# The Smith-Root eDNA Sampler system

Austen Thomas Ph.D. – Smith-Root Inc.

# Smith-Root

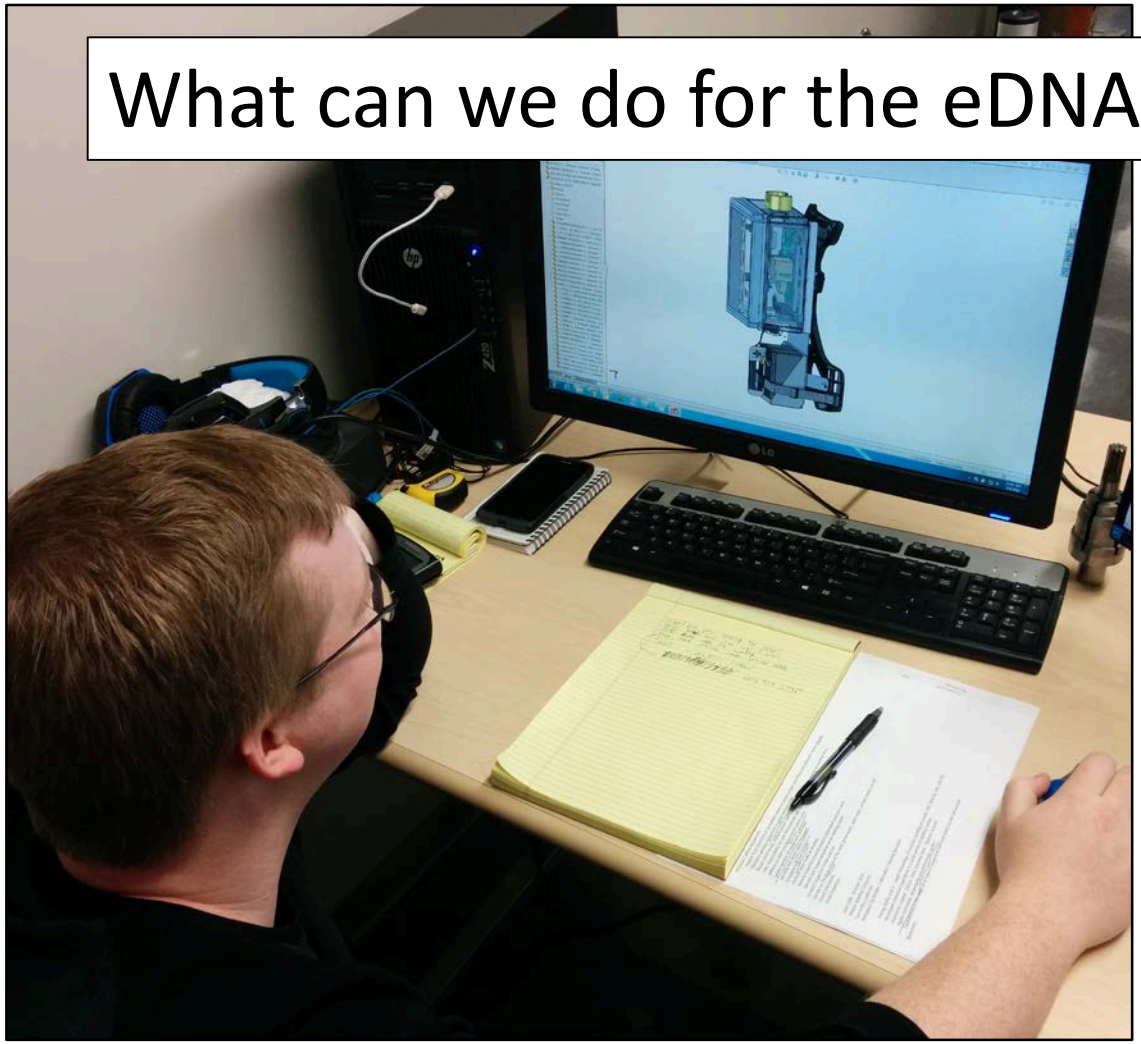
*Technology for fisheries conservation*

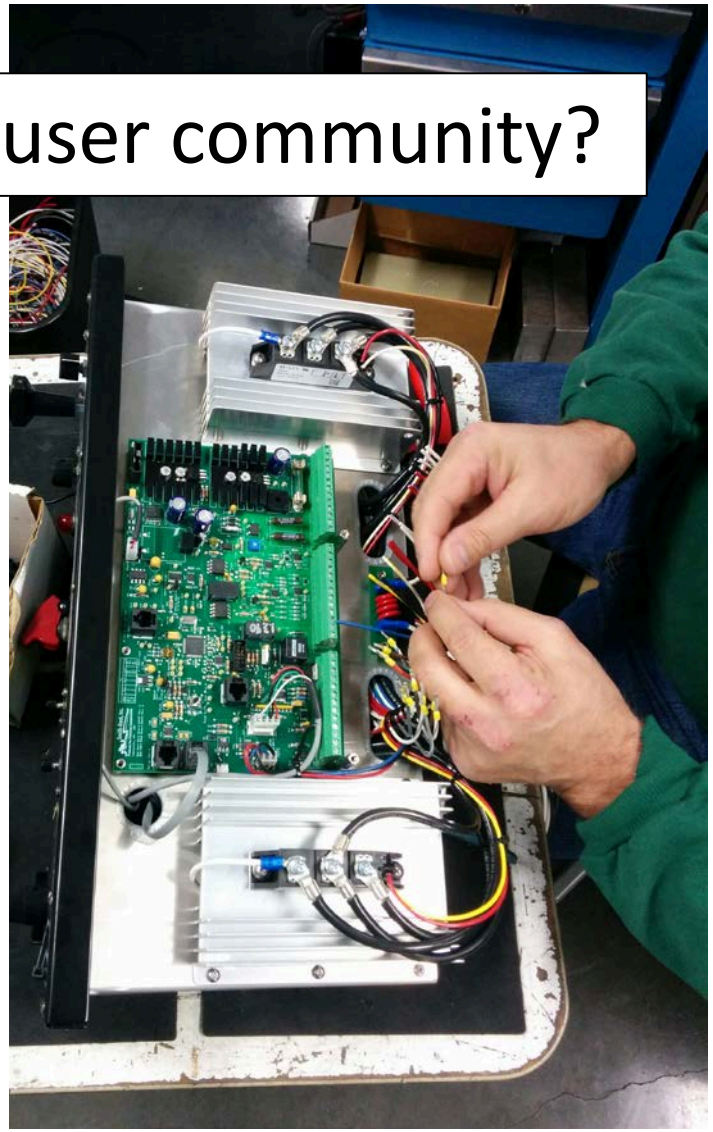
# Smith-Root

*Technology for fisheries conservation*

# SRI Engineering Team

What can we do for the eDNA user community?





USGS
science for a changing world

Laramie et al., 2015

Prepared in cooperation with Washington State University

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

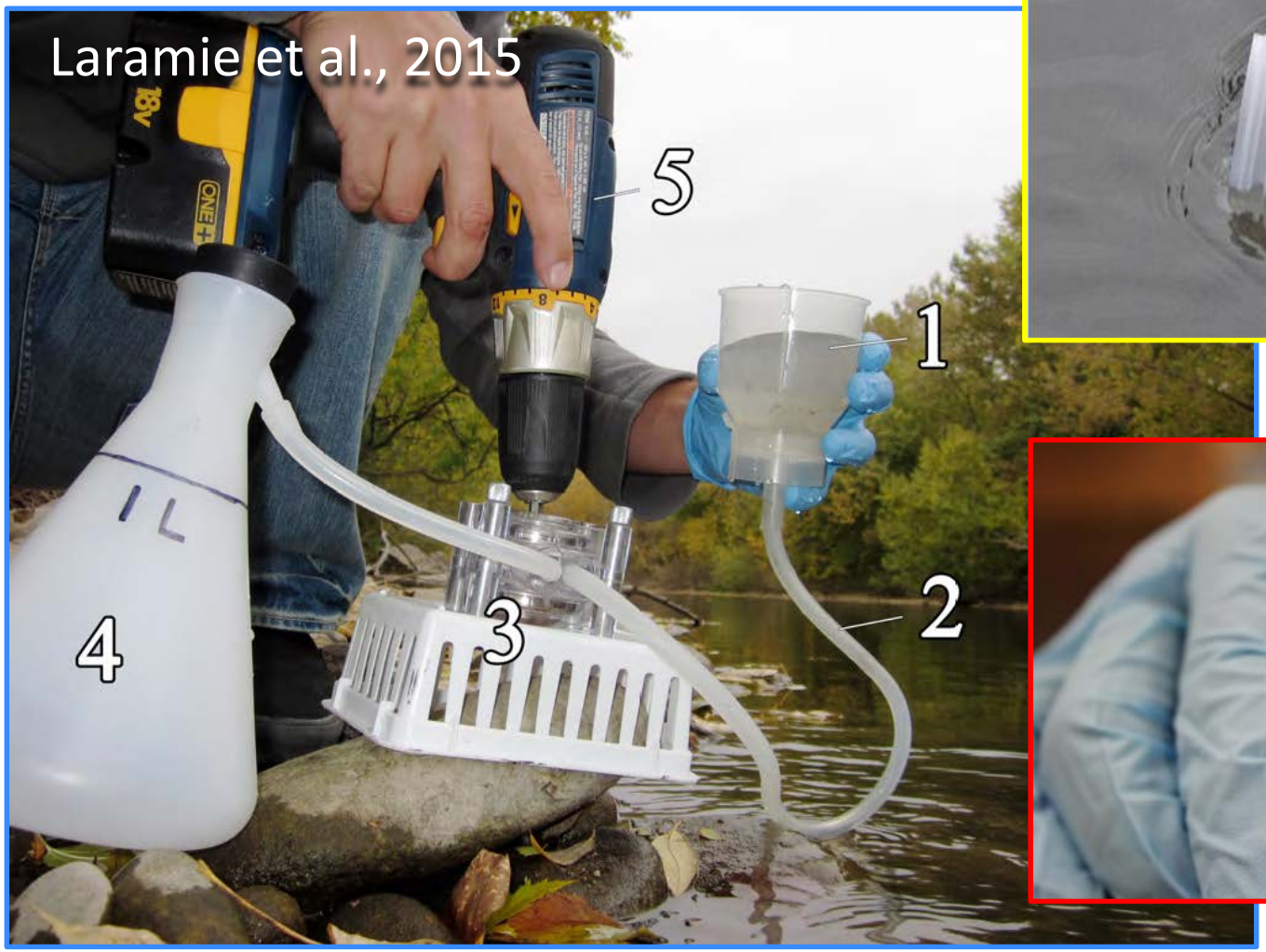
Carim et al., 2015

Rocky Mountain Research Station

## Protocol for collecting eDNA samples from streams

## Version 2.3- July 2015

# eDNA sampling methods

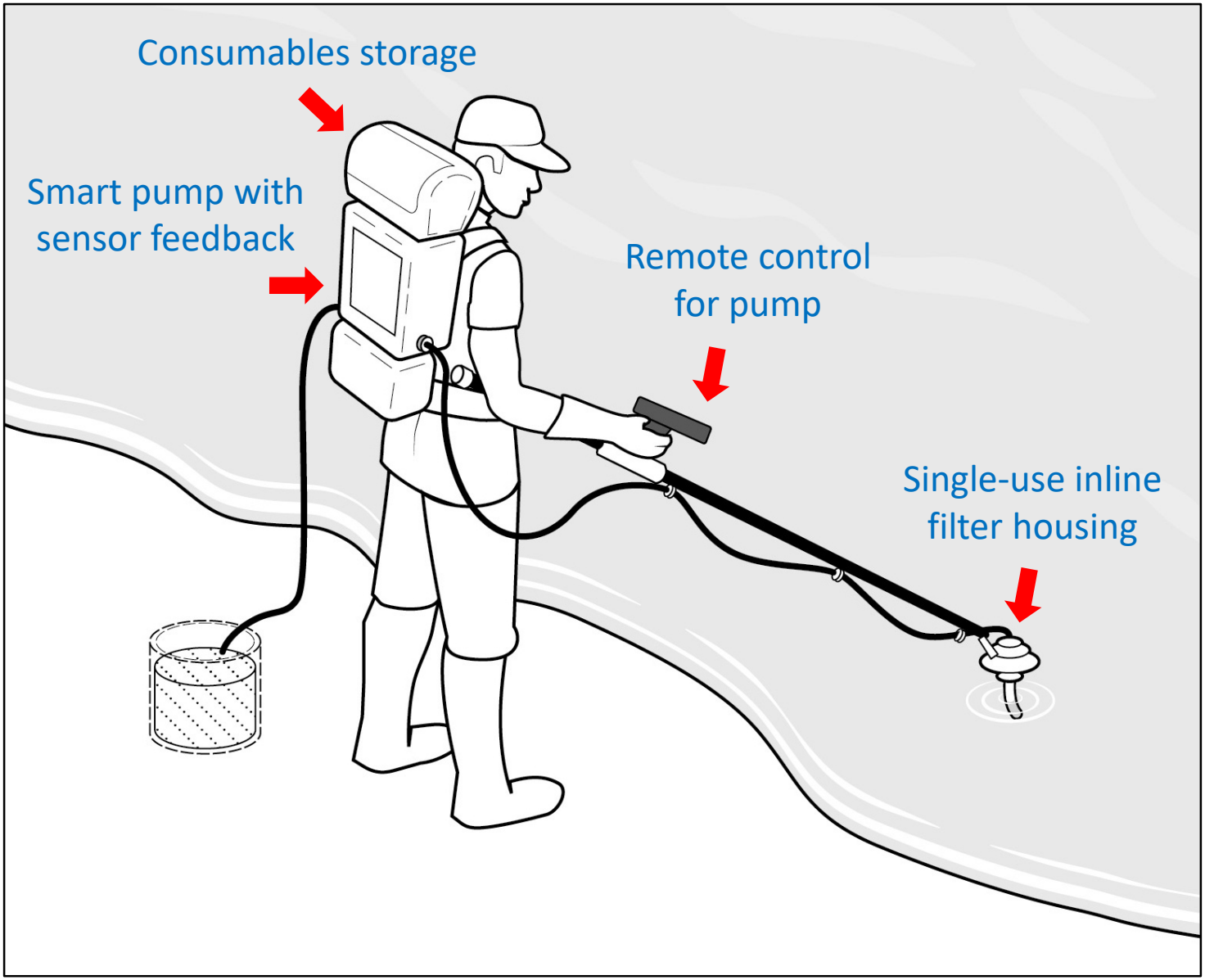



# Current Tool Limitations

- Pore sizes not designed for eDNA
- Minimal control over filtration process
- Sampling gear is not purpose-built (somewhat cumbersome)

