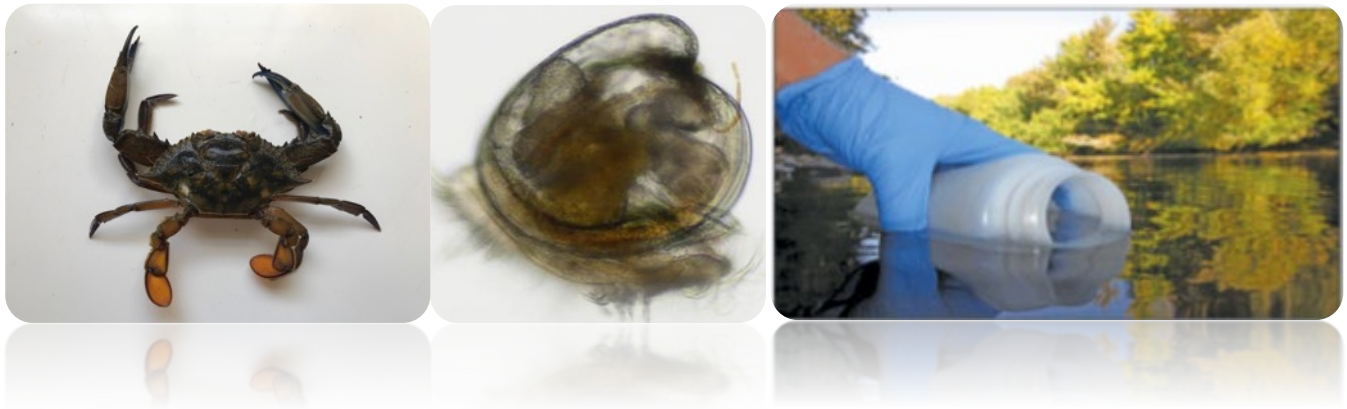
Bio-monitoring 2.0: the power and promise of environmental DNA (eDNA) sampling



Louis Plough
Horn Point Laboratory,
University of Maryland Center for Env. Science

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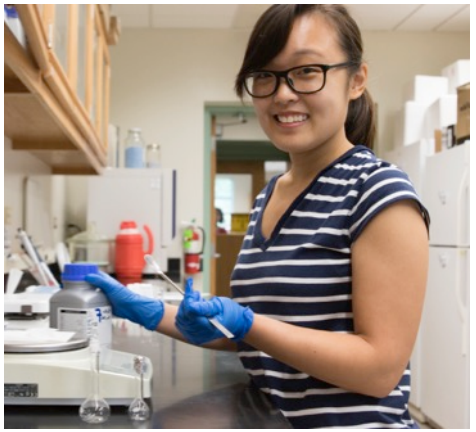
Restoring Coastal Health Through Science and Discovery

Marsh Restoration & Coastal Resiliency	Sustainable Land Practices & Water Quality	Aquaculture & Oyster Restoration	Educating Future Environmental Leaders



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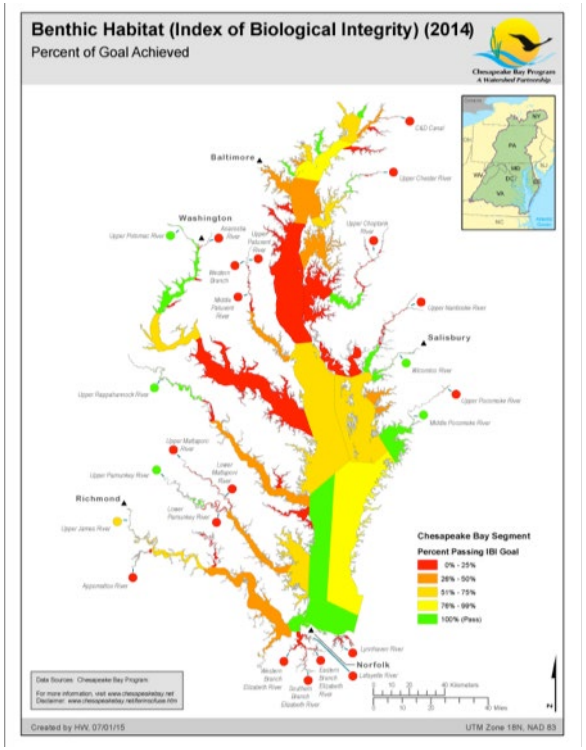
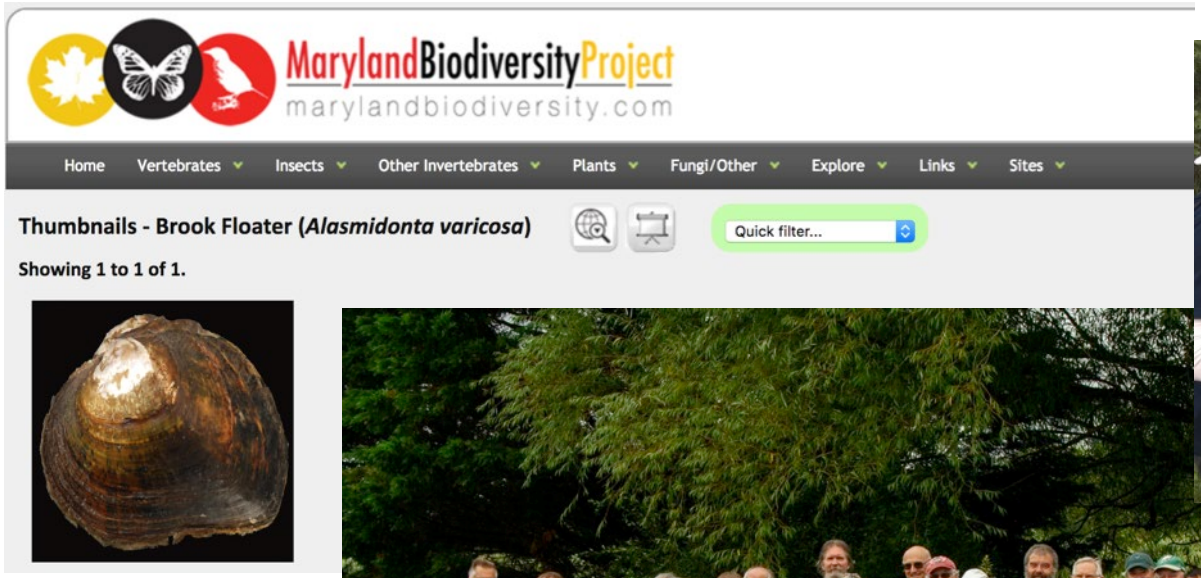
Biodiversity is important

Supports healthy ecosystems and natural resources

Threats from:

Overharvesting
Climate variation
Invasive species



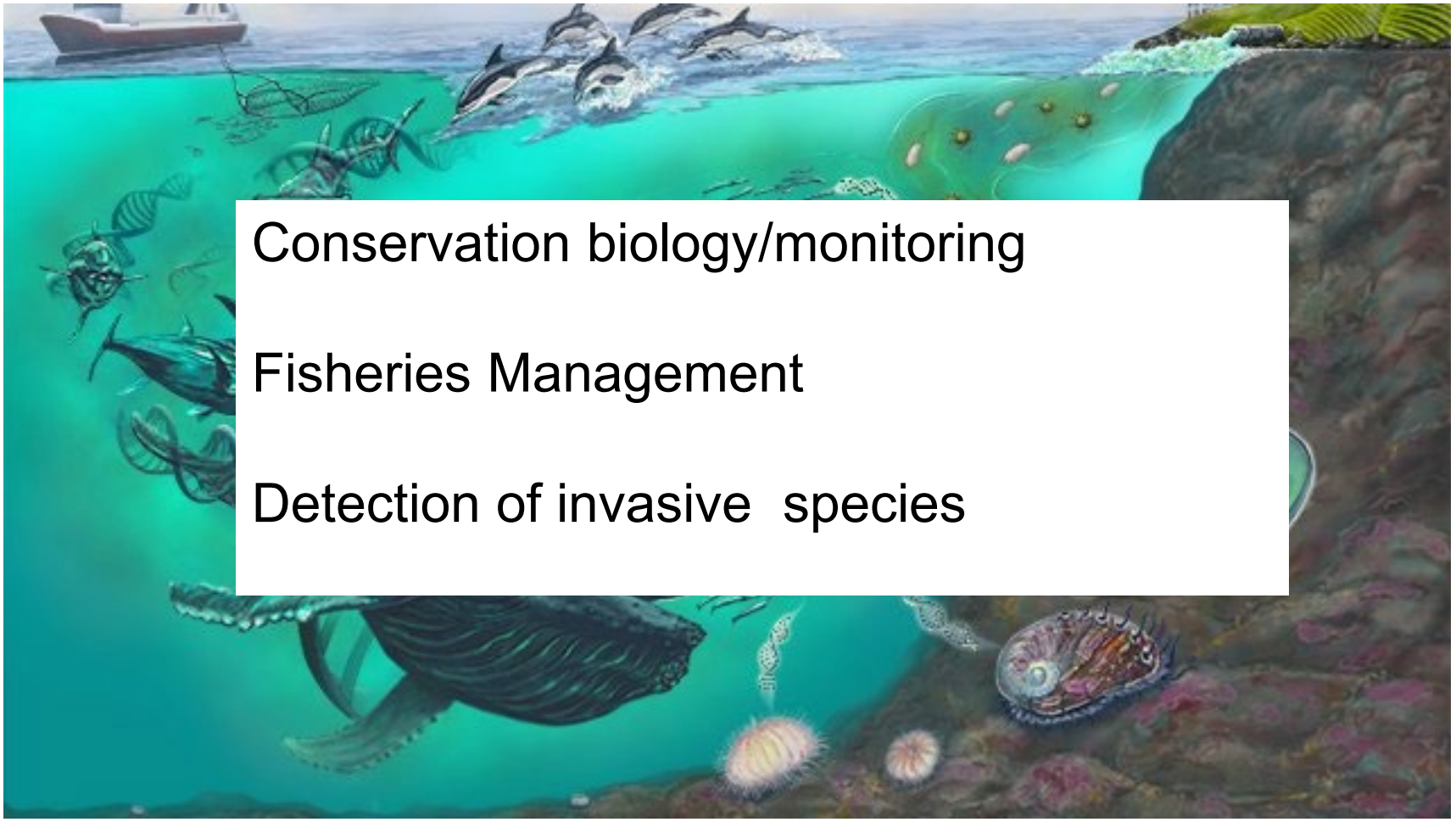


Bioblitz @ HPL, September 2018

⇒ Increased or renewed focus on cataloguing our local biodiversity (baseline)

⇒ Monitoring for invasive species

Bio-monitoring by surveying DNA in the environment?



Conservation biology/monitoring

Fisheries Management

Detection of invasive species

What is eDNA?

- The collection of DNA from any species, found in the environment (aquatic, soil, air).
- ***Our focus*** is on macrobial DNA – fish, inverts, plankton

- Produced by sloughing of cells, mucous, feces, gametes, hair, etc…

Advantages of eDNA sampling

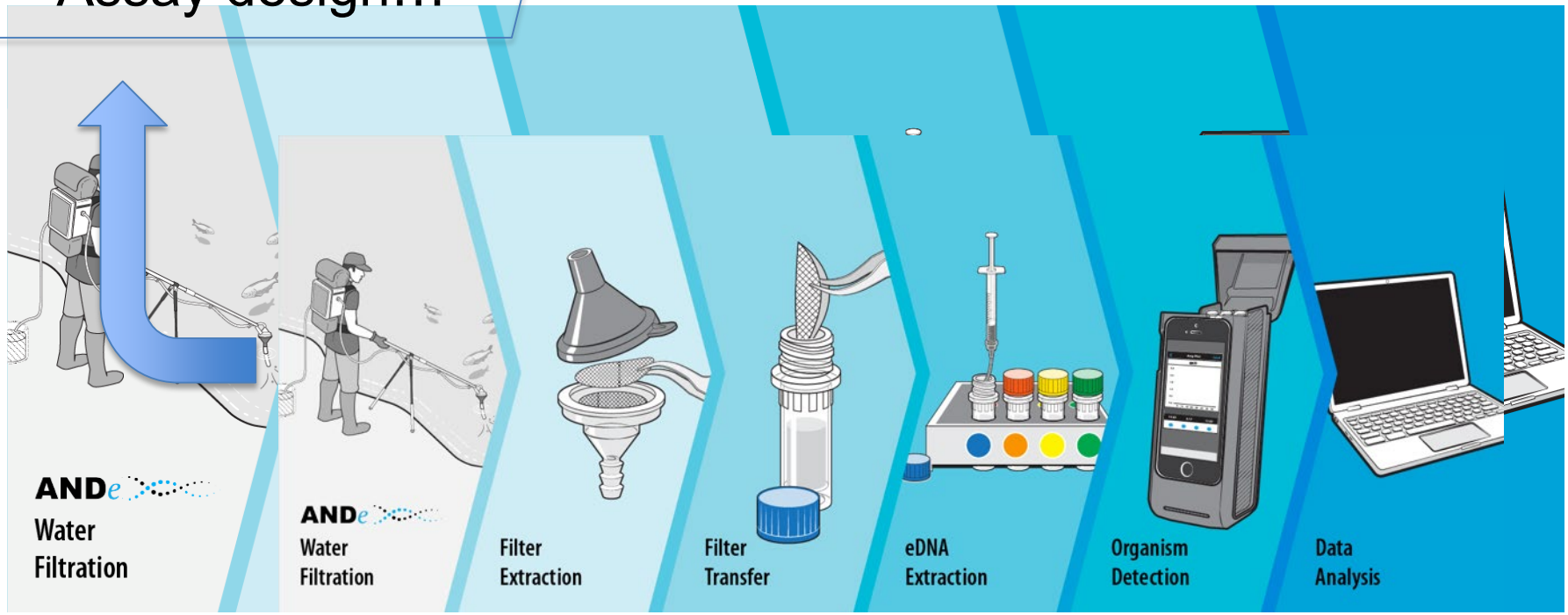
- Non-invasive sampling (just take water)
- Ability to sample remotely, or in hard to reach places
- Sequence-based ID (“DNA barcode”) vs morphological ID

