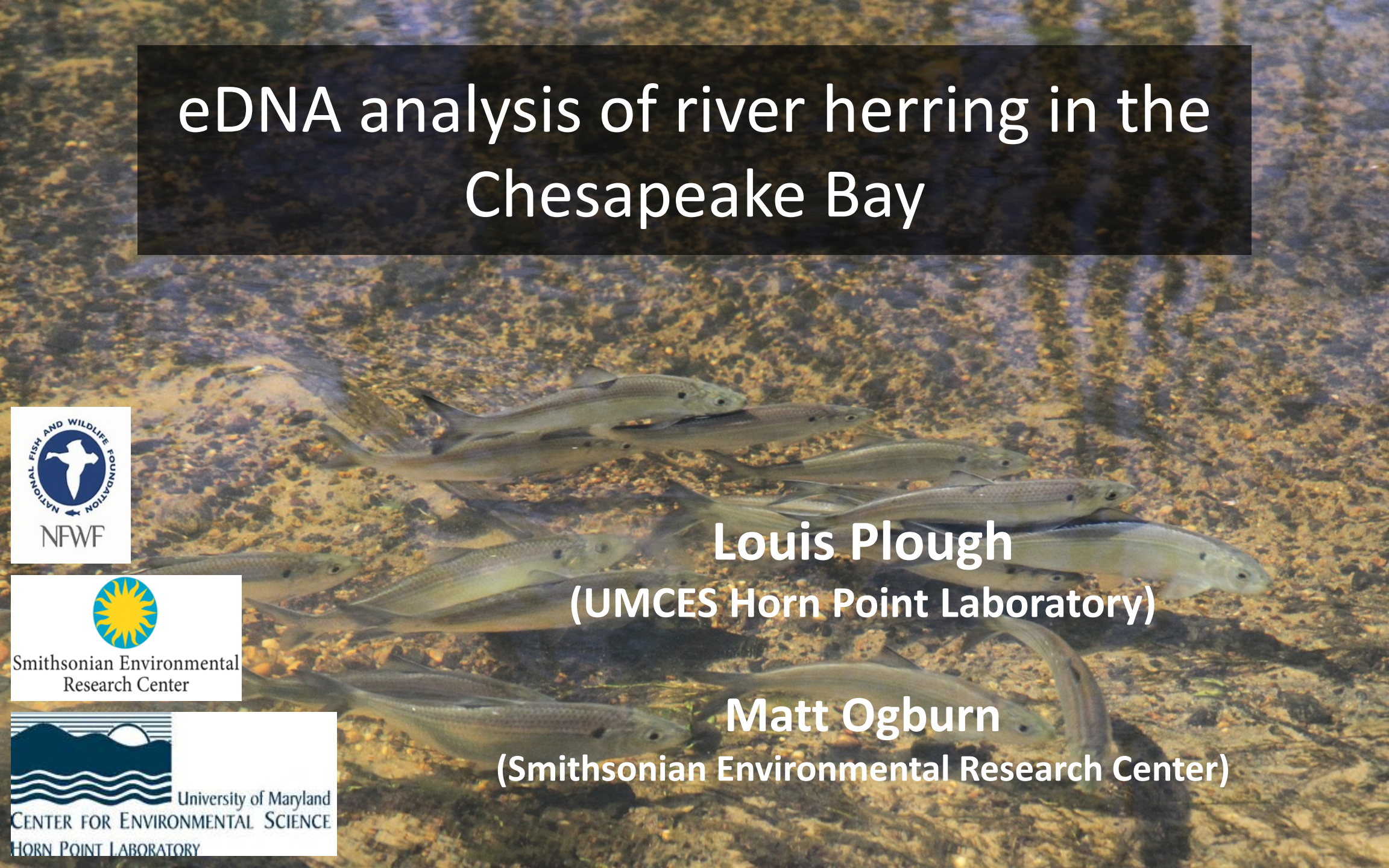
eDNA analysis of river herring in the Chesapeake Bay



Louis Plough
(UMCES Horn Point Laboratory)

Matt Ogburn
(Smithsonian Environmental Research Center)

