

The USACE Invasive Species Leadership Team Presents:

Environmental DNA and Invasive Aquatic Species: 9 Years In

By Dr. Richard Lance, ERDC Environmental Laboratory

The meeting will start at 1:00 PM Central Time

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- “Listen Only” is activated, only the host can be heard
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Providing solutions for tomorrow's environmental challenges



Environmental DNA and Invasive Aquatic Species: 9 Years In

Richard Lance, PhD

Lead, Conservation & Ecological Genetics Team

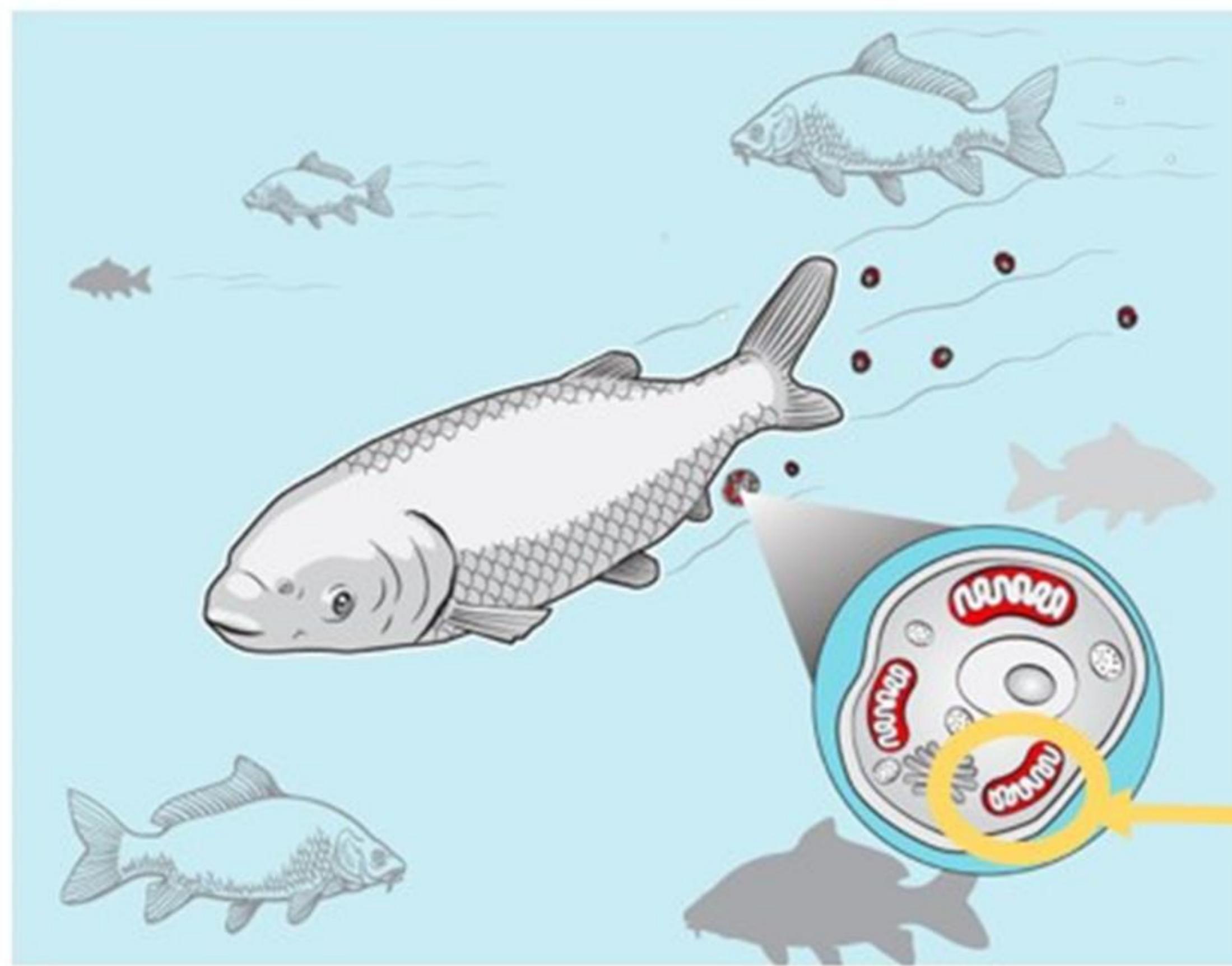
Senior PI, Center for eDNA Development & Research (CeDAR)

Research Biologist, Ecological Processes Branch

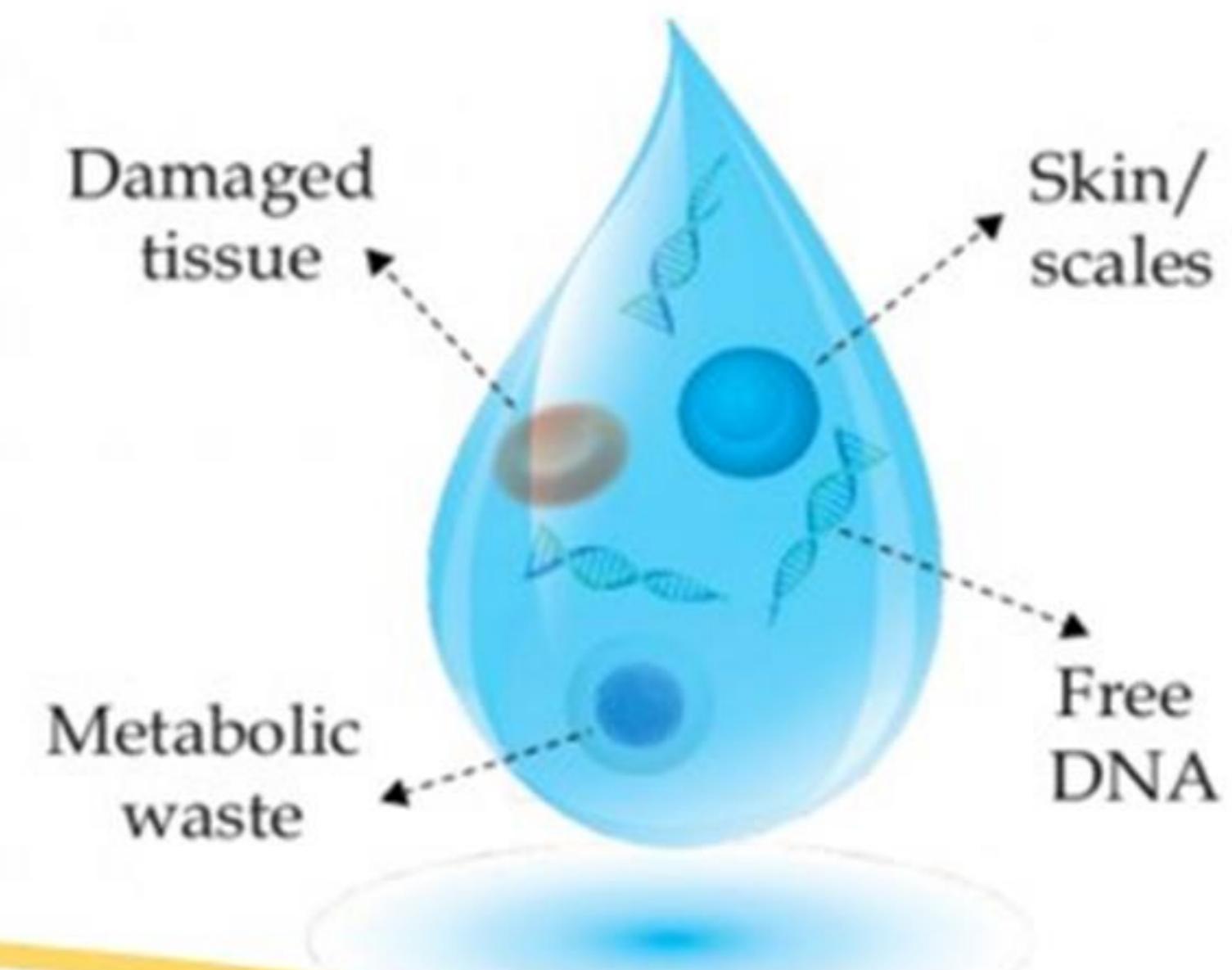
08 Aug 2018



What is eDNA?



www.smith-root.com

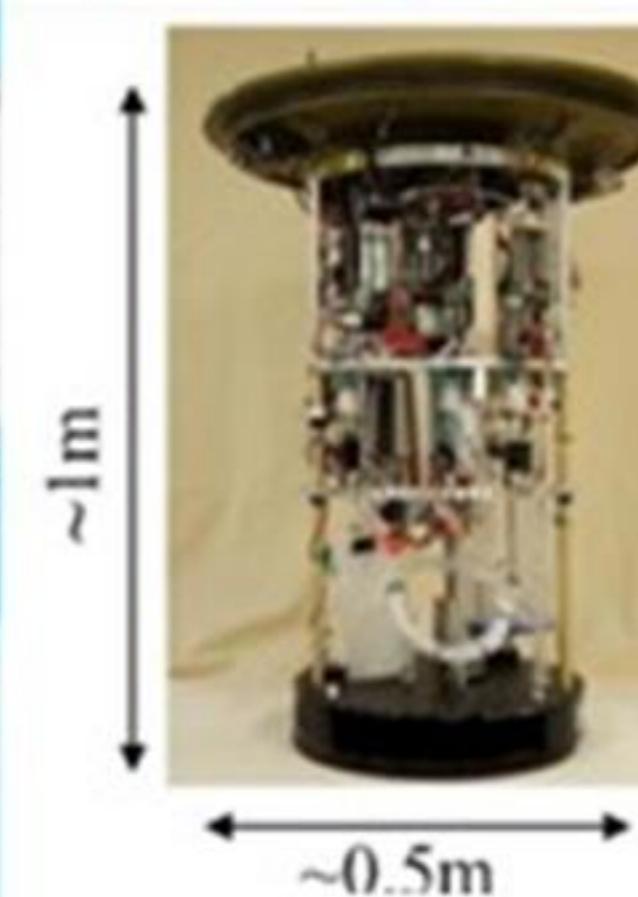
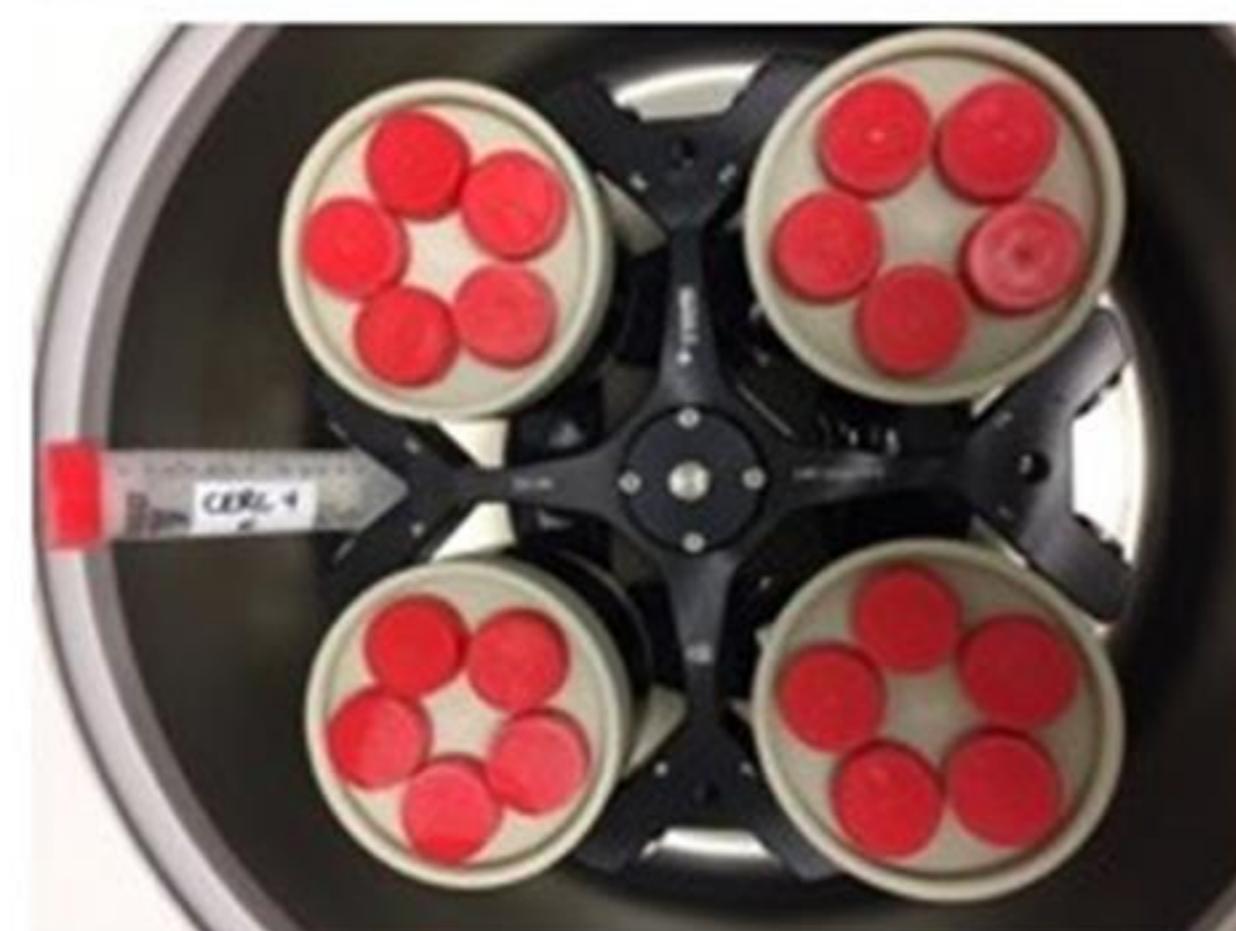
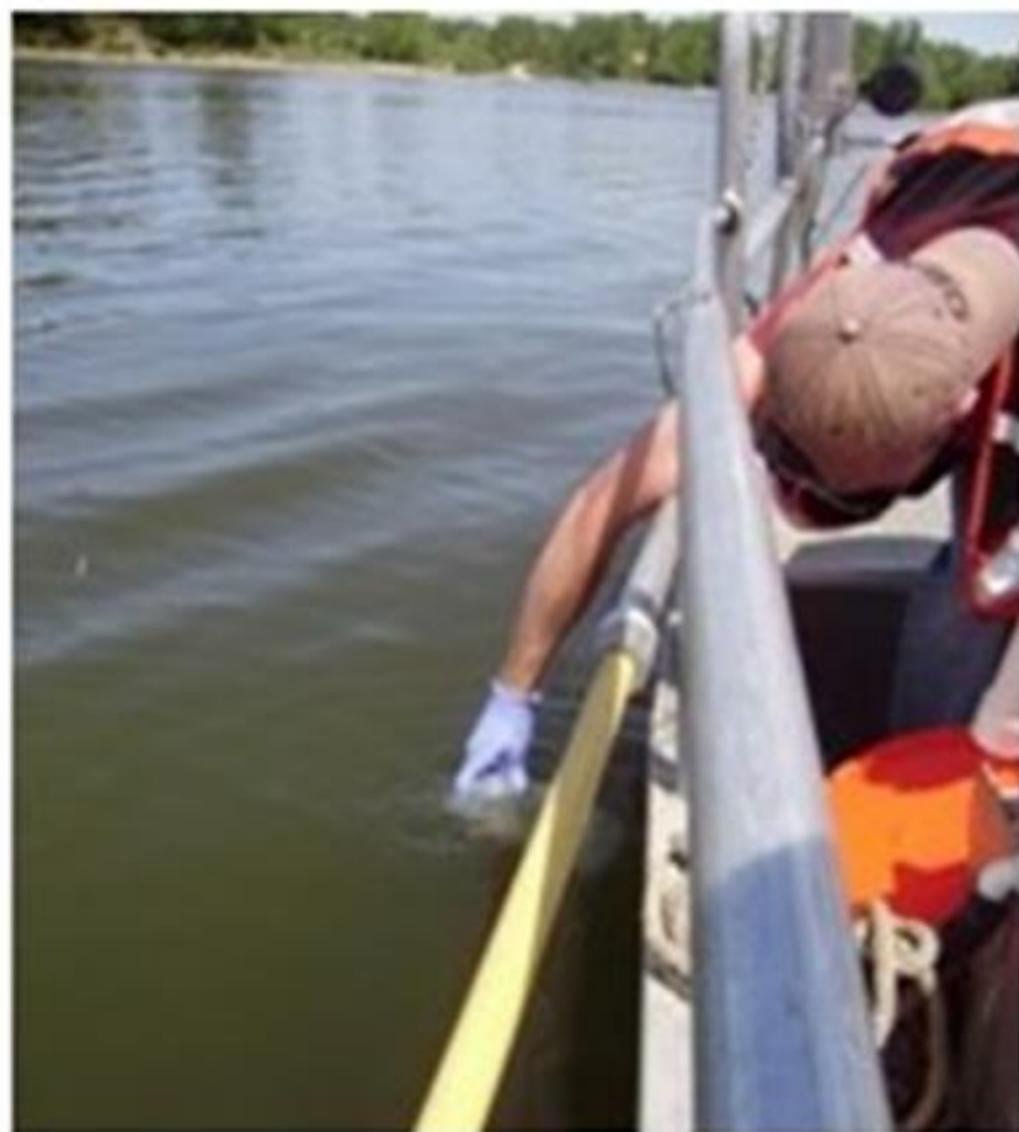