Outline

- Introduction to eDNA sampling/analysis
- Methodological challenges
- Two examples from Chesapeake Bay
  - River herring monitoring
  - Atlantic sturgeon monitoring
- Final thoughts

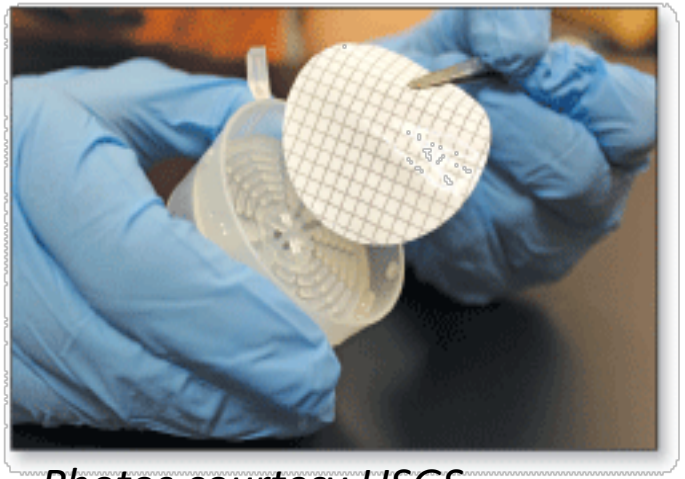
Basic eDNA sampling workflow

Assay design…



eDNA development/sampling

- Workflow is simple
  - Sample water, filter, extract DNA, perform PCR/sequencing
- Possible to sample many sites, cheaply
  - Citizen-science potential
  - High-school science curriculum
- **PCR-based assay amplifies target species or community**



Photos courtesy USGS

Sampling

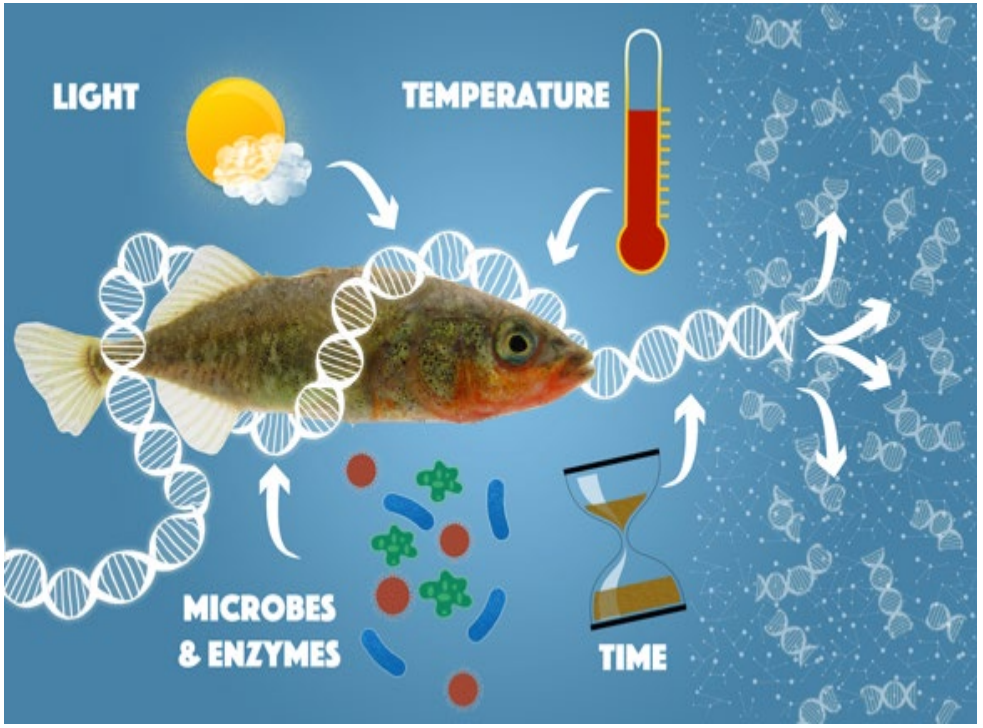
In-field filtering…

Lab filtering…

What volumes are needed?

eDNA sampling challenges

- Filtering of water in sites of variable water quality
  - **Degradation/inhibition**
- Assay development (qPCR) bioinformatics (metabarcoding)
- Relationship between molecular/sequence abundance and true abundance? Fate and transport?



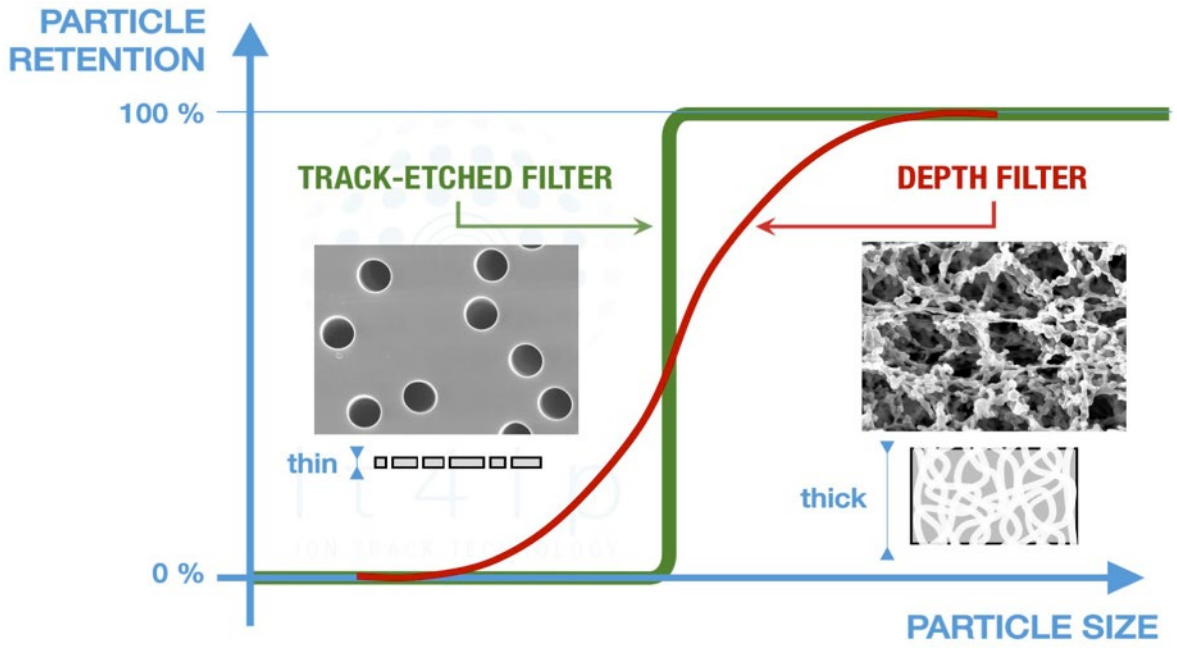
http://fishbio.com/field-notes/the-fish-report/true-or-false-challenges-of-edna-species-detection

Extraction of DNA from filter

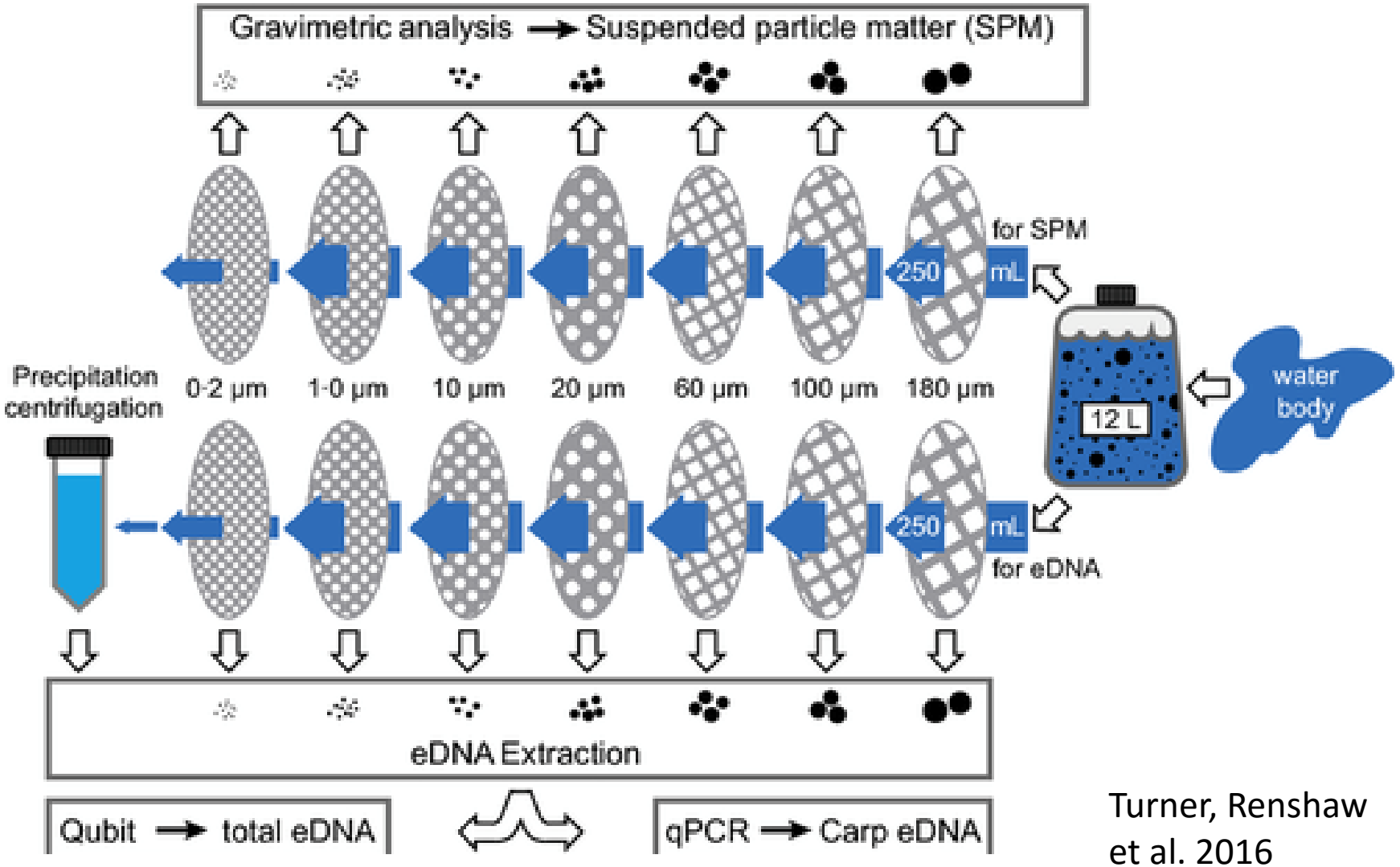
- What kind of filter? What pore size?