NFWF

Smithsonian Environmental Research Center

University of Maryland
Center for Environmental Science
Horn Point Laboratory

Baywide monitoring goals

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

Chesapeake Bay River Herring Monitoring Plan

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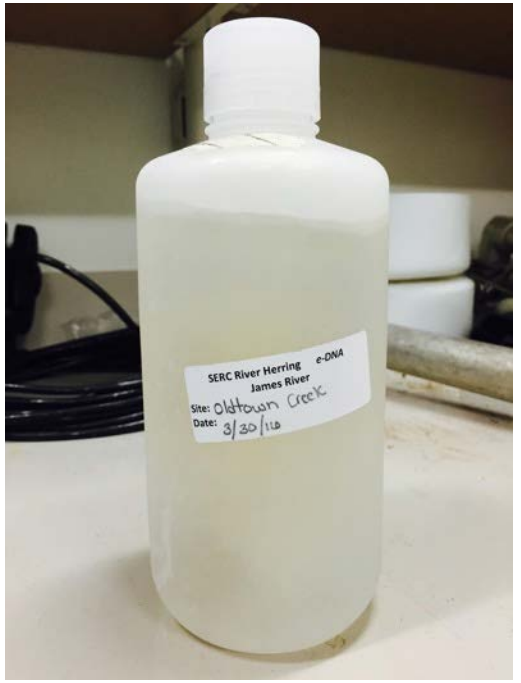
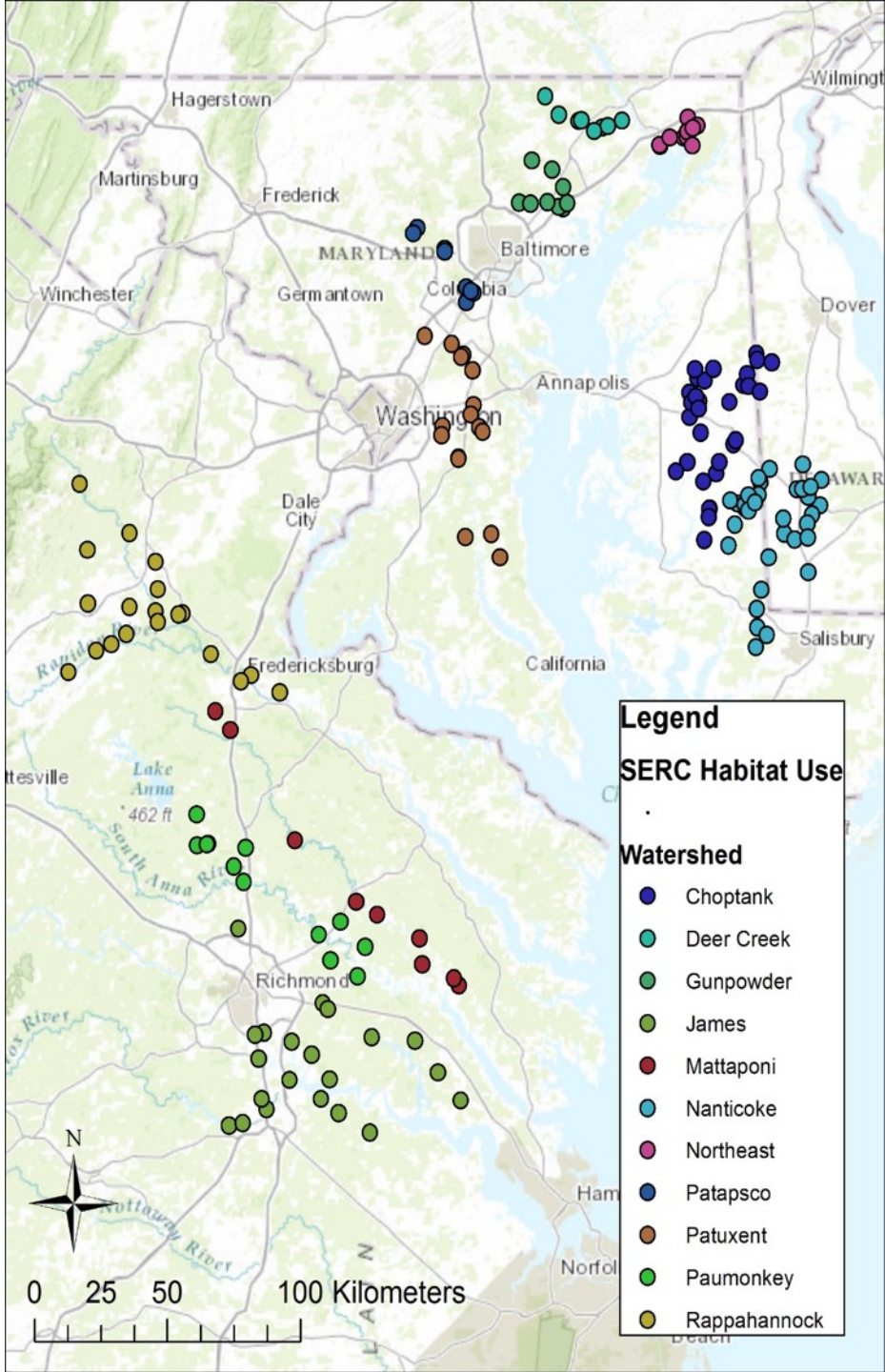
Version 2
6.11.2015

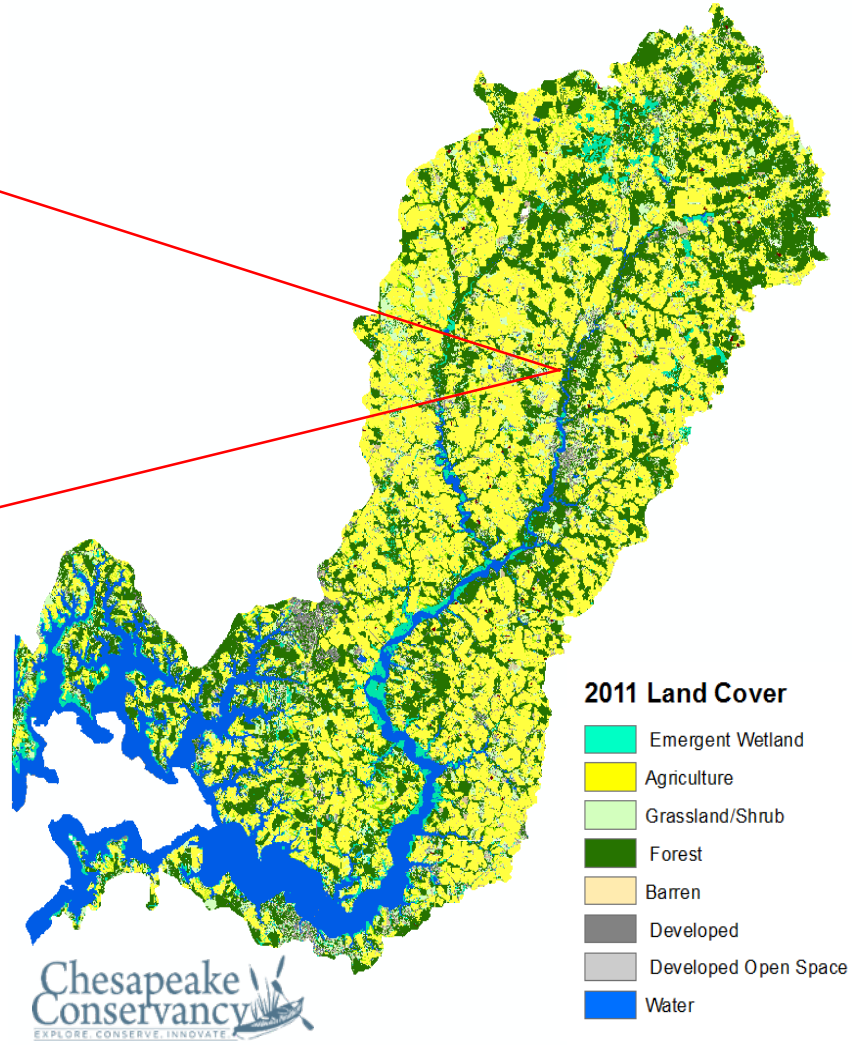
Prepared for:
National Fish and Wildlife Foundation
River Herring Keystone Initiative