<http://seaharmony.org/>

- All organisms leave behind DNA in their environment
- Mitochondrial DNA (mtDNA) particularly useful

What is eDNA?

- This DNA, or environmental DNA, or eDNA, may be collected in water samples using a variety of techniques

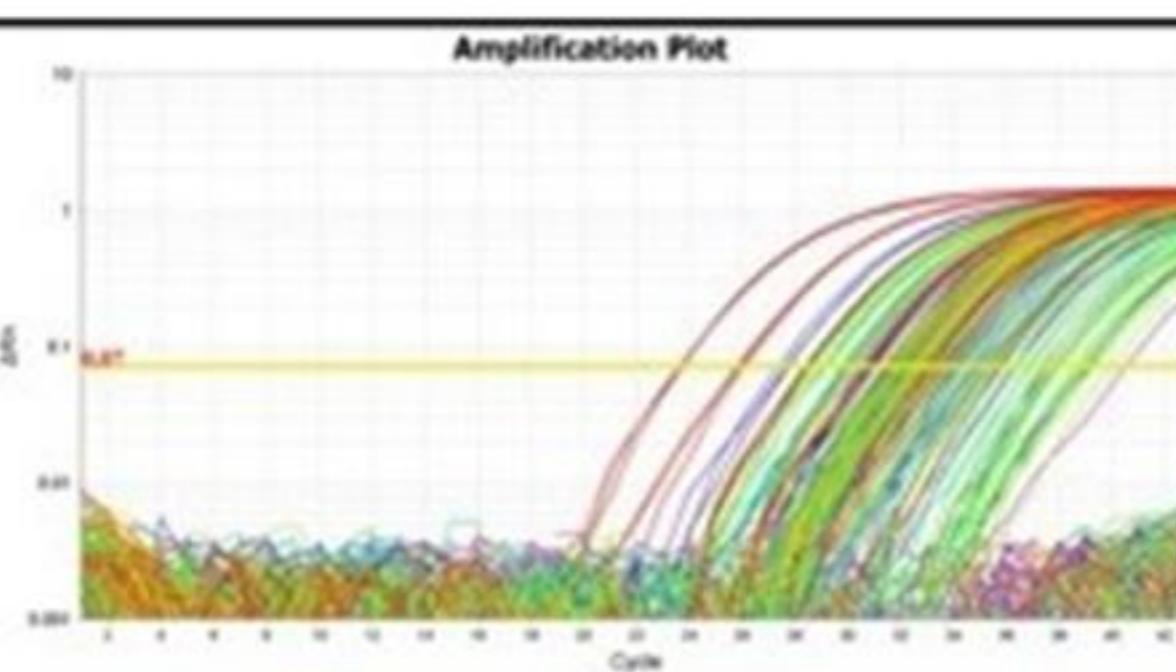
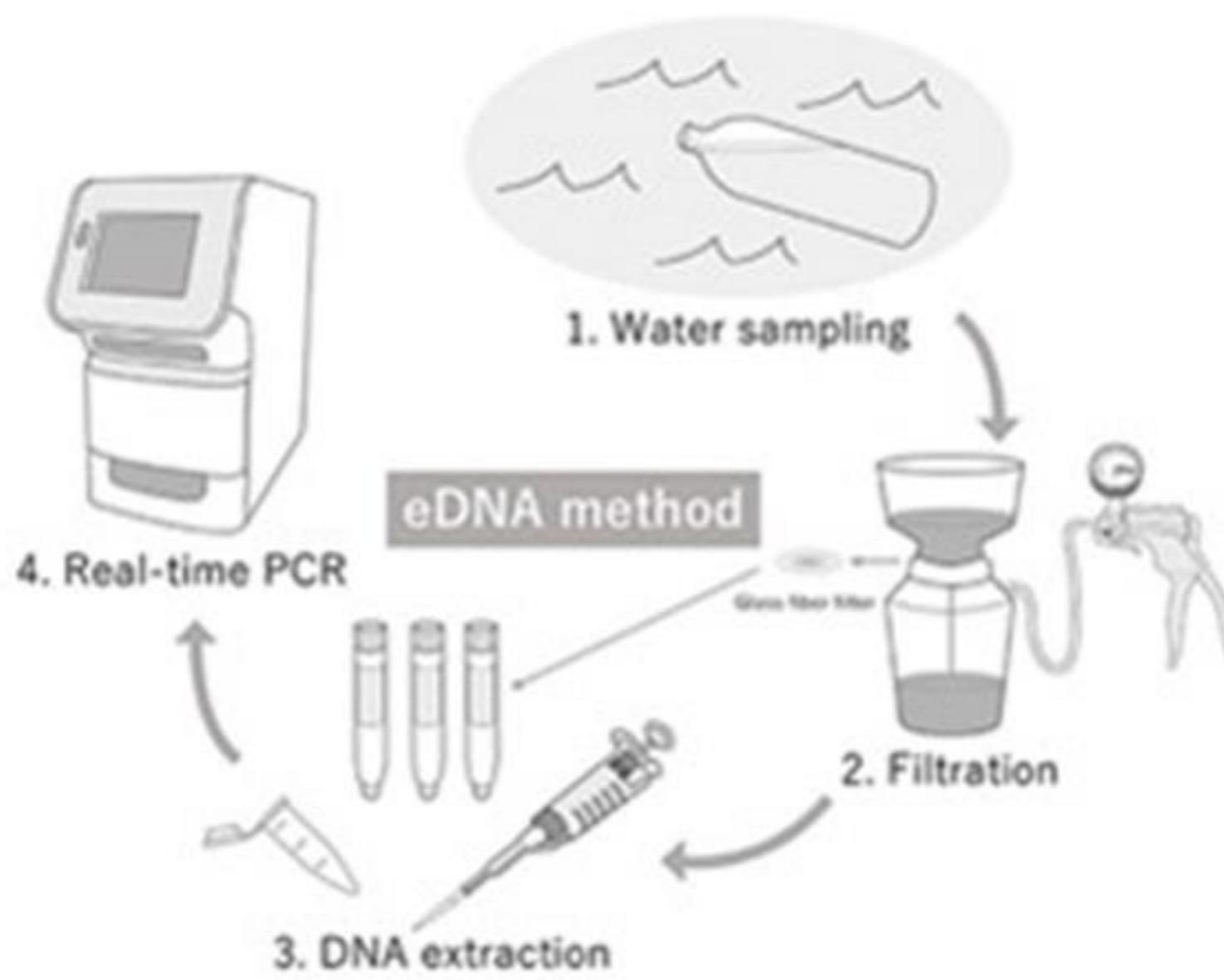


Environmental
Sample
Processor
(MBARI;
McLane
Research
Laboratories)

What is eDNA?

- eDNA can be detected using one or more of several approaches.

Hashizume et al. 2017
Acta Tropica 169:1-7



www.smith-root.com

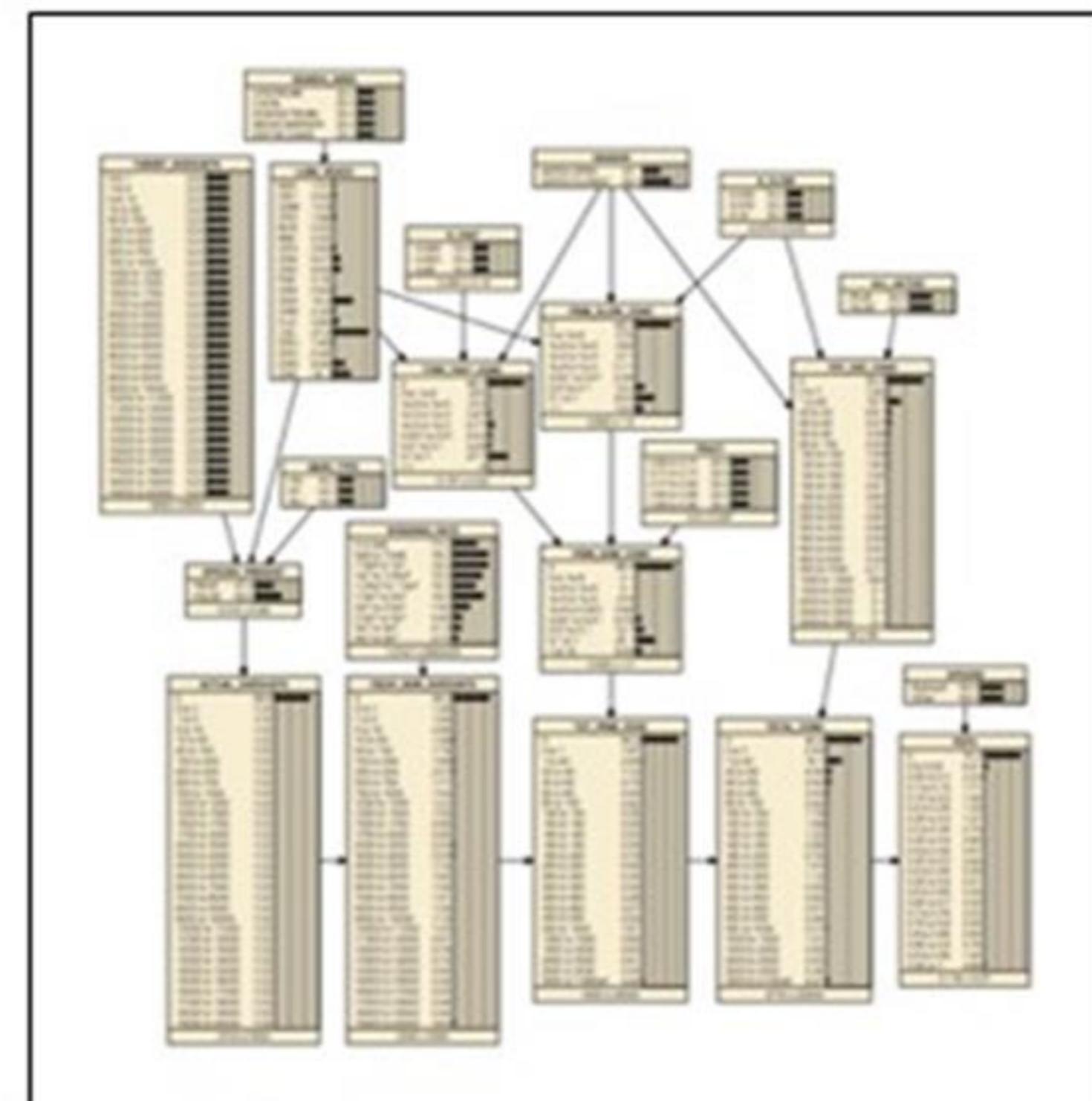


nanoporetech.com

Sequence data from 25 samples (M00483.89.00.00 to M00483.89.00.25) showing DNA base pairings (A, T, C, G) across 160 cycles.

What to do with eDNA?

- Data must be interpreted in light of:
 - Quality assurance and quality control (QA/QC)
 - Negative and positive controls
 - What you know about species ecology
 - Your risk tolerance profile
 - Relative costs of with false positives vs false negatives?