Filter material and pore size affects retention

Membrane Filters: Thin plastic membranes with different pore sizes and material that retain particles of a certain size



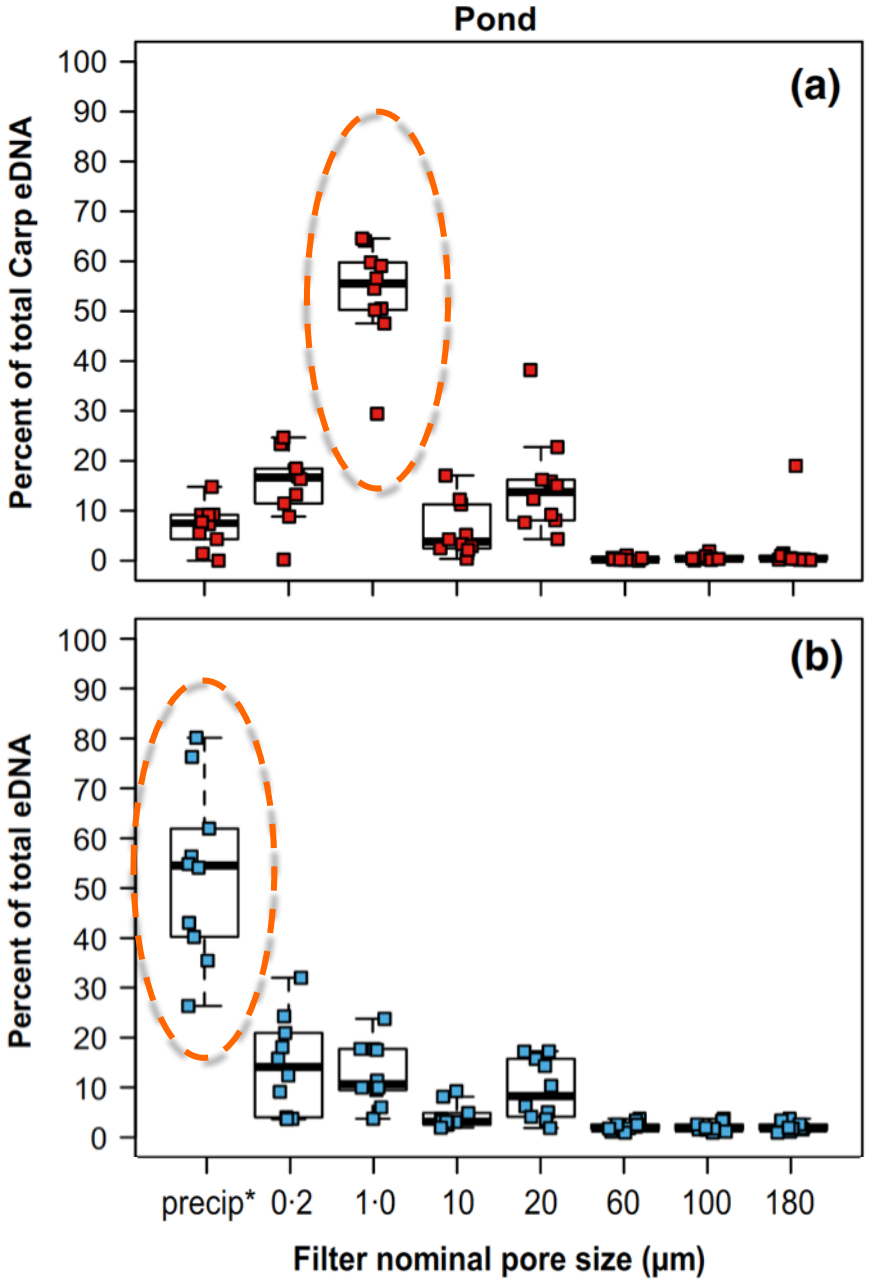
Which filters to use for eDNA?

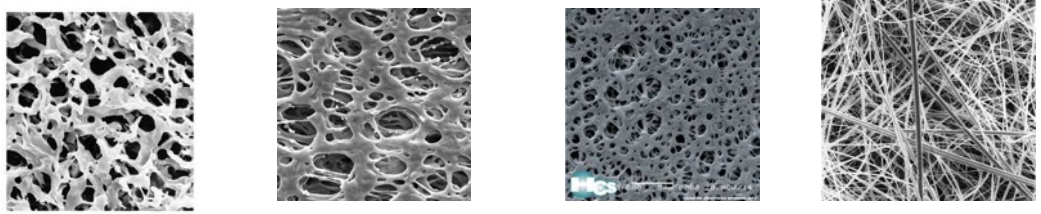


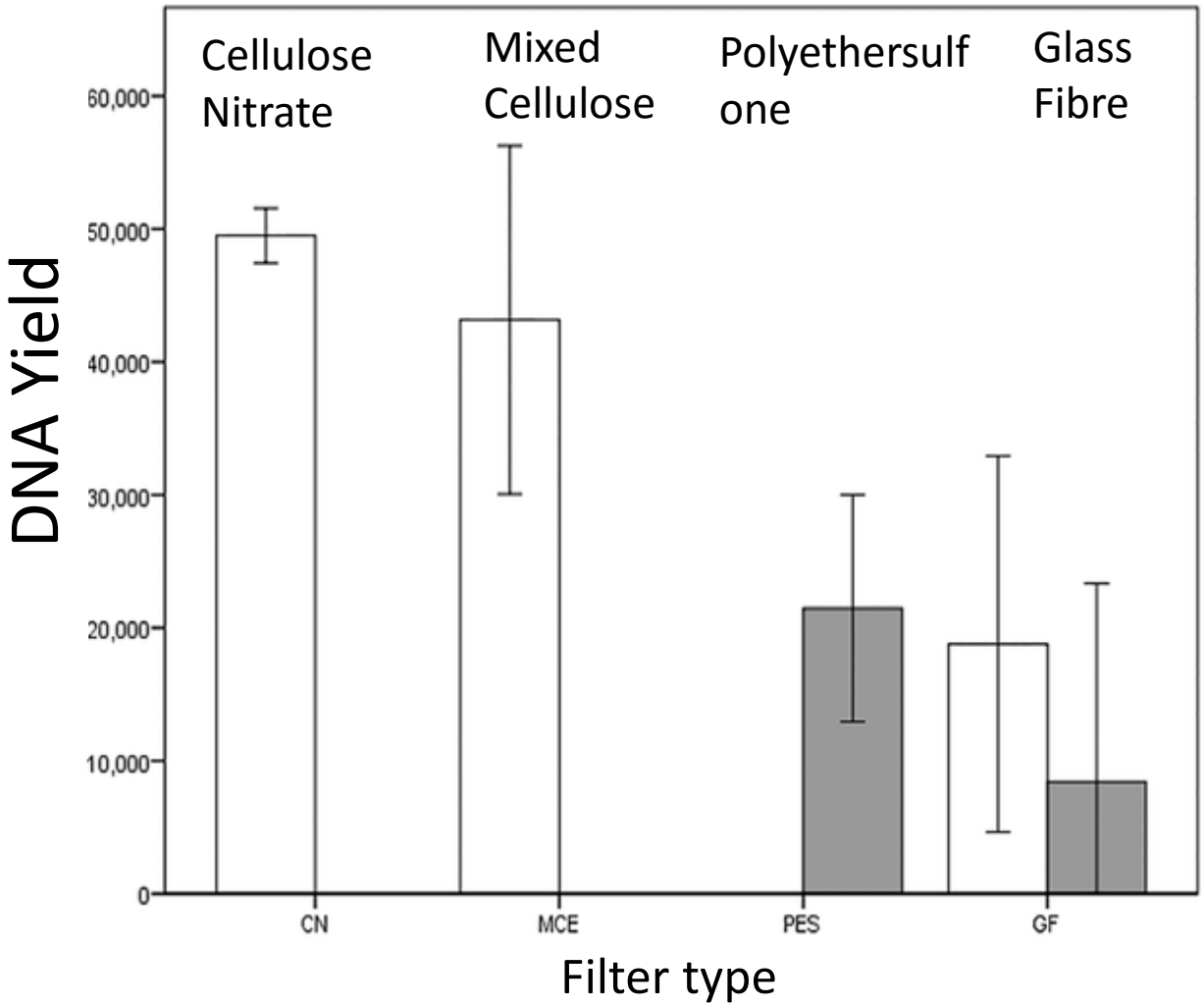
Turner, Renshaw et al. 2016

~90% of the macrobial (fish) DNA is between 1 and 10µm

~60% of the total eDNA is in the smallest size fraction (<0.2 µm)







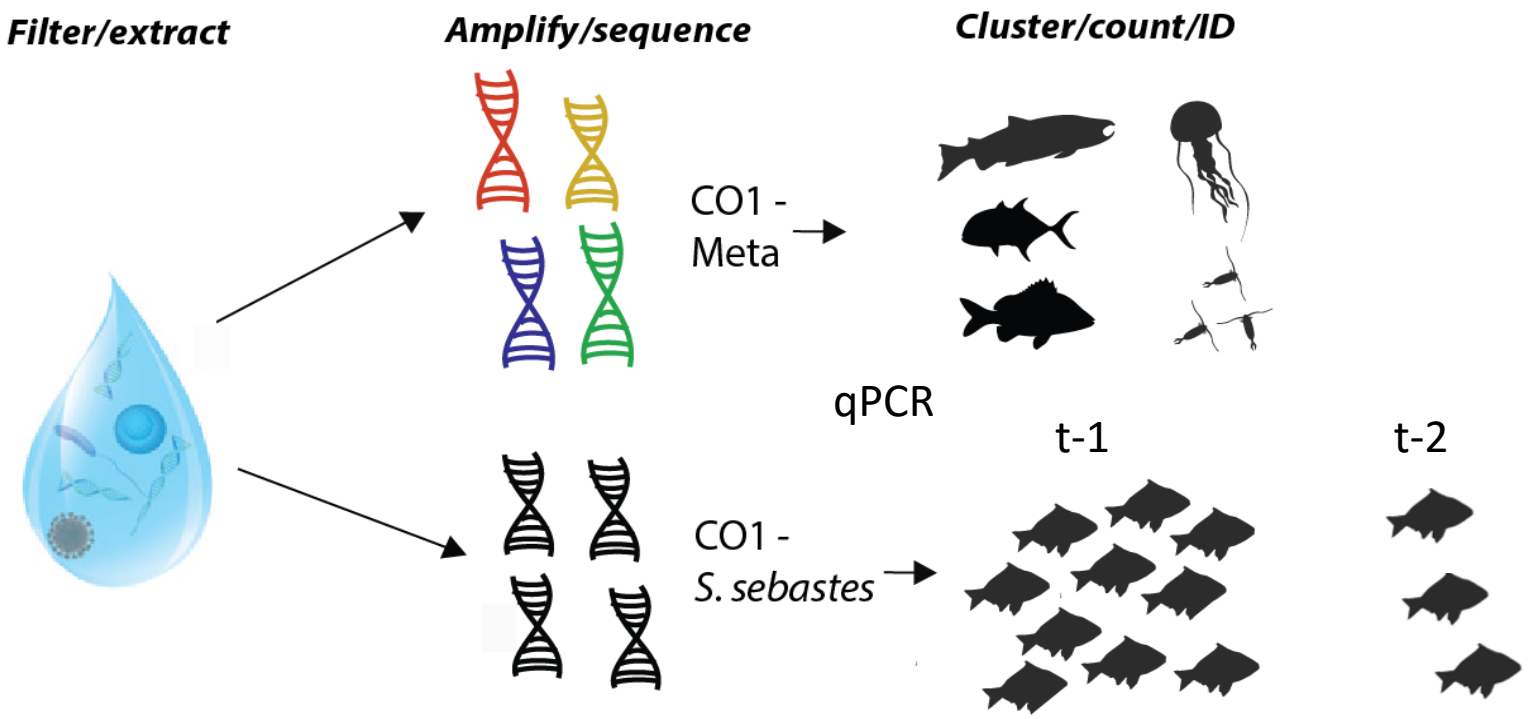
Hinlo R, Gleeson D, Lintermans M, Furlan E (2017) Methods to maximise recovery of environmental DNA from water samples. PLOS ONE 12(6): e0179251. https://doi.org/10.1371/journal.pone.0179251