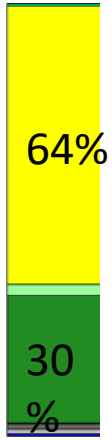
Habitat use sampling



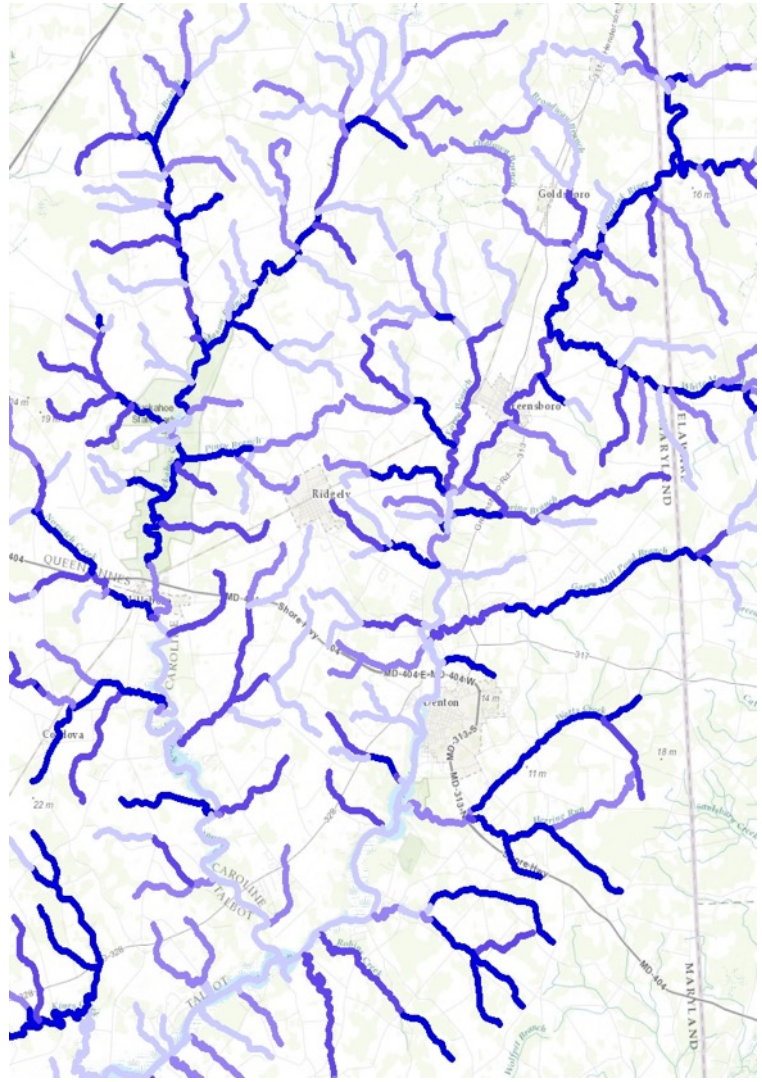


Habitat modeling for restoration prioritization at the sub-watershed or stream-reach scale





- Percent Land Cover type for entire Choptank watershed
- Data from NLCD and Chesapeake Conservancy



Environmental DNA (eDNA) monitoring

Monitor/detect the presence of species based on molecular detection of aqueous DNA

- Environmental DNA (eDNA) is nuclear or mitochondrial DNA that is released into the environment
  - ***Sources****:* secreted feces, mucous, and gametes; shed skin and hair; carcasses.
  - In aquatic environments, eDNA is diluted, lasts about 7–21 days, depending on environmental conditions (Dejean and others, 2011).

- Initially developed to detect **invasive** fish (Asian carp in Great Lakes) and **rare/threatened** species (amphibians)

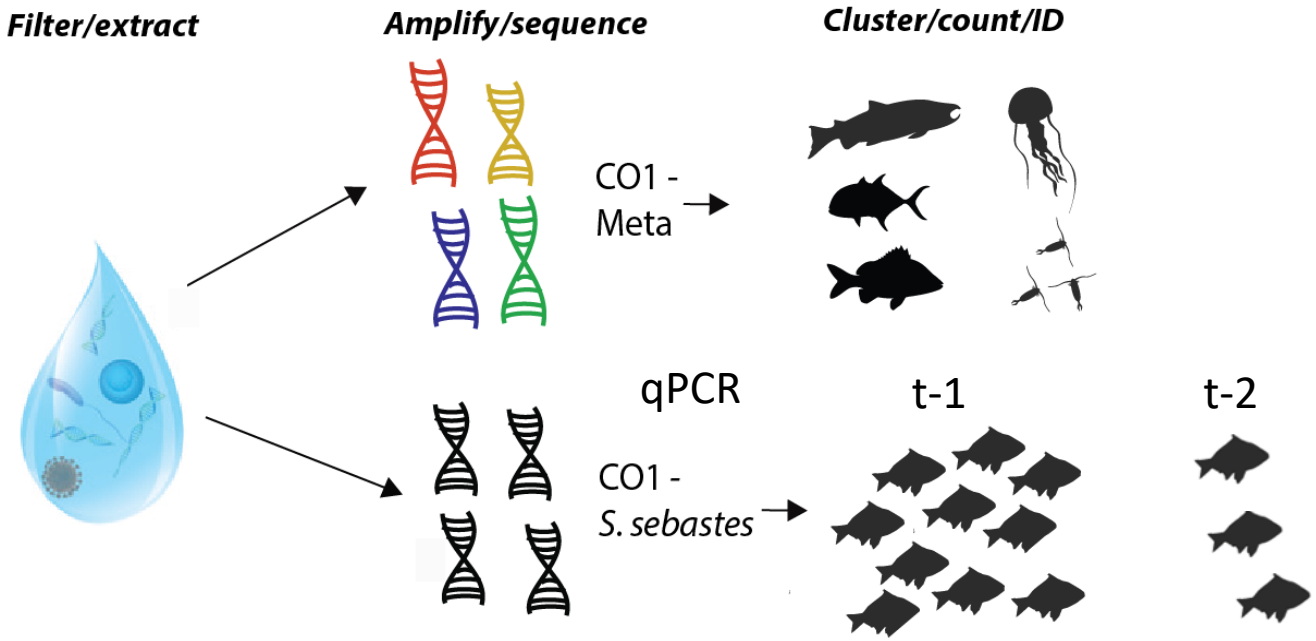
Targeted vs. species specific (eDNA) monitoring