# The eDNA Sampler Backpack

(A fully integrated eDNA sampling system)

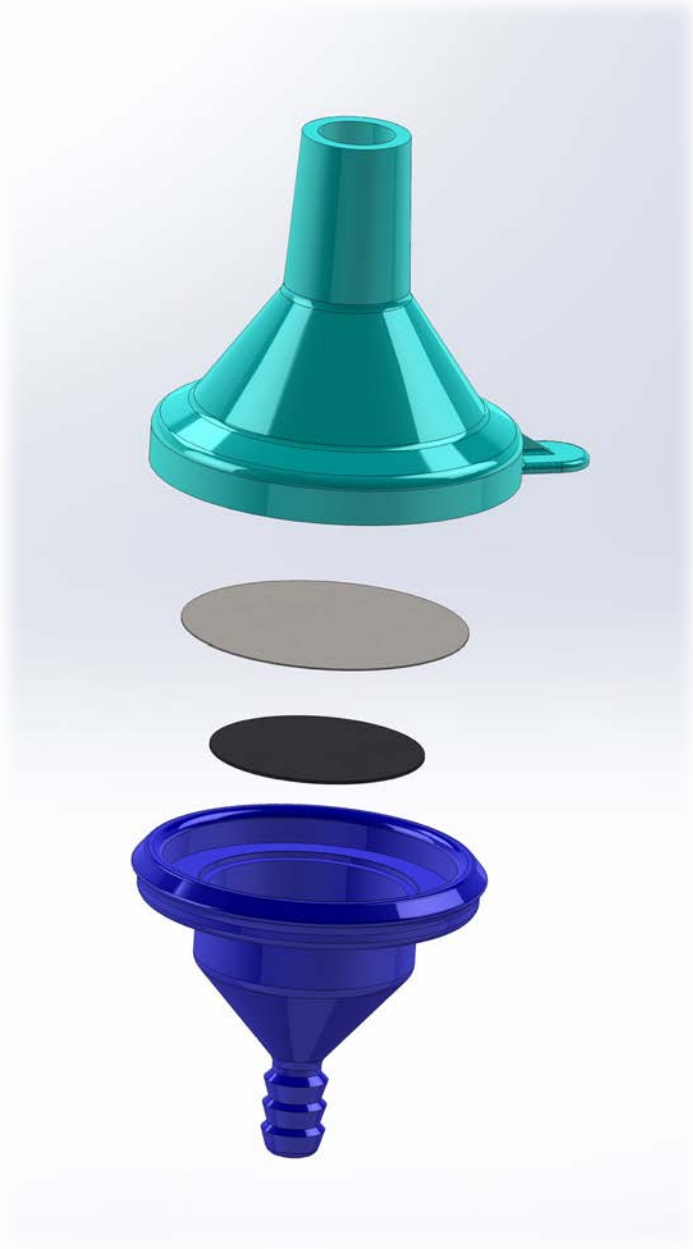


# eDNA

## SAMPLER BACKPACK

# Filter housings:

- Single-use inline filter housing
- Takes any 47mm membrane filter
- Minimizes sediment accumulation (high pressure, low velocity)

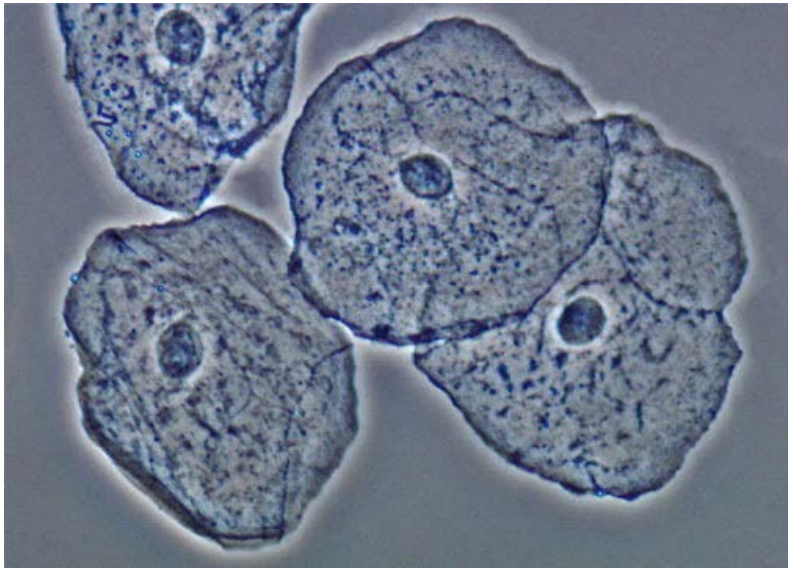
# Why do on-site filtration?

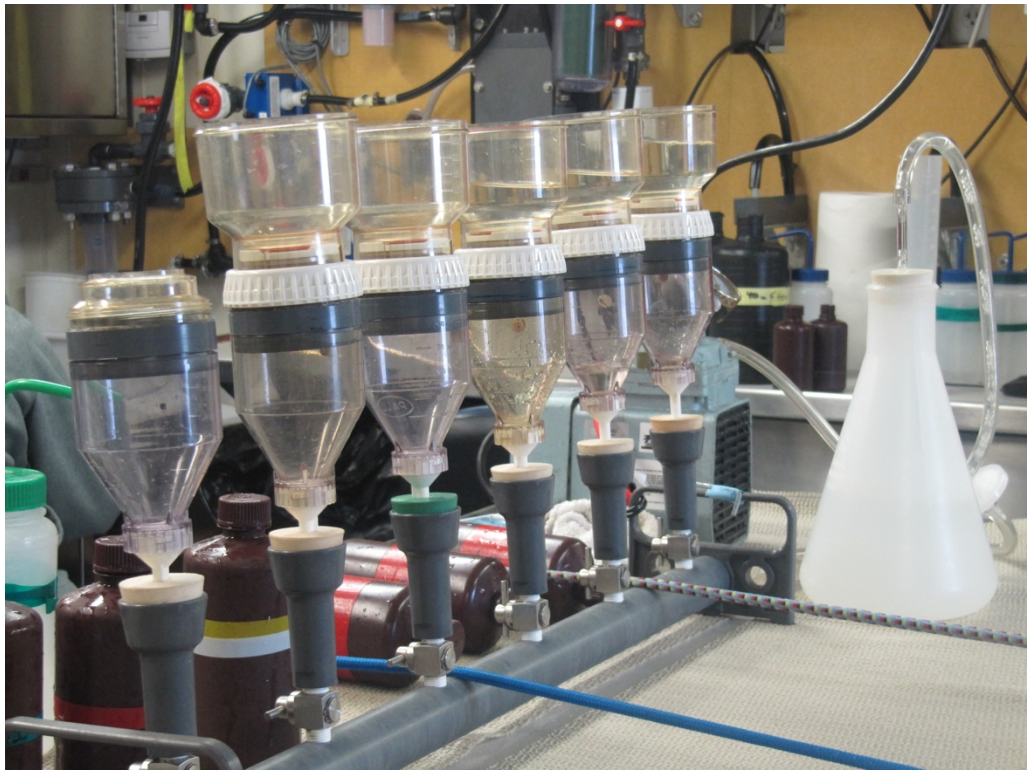
Immediate DNA preservation
(prevents loss during transport)

Concentrate a large water volume
(improves detectability)

Water transport can be prohibitive
(limits sample size)

# Why use a pressure threshold?





"...delicate particles might break when the pressure is too high. A pressure of 300-400 mmHg (~ 8 psi) is recommended."

Neukermans et al.,(2012) Limnology and Oceanography: Methods, 10(12), 1011-1023.

# Why include a flow meter?

1. Meter volume filtered
2. Set a target flow rate
3. Filter clogging alert

# “What settings should I use?”

Methods in Ecology and Evolution
BRITISH ECOLOGICAL SOCIETY

APPLICATION | Open Access

# ANDe™: A fully integrated environmental DNA sampling system

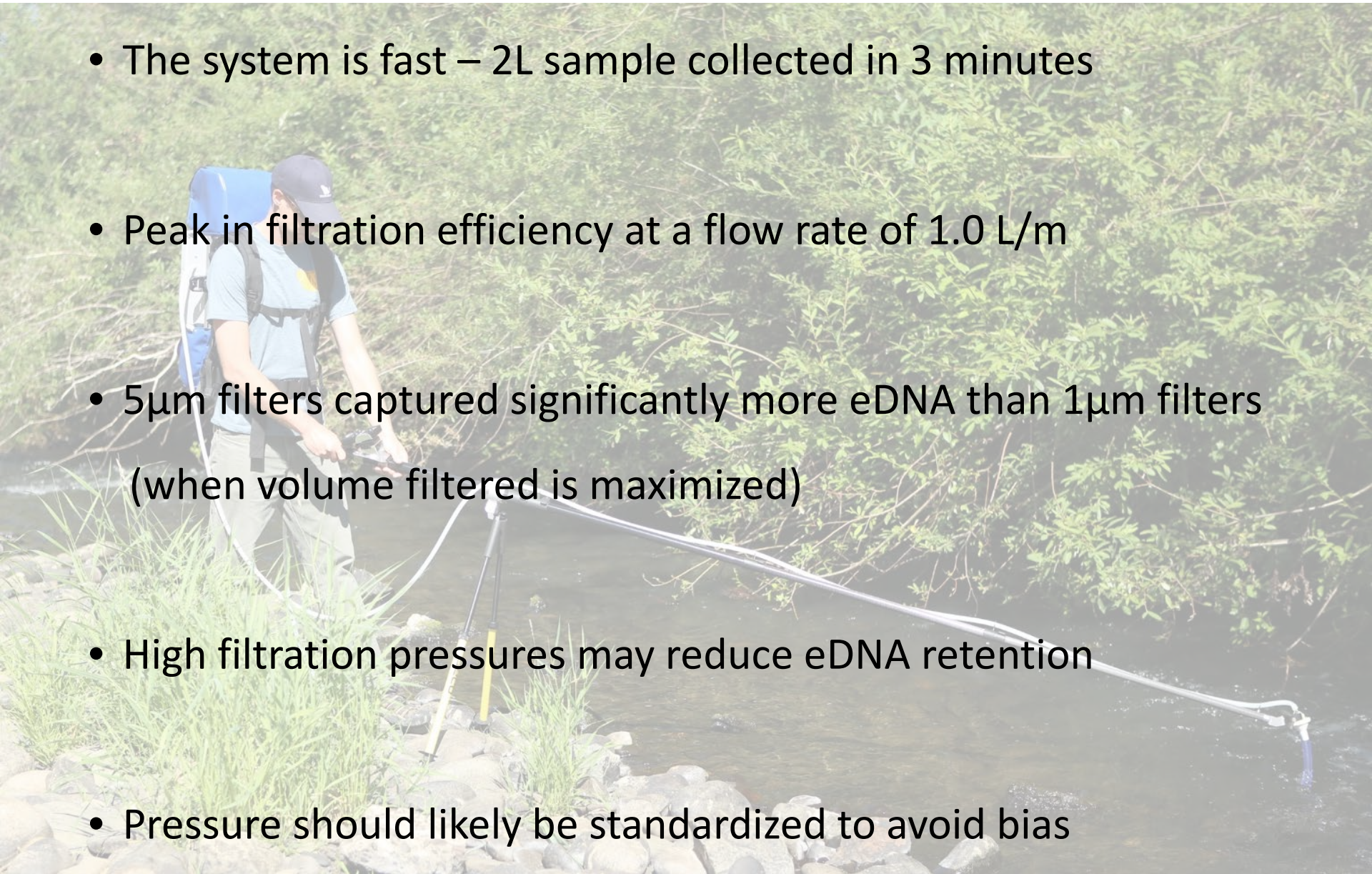
Austen C. Thomas, Jesse Howard, Phong L. Nguyen, Tracie A. Seimon, Caren S. Goldberg

First published: 28 March 2018 | https://doi.org/10.1111/2041-210X.12994



eDNA SAMPLER BACKPACK

# Paper conclusions:

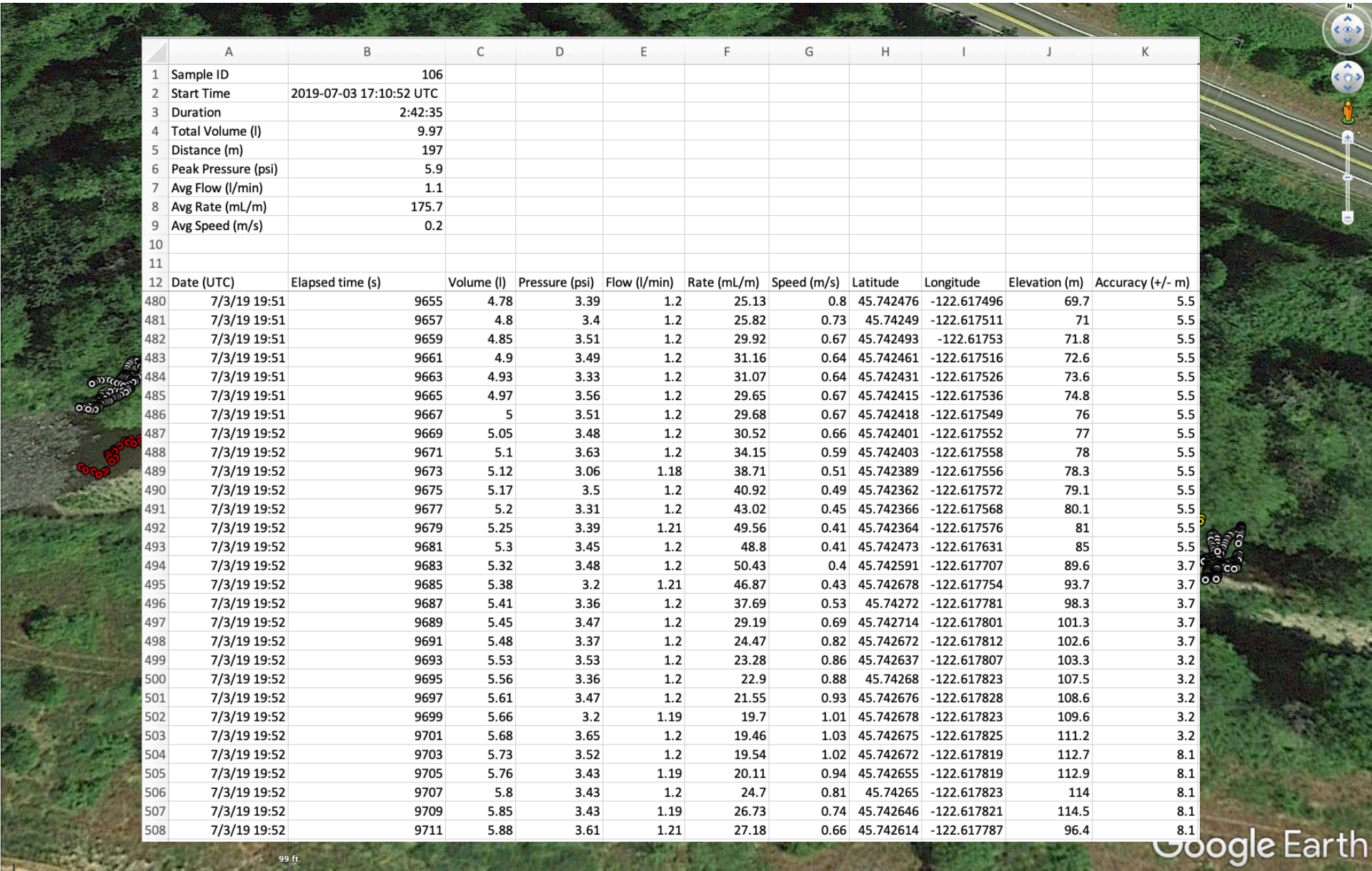


- The system is fast – 2L sample collected in 3 minutes
- Peak in filtration efficiency at a flow rate of 1.0 L/m
- 5µm filters captured significantly more eDNA than 1µm filters (when volume filtered is maximized)
- High filtration pressures may reduce eDNA retention
- Pressure should likely be standardized to avoid bias