Trending eDNA

- Ficetola et al. 2009 – American Bullfrog in France
- 2009 Univ. Notre Dame, Nature Conservancy , US Army Corps of Engineers – Bigheaded carp in Chicago Area Waterway System
 - Lodge et al. 2011
- Goldberg et al. 2011 – endangered amphibians



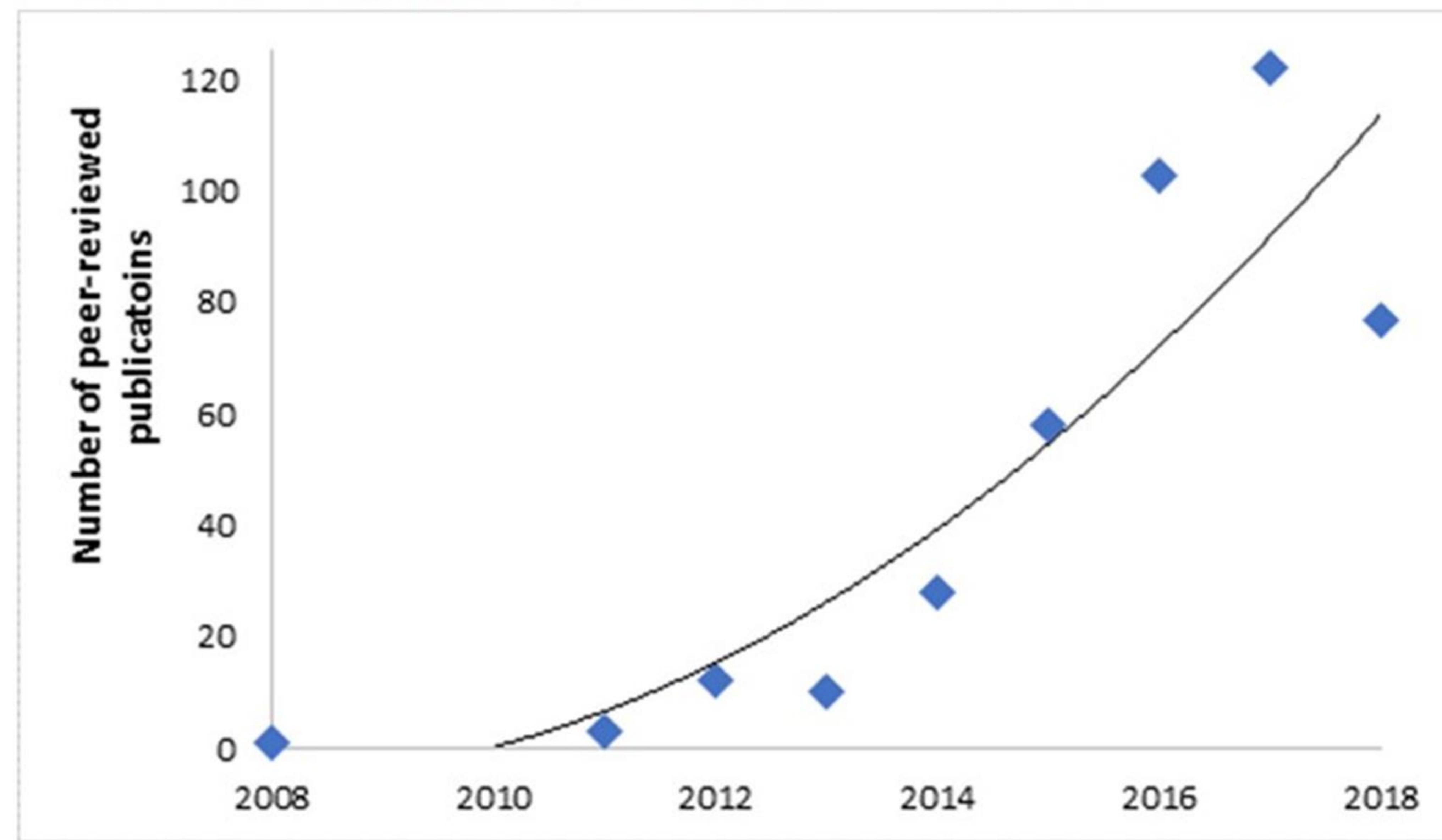
J. Cossel JR.



R. Killackey
ID Fish & Game

Trending eDNA

- 120+ eDNA papers published just in 2017.



C. Goldberg & K. Strickler, Washington State Univ. - <https://labs.wsu.edu/edna/>

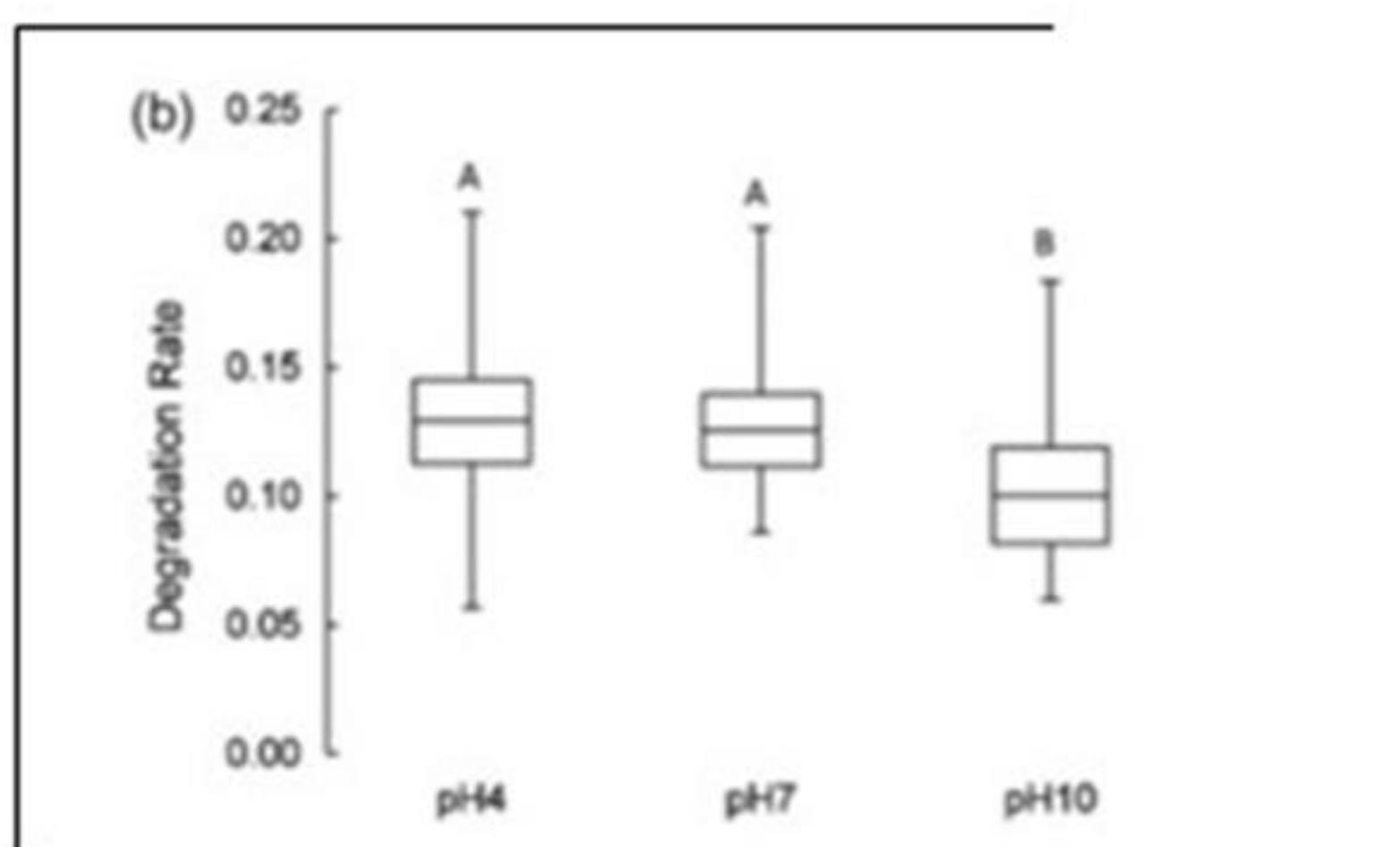
Trending eDNA - species

Acheilognathus typus	Chilodonella hexasticha	Hula painted frog	Ornate chorus frog	Smooth newt
Aedes mosquitos	Chinese giant salamander	Idaho giant salamander	Pacific chub mackerel	Sockeye salmon
African clawed frog	Chinook salmon	Japanese giant salamander	Pacific coast lamprey	Southeast liver fluke
American bullfrog	Chinook salmon	Japanese jack mackerel	Pacific Lamprey	Spotted gar
Anopheles mosquito	Chiricahua leopard frog	Japanese rice fish	Pacific sardine	Striped bass
Arctic char	Coldwater crayfish	Japanese sea nettle	Painted turtle	Striped newt
Arctic grayling	Common carp	Kidneyshell mussel	Patch-nosed salamander	Tadpole shrimp
Arctic grayling	Common carp	Killer whale	Rainbow trout/steelhead	Temperate seabass
Arizona treefrog	Common carp	Lacustrine shrimp	Rainbow trout/steelhead	Threadfin shad
Asian clam	Common gartersnake	Lake trout	Redfin perch	Three-spined stickleback
Atlantic herring	Common snapping turtle	Large white-faced darter	Rocky mountain tailed frog	Tidewater goby
Atlantic salmon	Common spadefoot toad	Largemouth bass	Rosette agent (parasite)	Tiger salamander
Ayu sweetfish	Dolly Varden trout	Longfin smelt	Round goby	Walleye
Bighead carp	Eastern pondmussel	Manatees	Rusty crayfish	water flea
Bighead carp	Eurasian otter	Mapleleaf mussel	Sacramento pikeminnow	Water thyme
Blanding's turtle	European eel	mayfly	Sacramento splittail	Wavy-rayed lampmussel
Bluegill sunfish	European flounder	Mekong giant catfish	Sakhalin taimen	Western pearlshell mussel
Boa constrictor	European weather loach	Narrow-clawed crayfish	Salmon fluke	Westslope cutthroat
Brazilian waterweed	Flathead grey mullet	Narrow-clawed crayfish	Sauger	White colonial sea squirt
Brook trout	Flatwoods salamander	Nepa hoffmanni	Sea lamprey	White sturgeon
Brown trout	Gizzard shad	New Zealand mudsnail	Sea squirt	Wood frog
Brown trout	Golden tree frog	Noble crayfish	Shorthorn sculpin	Wood turtle
Bryozoan	Goldsinny-wrasse	North African python	Signal crayfish	Yellow anaconda
Bull trout	Gopher frog	Northern anchovy	Signal crayfish in part	Yellowstone cutthroat
Burbot	Great cormorant	Northern medaka	Silver and bighead carp	
Burmese python	Great crested newt	Northern pike	Silver carp	
California red-legged frog	Green anaconda	Northern red-legged frog	Silver carp	
Carpet sea squirt	Green sturgeon	Northern watersnake	Silver carp	
Carpet sea squirt	Harbor porpoise	Opossum shrimp	Slimy sculpin	
Chilean devil ray	Hellbender	Oriental weatherloach	Smallmouth bass	

Advancing eDNA

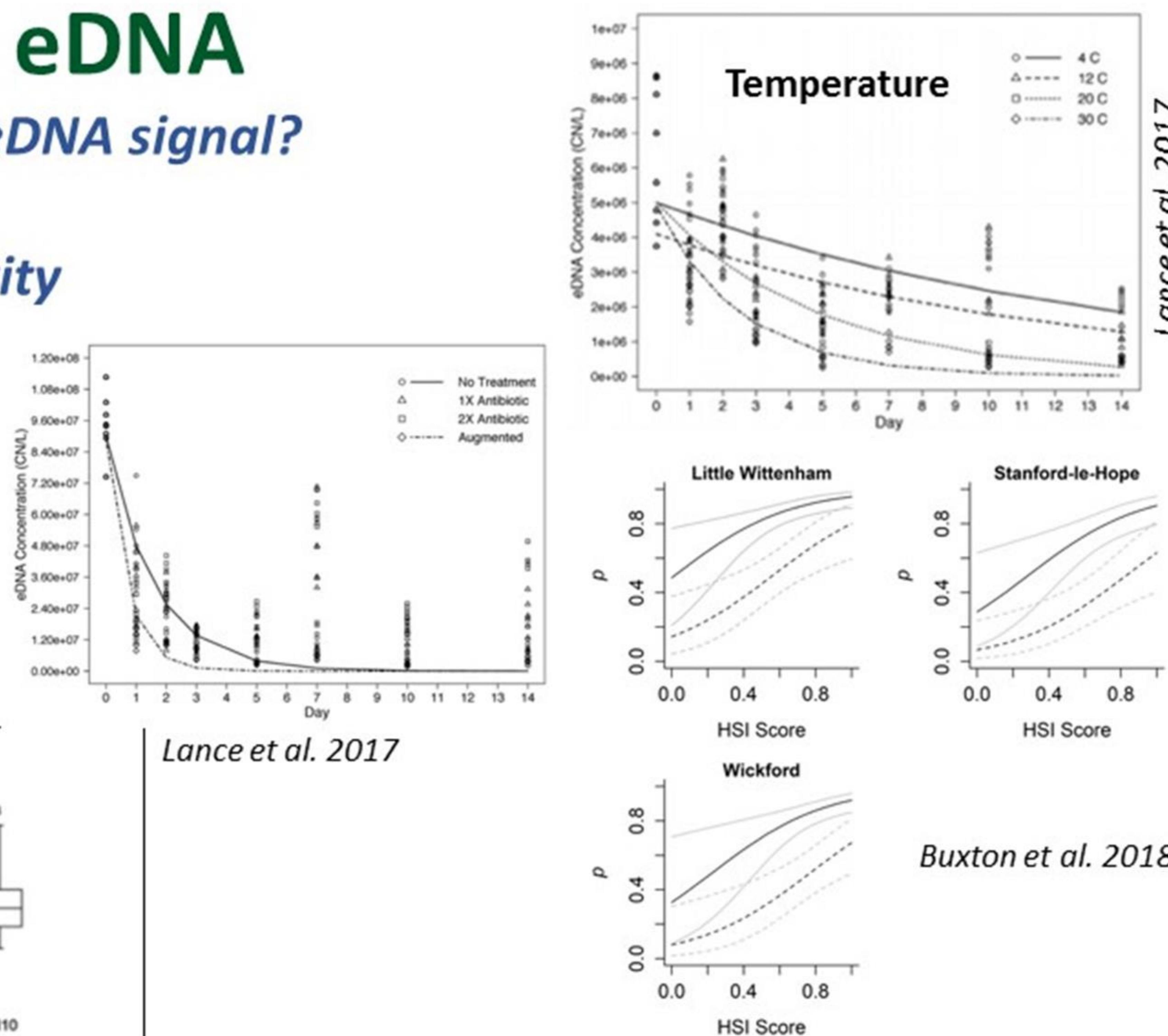
What influences eDNA signal?

