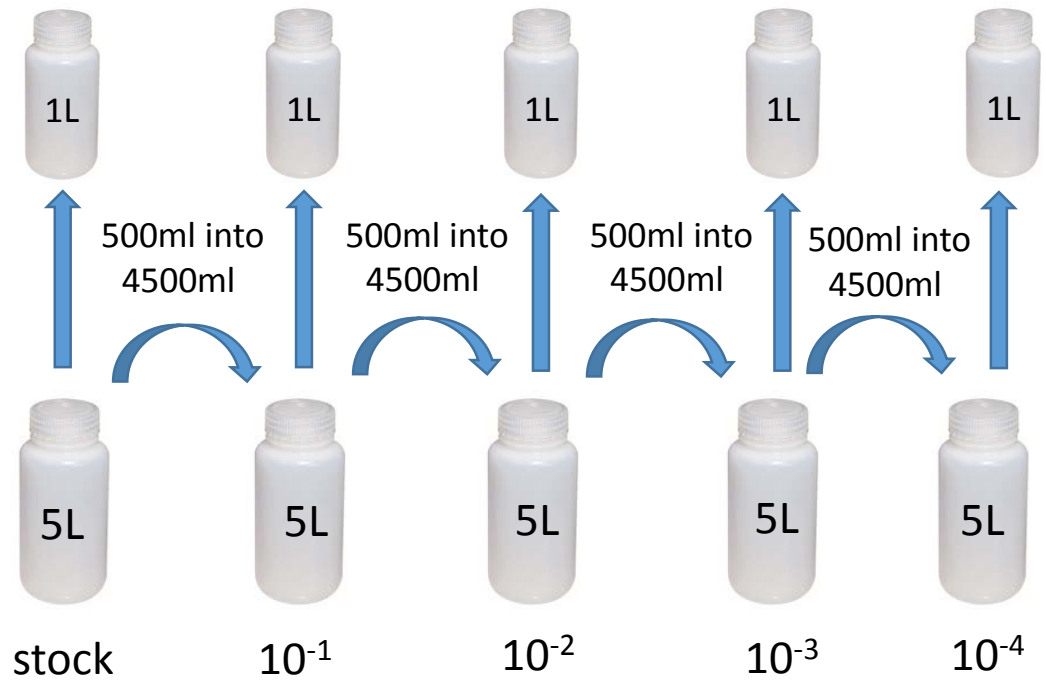
# Data portal for real-time analysis