1.)Metabarcoding employs a 'universal' gene or marker

⇒ survey entire communities, but not as quantitative

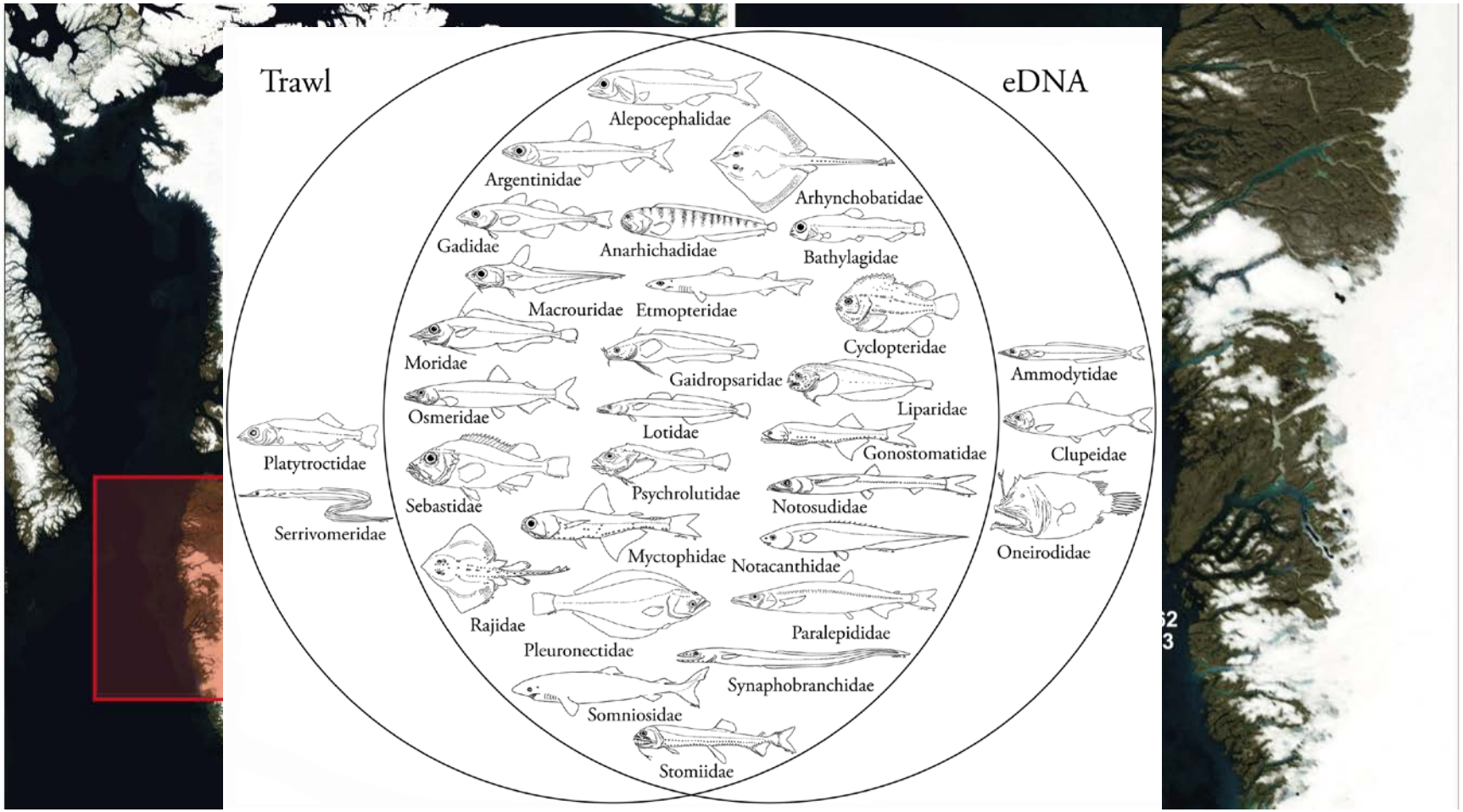
2.) qPCR targets a sequences from a single species

⇒ very quantitative, but single species



Trawl Survey with eDNA (metabarcoding)

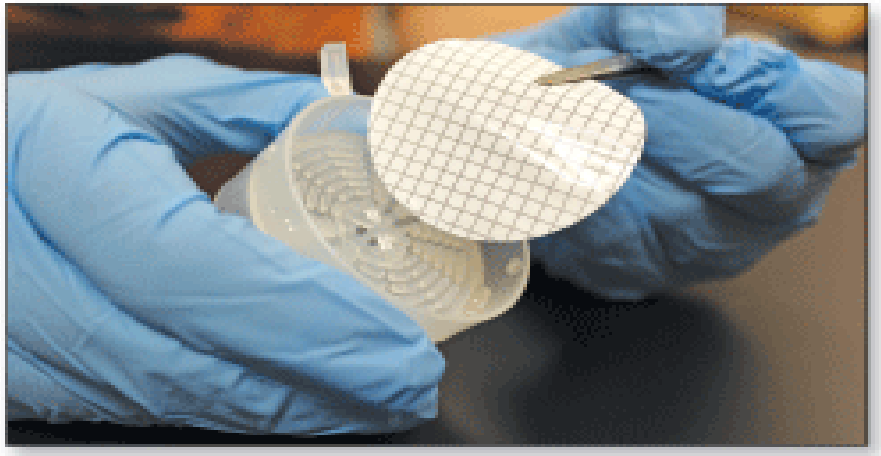
Thomsen et al 2016 PloS ONE.



eDNA development/sampling

<u>A technology with great promise</u>

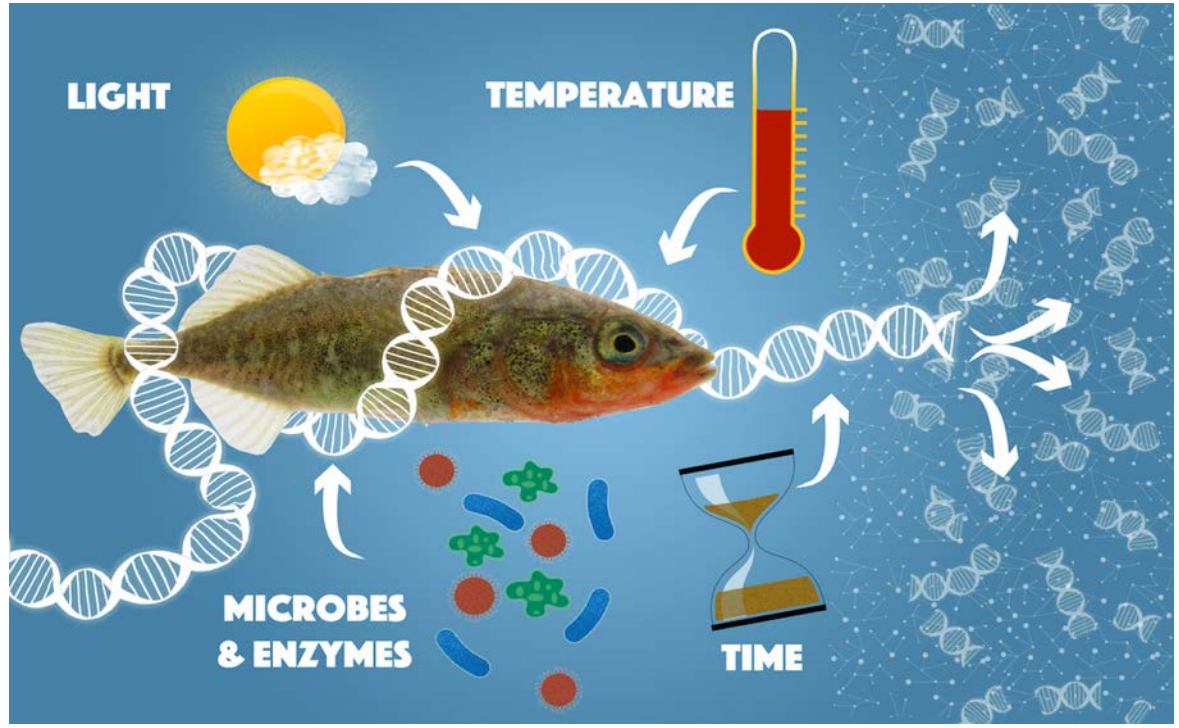
- **PCR-based assay amplifies target species but NOT other species**
- Workflow is simple
  - Sample water, filter, extract DNA, perform PCR
- Possible to sample many sites, cheaply
  - Citizen-science potential