# Evolution of the eDNA Sampler

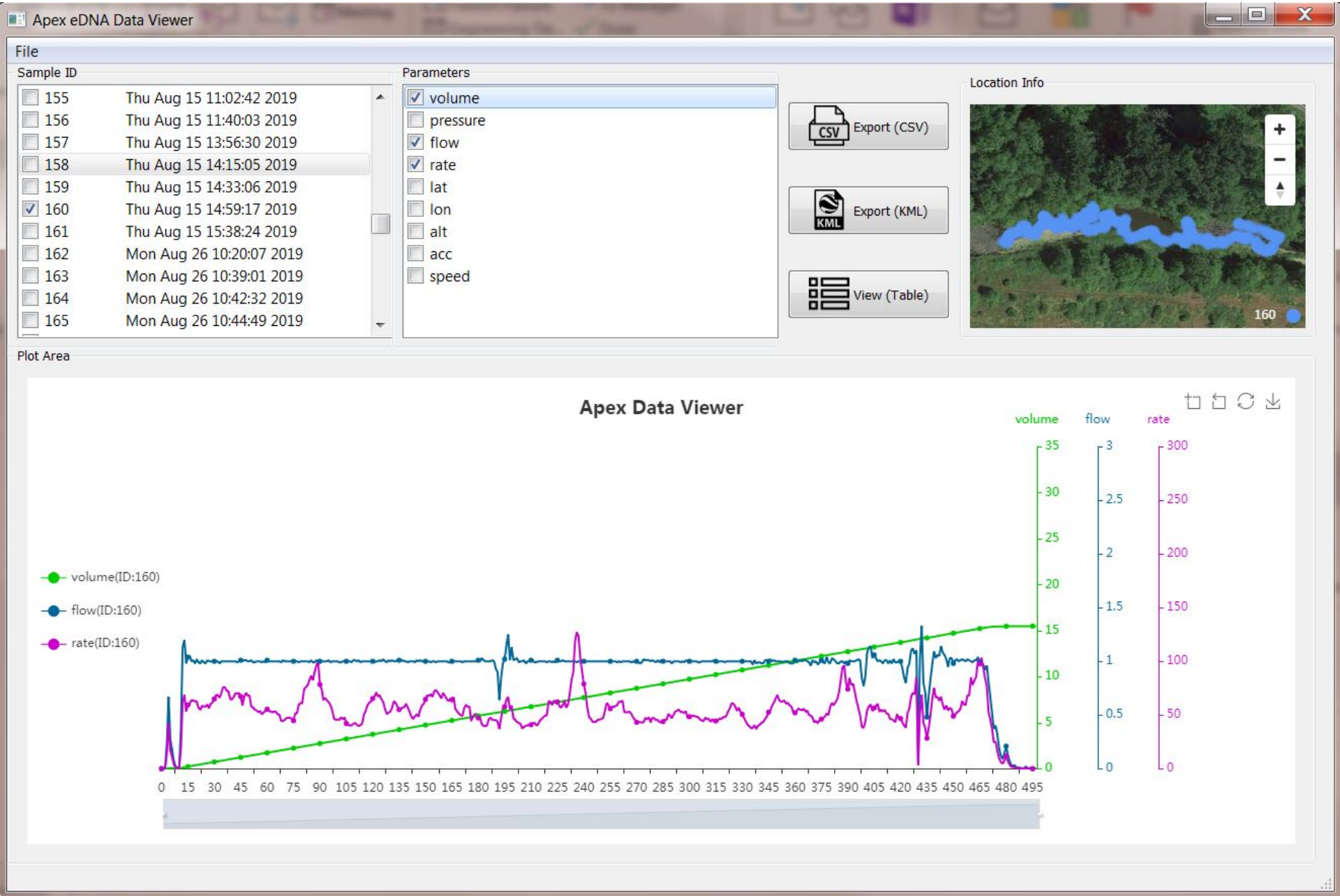
# Generating metadata record for eDNA

# Generating metadata record for eDNA



| | A | B | C | D | E | F | G | H | I | J | K |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Sample ID | 106 | | | | | | | | | |
| 2 | Start Time | 2019-07-03 17:10:52 UTC | | | | | | | | | |
| 3 | Duration | 2:42:35 | | | | | | | | | |
| 4 | Total Volume (l) | 9.97 | | | | | | | | | |
| 5 | Distance (m) | 197 | | | | | | | | | |
| 6 | Peak Pressure (psi) | 5.9 | | | | | | | | | |
| 7 | Avg Flow (l/min) | 1.1 | | | | | | | | | |
| 8 | Avg Rate (mL/m) | 175.7 | | | | | | | | | |
| 9 | Avg Speed (m/s) | 0.2 | | | | | | | | | |
| 10 | | | | | | | | | | | |
| 11 | | | | | | | | | | | |
| 12 | Date (UTC) | Elapsed time (s) | Volume (l) | Pressure (psi) | Flow (l/min) | Rate (mL/m) | Speed (m/s) | Latitude | Longitude | Elevation (m) | Accuracy (+/- m) |
| 480 | 7/3/19 19:51 | 9655 | 4.78 | 3.39 | 1.2 | 25.13 | 0.8 | 45.742476 | -122.617496 | 69.7 | 5.5 |
| 481 | 7/3/19 19:51 | 9657 | 4.8 | 3.4 | 1.2 | 25.82 | 0.73 | 45.74249 | -122.617511 | 71 | 5.5 |
| 482 | 7/3/19 19:51 | 9659 | 4.85 | 3.51 | 1.2 | 29.92 | 0.67 | 45.742493 | -122.61753 | 71.8 | 5.5 |
| 483 | 7/3/19 19:51 | 9661 | 4.9 | 3.49 | 1.2 | 31.16 | 0.64 | 45.742461 | -122.617516 | 72.6 | 5.5 |
| 484 | 7/3/19 19:51 | 9663 | 4.93 | 3.33 | 1.2 | 31.07 | 0.64 | 45.742431 | -122.617526 | 73.6 | 5.5 |
| 485 | 7/3/19 19:51 | 9665 | 4.97 | 3.56 | 1.2 | 29.65 | 0.67 | 45.742415 | -122.617536 | 74.8 | 5.5 |
| 486 | 7/3/19 19:51 | 9667 | 5 | 3.51 | 1.2 | 29.68 | 0.67 | 45.742418 | -122.617549 | 76 | 5.5 |
| 487 | 7/3/19 19:52 | 9669 | 5.05 | 3.48 | 1.2 | 30.52 | 0.66 | 45.742401 | -122.617552 | 77 | 5.5 |
| 488 | 7/3/19 19:52 | 9671 | 5.1 | 3.63 | 1.2 | 34.15 | 0.59 | 45.742403 | -122.617558 | 78 | 5.5 |
| 489 | 7/3/19 19:52 | 9673 | 5.12 | 3.06 | 1.18 | 38.71 | 0.51 | 45.742389 | -122.617556 | 78.3 | 5.5 |
| 490 | 7/3/19 19:52 | 9675 | 5.17 | 3.5 | 1.2 | 40.92 | 0.49 | 45.742362 | -122.617572 | 79.1 | 5.5 |
| 491 | 7/3/19 19:52 | 9677 | 5.2 | 3.31 | 1.2 | 43.02 | 0.45 | 45.742366 | -122.617568 | 80.1 | 5.5 |
| 492 | 7/3/19 19:52 | 9679 | 5.25 | 3.39 | 1.21 | 49.56 | 0.41 | 45.742364 | -122.617576 | 81 | 5.5 |
| 493 | 7/3/19 19:52 | 9681 | 5.3 | 3.45 | 1.2 | 48.8 | 0.41 | 45.742473 | -122.617631 | 85 | 5.5 |
| 494 | 7/3/19 19:52 | 9683 | 5.32 | 3.48 | 1.2 | 50.43 | 0.4 | 45.742591 | -122.617707 | 89.6 | 3.7 |
| 495 | 7/3/19 19:52 | 9685 | 5.38 | 3.2 | 1.21 | 46.87 | 0.43 | 45.742678 | -122.617754 | 93.7 | 3.7 |
| 496 | 7/3/19 19:52 | 9687 | 5.41 | 3.36 | 1.2 | 37.69 | 0.53 | 45.74272 | -122.617781 | 98.3 | 3.7 |
| 497 | 7/3/19 19:52 | 9689 | 5.45 | 3.47 | 1.2 | 29.19 | 0.69 | 45.742714 | -122.617801 | 101.3 | 3.7 |
| 498 | 7/3/19 19:52 | 9691 | 5.48 | 3.37 | 1.2 | 24.47 | 0.82 | 45.742672 | -122.617812 | 102.6 | 3.7 |
| 499 | 7/3/19 19:52 | 9693 | 5.53 | 3.53 | 1.2 | 23.28 | 0.86 | 45.742637 | -122.617807 | 103.3 | 3.2 |
| 500 | 7/3/19 19:52 | 9695 | 5.56 | 3.36 | 1.2 | 22.9 | 0.88 | 45.74268 | -122.617823 | 107.5 | 3.2 |
| 501 | 7/3/19 19:52 | 9697 | 5.61 | 3.47 | 1.2 | 21.55 | 0.93 | 45.742676 | -122.617828 | 108.6 | 3.2 |
| 502 | 7/3/19 19:52 | 9699 | 5.66 | 3.2 | 1.19 | 19.7 | 1.01 | 45.742678 | -122.617823 | 109.6 | 3.2 |
| 503 | 7/3/19 19:52 | 9701 | 5.68 | 3.65 | 1.2 | 19.46 | 1.03 | 45.742675 | -122.617825 | 111.2 | 3.2 |
| 504 | 7/3/19 19:52 | 9703 | 5.73 | 3.52 | 1.2 | 19.54 | 1.02 | 45.742672 | -122.617819 | 112.7 | 8.1 |
| 505 | 7/3/19 19:52 | 9705 | 5.76 | 3.43 | 1.19 | 20.11 | 0.94 | 45.742655 | -122.617819 | 112.9 | 8.1 |
| 506 | 7/3/19 19:52 | 9707 | 5.8 | 3.43 | 1.2 | 24.7 | 0.81 | 45.74265 | -122.617823 | 114 | 8.1 |
| 507 | 7/3/19 19:52 | 9709 | 5.85 | 3.43 | 1.19 | 26.73 | 0.74 | 45.742646 | -122.617821 | 114.5 | 8.1 |
| 508 | 7/3/19 19:52 | 9711 | 5.88 | 3.61 | 1.21 | 27.18 | 0.66 | 45.742614 | -122.617787 | 96.4 | 8.1 |

# Visualization and data management




# Compact unit designed for portability and Aquaculture applications

# Why single-use filter packs?

1. Bleach sterilization can introduce contamination risk

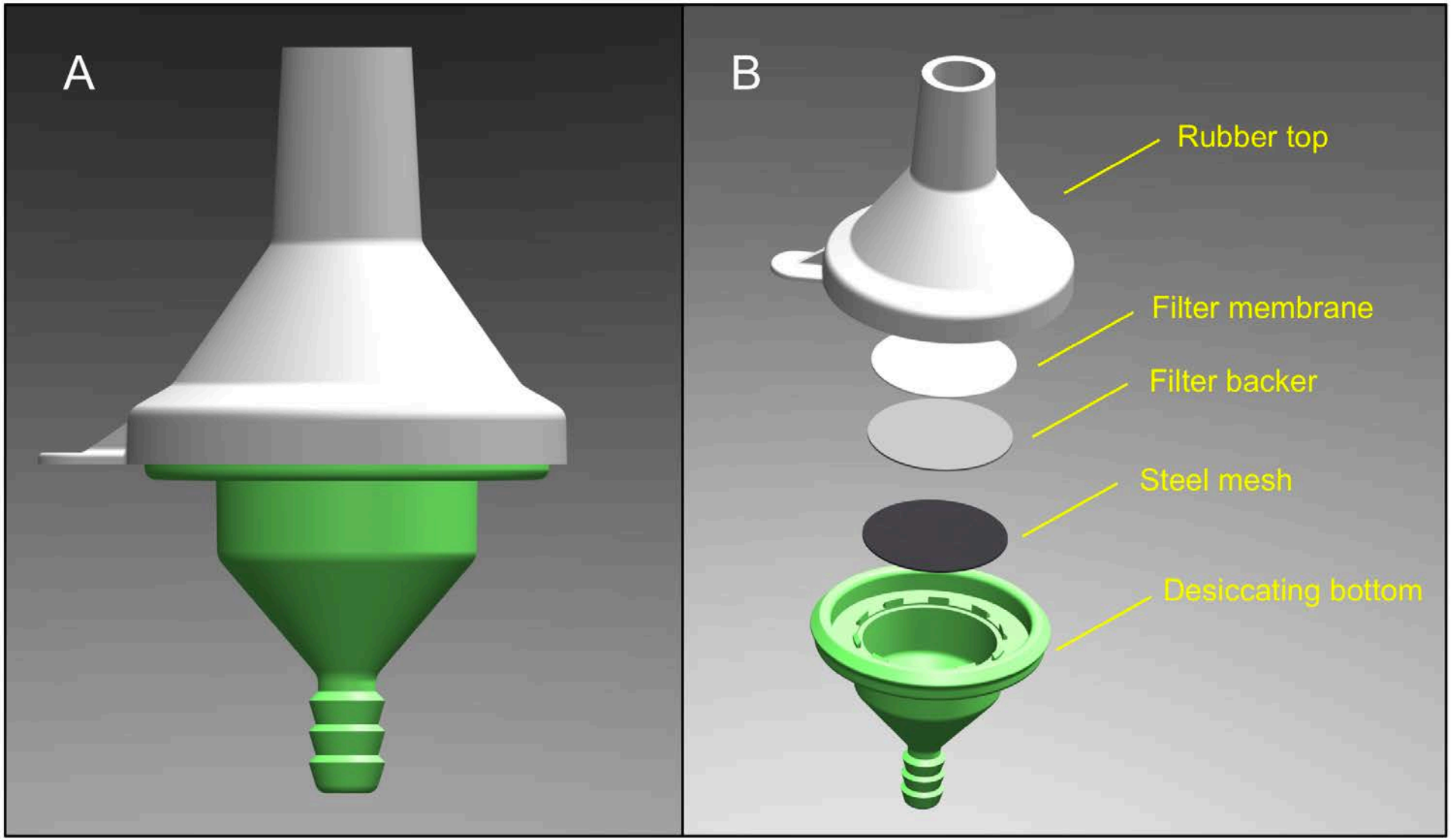
2. Sterilization procedures are time consuming (costly)