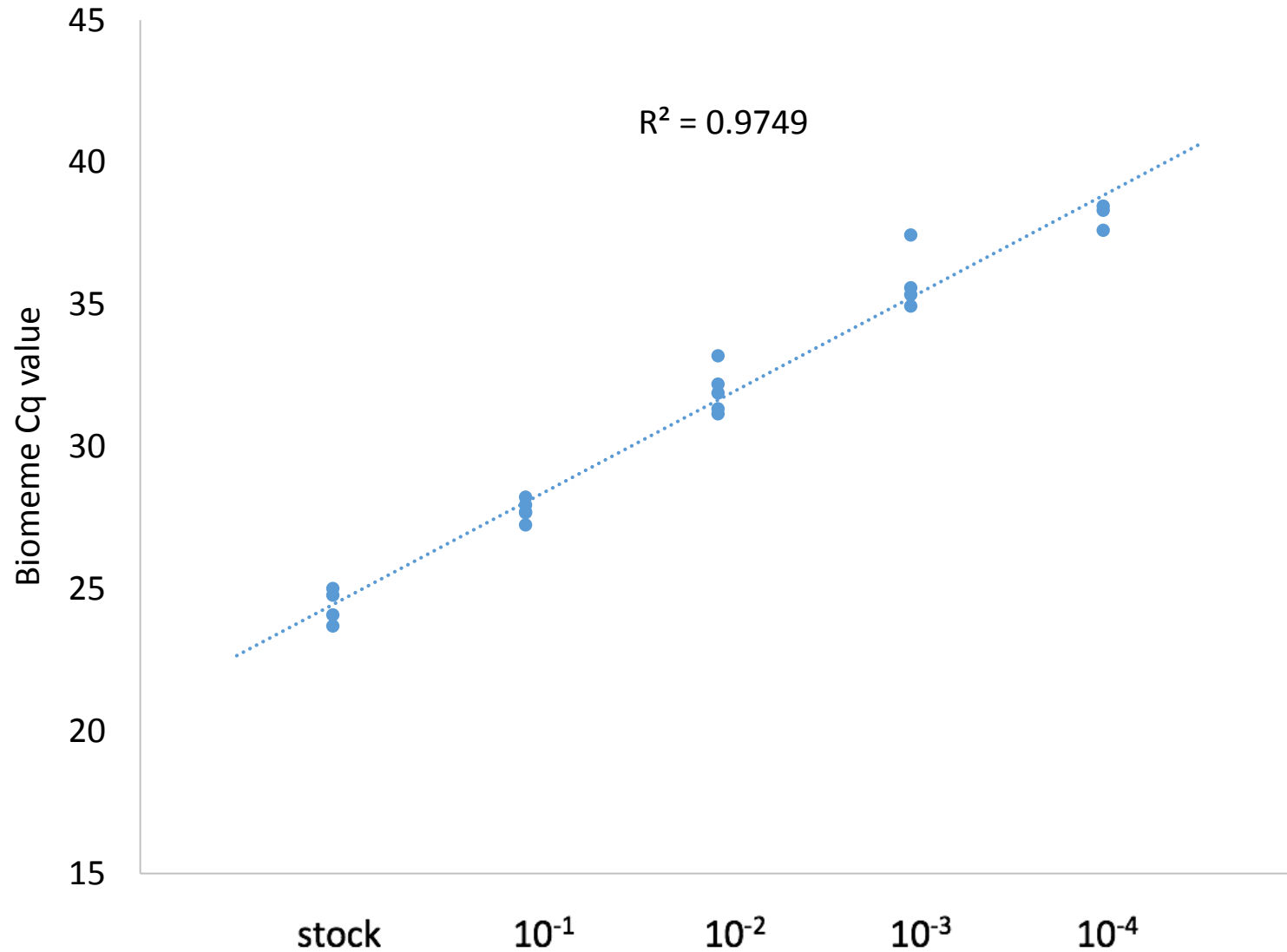
# New Zealand Mudsnail exp.