- *Temperature*
- *Microbial activity*
- *pH*
- *Sunlight/UV?*
- *Location*



Strickler et al. 2015

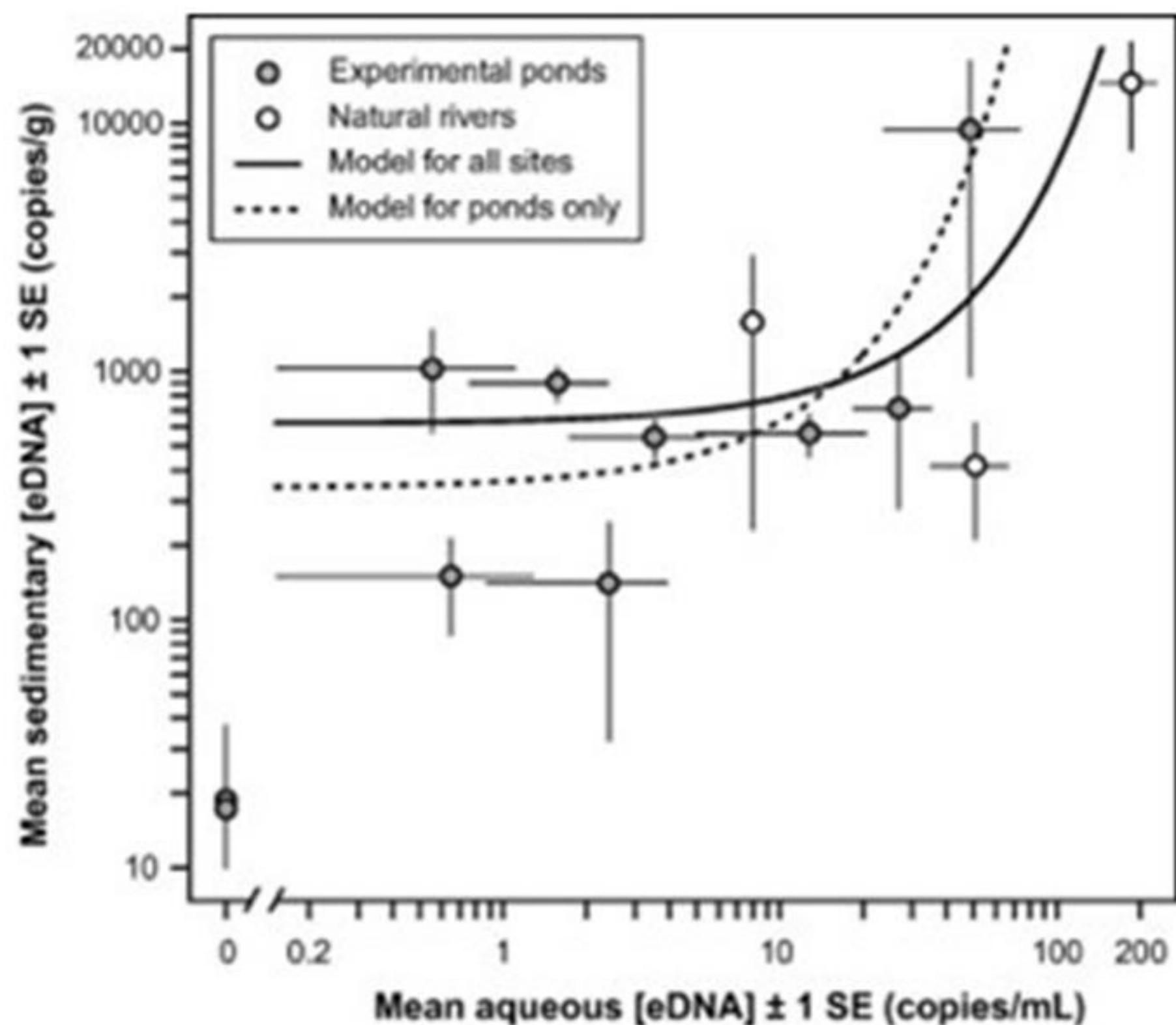
Lance et al. 2017



Advancing eDNA

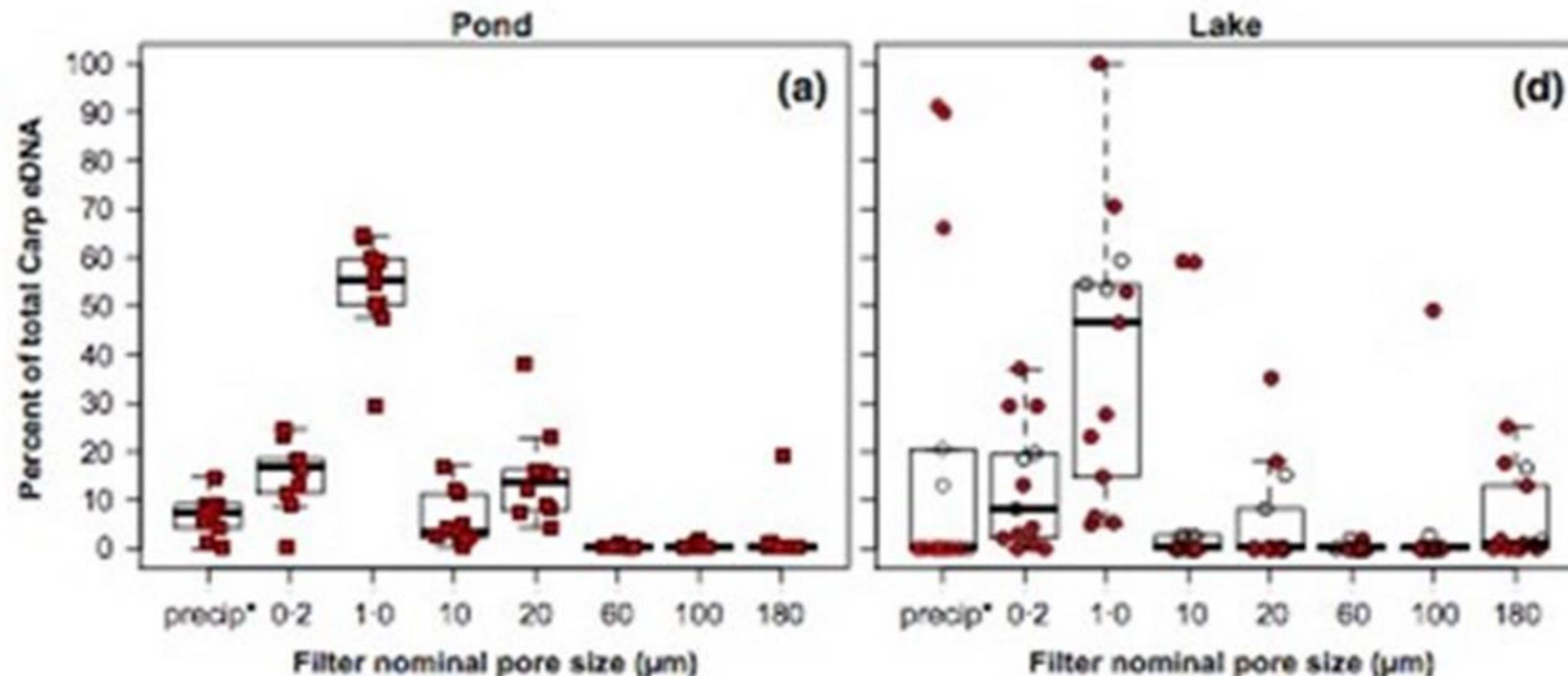
What influences eDNA signal?

- *Substrate*
- *Particulate Fraction*

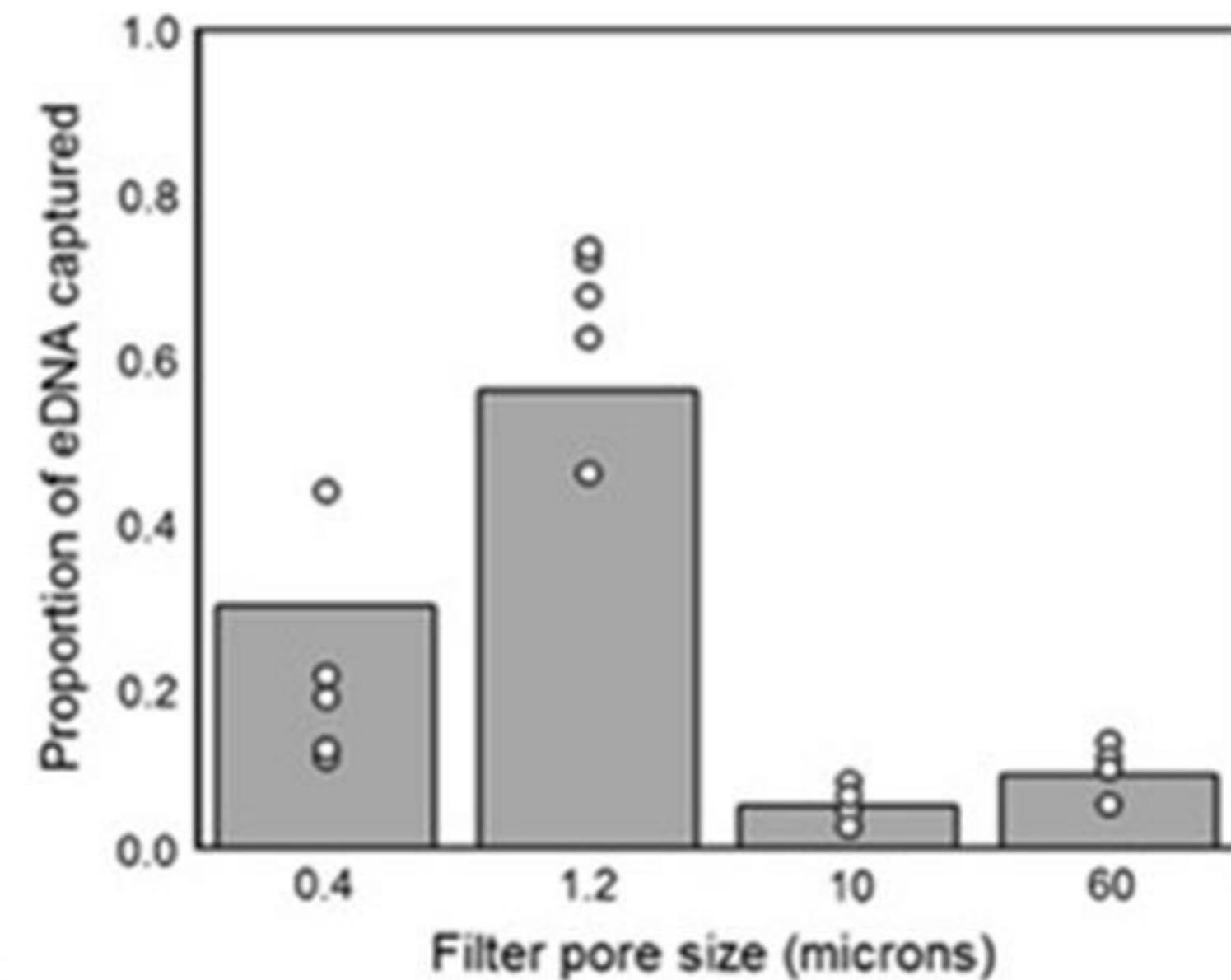


Turner et al. 2015

- *eDNA 8-1800X more concentrated in sediment*
- *eDNA lasted 3 months in sediment*



Turner et al. 2014



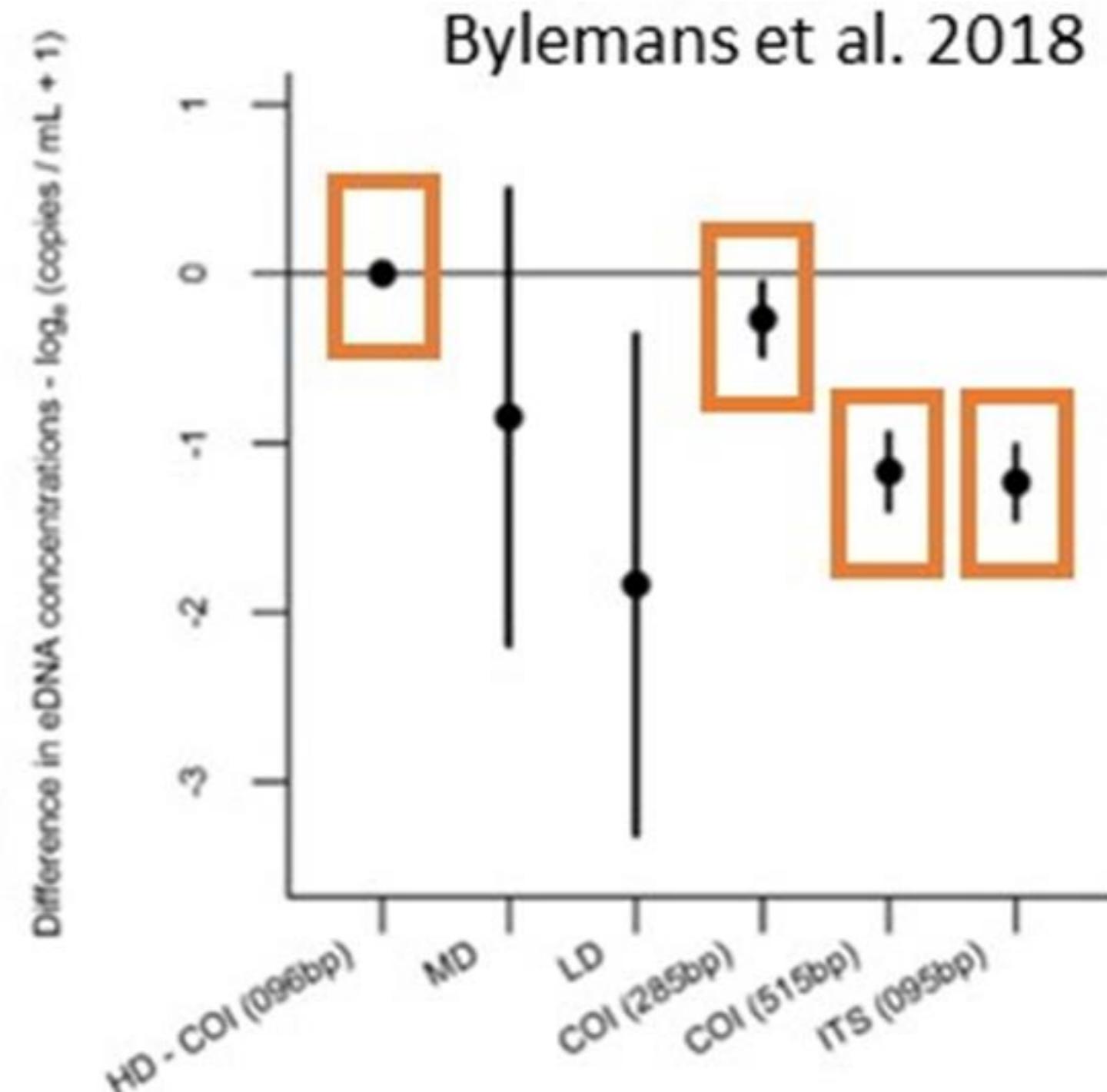
Wilcox et al. 2015

Advancing eDNA

Is it the target alive and here now?