Photos courtesy USGS

eDNA -- challenges

- Filtering of water in sites of variable water quality
  - **Degradation/inhibition**
- Assay development – requires molecular data and testing
- Relationship between molecular abundance and true abundance?
  - Quantitative (q)PCR



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

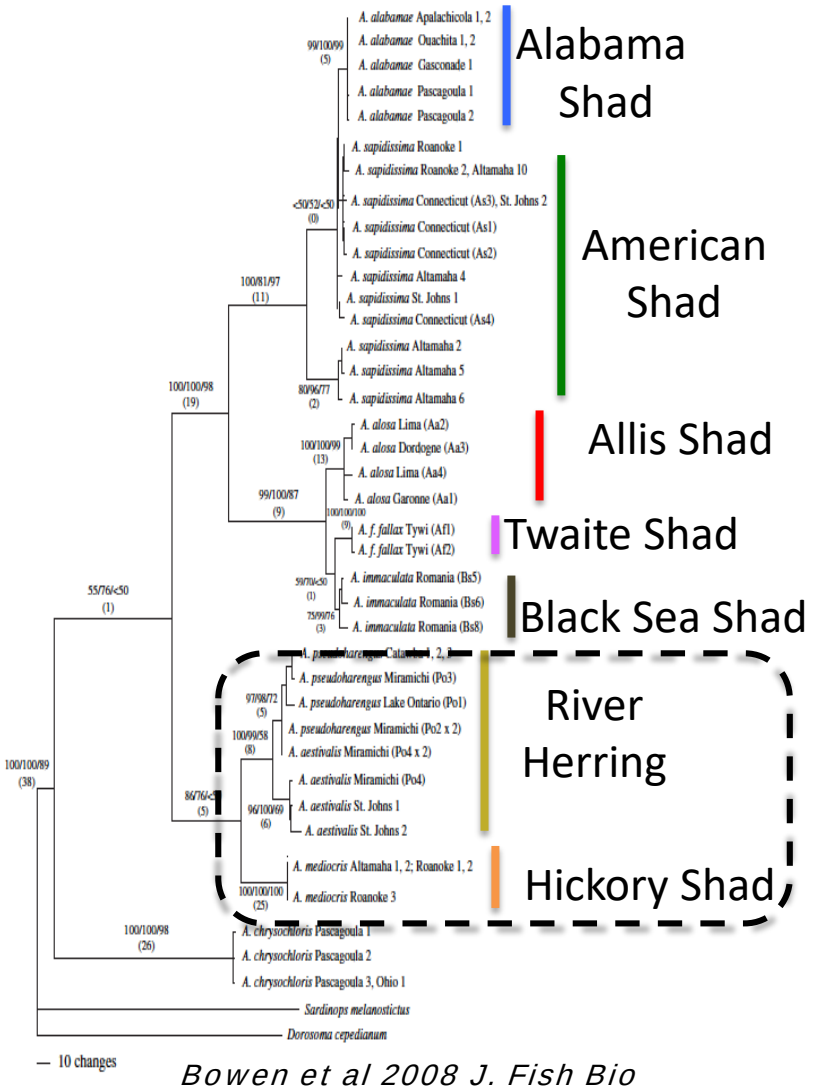
Objectives