- 50 snails in aquarium
- Diluted with river water



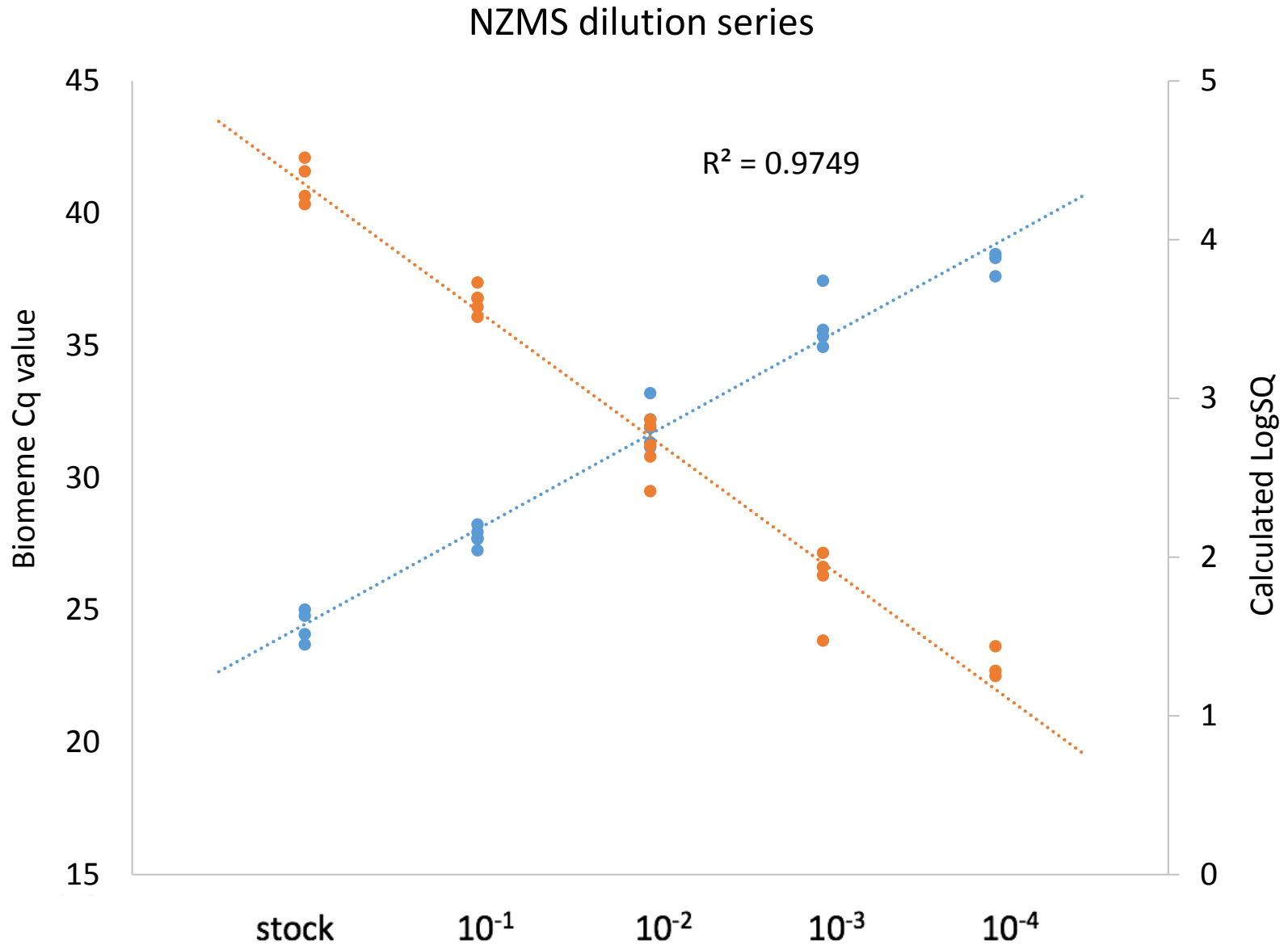
# Proof of concept study with mudsnails

NZMS dilution series



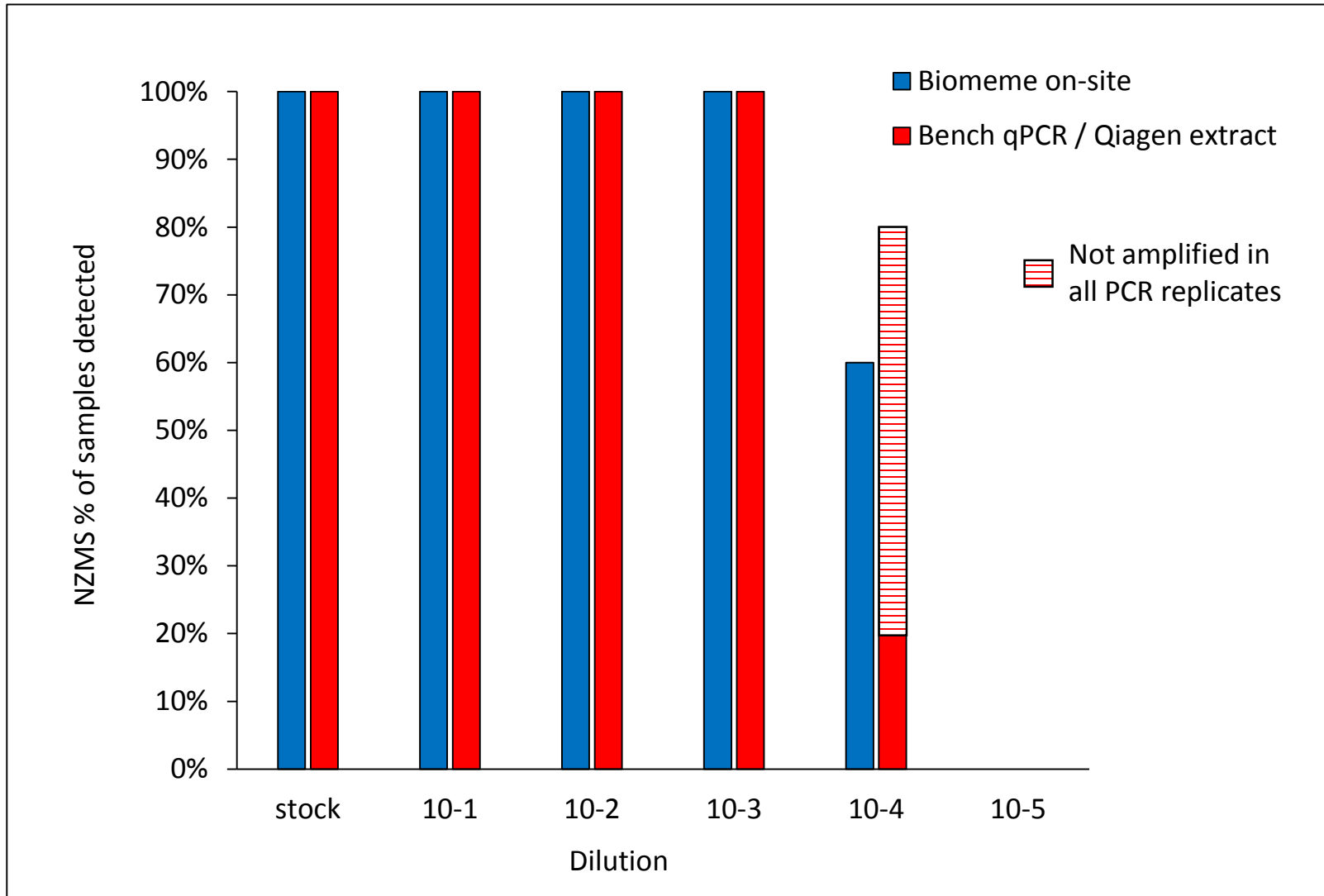


# Proof of concept study with mudsnails



# Biomeme compared to Bench qPCR

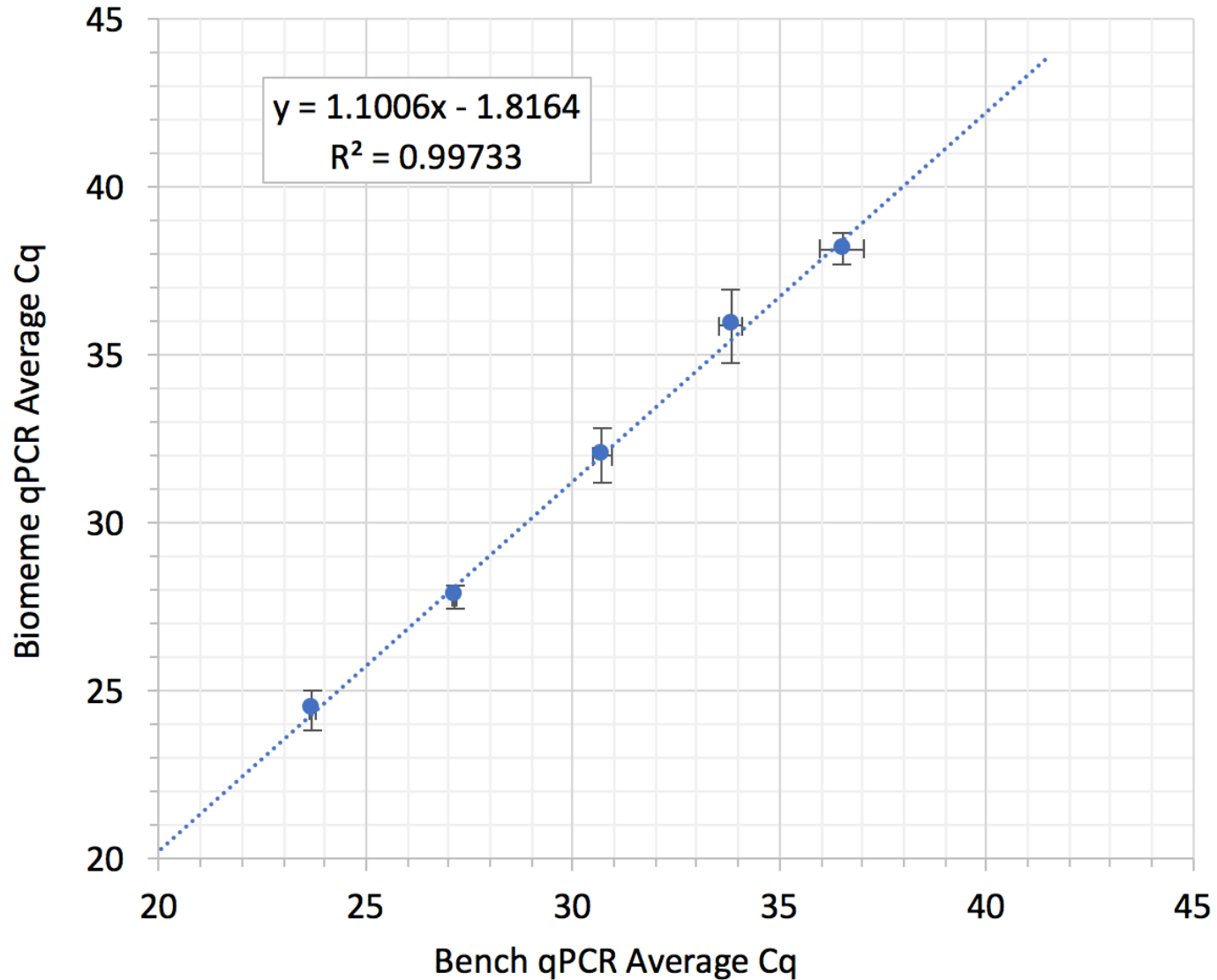
Bench qPCR credit: Goldberg lab



# Biomeme compared to Bench qPCR

Bench qPCR credit: Goldberg lab

## NZMS dilution series



# Michigan DNR NZMS detection with ANDe/Biomeme

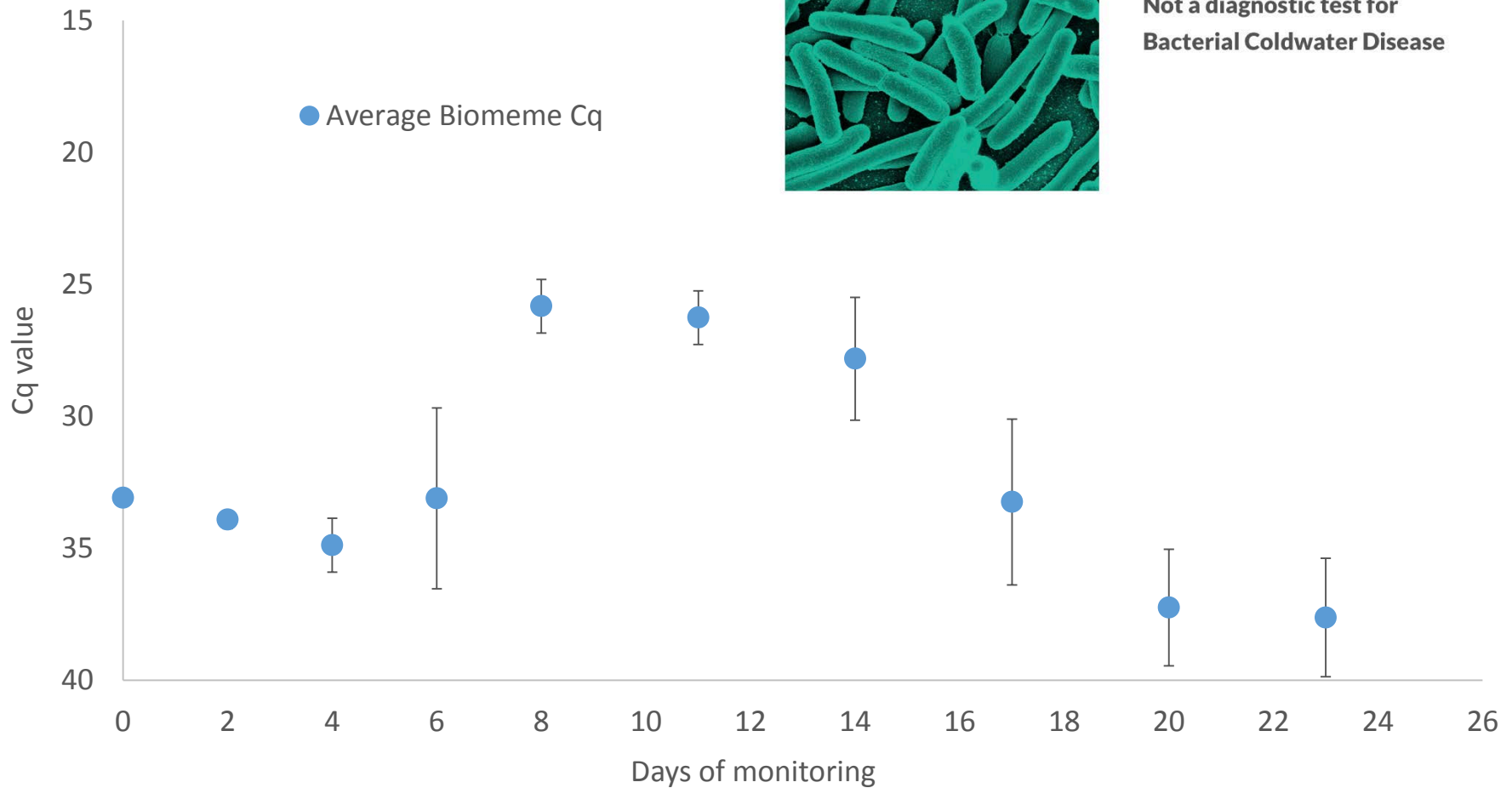


# Environmental fish pathogen monitoring

*Flavobacterium psychrophilum* Test Kit