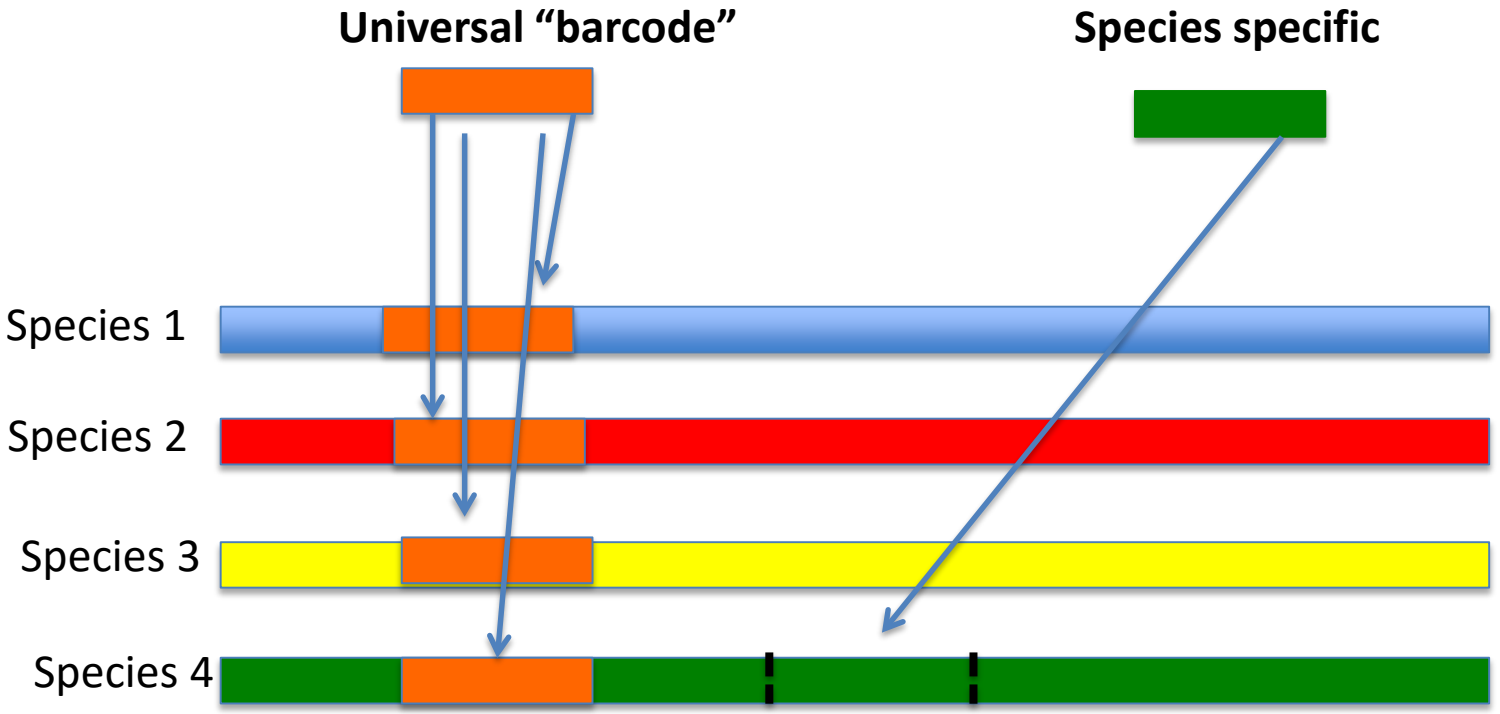
PLOS ONE

Targeted vs. species specific (eDNA) monitoring

1.)Metabarcoding employs a ‘universal’ gene or marker

2.) qPCR targets a sequences from a single species



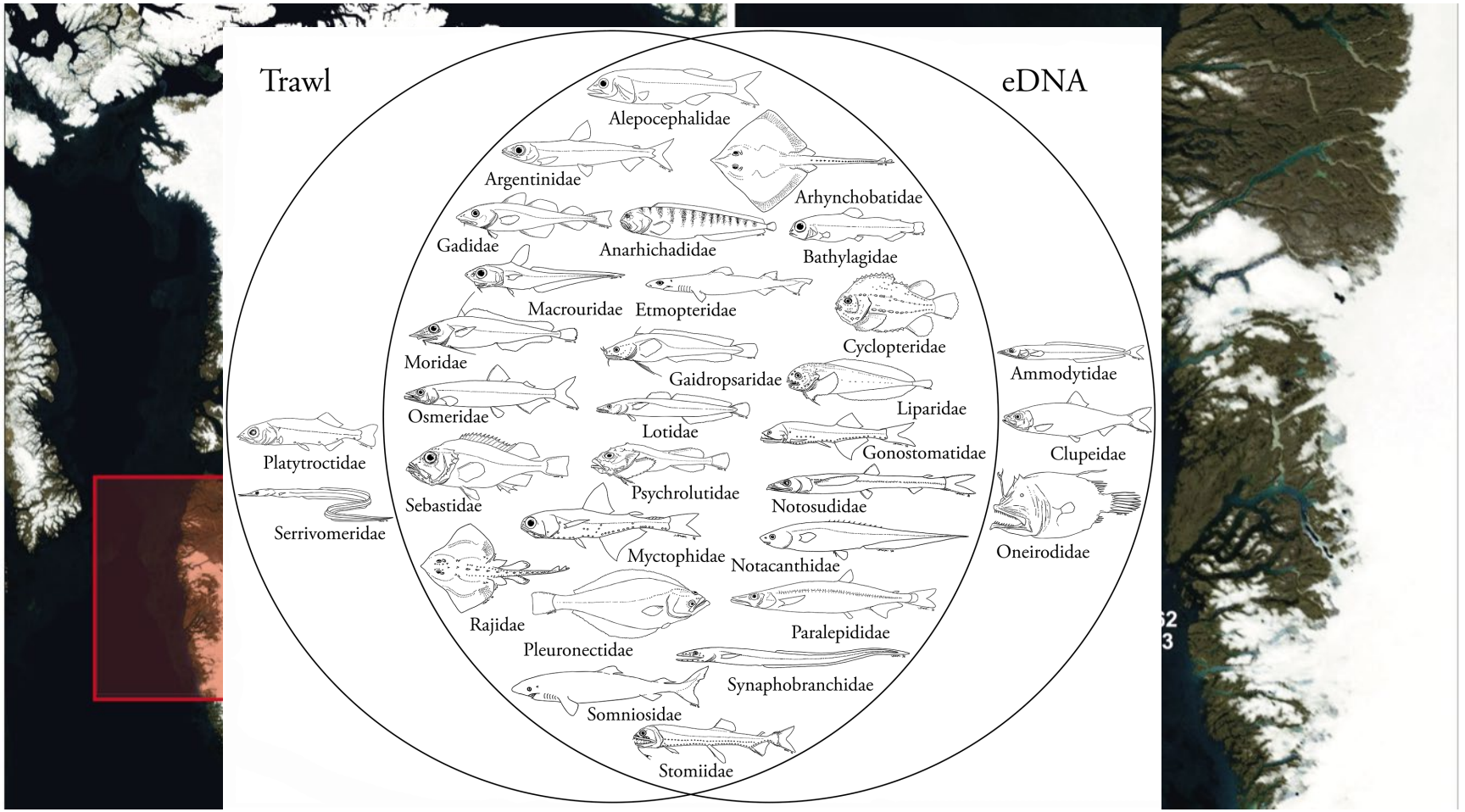


Choice of gene or marker?

Mitochondrial DNA is popular – high copy number, and significant online resources (databases)

Trawl Survey with eDNA (metabarcoding)

Thomsen et al 2016 PloS ONE.



Hunt for the Loch Ness monster with eDNA?

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Home › Nature › **The genetic hunt for the Loch Ness Monster – Neil Gemmell**

The genetic hunt for the Loch Ness Monster – Neil Gemmell

Professor Neil Gemmell on his project to survey the genetic diversity of Loch Ness using cutting-edge environmental DNA techniques, and maybe find clues about the Loch Ness Monster.

https://www.thedailybeast.com/loch-ness-monsters-existence-could-be-proven-with-edna

MONSTROUS

Loch Ness Monster's Existence Could Be Proven With eDNA

Environmental DNA could put the legend of the Loch Ness monster to rest, once and for all.

Tanya Basu 05.23.18 2:12 PM ET

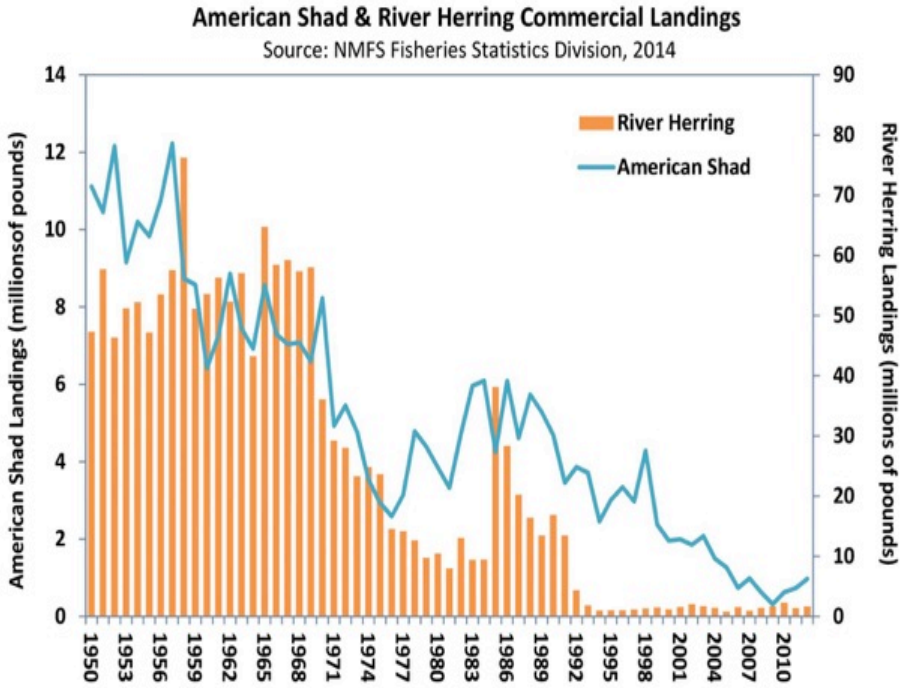
Monster hunt: using environmental DNA to survey life in Loch Ness

June 26, 2018 3.49pm EDT

With the help of environmental DNA, scientists are compiling a census of life in Loch Ness, which should establish if there is any scientific basis to the centuries-old legend of the Loch Ness monster.

Supplied, CC BY-SA

eDNA analysis of River Herring in Chesapeake Bay



ASMFC.org

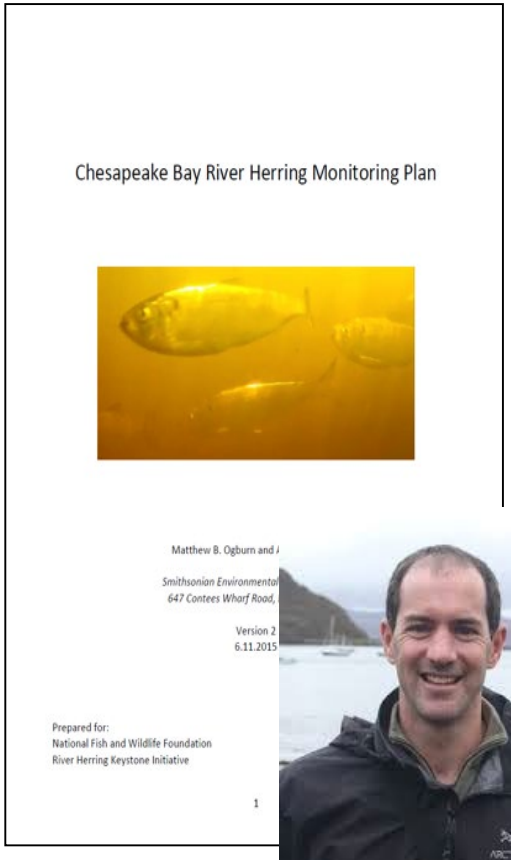
Hudson River herring - Erica Capuana/AP

⇒ **Petition to list River herring on threatened species list denied, 2013**

Keystone Herring Initiative – A monitoring plan

- Habitat use in at least 10 tributaries
- Run counts in 5 tributaries
- Fish passage assessment

fws.org



Matt Ogburn and Tuck Hines, Smithsonian Environmental Research Center (SERC)

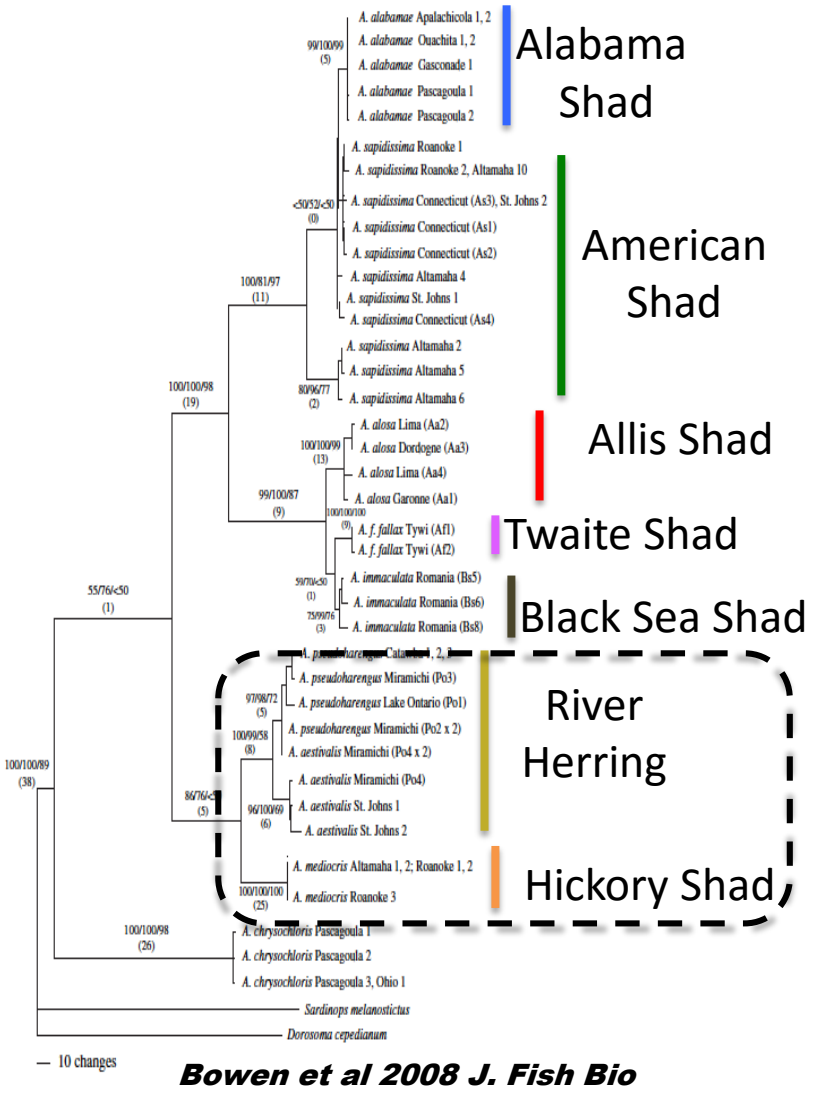
Objectives

- Develop a robust eDNA assay for detection and quantification of river herring (*alewife & blueback herring)*
  - *No amplification of other Alosines*

- Examine species-specific patterns of presence and abundance across the Chesapeake Bay