# Highly hydrophilic



Filters are often preserved in desiccant

# A self-preserving eDNA filter



# A self-preserving eDNA filter

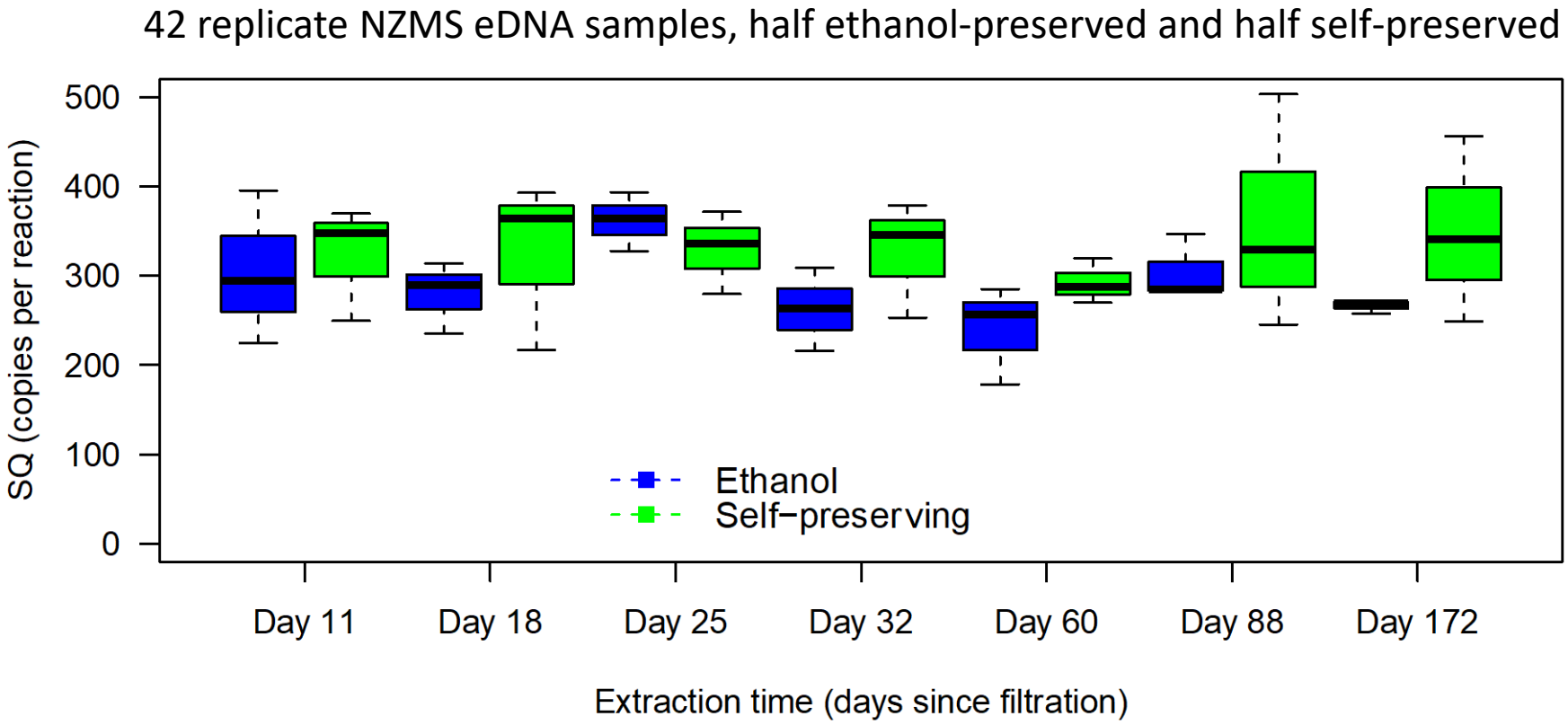
# Advantages of a self-preserving eDNA filter

Pre-extraction storage


Lab processing


- No filter membrane transfer step
- Reduce chance of contamination
- No chemical or cold storage
- Reduces per sample field time

# Results from 6-month preservation trial




No significant difference in eDNA quantity over 6 months
- slightly higher eDNA recovery from self-preserved

# Results from field trial

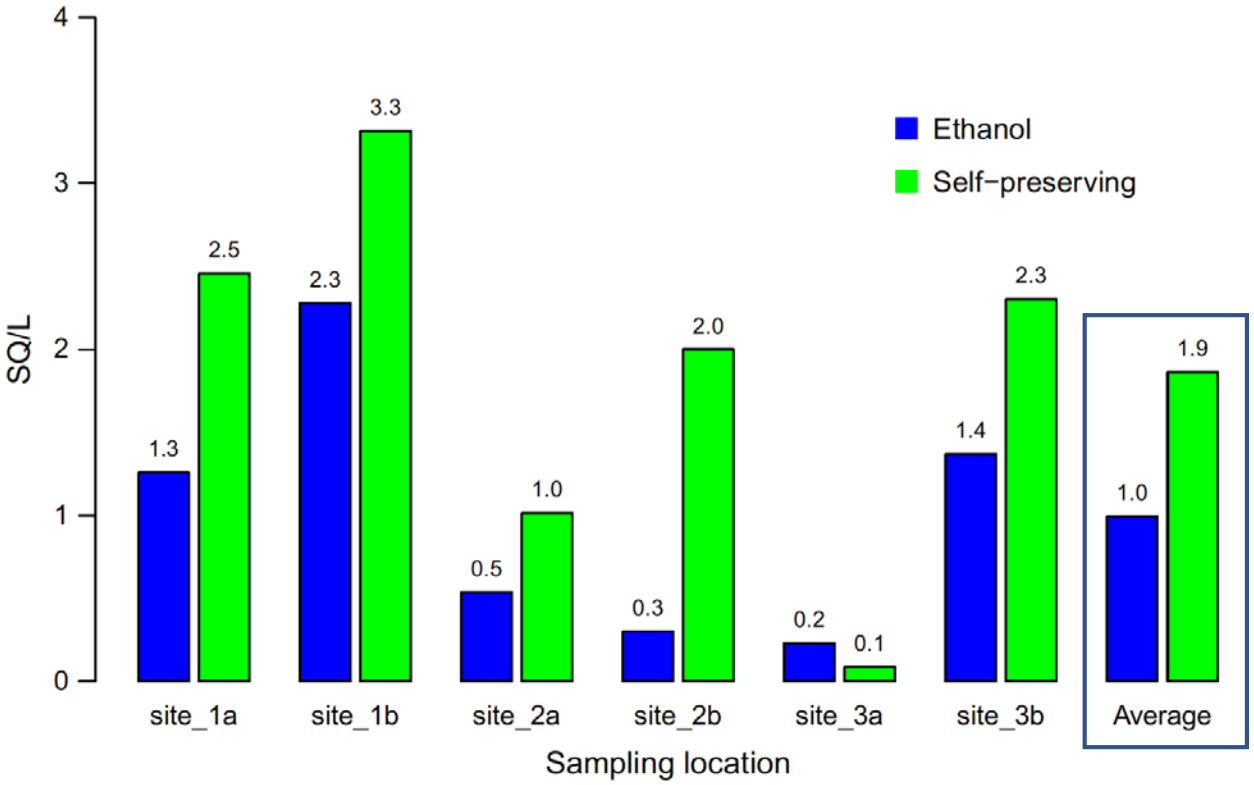



**FIGURE 4** Results from paired eDNA field sampling with both preservation methods: ethanol (blue) and self-preserving (green). Six total locations were sampled from three different ponds targeting spotted frog eDNA. SQ/L values indicate an index of target eDNA quantity based on a tissue extract standard curve and divided by volume filtered

Self-preserving filters contained approximately 2X the eDNA of ethanol-preserved samples on average (paired t test, p = 0.020)

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PRACTICAL TOOLS

Methods in Ecology and Evolution BRITISH ECOLOGICAL SOCIETY

# A self-preserving, partially biodegradable eDNA filter

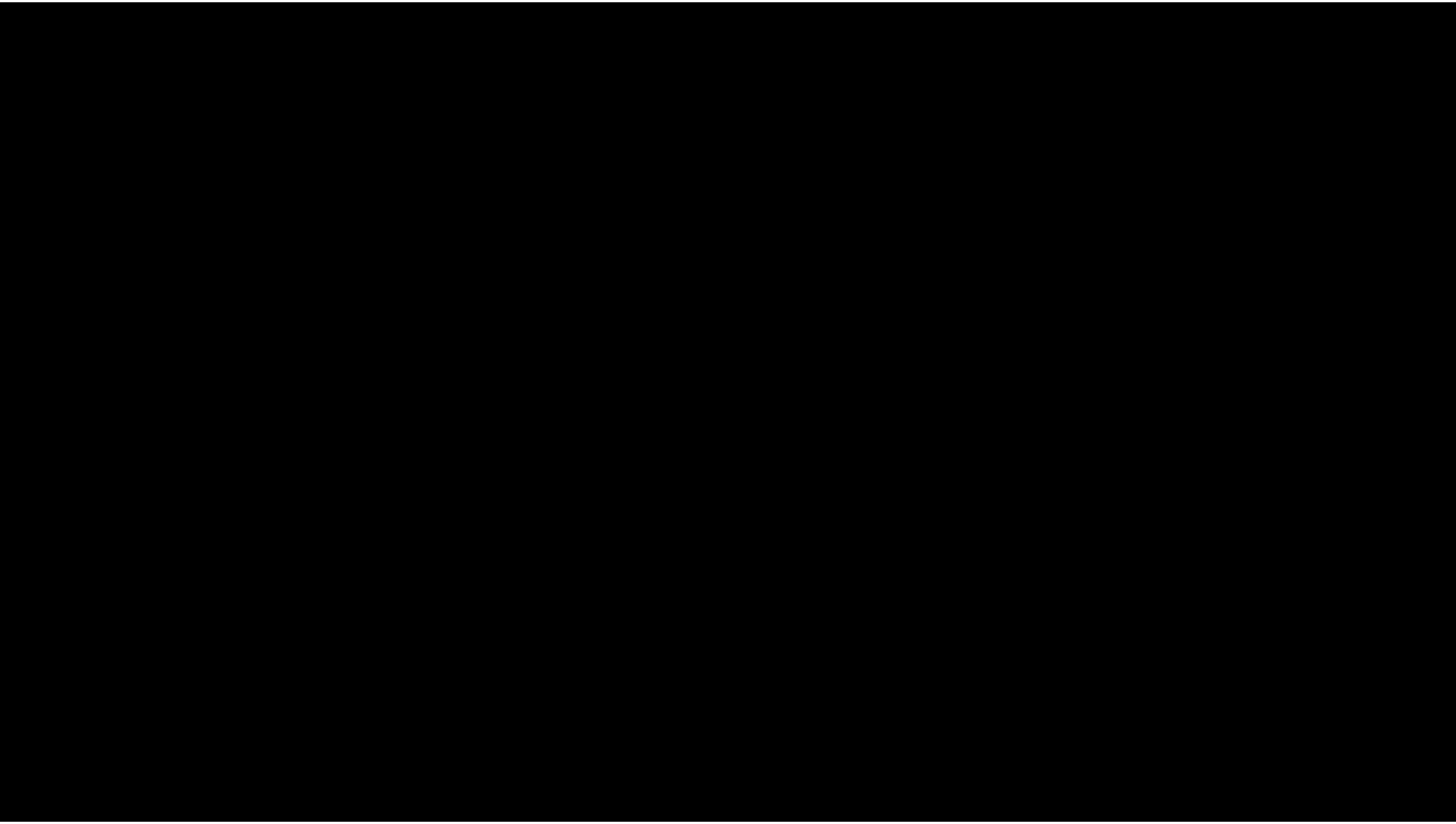
Austen C. Thomas[1] | Phong L. Nguyen[1] | Jesse Howard[1] | Caren S. Goldberg[2]

Sissel Jentoft

# The future of eDNA Sampling

# A low-cost/rental sampler for citizen science projects

# What about boat sampling?

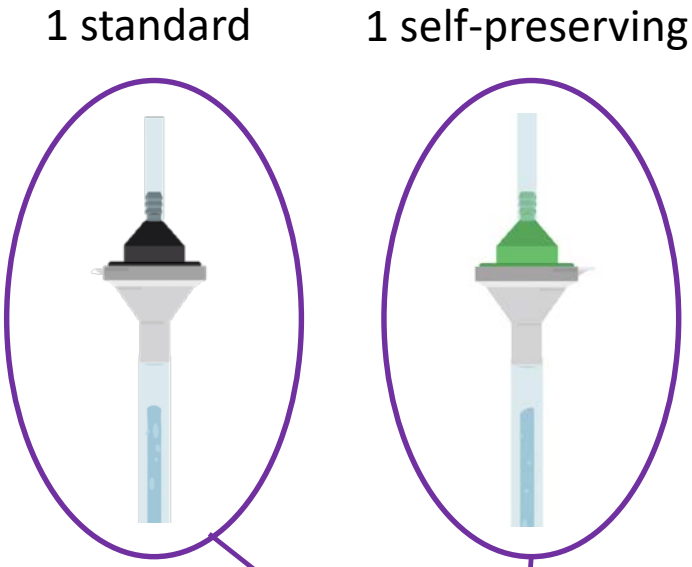



Thank you

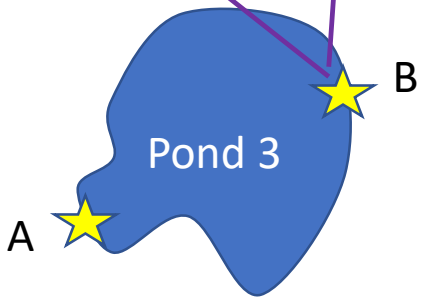

Demo

# Pilot field experiment

Columbia Spotted Frog - *Rana luteiventris*






6 Free eDNA filter packets
1.2um
1.2um
1.2um
5um
5um
5um
Box
SMITH ROOT
INSTRUCTIONS
SELF-PRESERVING FILTERS
Beta testing
Instruction sheet