Fragment Length and Origin

- Longer fragments are released at lower rates
- Nuclear DNA (nDNA) fragments also released at lower rates
- Longer fragments may degrade more quickly
- Longer fragments and nDNA should disappear more rapidly
- So, relative abundances of long fragments (> 500 bp?) and nDNA fragments may provide insights into target presence

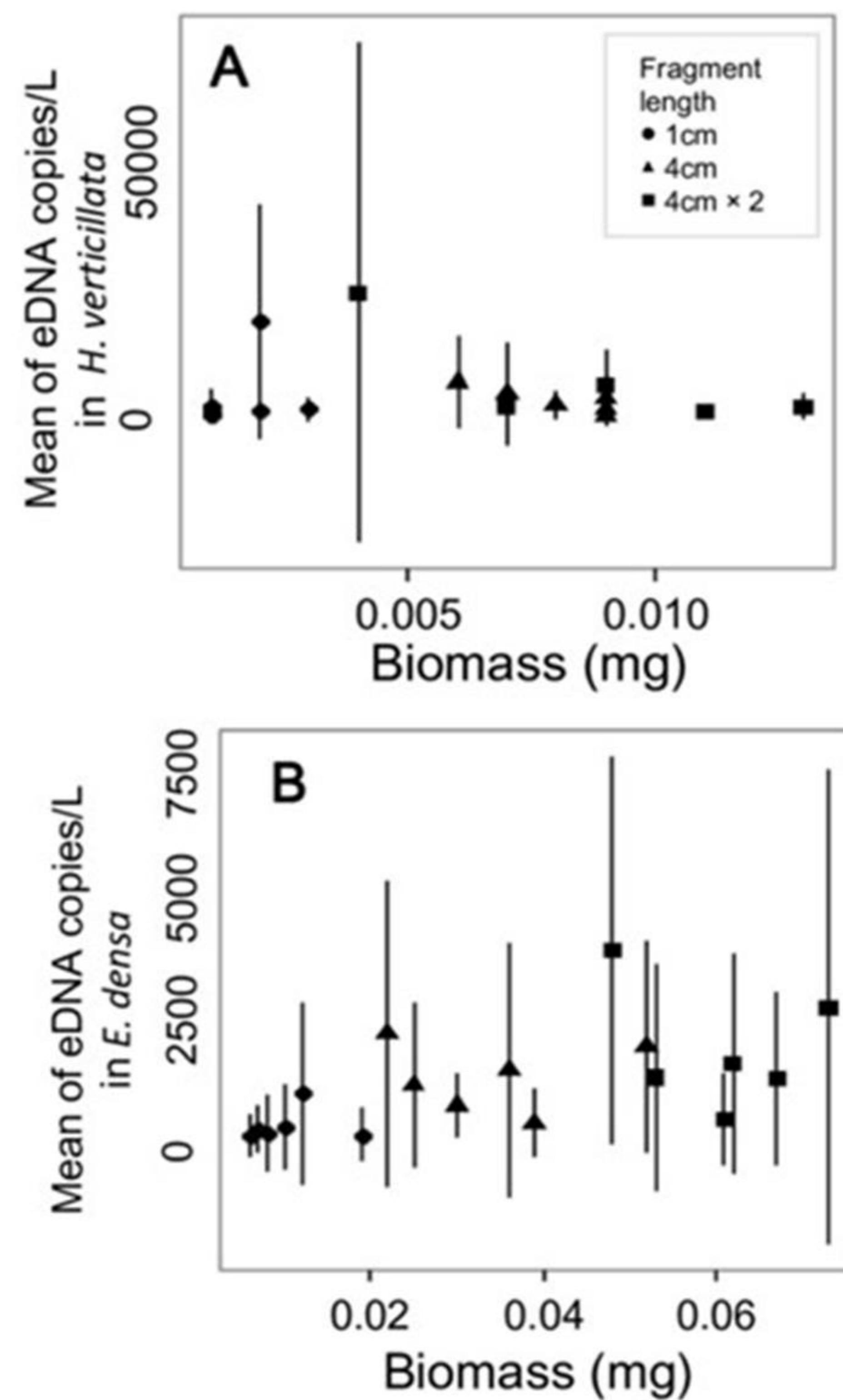
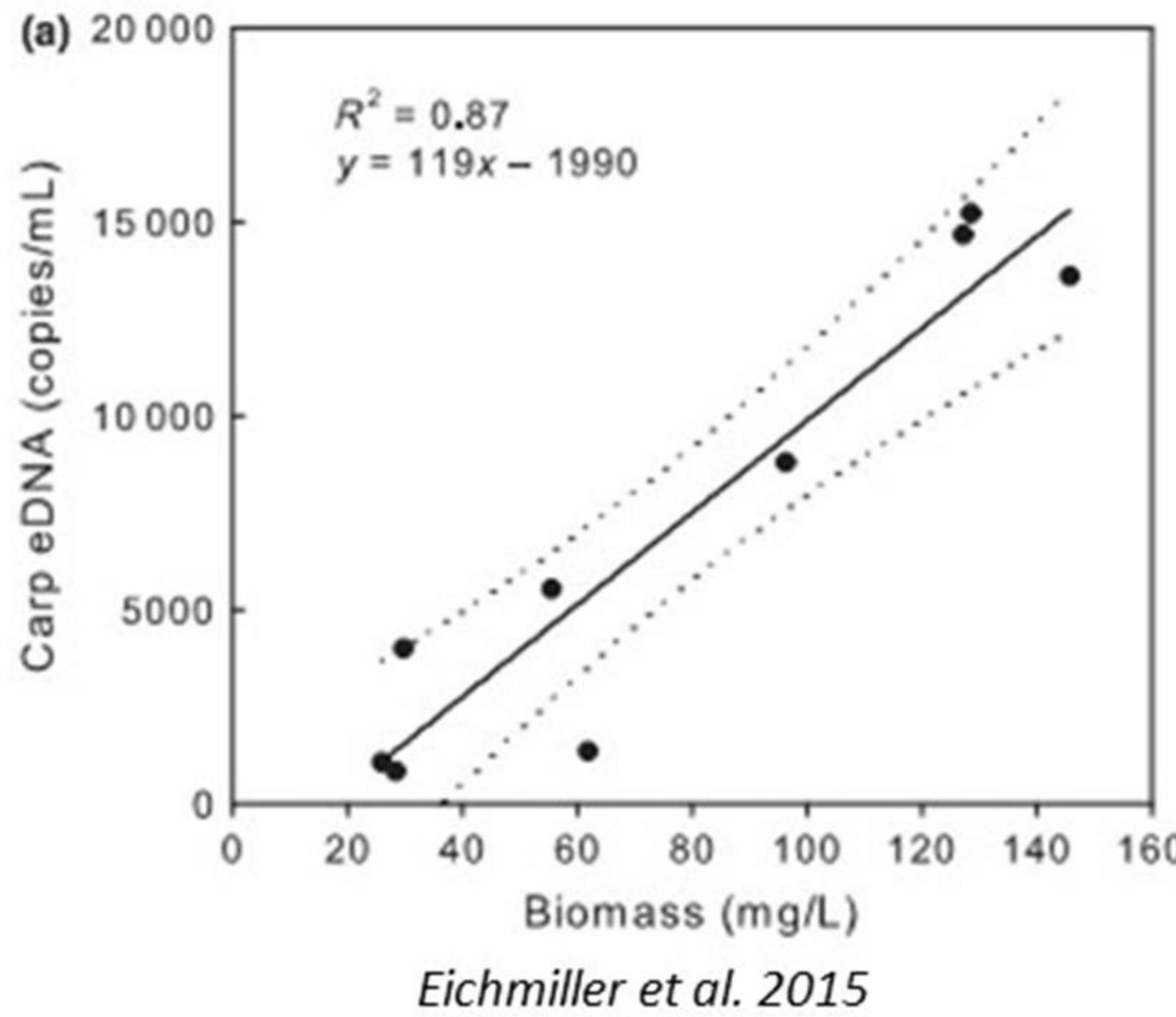


Environmental RNA (eRNA)

- RNA, stereotypically, has much shorter lifespan than DNA
- Can eRNA better indicate recent presence of living target organism?

Advancing eDNA

How many or how much?