- 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

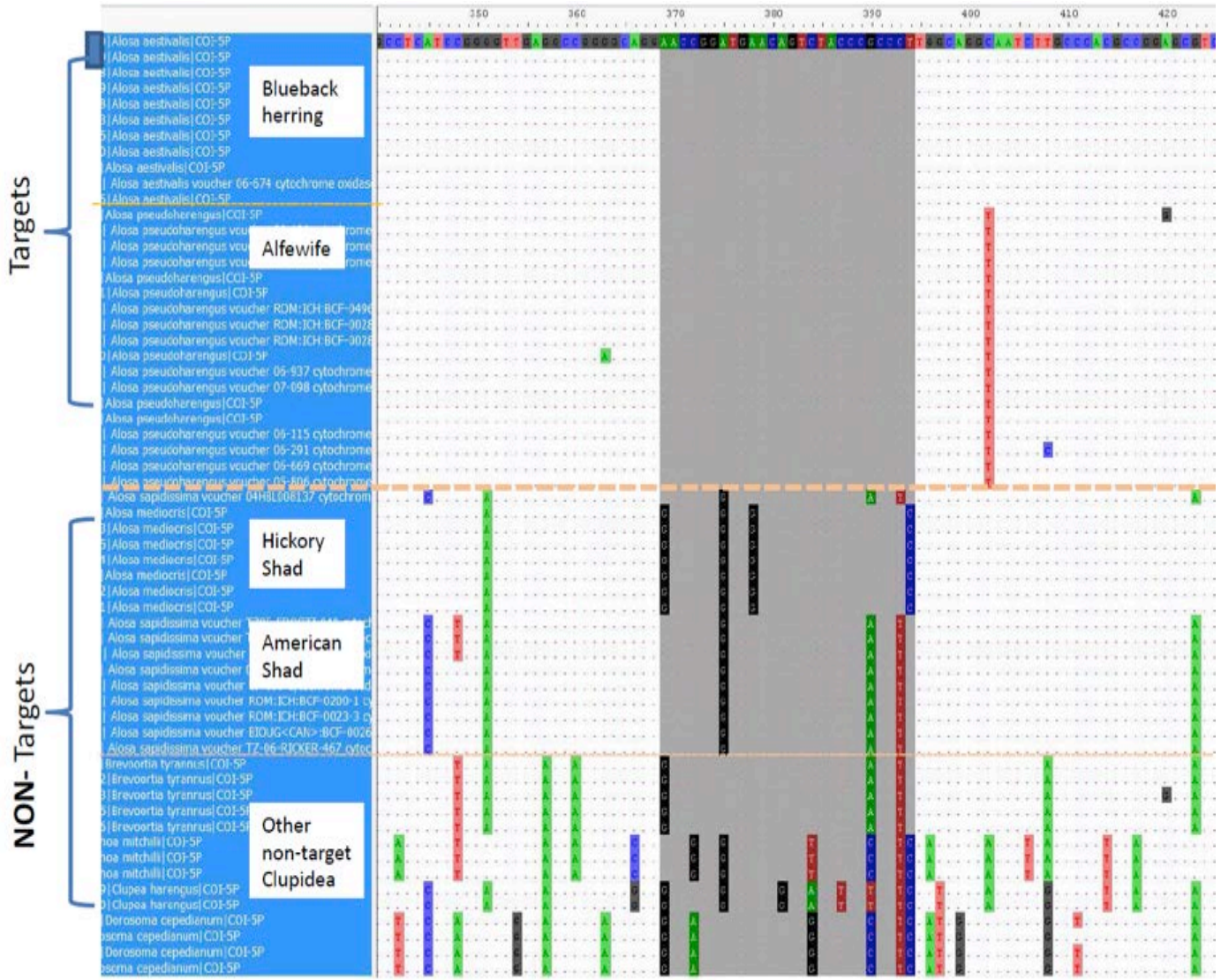
Design/efficiency of the assay

- 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.



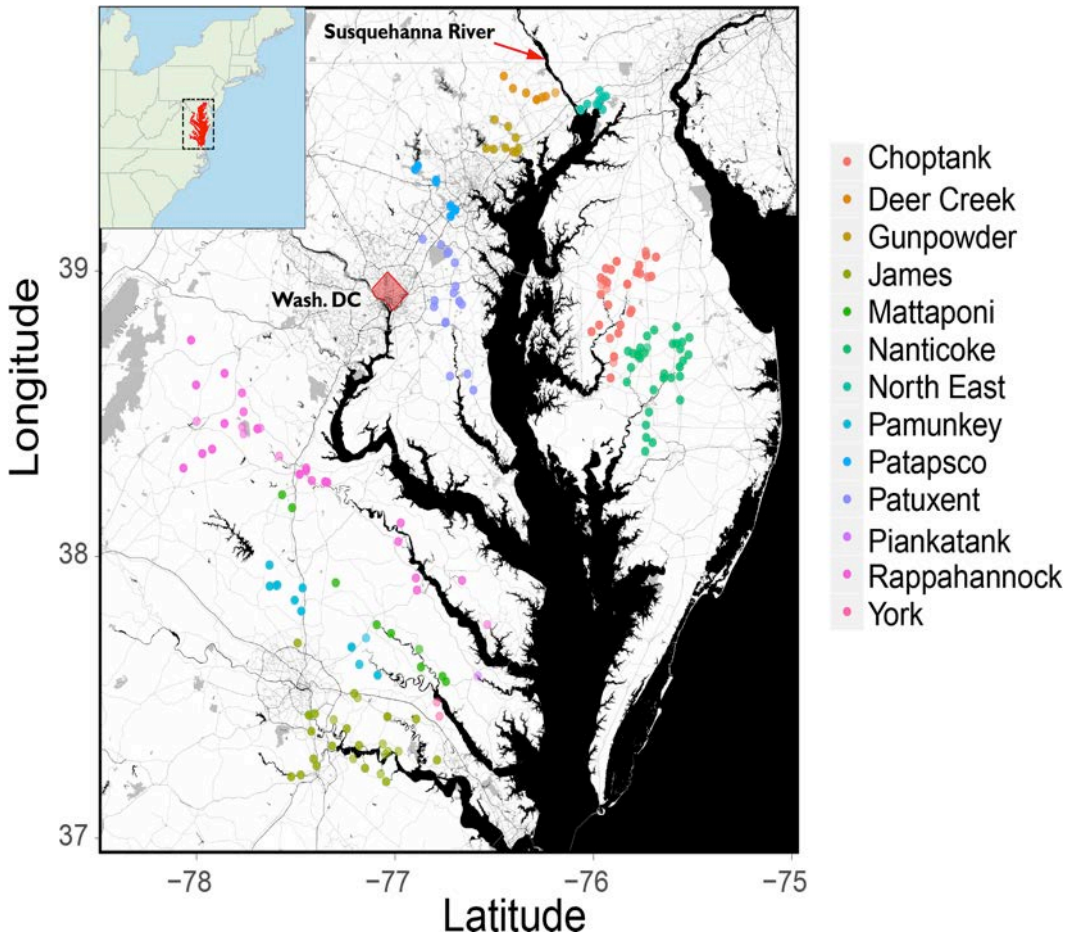
Bowen et al 2008 J. Fish Bio

Sequence alignment for the assay

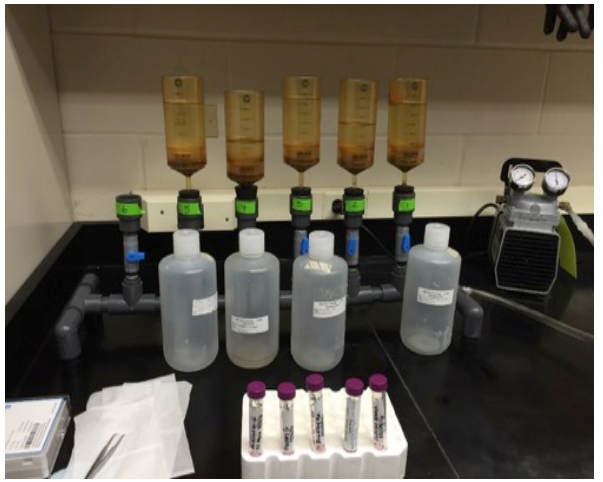


Cytochrome Oxidase subunit 1 (CO1)