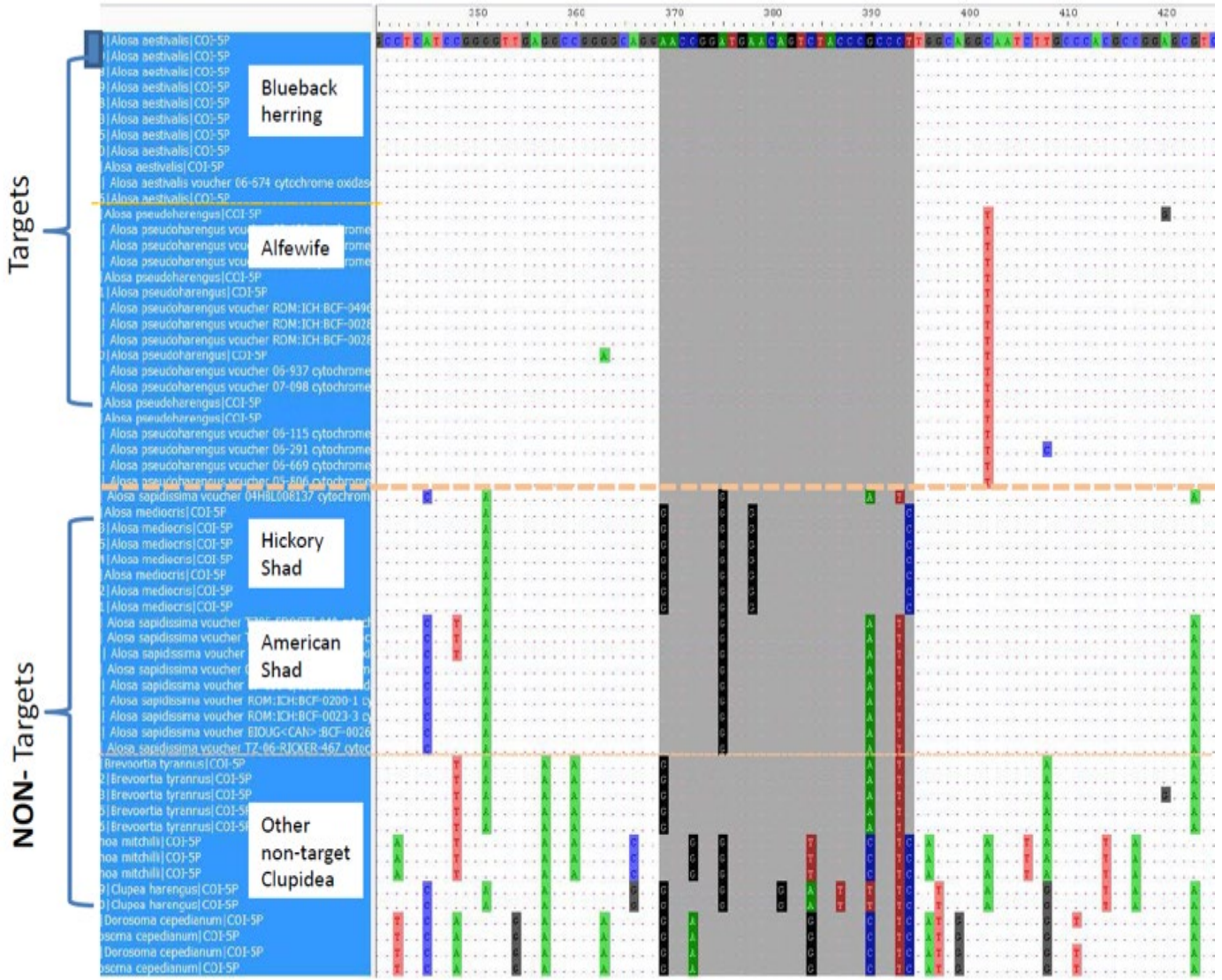
Design/efficiency of the assay

- Mitochondrial data publicly available for Alosines and Clupeidae
  - 98% similarity to hickory shad
- qPCR assay tested against DNA of ~15 estuarine/freshwater fish
- **Assay is River herring specific**: ID of alewife vs. blueback via sequencing post-detection.



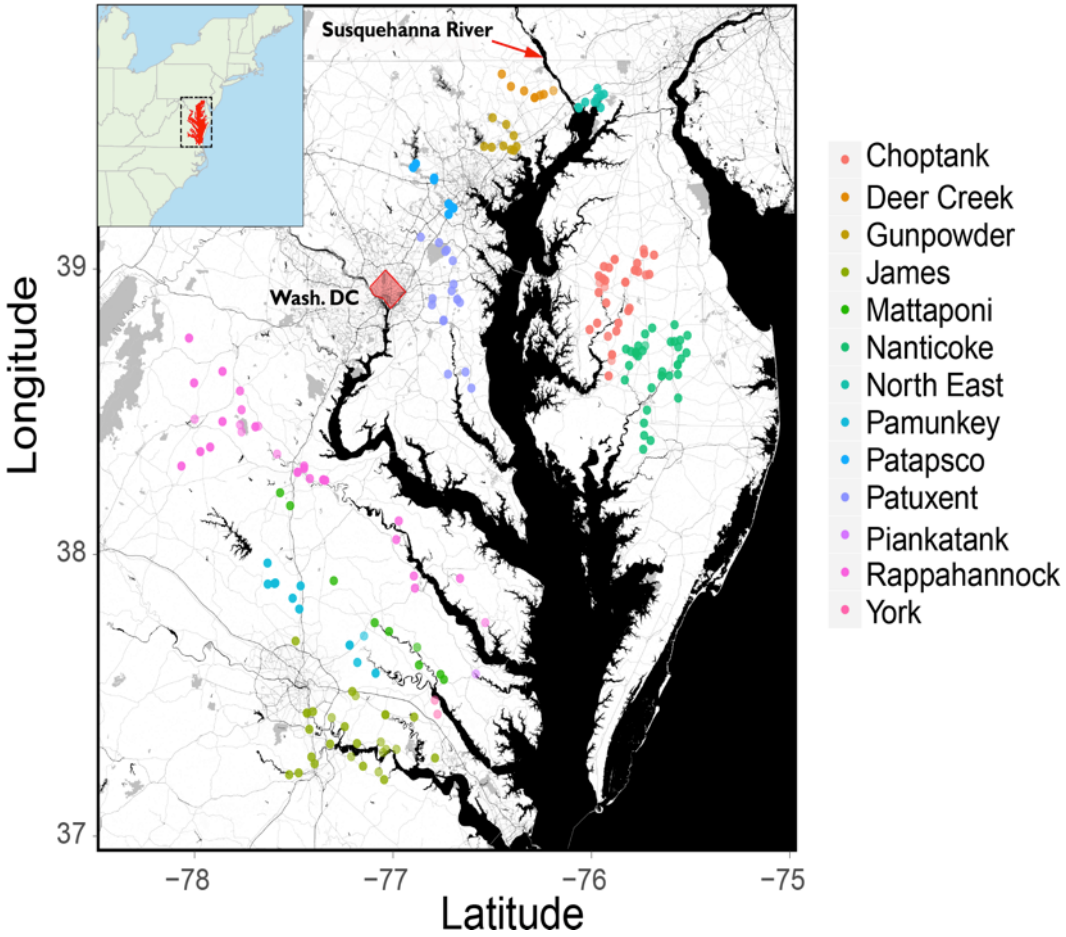
Bowen et al 2008 J. Fish Bio

Sequence alignment for the assay

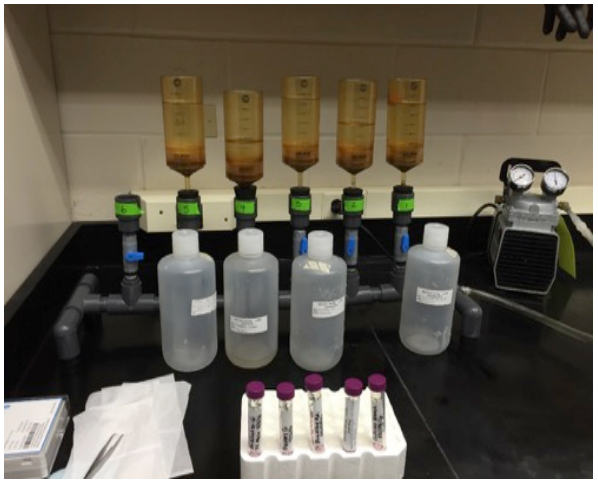


Cytochrome Oxidase subunit 1 (CO1)

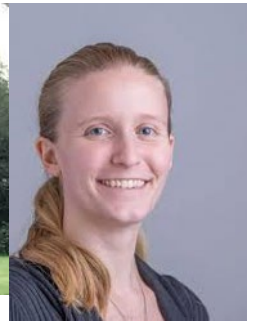
Assay validation and field testing in Chesapeake Bay



Plough et al. 2018 *PLoSOne*

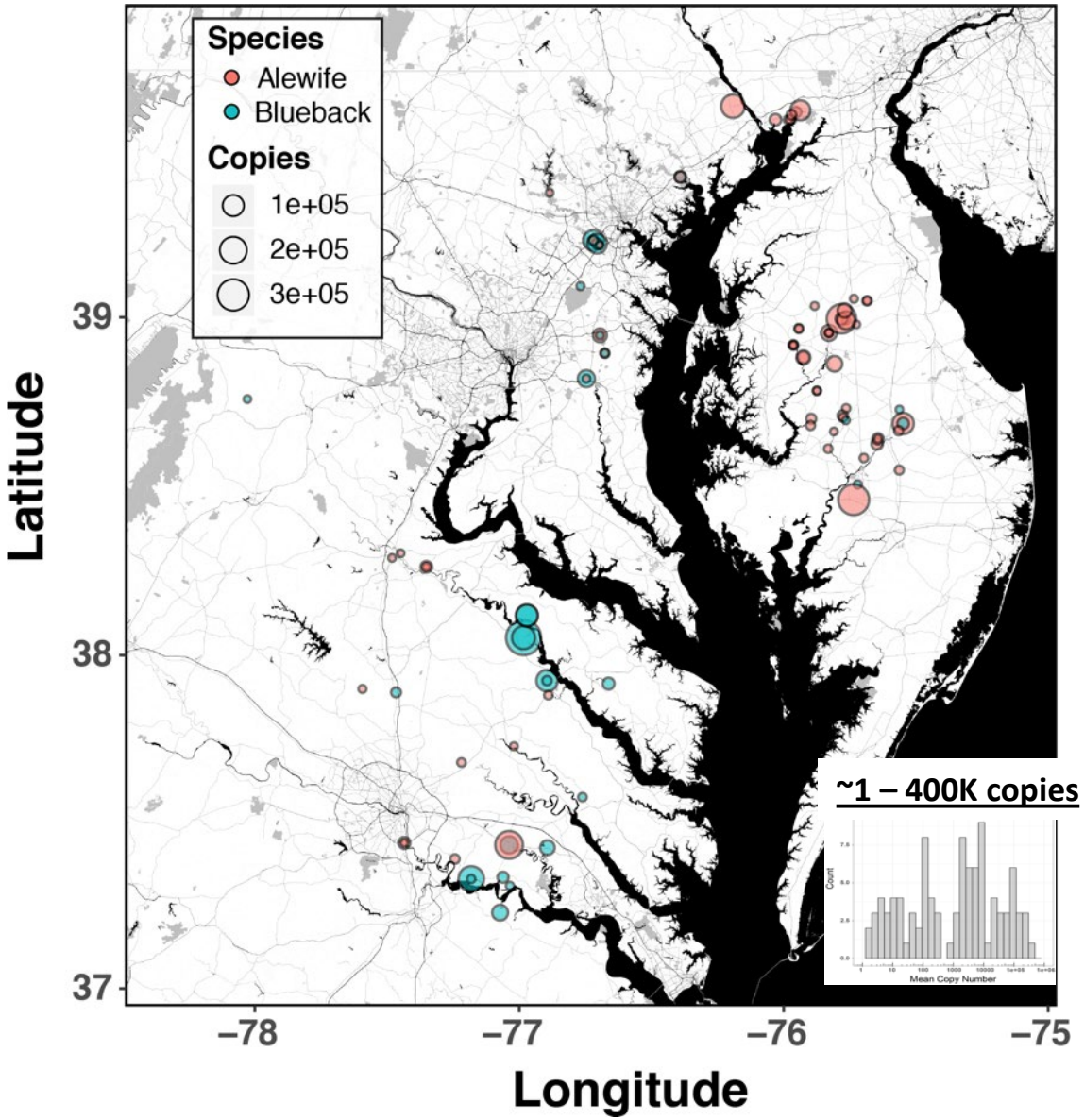


Rose Geranio



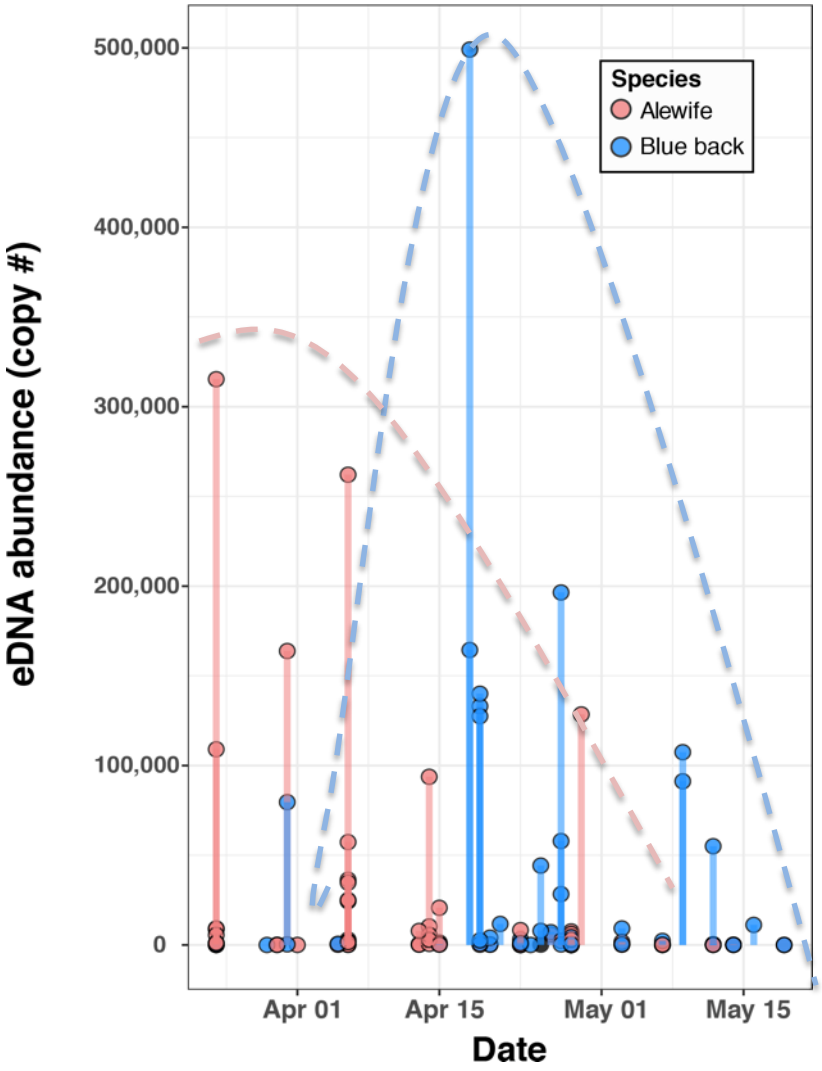
Shore-specific patterns of river herring habitat use