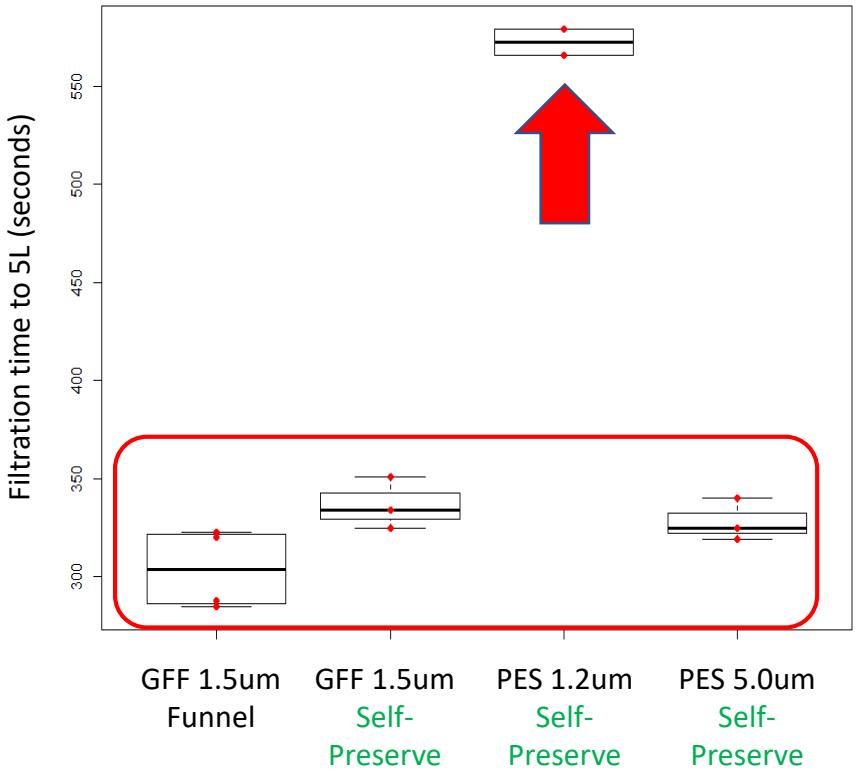
# Beta testing results

Dr. Taylor Wilcox

National Genomics Center

U.S. FOREST SERVICE
Caring for the land and serving people

Brook trout




- 1.2um self-preserving filters took longer to filter (GeoTech pump)
- GFF and PES 5.0um self-preserving were similar to standard method

# Beta testing results

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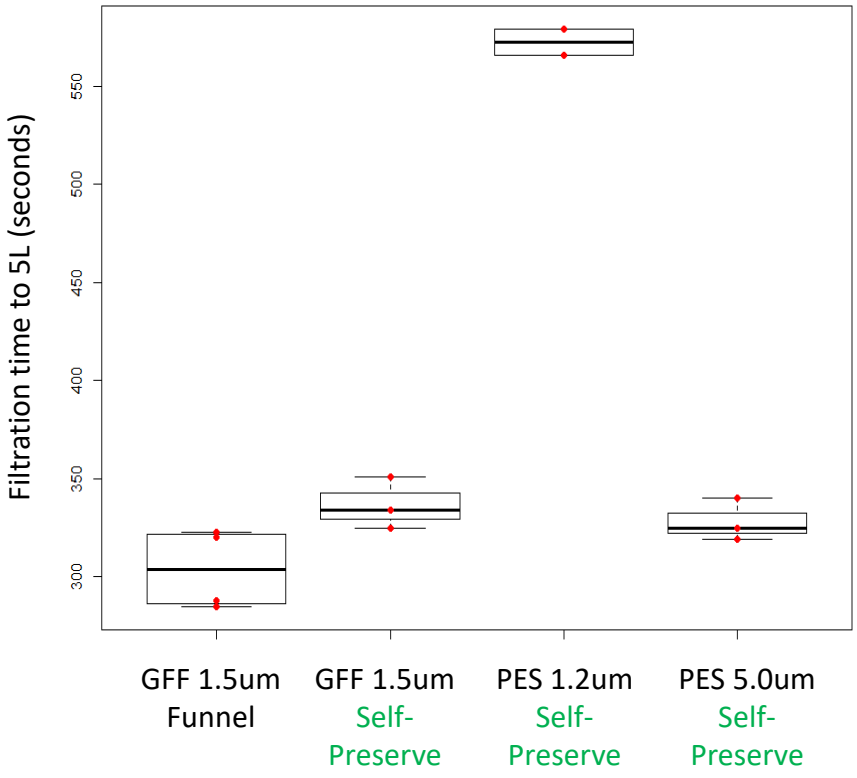
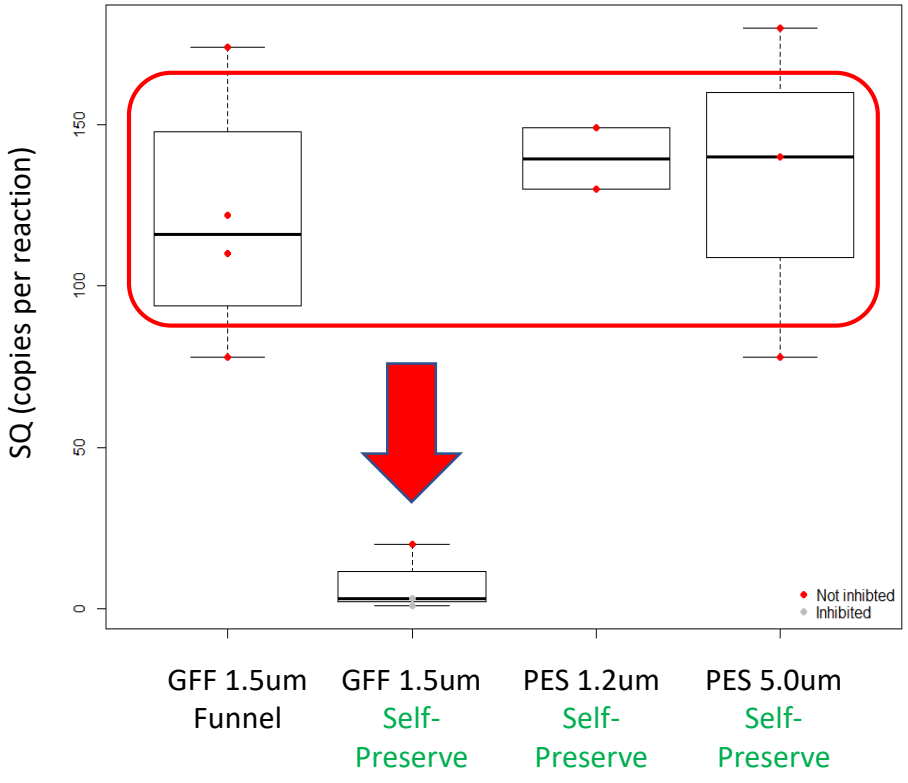
Brook trout




- GFF self-preserving were inhibited and degraded (thick membrane)
- 1.2um and 5.0um PES self-preserving comparable yield to standard

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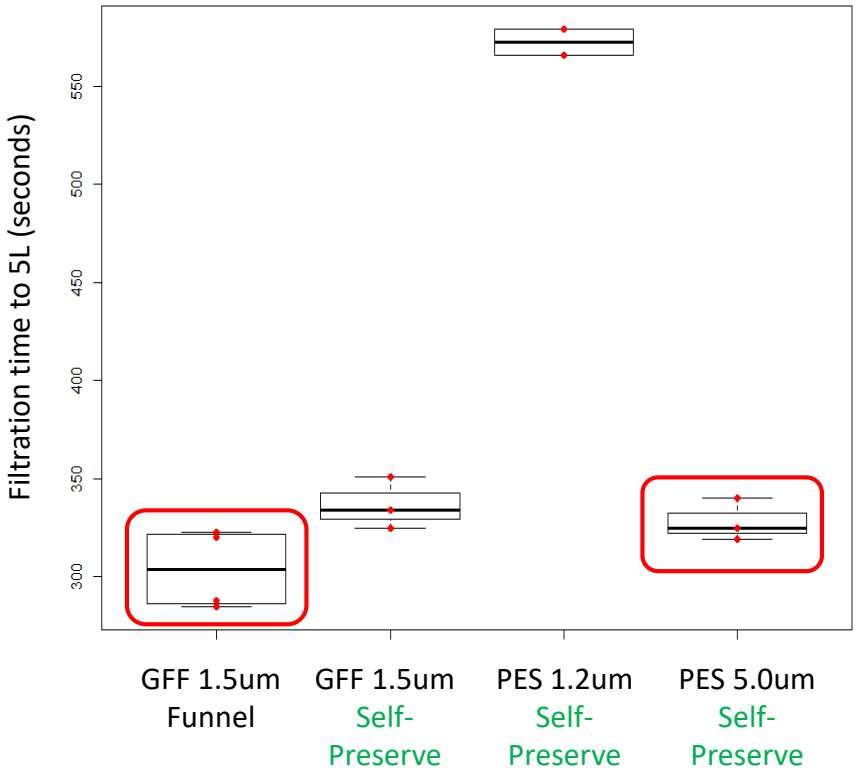
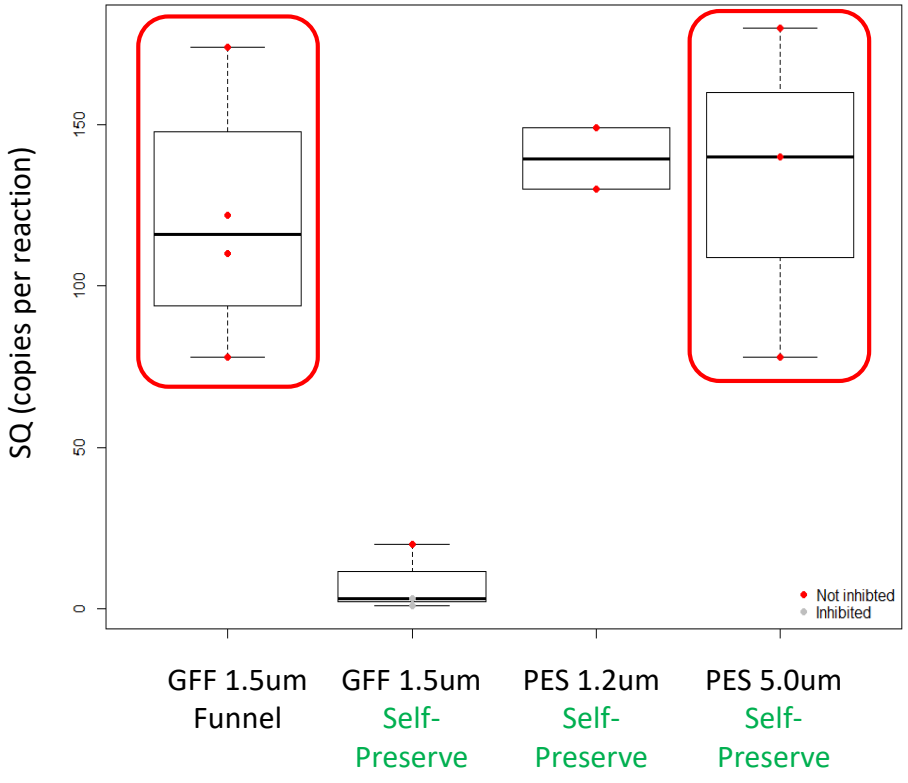



5.0um Self-preserving had comparable eDNA yield and filtration time to the standard NGC method