Assay validation and field testing in Chesapeake Bay



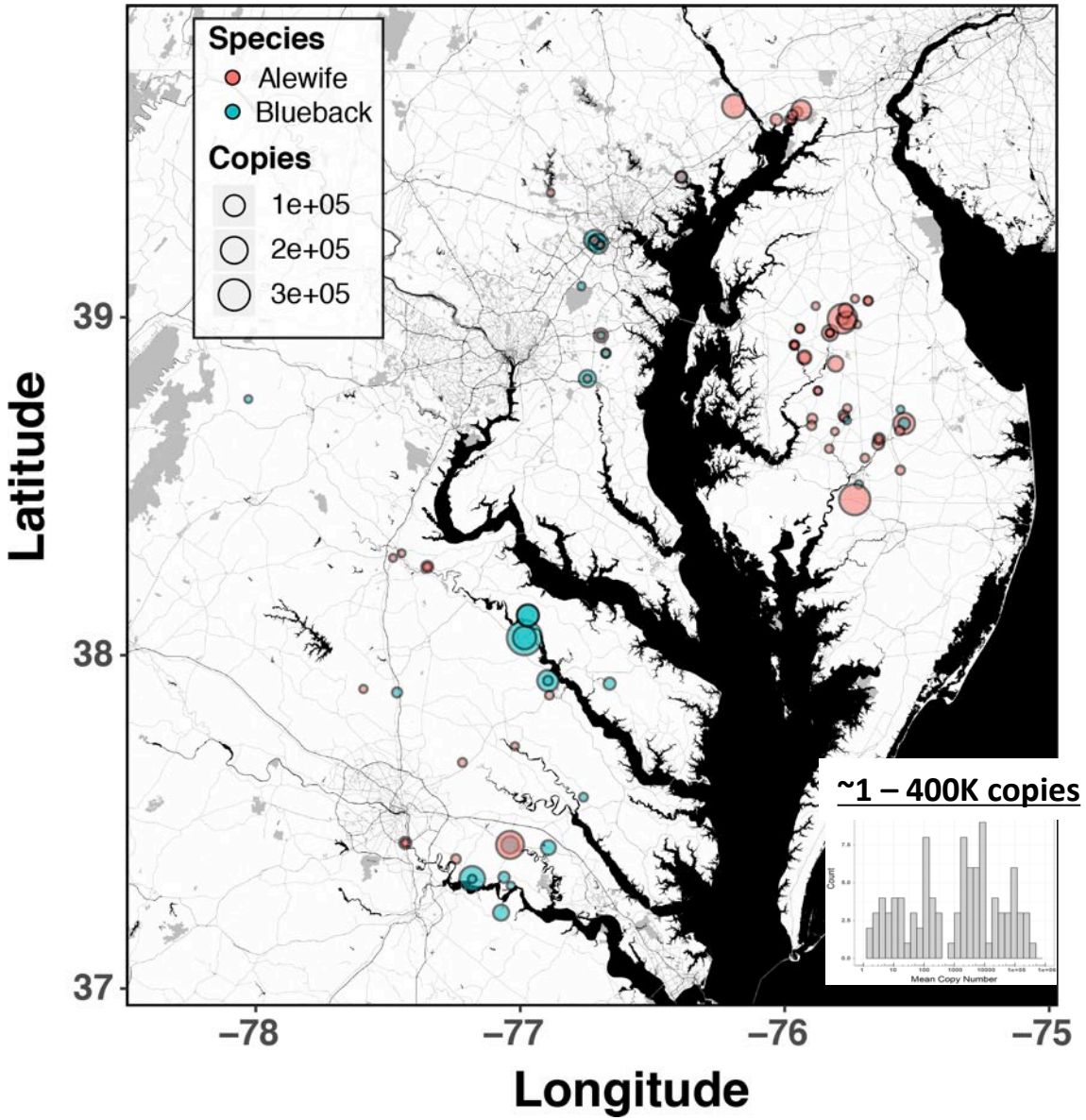
Plough et al. 2018 *PLoSOne*



Rose Geranio

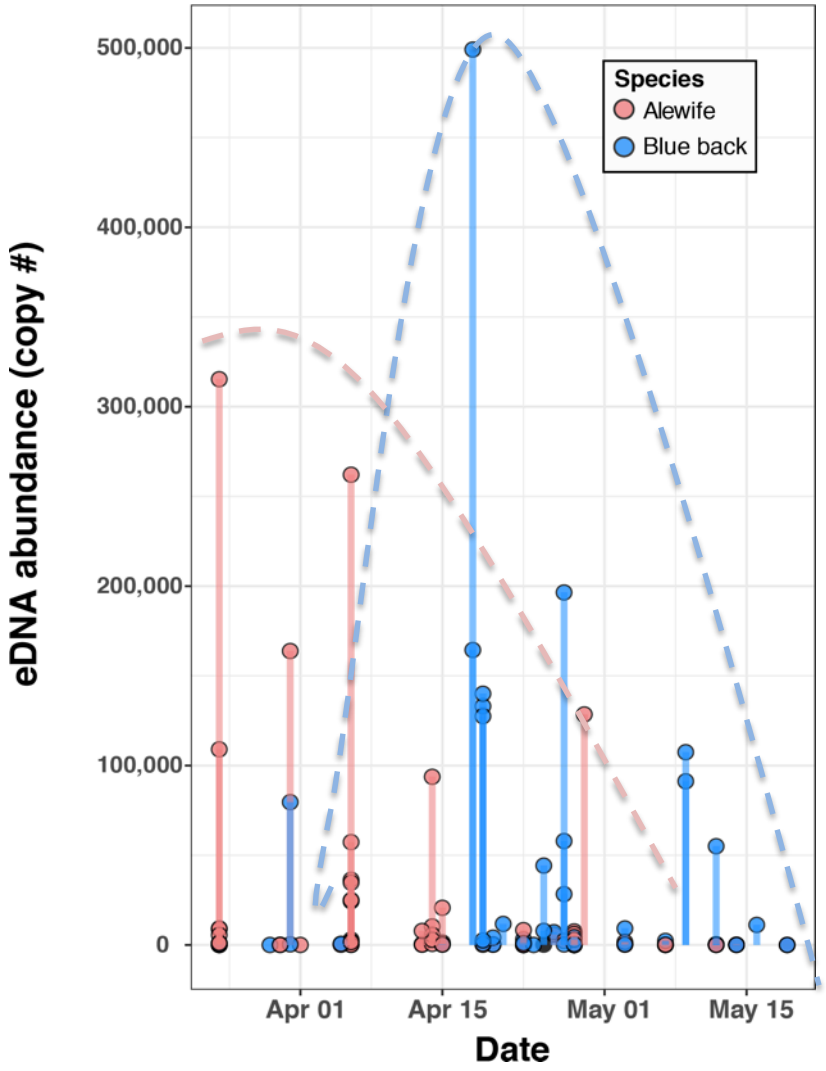
Shore-specific patterns of river herring habitat use