- eDNA detections for 112/445 (25%) samples
- Highly sensitive (down ~ 1 copy)
- Species ID's for 98%



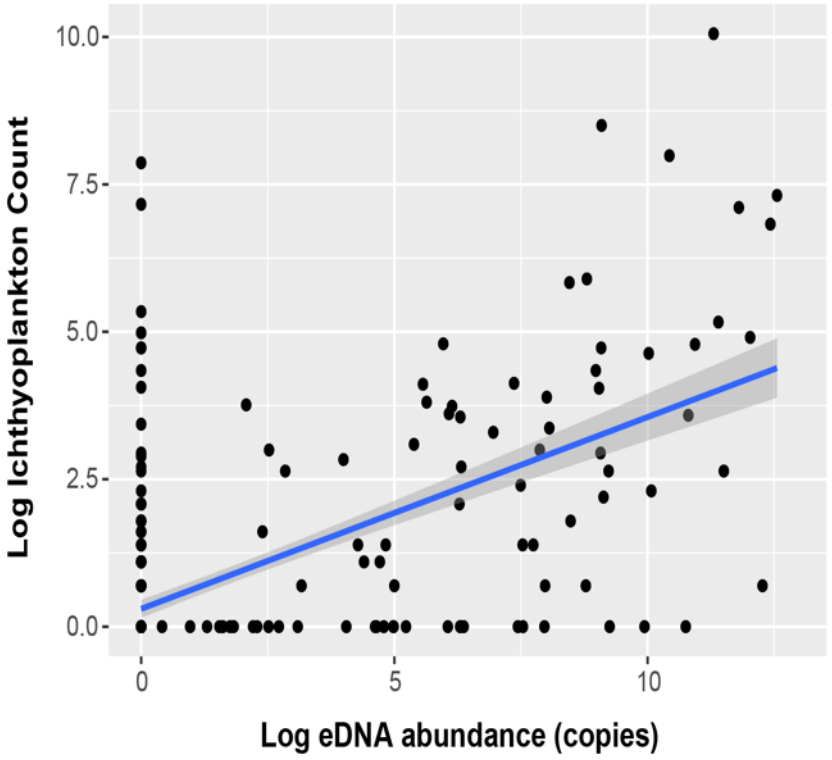
eDNA recovers timing of herring spawning

- Alewife spawn earlier in the spring (March-April)
- Blueback herring spawn later (May)



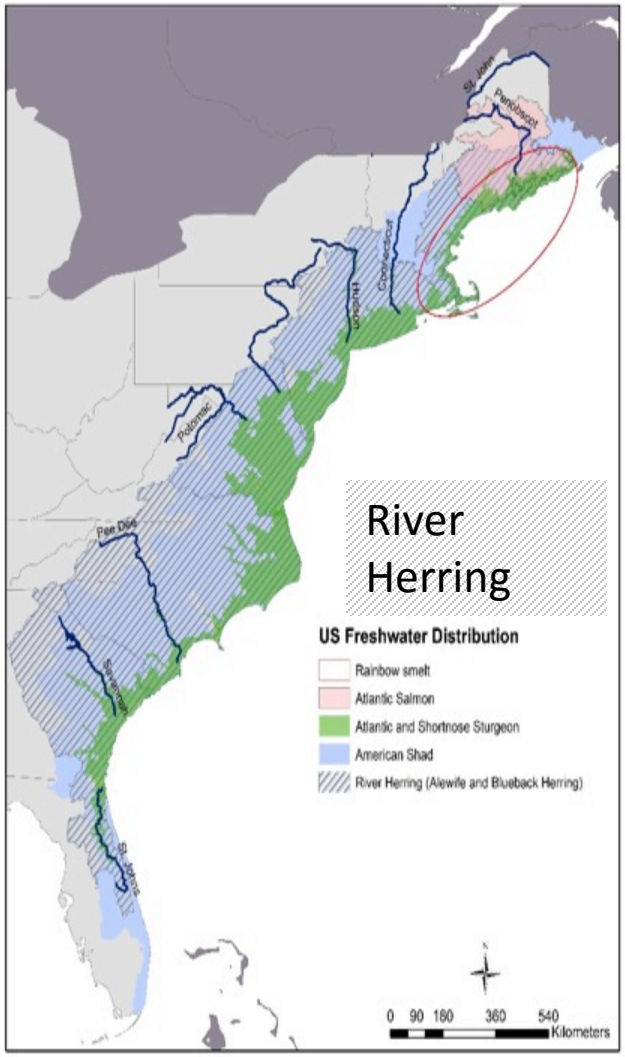
Comparison of eDNA with traditional survey methods

- High correlation between **Icthtyoplankton (net samples)** and **eDNA** datasets (N=362)
  - Spearman's Rho = 0.60
- Log-log plot eDNA vs Ichthyoplankton $R^2 = 0.48$
- A fair comparison?



Herring eDNA summary

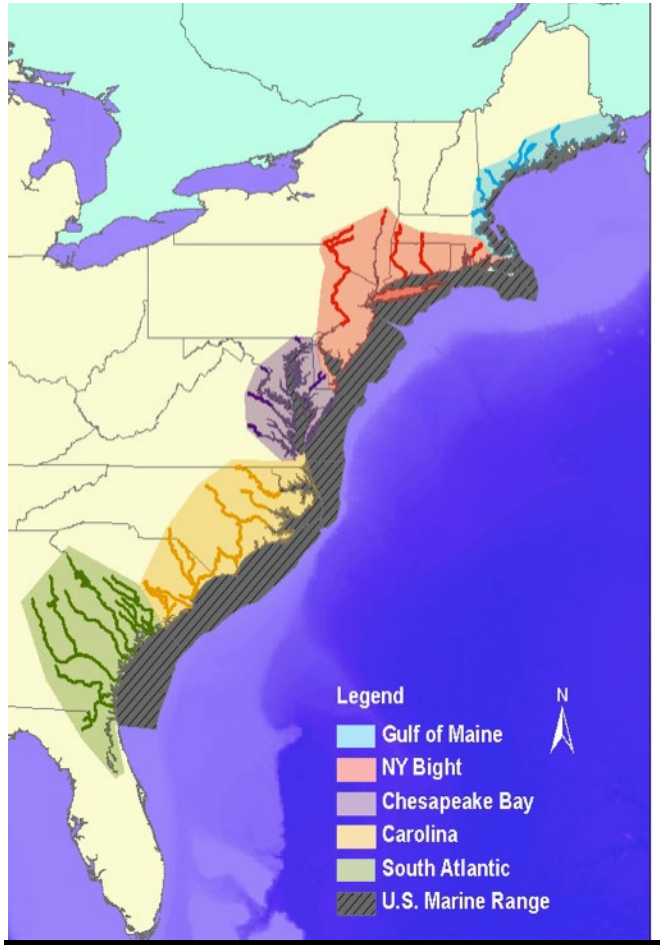
- eDNA is a robust and sensitive approach to quantify the relative abundance of river herring (*Plough et al. 2018 PLoS)*
  - Highly correlated w/ other 'catch' survey data
  - Recovers run-timing differences between species
  - eDNA data incorporated into habitat use model (Ogburn, Plough et al. *in prep*)

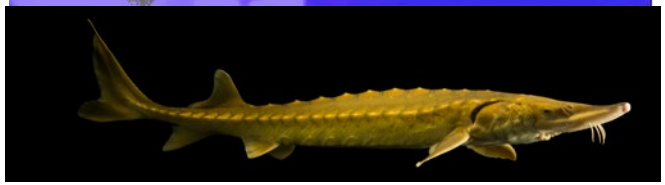


Atlantic sturgeon eDNA

- Over fishing and habitat degradation led to the decline of spawning runs along the US East coast

- 5 ‘population segments’ are considered endangered

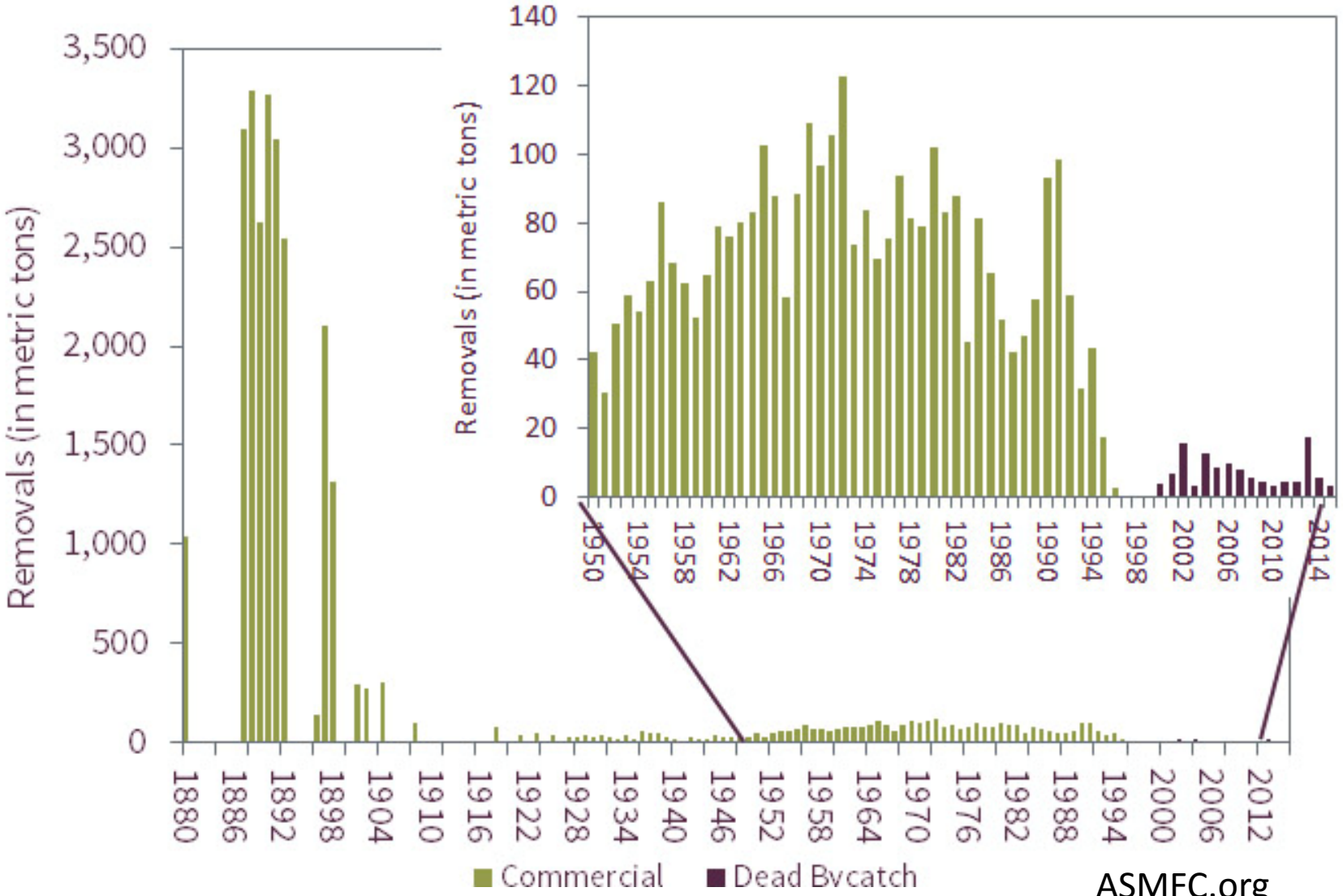




Coastwide Atlantic Sturgeon Commercial Landings and Dead Bycatch, 1880–2014

Source: ASMFC Atlantic Sturgeon Bechmark Stock Assessment, 2017

inserted graph provides same information but for a more recent timeframe, 1950–2014



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Tagging and monitoring of Atlantic Sturgeon in Chesapeake Bay

- Tags surgically implanted
- Acoustic arrays set up in the VA and MD portions of Chesapeake Bay
- Tracking movement provides insight into habitat use (potential spawning grounds)

Alternative strategies to monitoring Atlantic Sturgeon (ATS)?