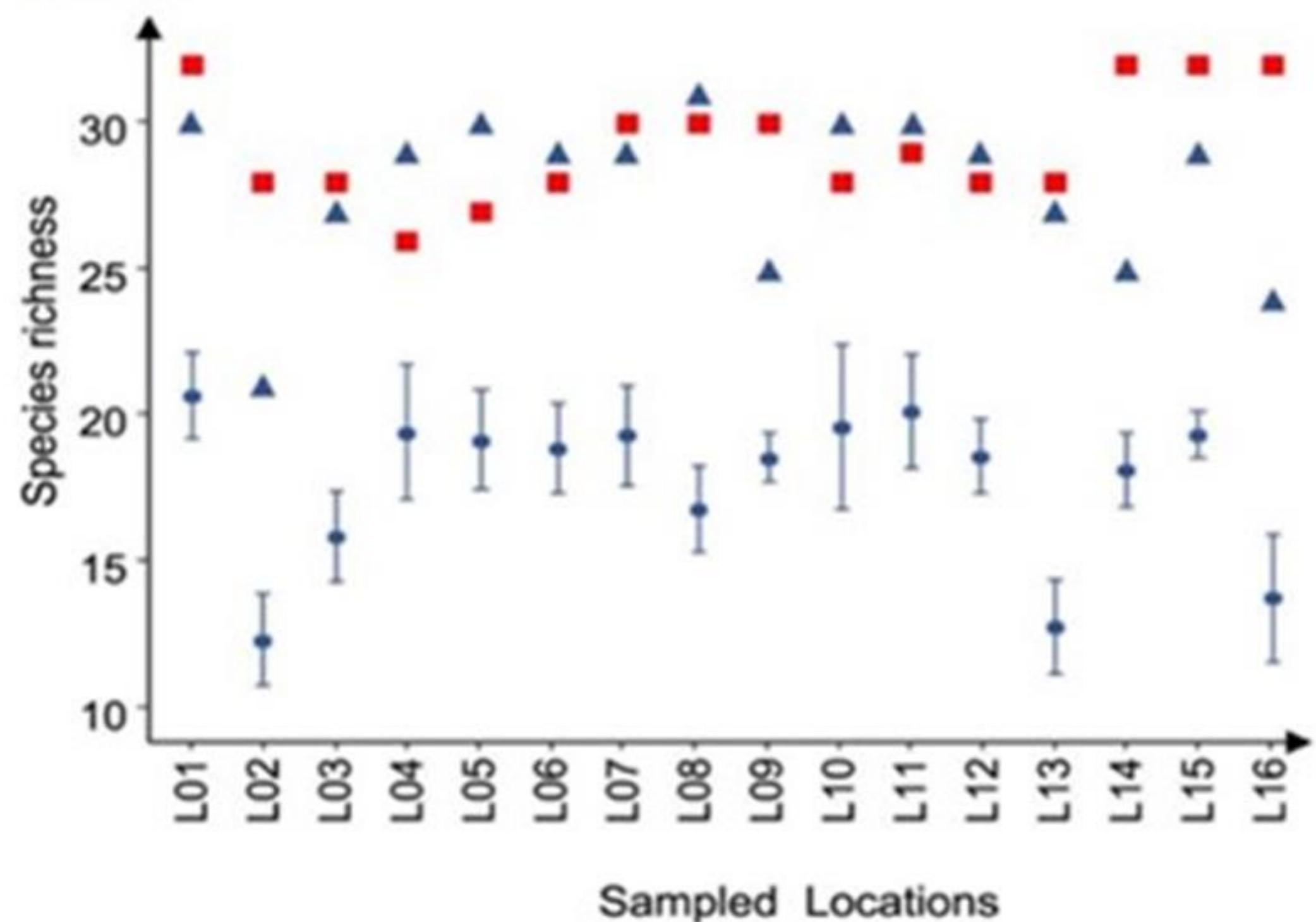


Matsuhashi et al. 2016

Advancing eDNA

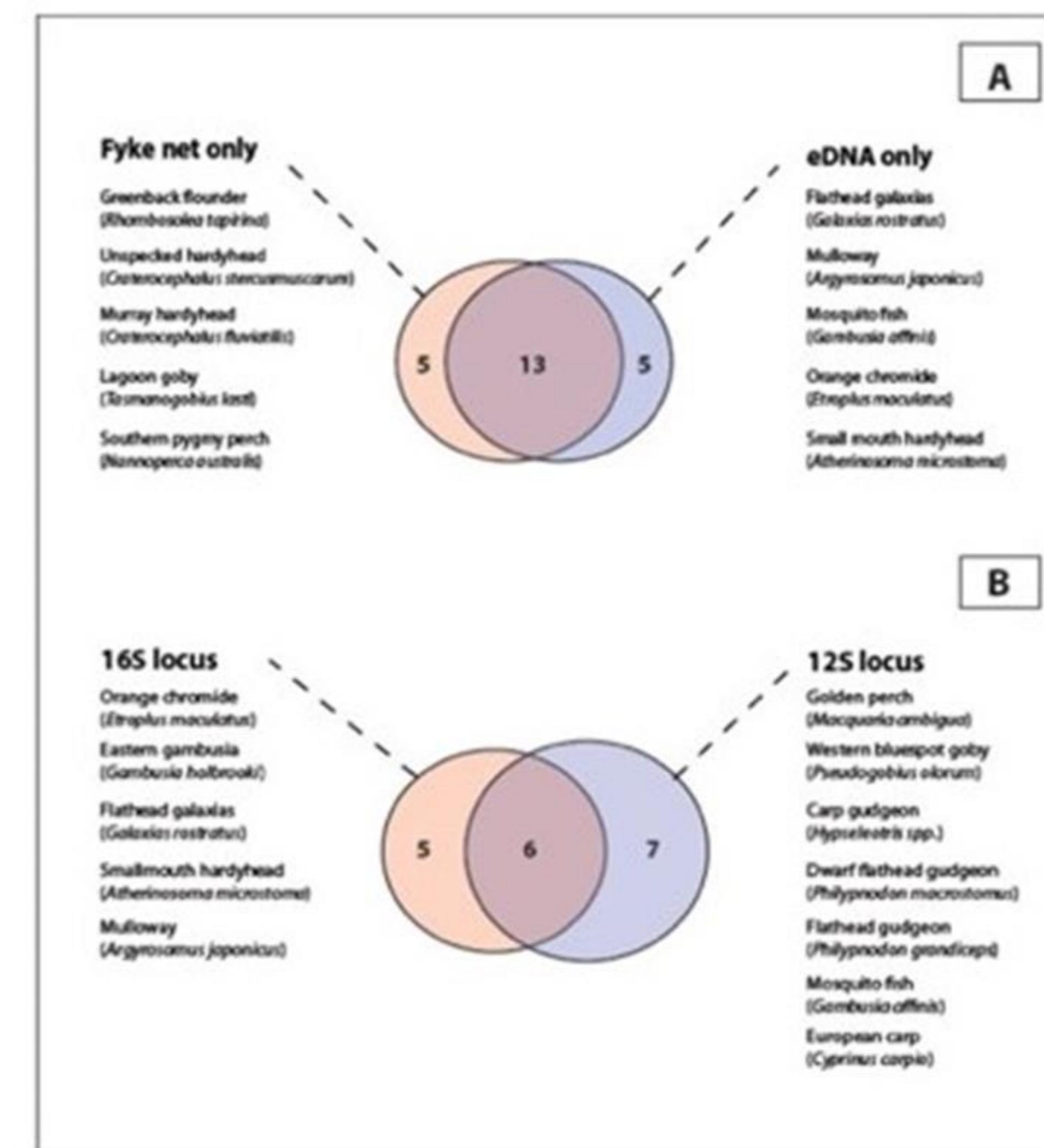
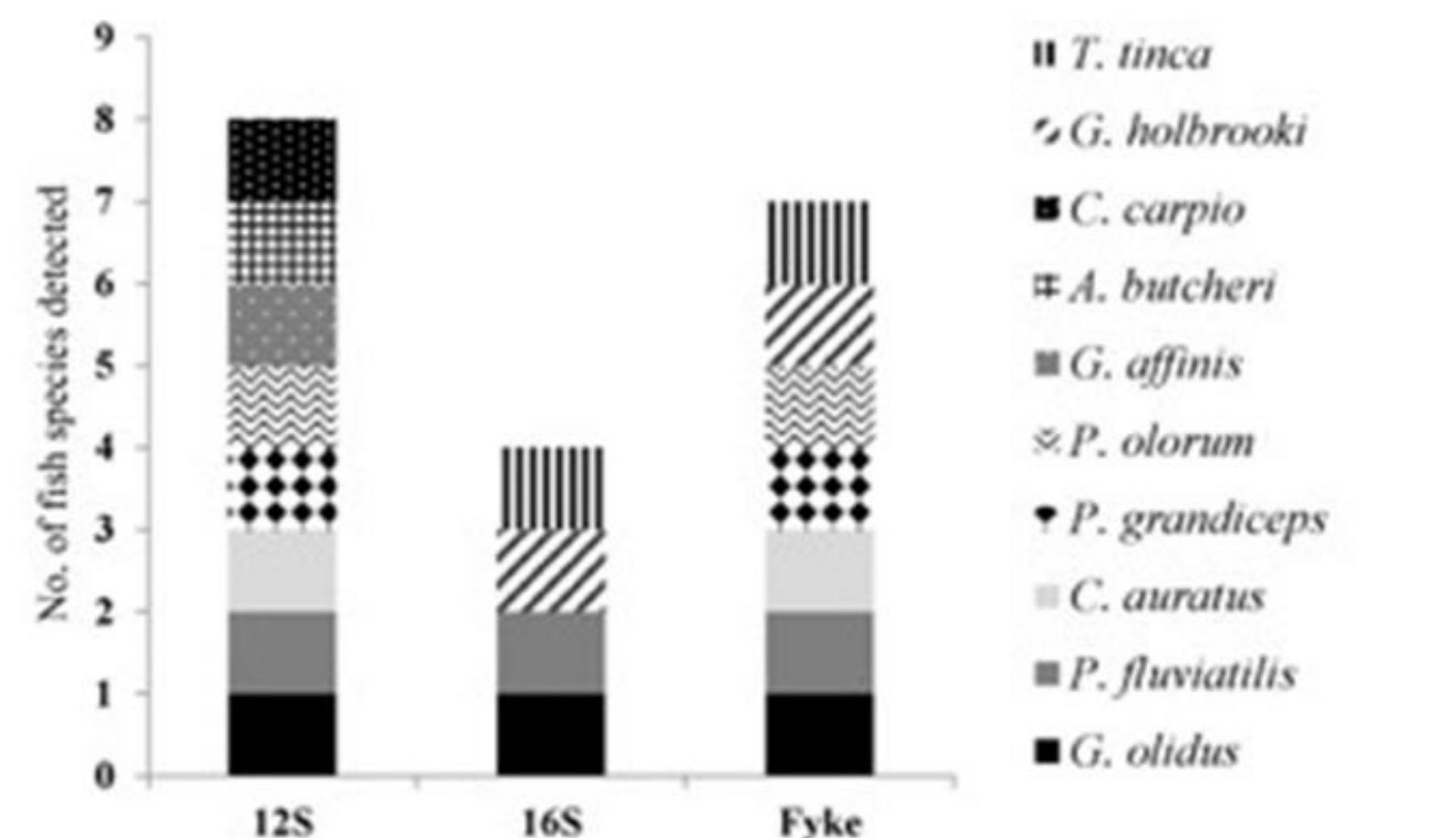
What's there?

Figure 3



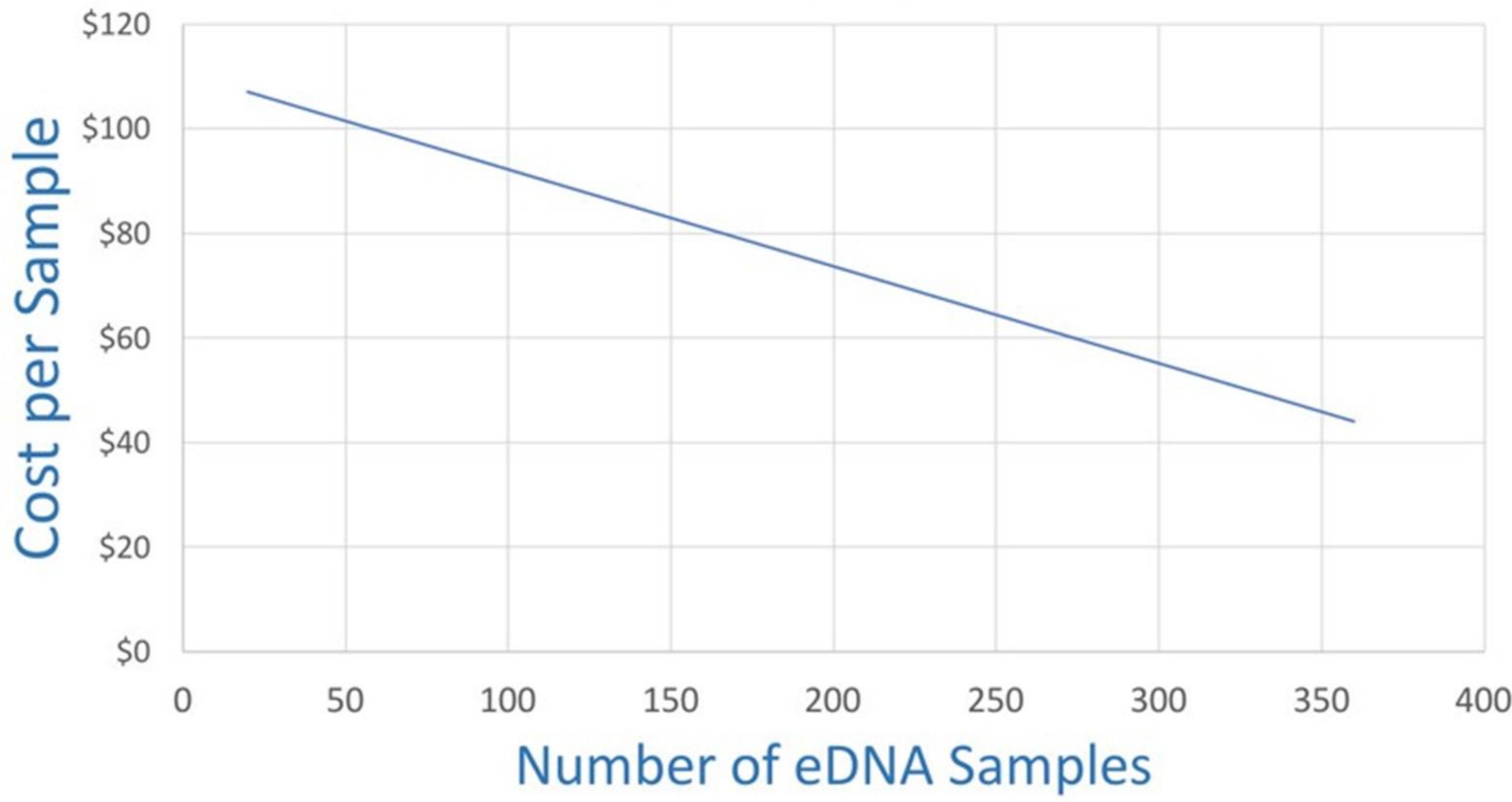
Comparison of the mean number of species ($\pm 95\%$ confidence interval) caught annually with TEF (blue circle), the total number of species (blue triangle) caught during the 10-year survey (2006–2016), and the number of species detected in eDNA samples (red squares) for each of the 16 locations (L01 to L16) sampled with both methods (see Supplementary Table 1).