Department of Primary Industries

Redfin perch eDNA

# Beta testing results

## Smith-Root Self-preserving eDNA filters

Dr. Meaghan Duncan

Jackson Wilkes Walburn

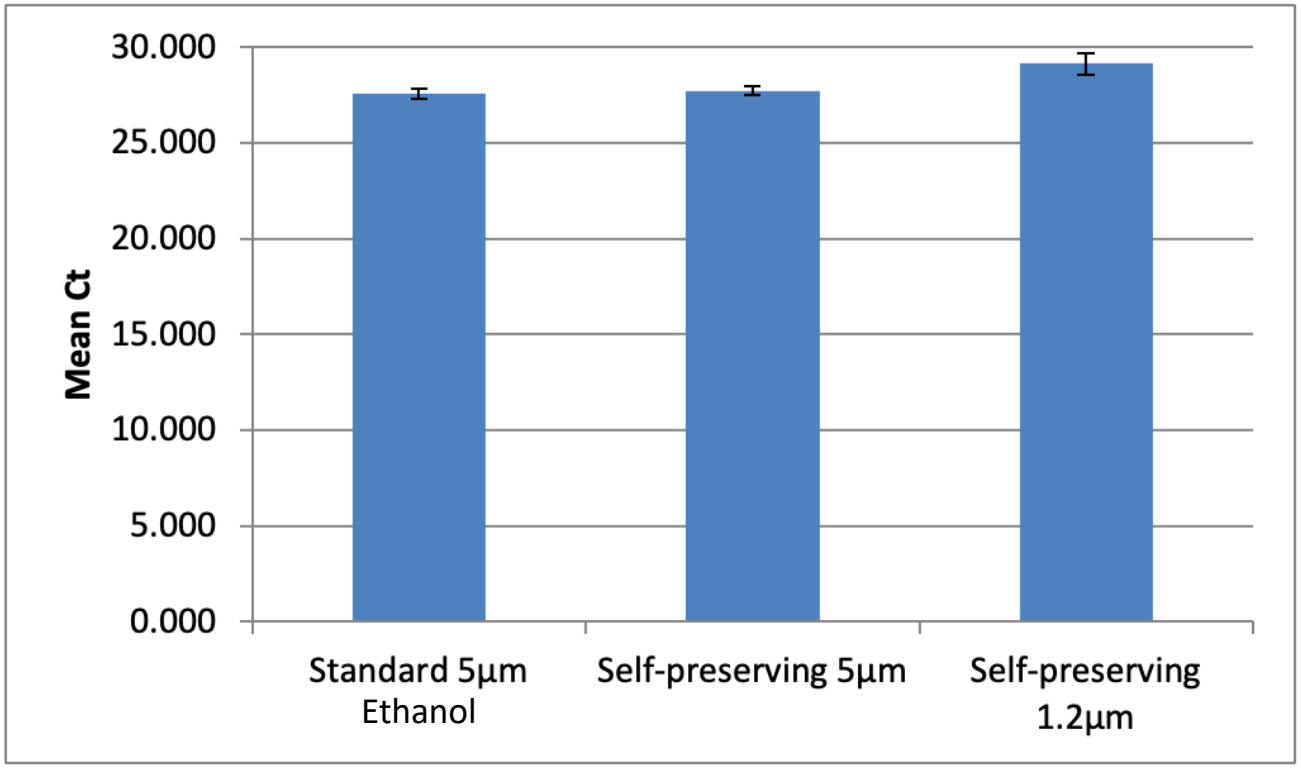


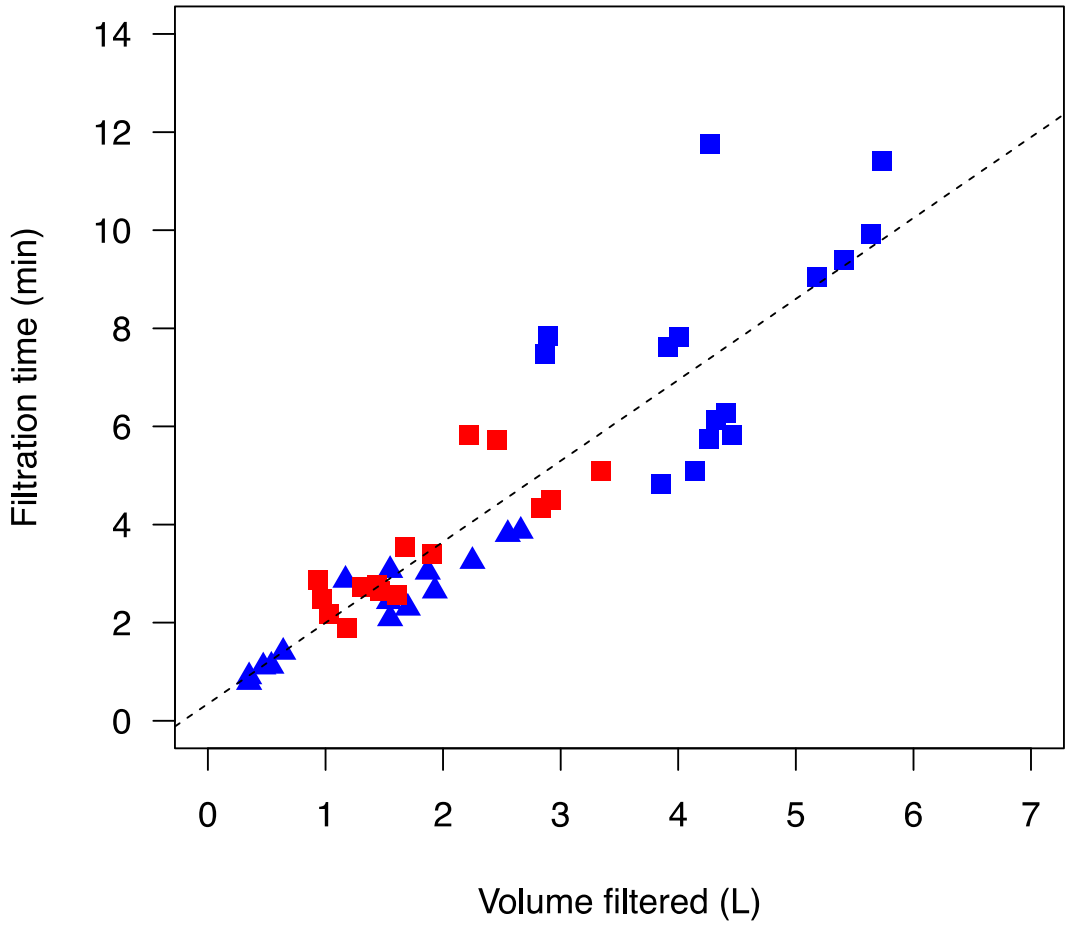

**Figure 1** – Mean Ct values recorded for standard 5µm, self-preserving 5µm and self-preserving 1.2µm filters. **Note that lower Ct = more template DNA.**

Found no significant difference in perch eDNA between self-preserved filters and ethanol preservation. Slightly more eDNA on 5µm.

# Conclusions

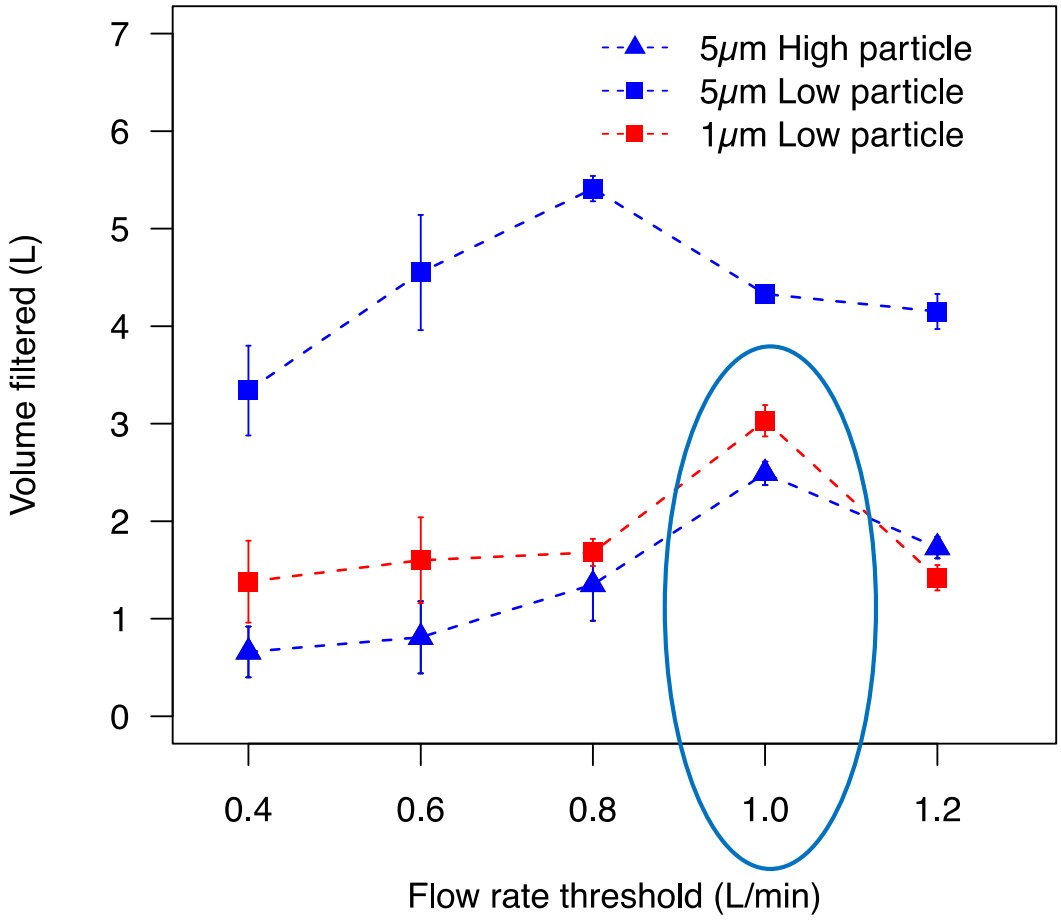
- eDNA preservation duration > 6months
- Comparable or better than ethanol in field trials
- 5µm filters performed similarly to GFF (flow rate, eDNA yield)
- GFF filters do not preserve well in housing
- Field trials with larger sample sizes are in the works

# The eDNA Sampler is fast




2 L of water can be filtered in approximately 3 min

# Flow experiment results



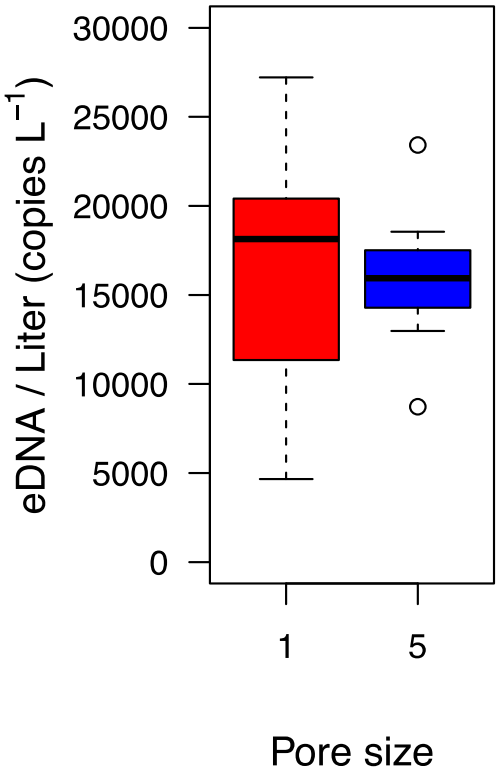

1.0 L/min set point more than doubles the filterable water volume

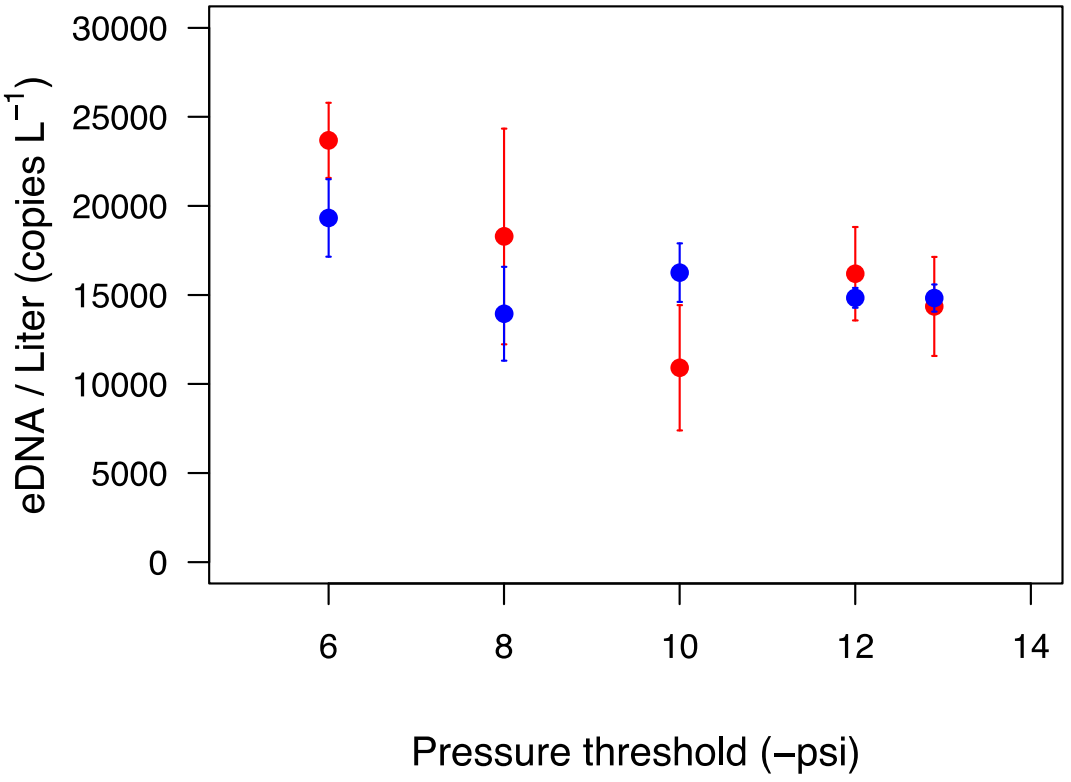
# Pressure experiment results

$eDNA_{Total} =$ Index of detection sensitivity

$eDNA/L =$ Relative index of eDNA capture efficiency

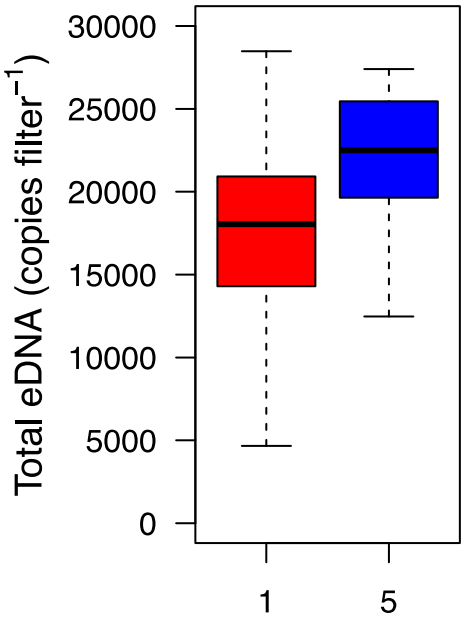
# Pressure experiment results

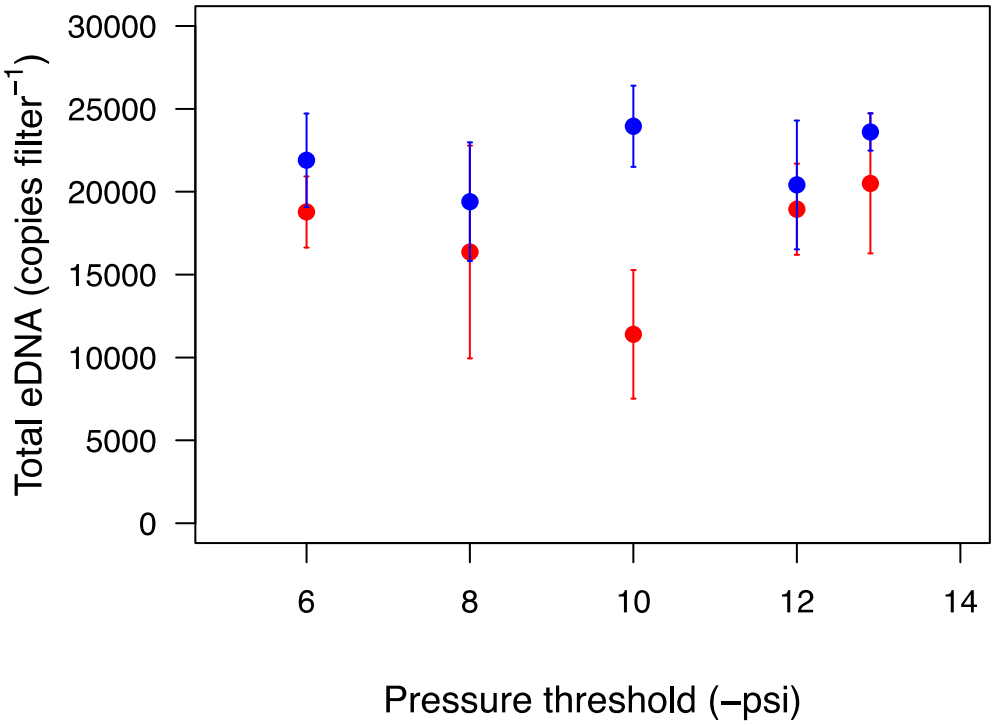



eDNA per Liter decreases with increasing filtration pressure

# Pressure experiment results




Captured significantly more NZ mudsnail eDNA on 5µm filters

# eDNA preservation experiment

**Filter preservation experiment**

- Single tank with suspended NZMS eDNA
- 42 replicate 0.5L samples collected
- Half self-preserved, half ethanol
- 3 extracted: 11 d - 172 d.
- Quantified NZMS eDNA by qPCR