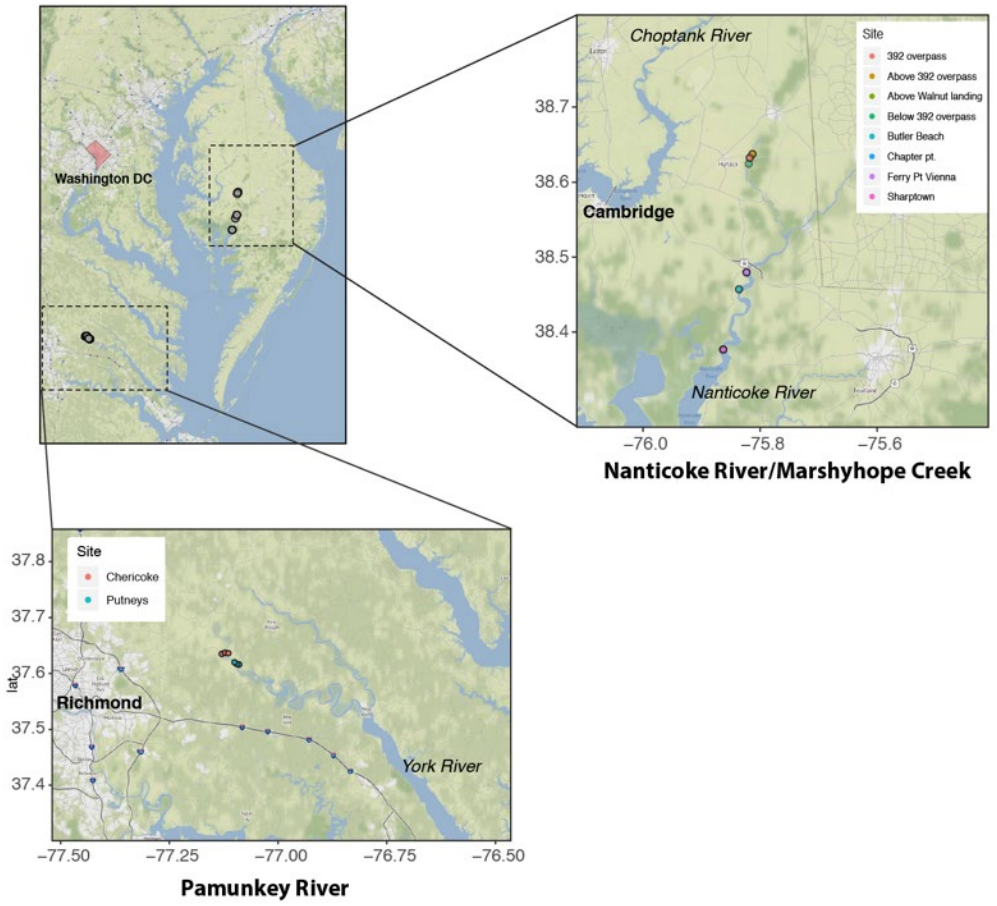
- eDNA had been considered but not thoroughly examined

- Concern about low abundance and low eDNA detection probability?

- Goal: develop and validate an eDNA assay for ATS
  - Are abundances sufficient for detection?
  - What is the shedding rate of ATS?

Development of an eDNA assay for Atlantic Sturgeon

- Single species (targeted) assay
- Test in aquaria, ponds, and in field
- Testing on Marshyhope Creek (Nanticoke river) and Pamunkey River (VA)



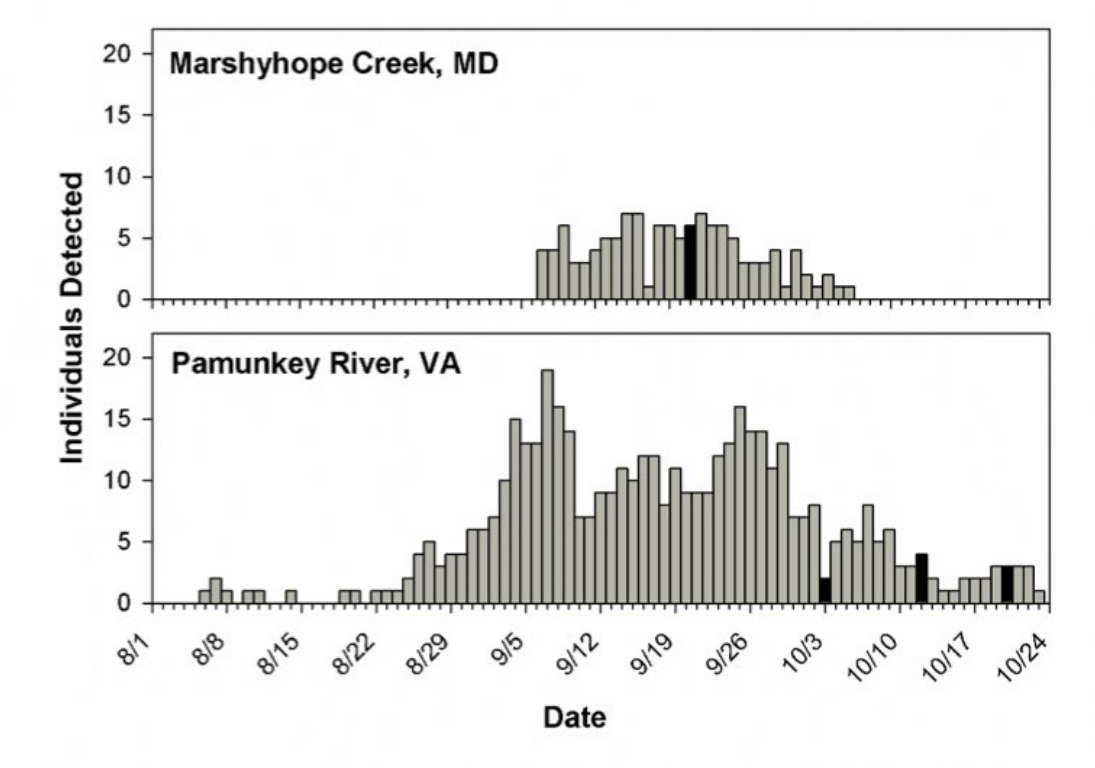
Lab-mesocosm results

Aquaria detections only with low flow

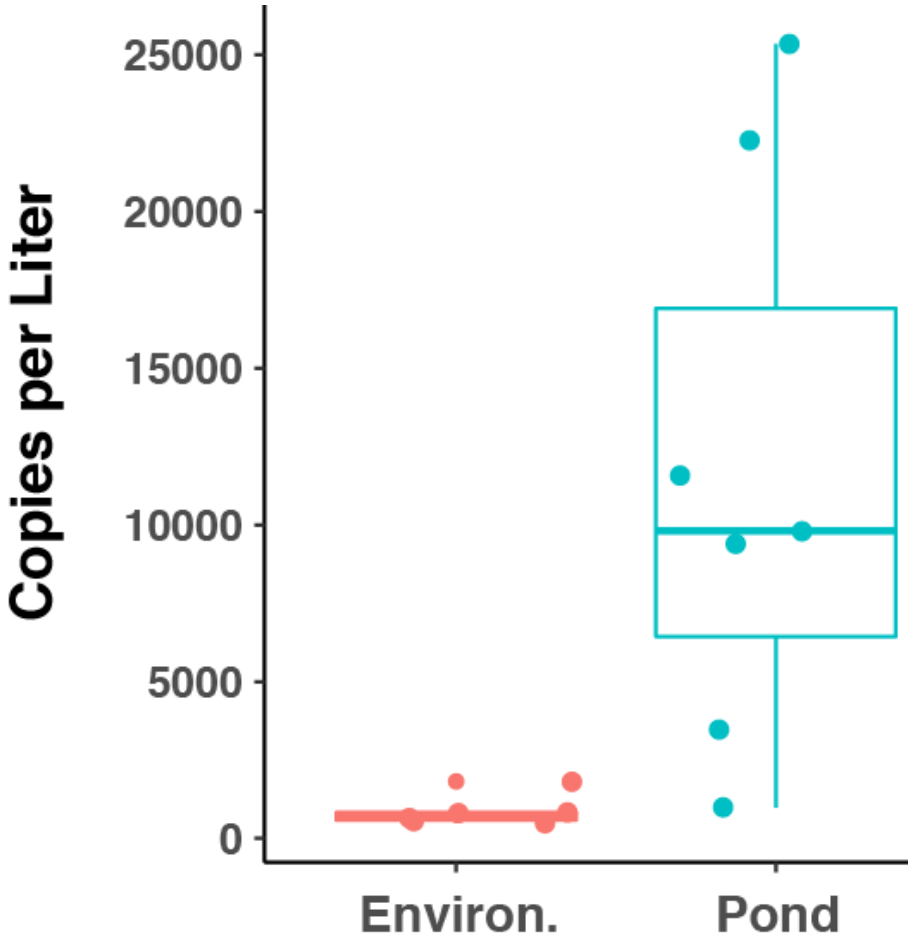
7/7 pond detections

Comparison of acoustic and eDNA detections

- 18% of field samples (6/34) detected sturgeon DNA
- eDNA detections coincide with acoustic detections (black bars)



Low eDNA concentrations in field…



- *What is the eDNA shedding rate of Atlantic sturgeon?*
- *Can eDNA of Atlatnic sturgeon be used to quauntify relative abundance?*

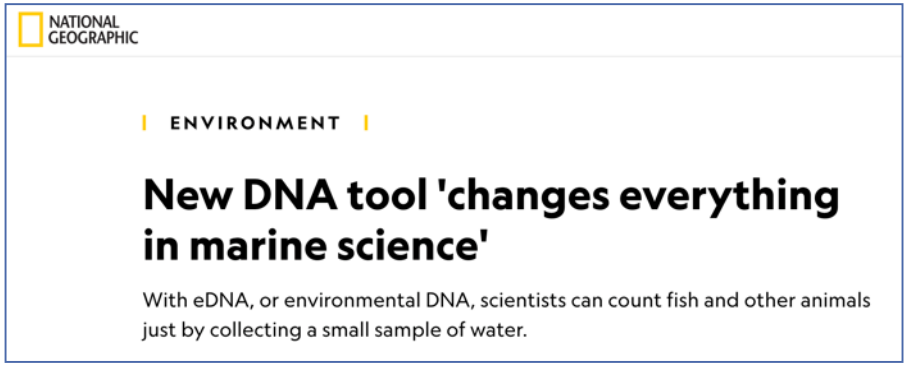
Summary of Atlantic Sturgeon eDNA work

- The assay works in lab, pond, and field trials
- Low eDNA abundances relative to other species…

- *On going experiments to determine shedding rate*

eDNA: *great promise, lots of work to do…*

Nov. 20-30 2018


NATIONAL GEOGRAPHIC

ENVIRONMENT

New DNA tool 'changes everything in marine science'

With eDNA, or environmental DNA, scientists can count fish and other animals just by collecting a small sample of water.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Home / Annual Review of Ecology, Evolution, and Systematics / Volume 49, 2018 / Cristescu, pp 209-230

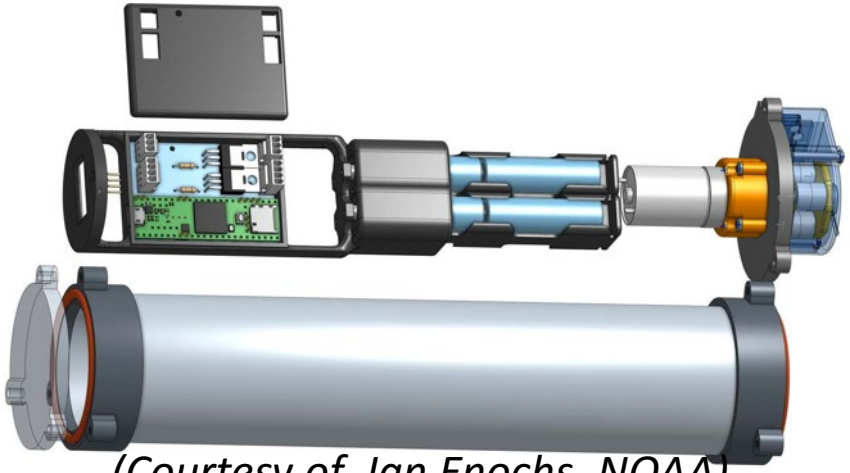
Uses and Misuses of Environmental DNA in Biodiversity Science and Conservation

Annual Review of Ecology, Evolution, and Systematics
Vol. 49:209-230 (Volume publication date November 2018)
First published as a Review in Advance on August 3, 2018
https://doi.org/10.1146/annurev-ecolsys-110617-062306

Ryan Kelly, Univ. Washington: ***"…It always amazes me when we get Orca DNA in our coastal datasets….but we actually know there are Orcas are out there… In fact, there are 85, and they all have names!!!"***

Automated and remote sampling of eDNA

Have…



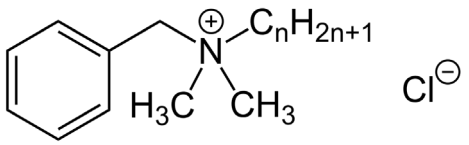
(Courtesy of Ian Enochs, NOAA)

SUBSURFACE AUTOMATIC SAMPLERS (SAS)

Getting soon!