Pont et al. 2018

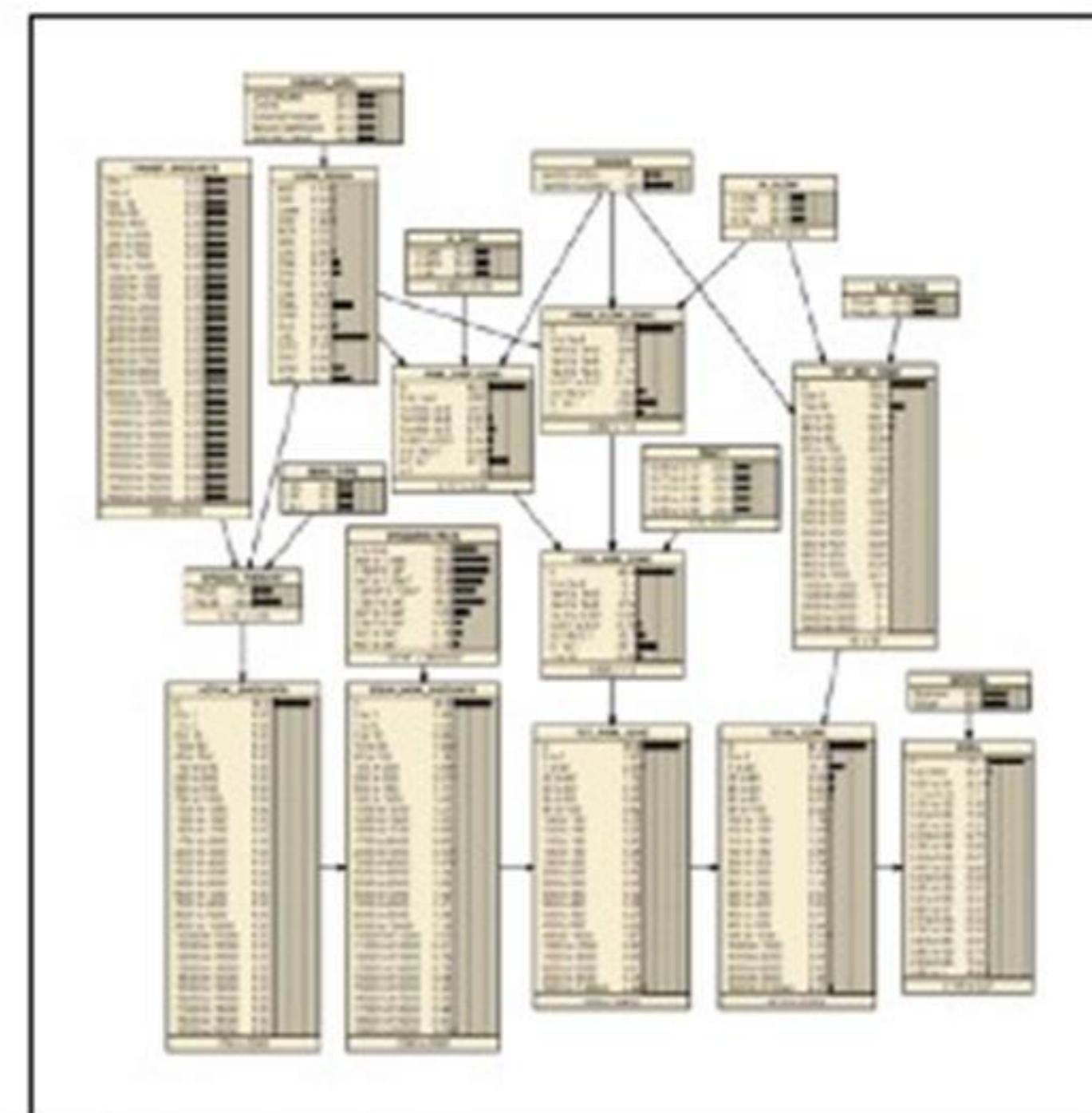
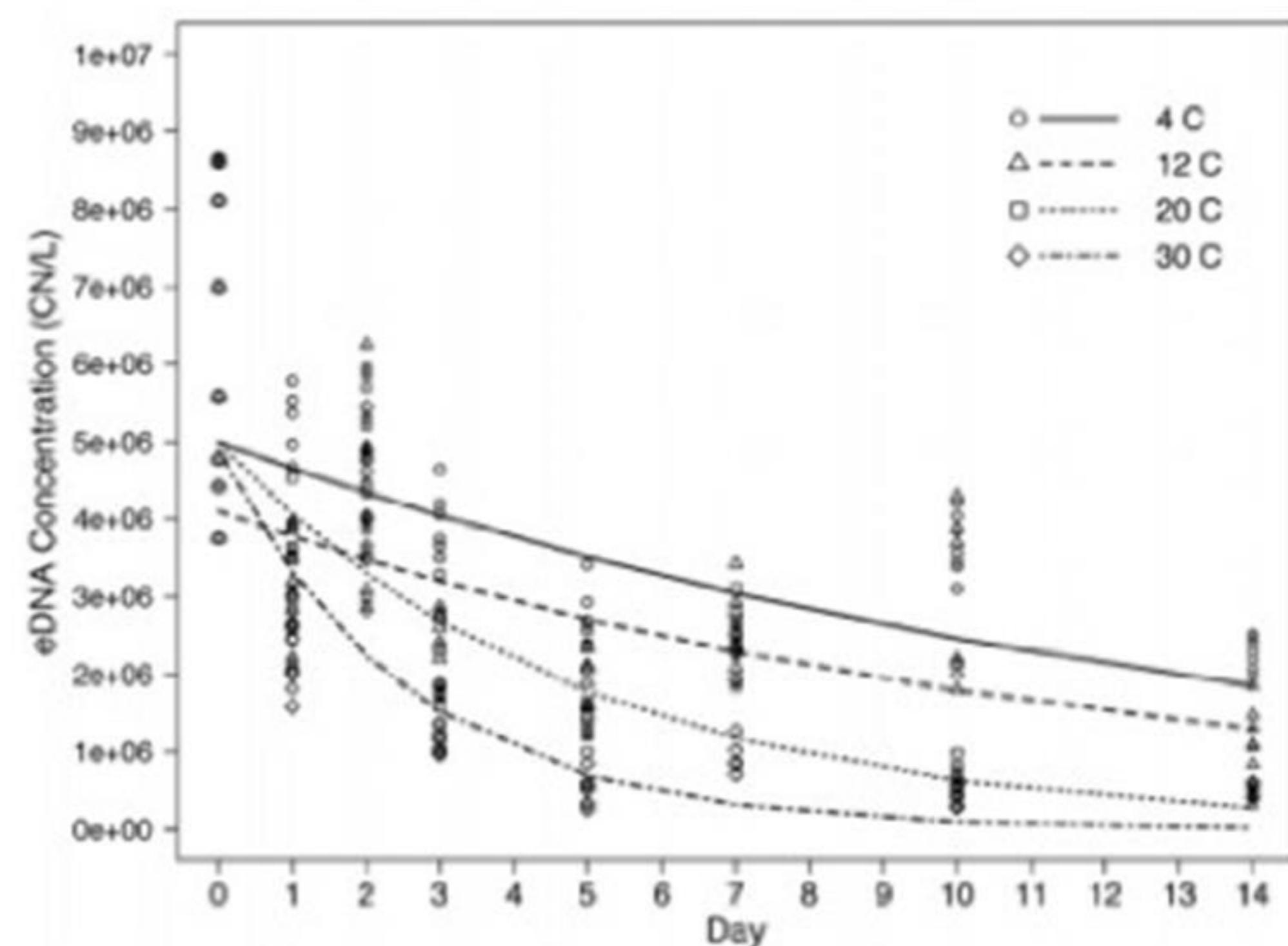
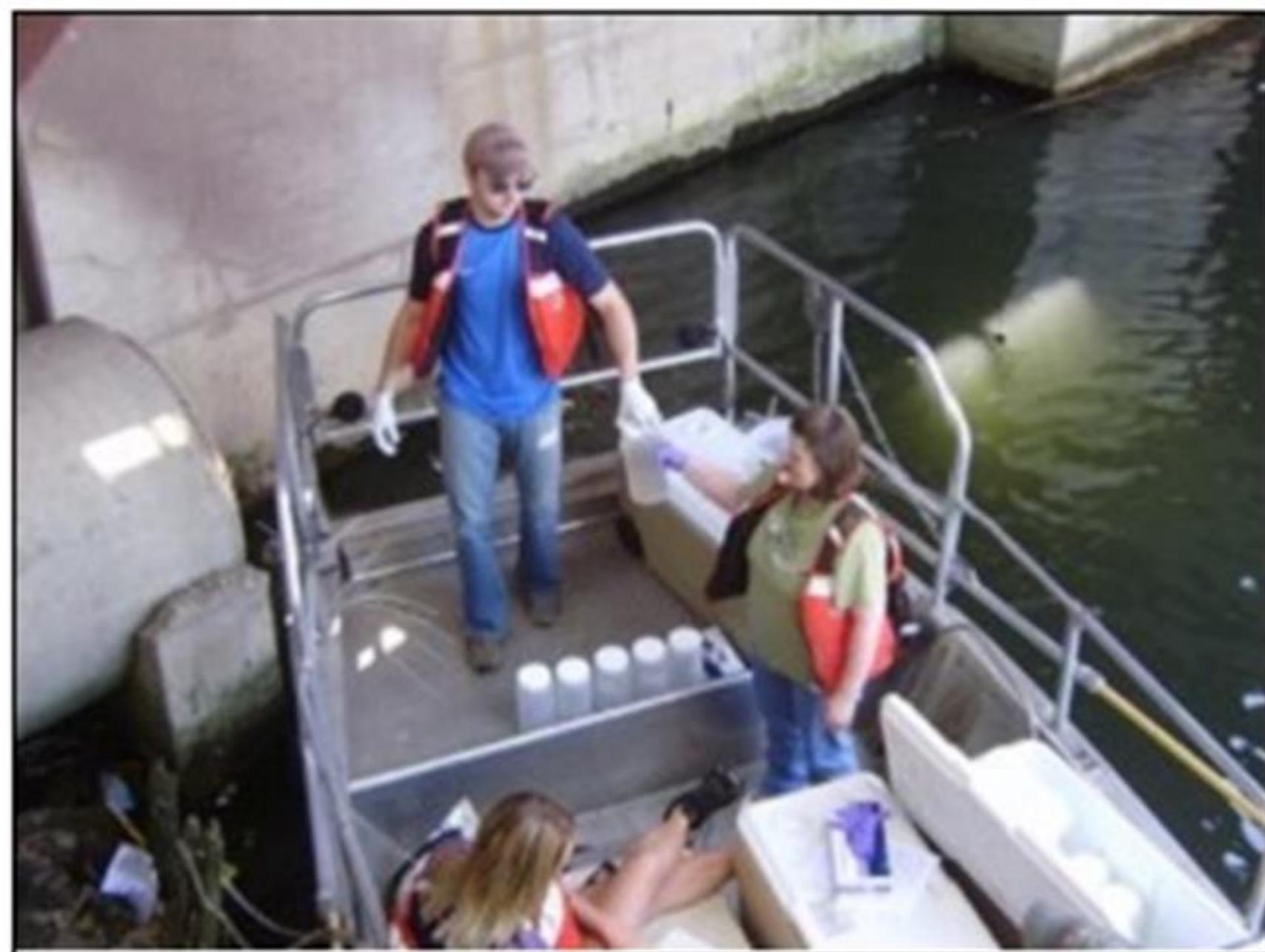


Cost of eDNA

Basic Estimate of Cost per Sample for eDNA Assays

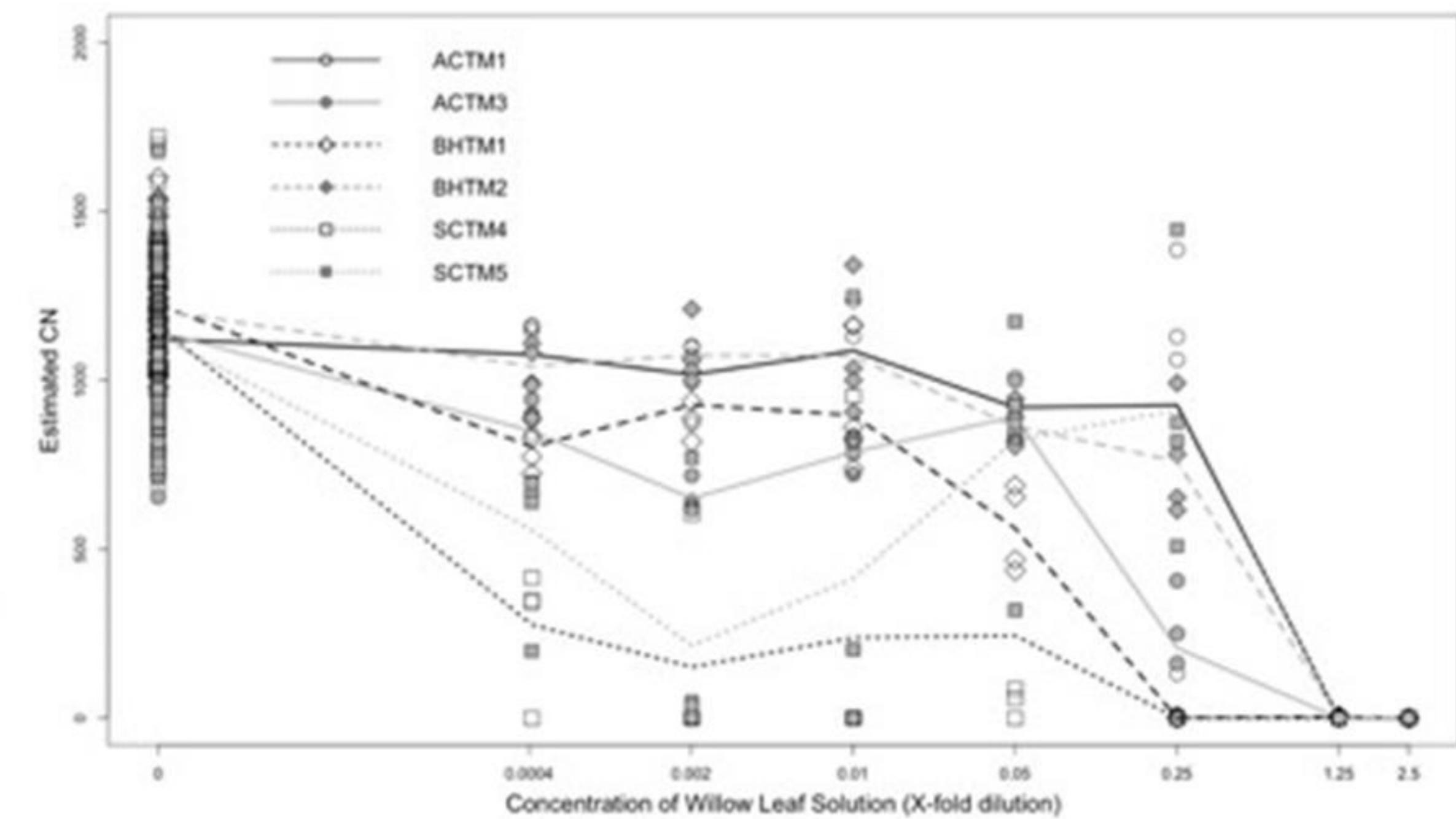
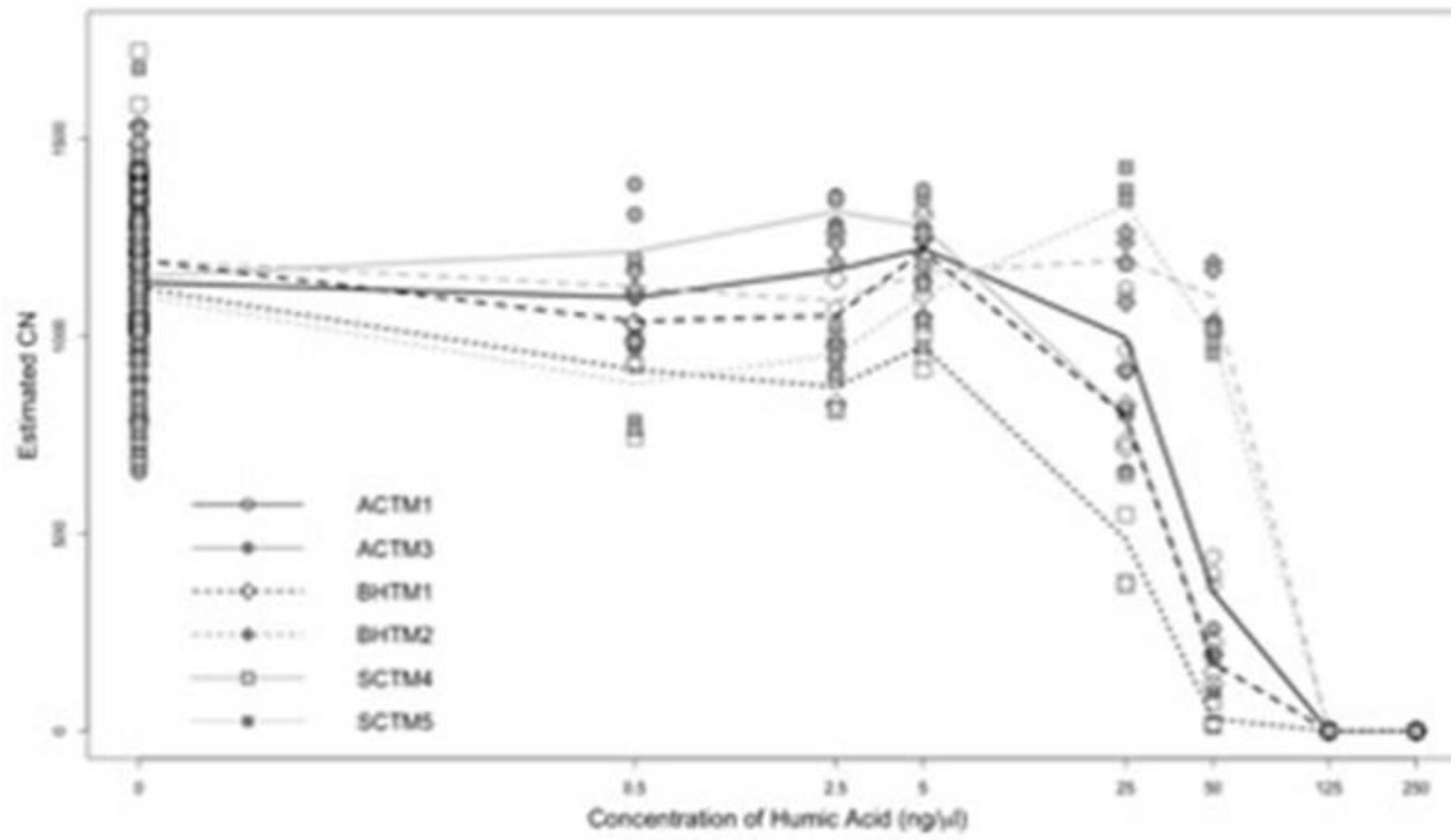
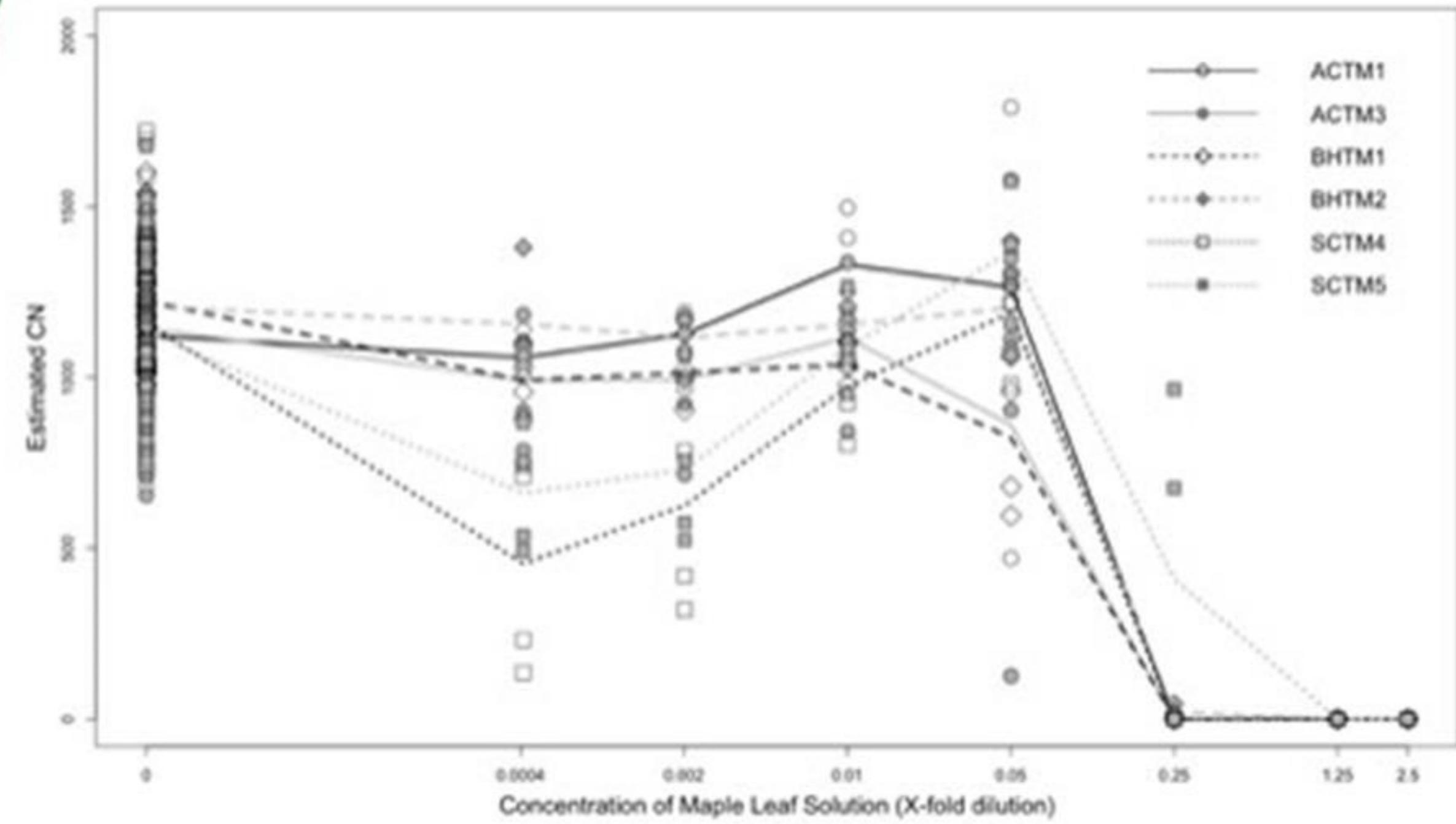