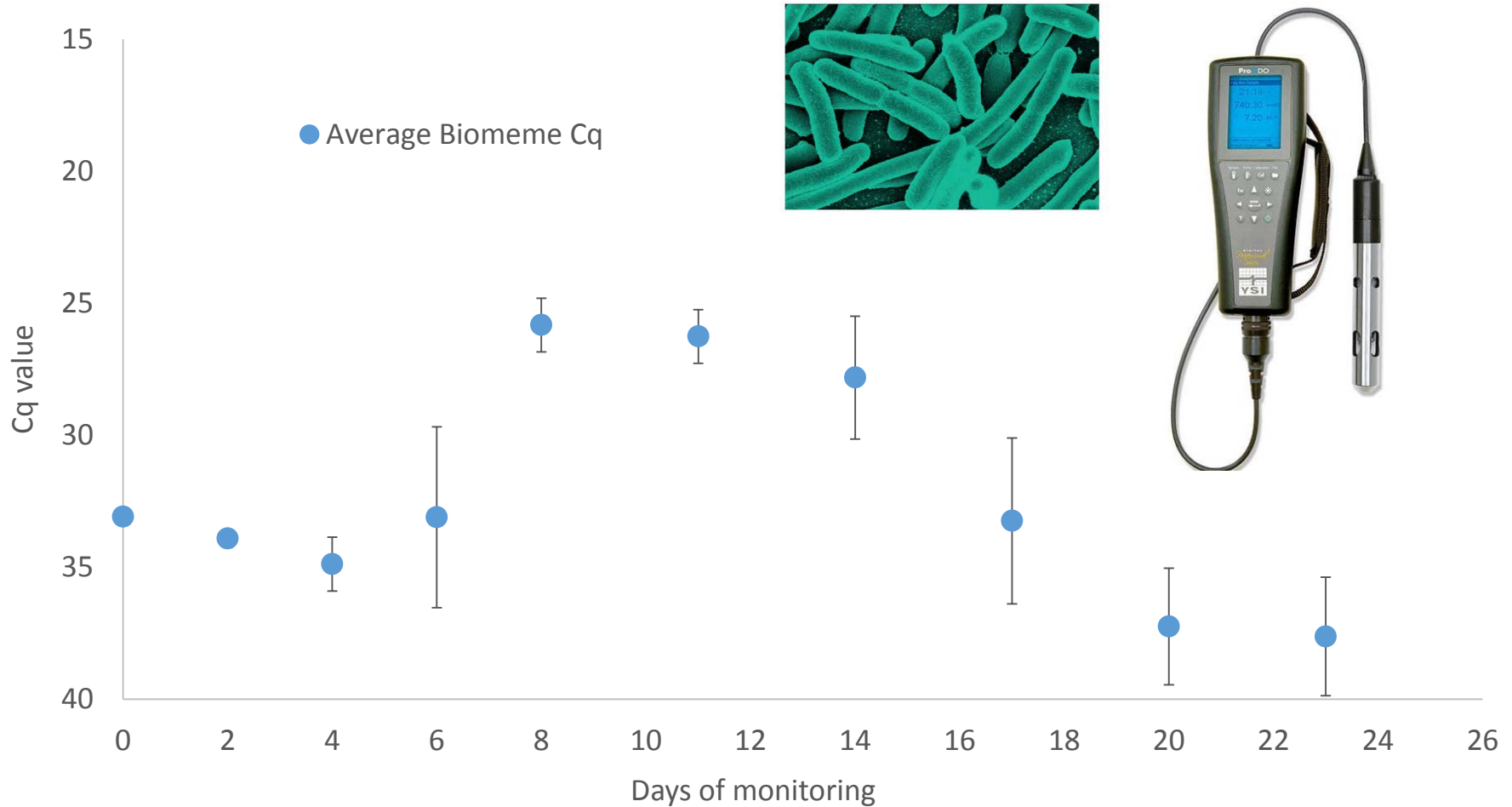


**Not a diagnostic test for  
Bacterial Coldwater Disease**



# Environmental fish pathogen monitoring

*Flavobacterium psychrophilum* Test Kit



# List of eDNA tests on Smith-Root website

## Environmental Microbes\*\*

### *Renibacterium salmoninarum* Test Kit



**Not a diagnostic test for  
Bacterial Kidney Disease**

Purchase Kit

## Aquatic Species

### Bighead Carp and Silver Carp Test Kit



*Hypophthalmichthys nobilis*  
and *Hypophthalmichthys*  
*molitrix*

Purchase Kit