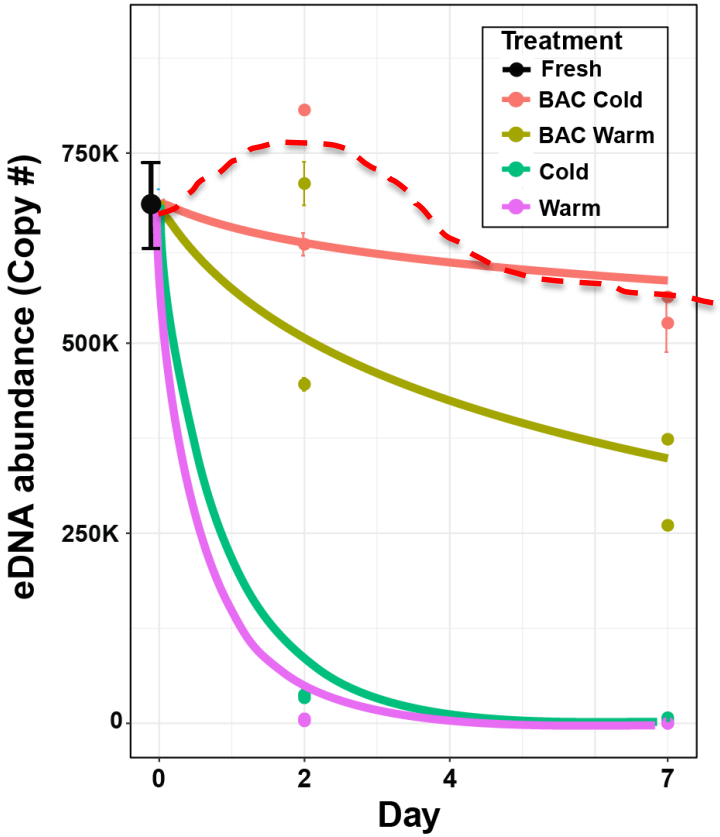
Preservation of eDNA in the field

- BAC (benzalkonium chloride)
  - cationic surfactant commonly used as an antiseptic/biocide

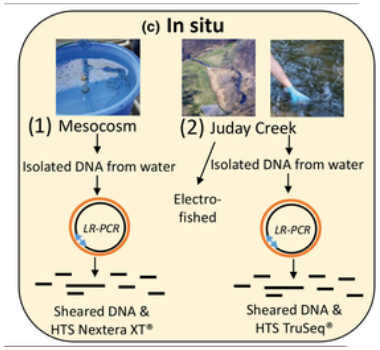


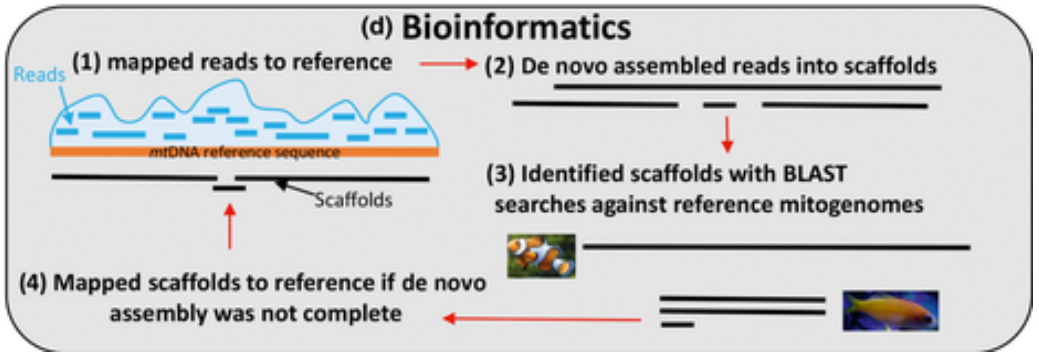
n = 8, 10, 12, 14, 16, 18

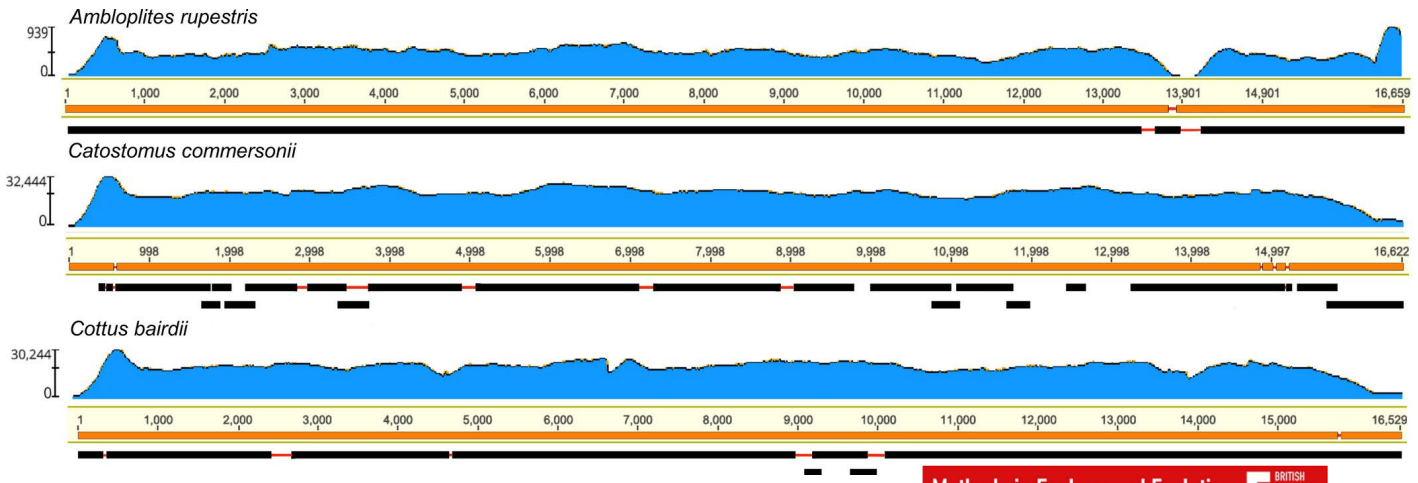
- Results of eDNA longevity experiment using BAC (0.01%) in cold (4C) or room temp (25C) over 1 week
  - ***BAC cold/warm show 50-80% of initial after 1 week***



Sequencing of entire mitogenomes from eDNA







Methods in Ecology and Evolution
BRITISH ECOLOGICAL SOCIETY
RESEARCH ARTICLE | Open Access

Long-range PCR allows sequencing of mitochondrial genomes from environmental DNA

Kristy Deiner, Mark A. Renshaw, Yiyuan Li, Brett P. Olds, David M. Lodge, Michael E. Pfrender

First published: 14 July 2017 | https://doi.org/10.1111/2041-210X.12836 | Cited by: 10

Deiner et al 2017

eDNA surveillance is a powerful monitoring tool for conservation biology and ecology…but it has limits

Acknowledgements

HPL

Students/staff/postdocs:

Catherine Fitzgerald
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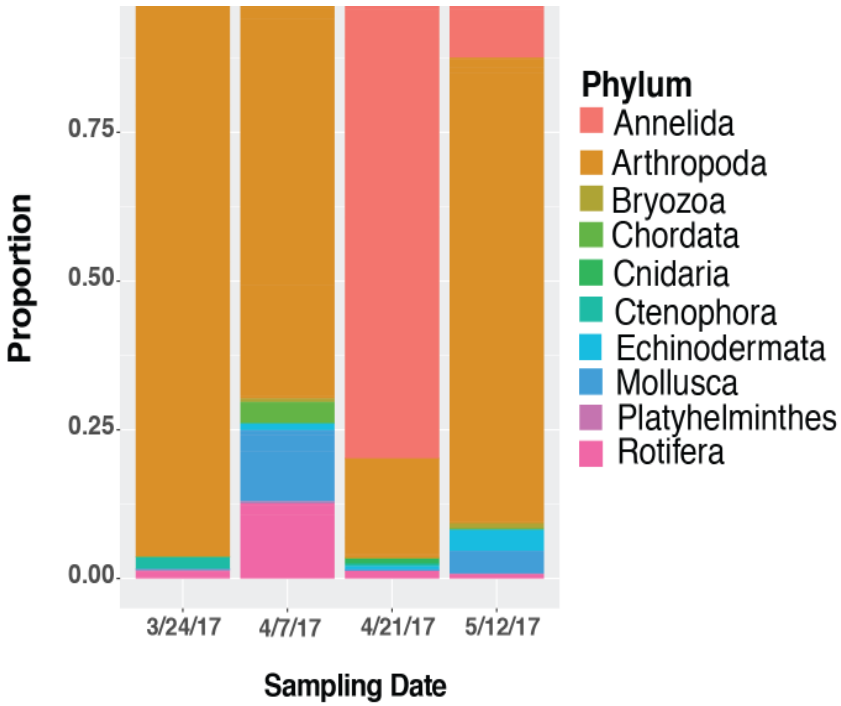
MARYLAND
DEPARTMENT OF NATURAL RESOURCES

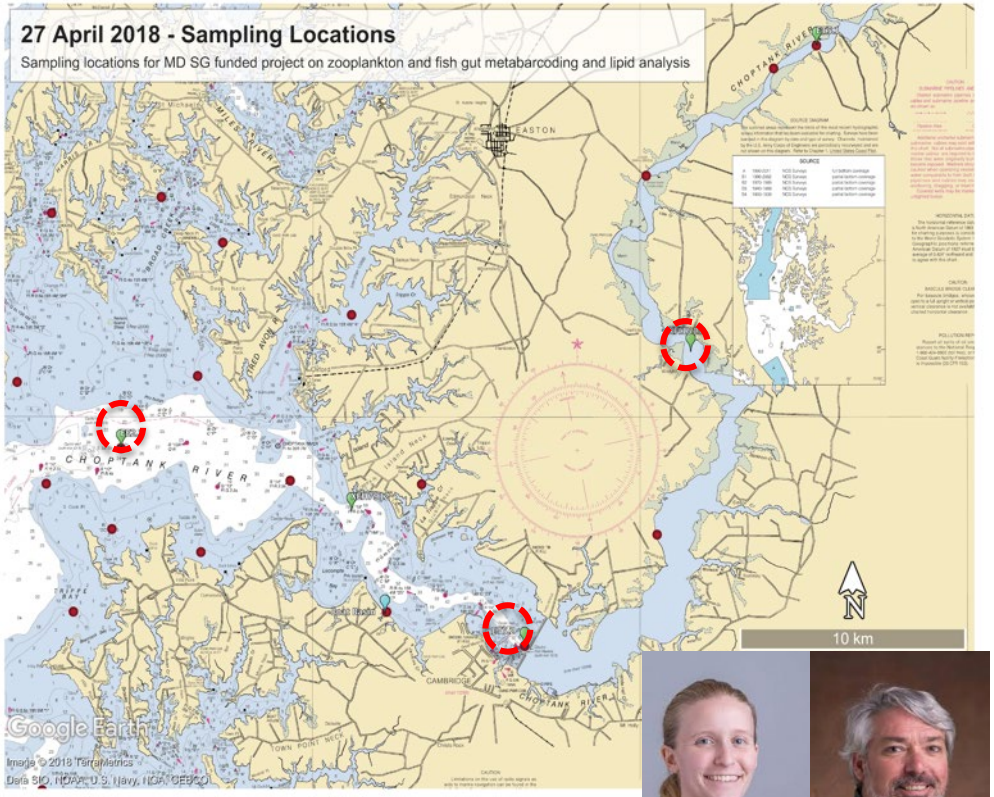
DEERBROOK CHARITABLE TRUST

Metabarcoding of zooplankton diversity in Chesapeake Bay



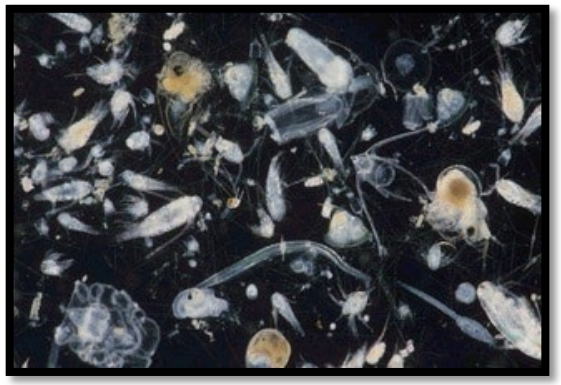
Tows at HPL dock March – May 2017

Choptank river zooplankton monitoring stations

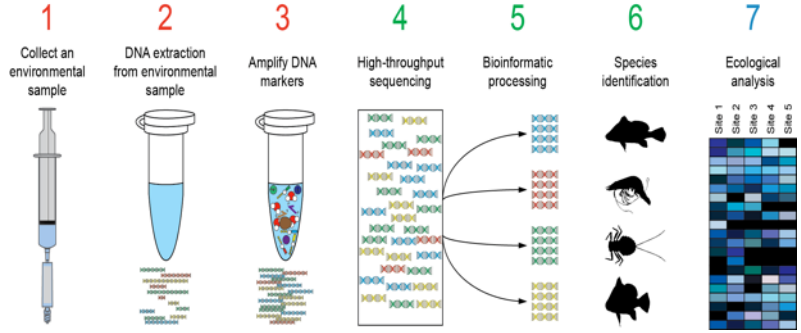


Towards genomic monitoring of zooplankton

- Is a genomic approach to monitoring feasible? Sensitive? Cost-saving? (fish diets)



vs.



https://www.naturemetrics.co.uk/

Data from HPL dock 2017