ERDC eDNA Studies



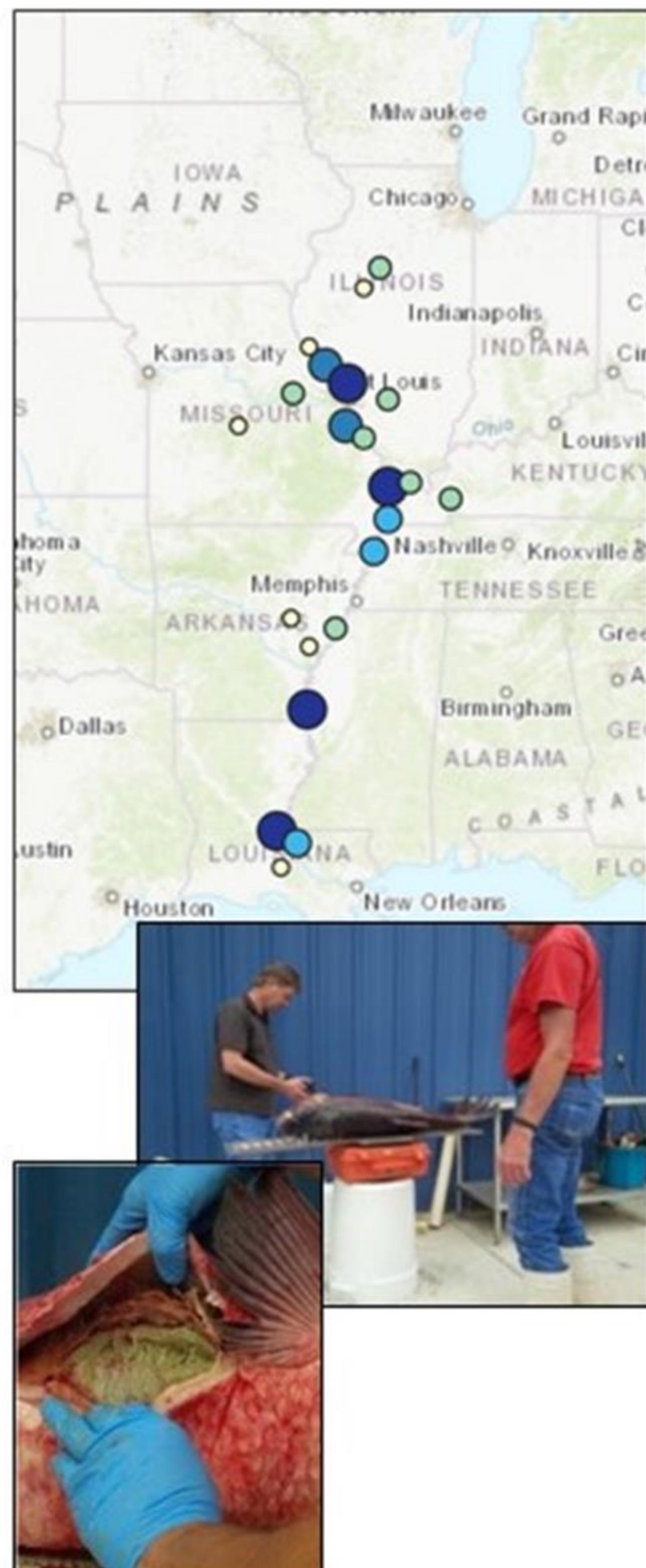
ERDC eDNA Studies

- eDNA Inhibitors Study



ERDC Black Carp – eDNA Markers

- Funded by USFWS (GLRI funds through Asian Carp)
Invasive molluscivore that threatens native
mussels and pea clams
 - Numerous rare, protected species in Great
Lakes and associated watersheds
 - 20 (11) IL, 39 (4) MI, 24 (4) WI
- Sequenced 29 mitogenomes from
N America, China, and Vietnam
- Developed four eDNA markers and
transitioned to USFWS
 - Using 3 markers in multiplex
assay



ERDC Black Carp – eDNA sampling

- Testing water particulate fractions
 - > 30, 3-29, 1-2, < 1 uM
- Tested in experimental tanks, aquaculture ponds, big river



ERDC Pike & Waterweed eDNA

- Joint Base Elmendorf Richardson, AK
- Invasive pike problematic for salmon stocks and beluga foraging
 - Pre- and post-rotenone treatment to help verify pike eradication
- Invasive *Elodea* waterweed spreading through AK
 - Sequenced chloroplast DNA and developed 4 eDNA markers
 - 2 to detect all *Elodea*, 1 to detect *E. canadensis*, 1 to detect *E. nuttallii*.



G Mittenecker



P Dynowski

Upcoming eDNA Efforts

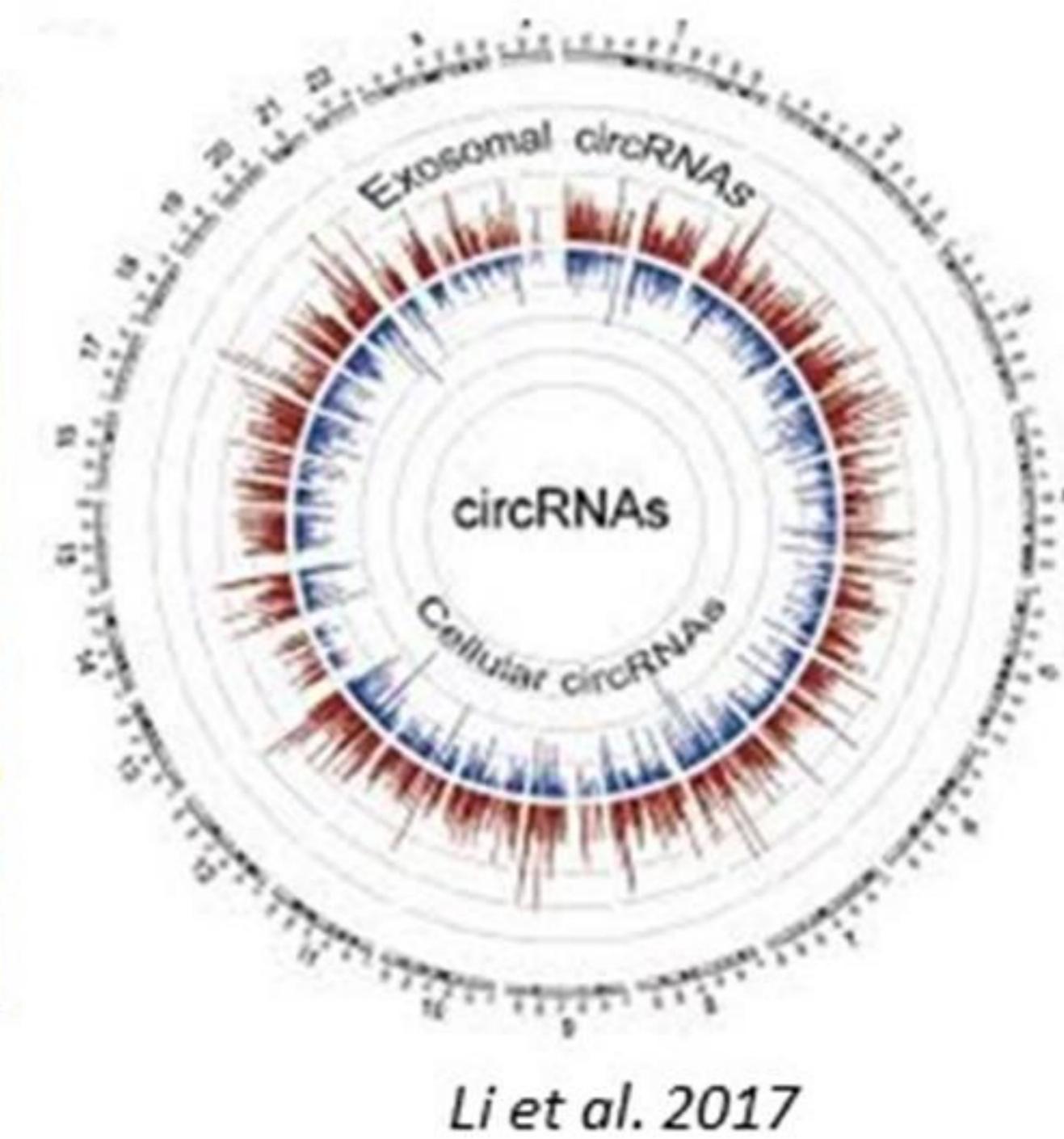
- Turtles on Fort Drum
- eDNAAtlas
- Wading birds
- Environmental RNA (eRNA) with nontraditional forms



JJ Mosesso, NBII



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Government eDNA Working Group

GEDWG

- 60 government eDNA researchers from numerous federal, state, provincial, municipal agencies
- Work together on eDNA issues
- eDNA Training and Technical Exchange Workshops
 - 1eDTTEW: Denver 2016
 - 75+ participants
 - 2eDTTEW: Virtual 2018
 - 150+ participants
 - 3eDTTEW: Tampa-St. Pete 2019

Questions?

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usace.army.mil**



QUESTIONS?

- A. Use Chat Feature; send to Everyone
- B. Select *6 on your phone to unmute your line and ask verbally
- C. After the meeting, email:
 - ▶ Richard.F.Lance@erdc.dren.mil
- D. Webinar suggestions, email:
 - ▶ Courtney.E.Chambers@usace.army.mil
- E. Recorded webinars:
 - ▶ <https://corpslakes.erdc.dren.mil/employees/invasive/exchange.cfm?Option=ArchiveSchedule&CoP=invasive>