### *Flavobacterium columnare* Test Kit



**Not a diagnostic test for  
Columnaris Disease**

Purchase Kit

### New Zealand Mudsail Test Kit



*Potamopyrgus antipodarum*

Purchase Kit

# Northern Pike test recently developed



## NORTHERN PIKE INVADE UPPER COLUMBIA RIVER

JUL 16, 2015 / JOHN HARRISON /

Northern pike, a voracious predator considered an invasive species in two of the four Northwest states, have been found in the Kettle River, a northeastern Washington tributary of the Columbia River, a sign that they are continuing their downstream migration from lakes and rivers in Idaho and Montana. What's worse, the pike found in and near the Kettle River were several different ages, indicating the species is breeding and proliferating.

That is bad news for the Columbia because if pike keep spreading downriver they could wind up below Grand Coulee and Chief Joseph dams where they could prey on threatened and endangered species of salmon and steelhead.

Council staff reported in July that between June 29 and July 3, 21 adult pike were captured at five locations around the mouth of the Kettle River, near Colville, Washington. Northern pike are classified as game fish in Montana and Idaho, and as prohibited species in Washington and Oregon, where they also are listed as aquatic invasive species. All four states prohibit live transport of northern pike.



This map by the Washington Department of Fish and Wildlife shows the known distribution of northern pike in the state. The northern-most red line represents the Pend Oreille River and the Columbia River to the mouth of the Kettle River. The southern-most red line represents the Spokane River. The northern and southern red circles represent Newman Lake and Liberty Lake, respectively, in Spokane County.



Can this be used to detect (*insert species*)?