Open packet


Collect sample


Replace and seal


Field storage


Pre-extraction storage

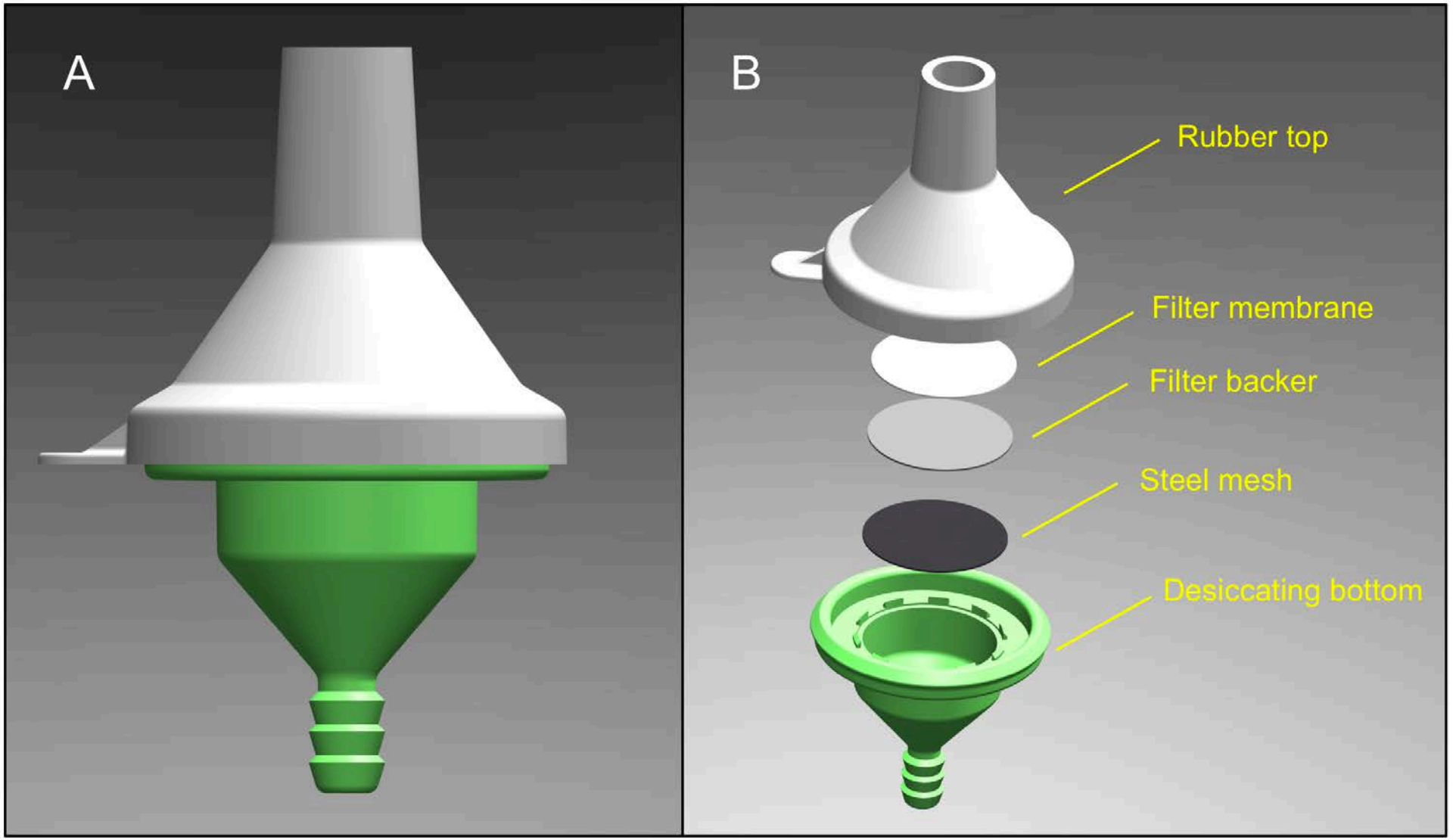

Lab processing


# The downside to eDNA

# Why single-use plastics?

Existing sterilization methods (bleach) can lead to false-positives when sterilization is insufficient, or false-negatives when residual bleach is carried over to subsequent samples.

# A self-preserving eDNA filter



# Advantages of a self-preserving eDNA filter

Pre-extraction storage


Lab processing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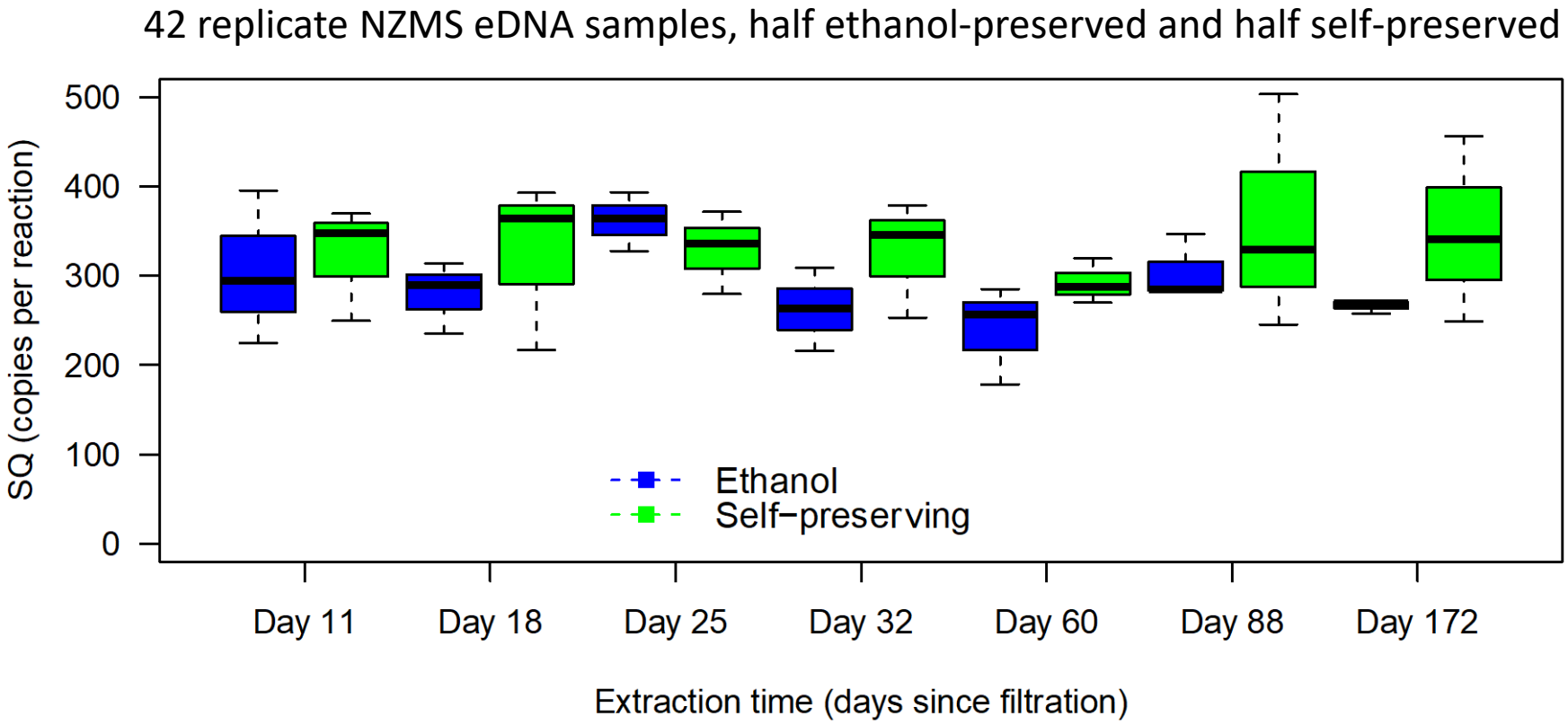
# Results from 6-month preservation trial

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# Results from field trial

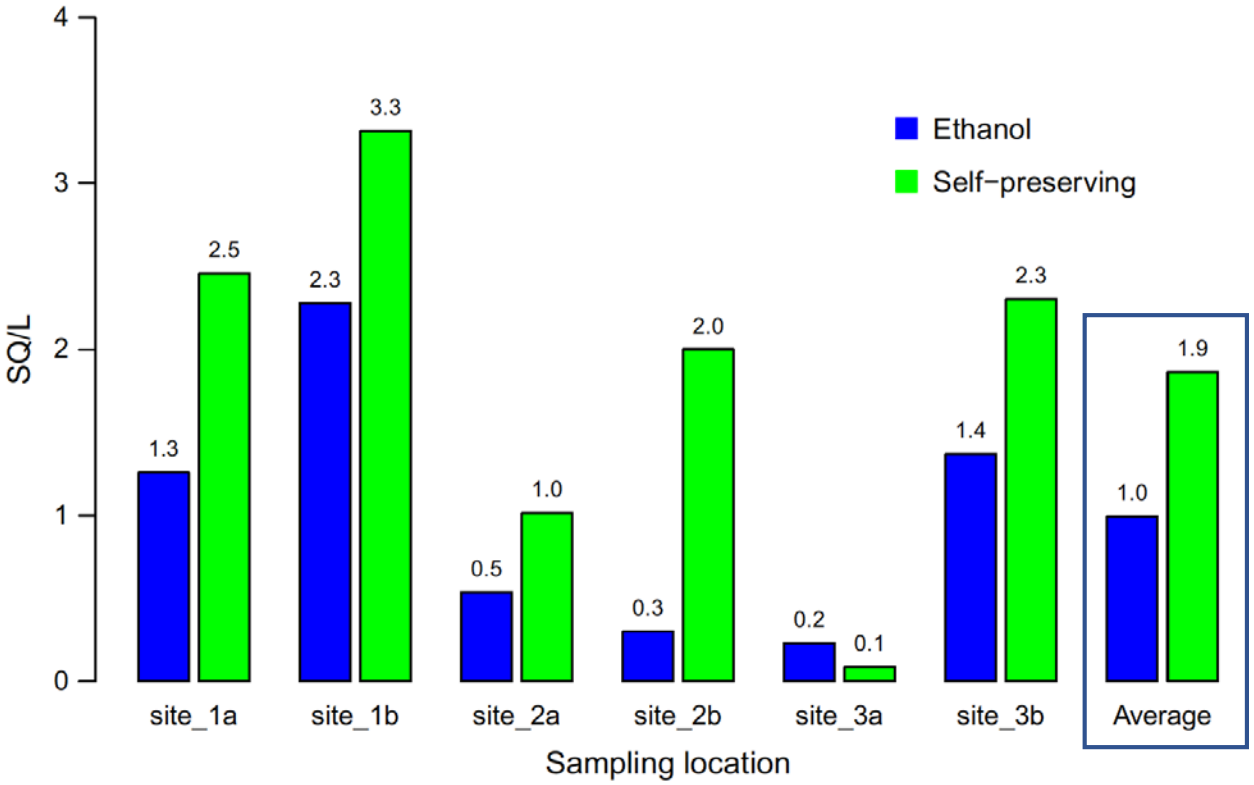


**FIGURE 4** Results from paired eDNA field sampling with both preservation methods: ethanol (blue) and self-preserving (green). Six total locations were sampled from three different ponds targeting spotted frog eDNA. SQ/L values indicate an index of target eDNA quantity based on a tissue extract standard curve and divided by volume filtered

Self-preserving filters contained approximately 2X the eDNA of ethanol-preserved samples on average (paired t test, p = 0.020)