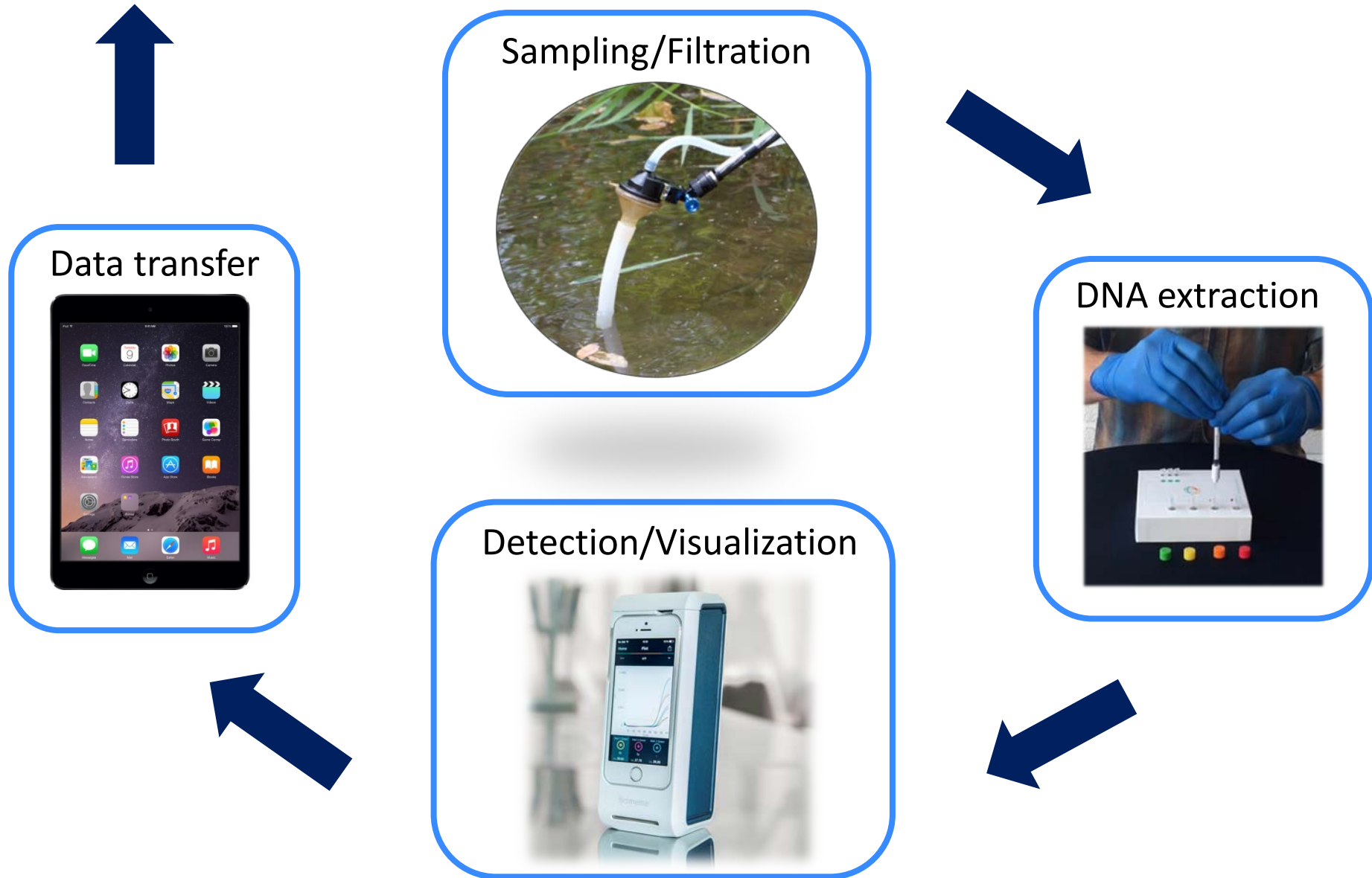


Can this be used to detect (*insert species*)?

YES



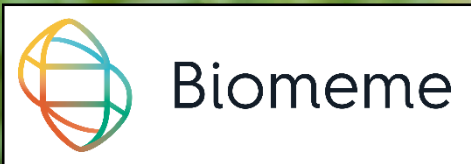
# End to end field eDNA process



Help us build the right tools for you



# Thank you!



# ANDe Beta testing program completed (April)

- Michigan DNR
- Wildlife Conservation Society
- Biodiversity Institute of Ontario
- Washington State University