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PRACTICAL TOOLS

Methods in Ecology and Evolution BRITISH ECOLOGICAL SOCIETY

# A self-preserving, partially biodegradable eDNA filter

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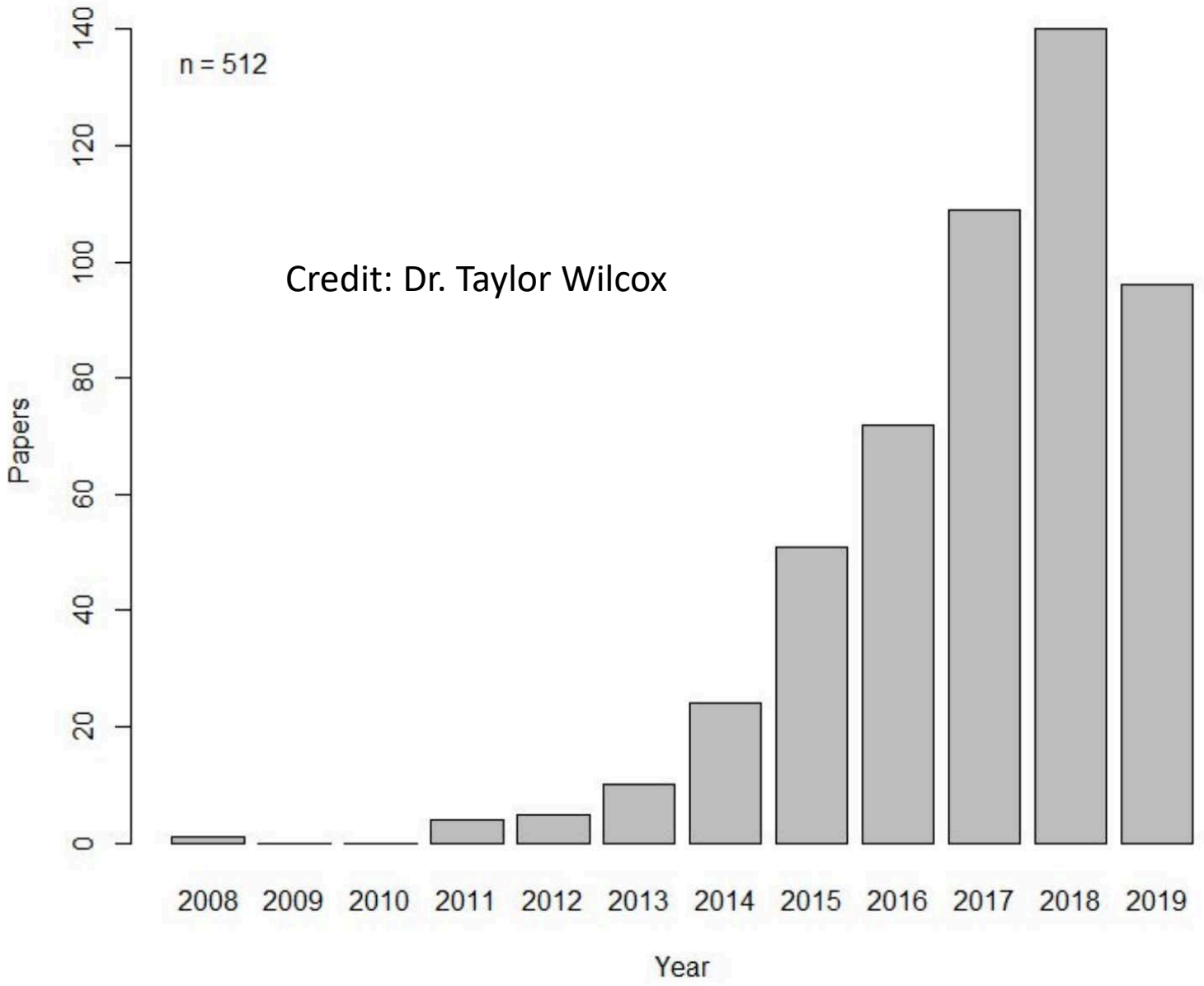
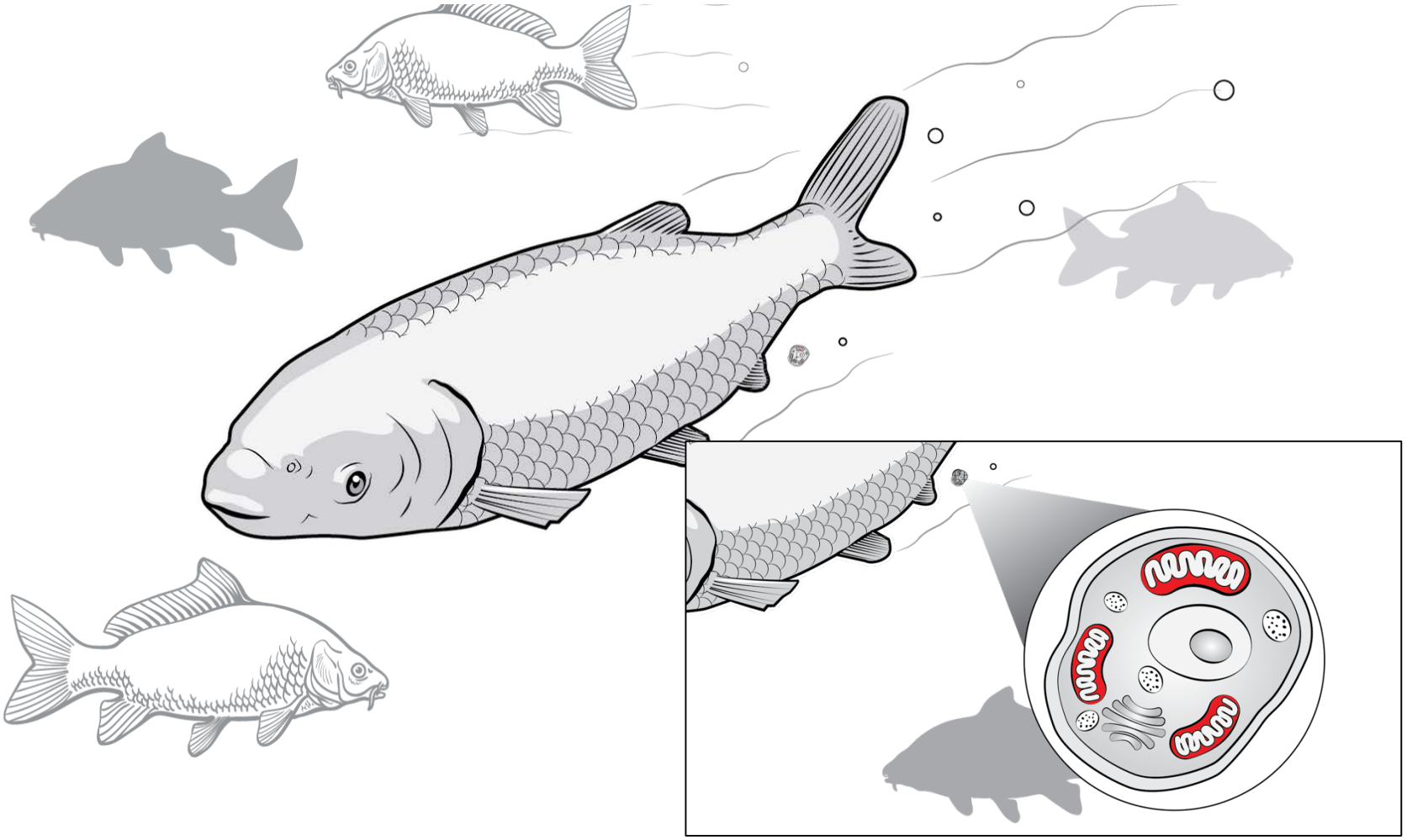
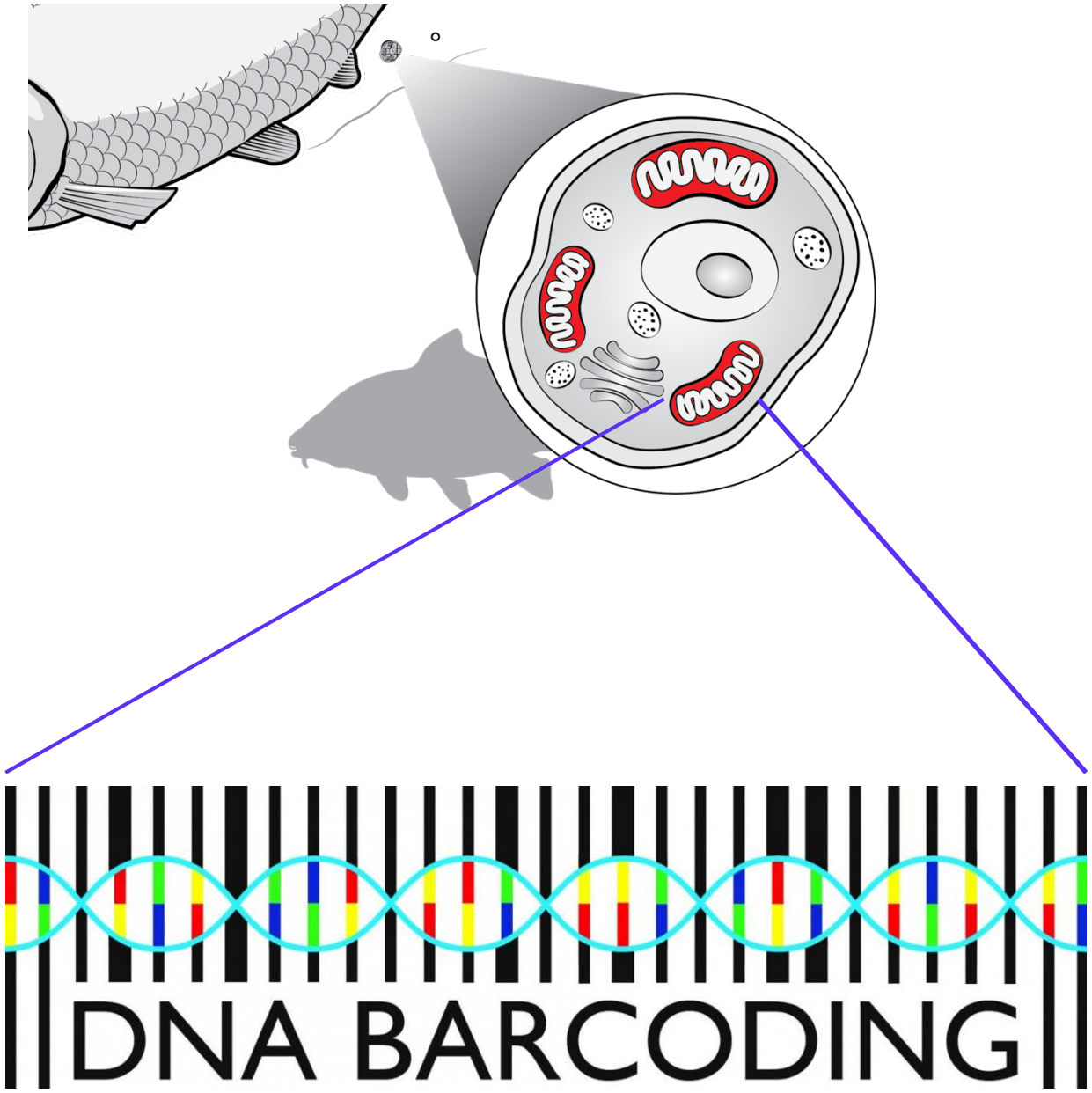
Sissel Jentoft

# eDNA Research papers (macrobial)



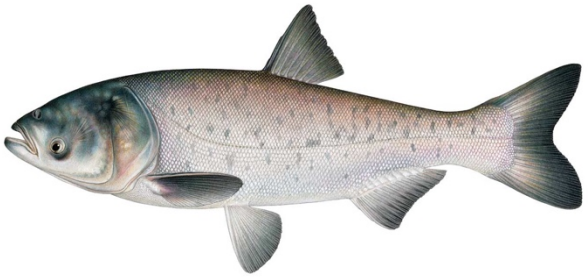
# What is environmental DNA?



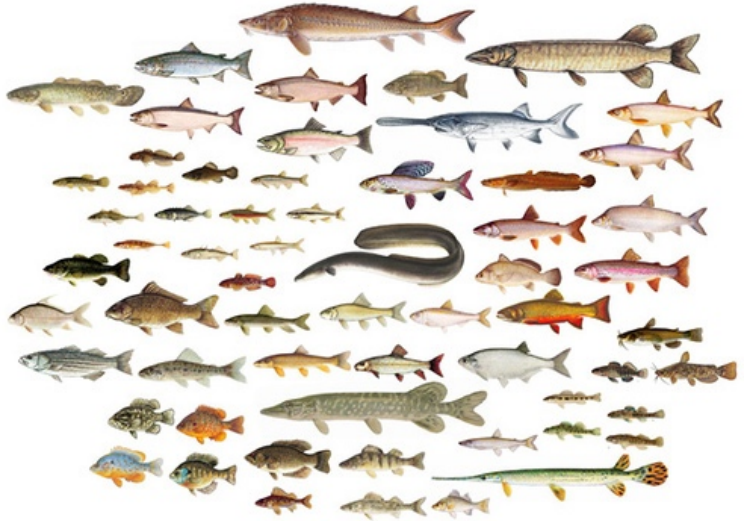

DNA BARCODING

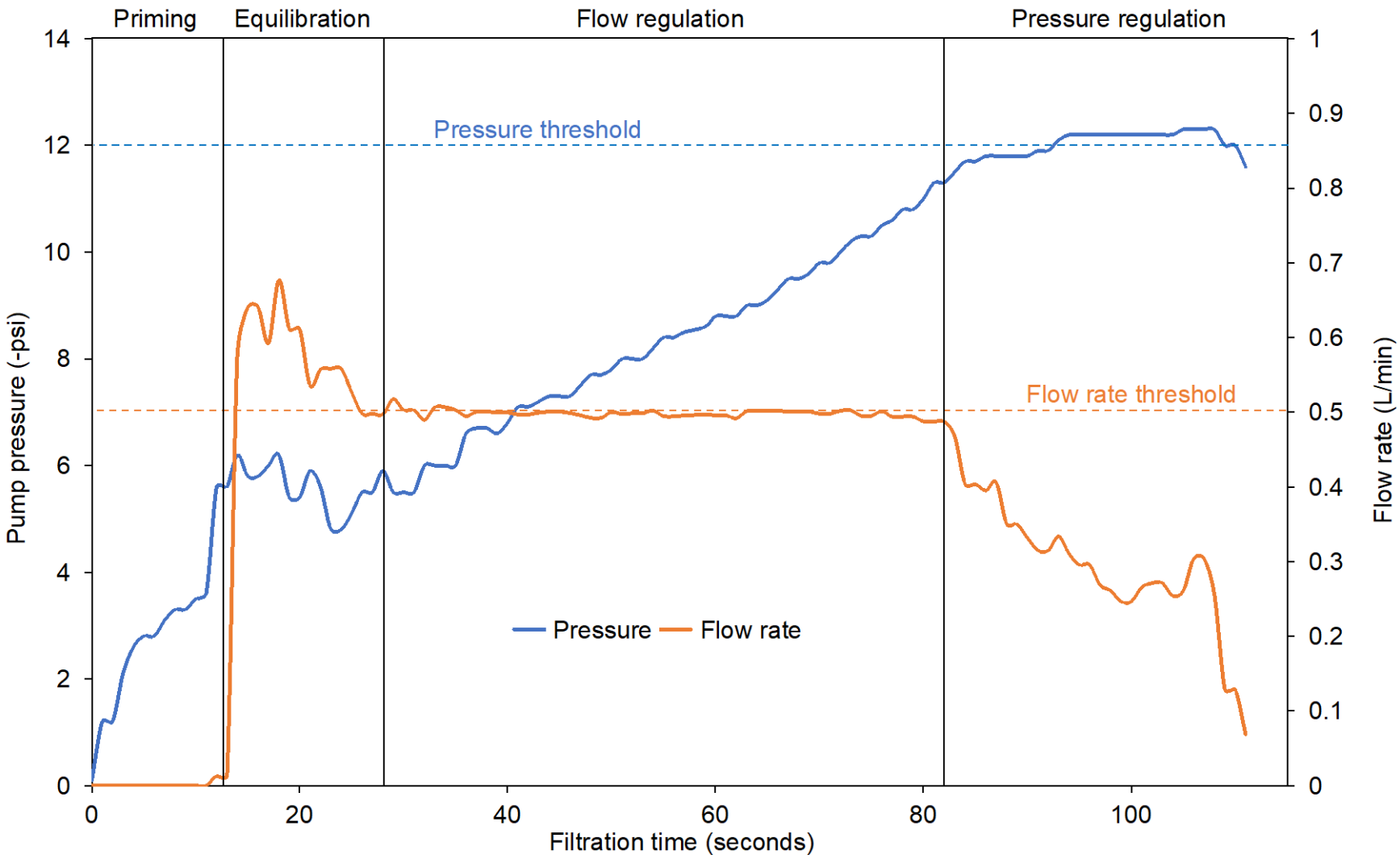
# Design eDNA tests for species or groups

Single species detection
(qPCR)



Community characterization
(DNA metabarcoding)




Priming
Equilibration
Flow regulation
Pressure regulation
Pressure threshold
Flow rate threshold
Pressure
Flow rate
Pump pressure (-psi)
Flow rate (L/min)
Filtration time (seconds)
0
2
4
6
8
10
12
14
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
20
40
60
80
100