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



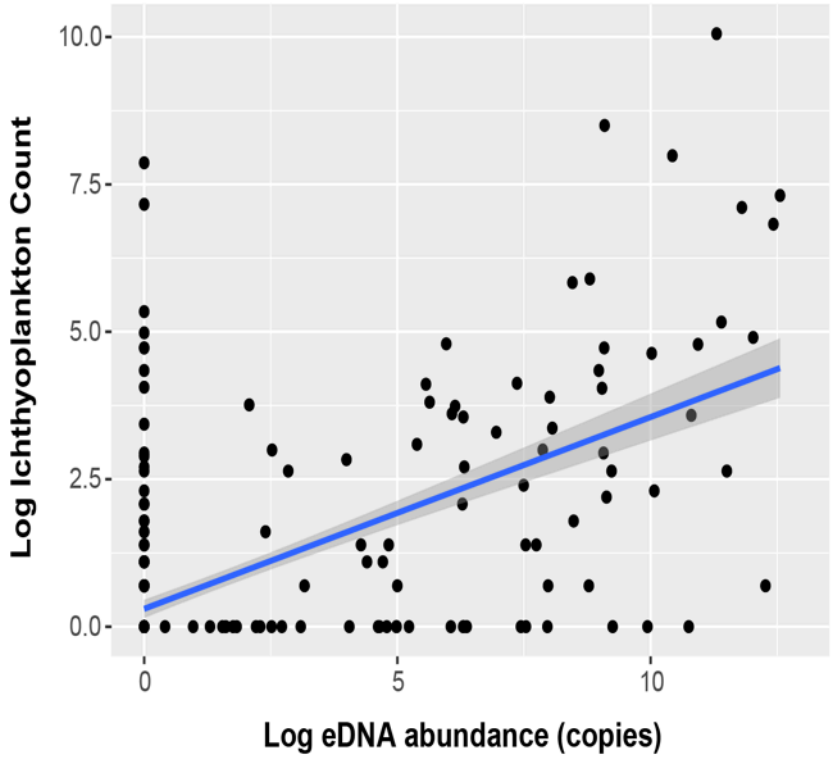
eDNA recovers timing of herring spawning

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



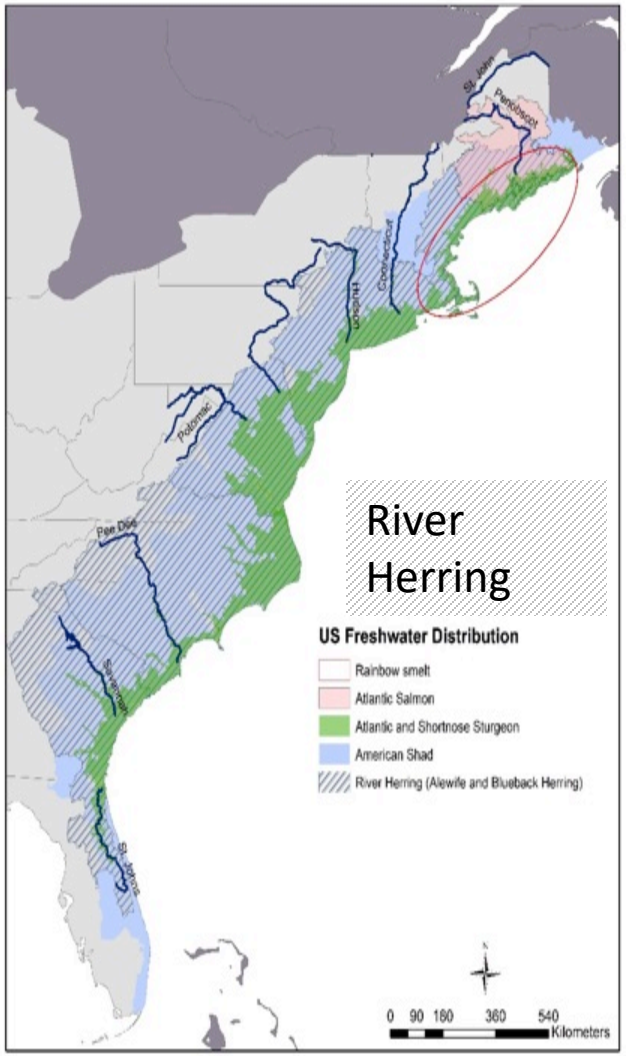
Comparison of eDNA with traditional survey methods

- 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*)



New directions….

- Using eDNA to examine habitat use of river herring in the Chesapeake Bay
  - Species distribution modeling (SDM) and analysis of fish passage

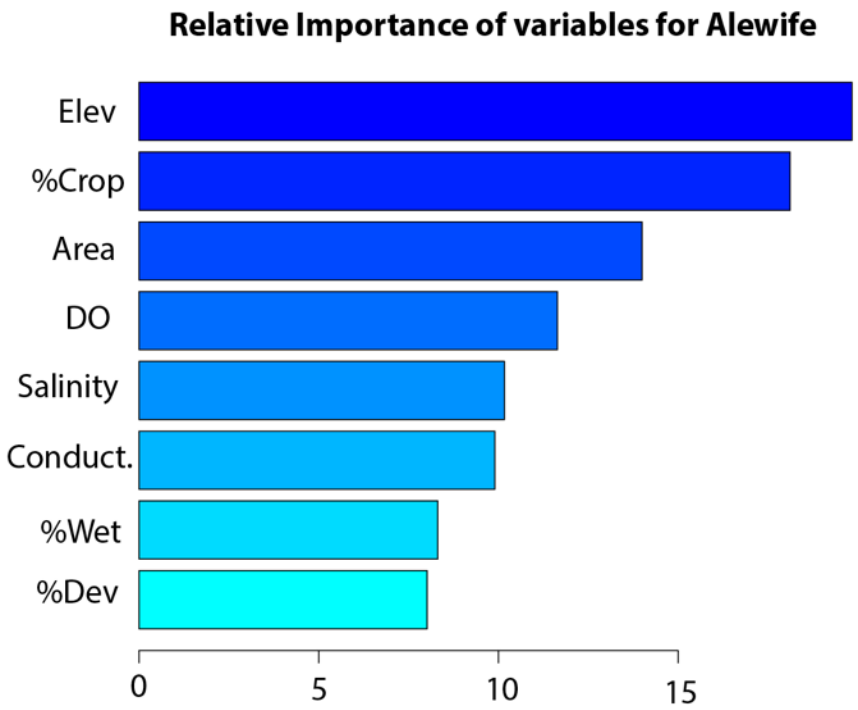
- Automated or remote sampling?

Species distribution modeling w/ eDNA

- Presence and eDNA abundance data to examine effect of landscape and environmental variables
  - environmental data (e.g. DO, salinity, temperature)
  - landscape variables (% land cover, watershed area, elevation)

- Non-linear modeling via boosted regression trees (BRT)
  - relative influence of each variable estimated

SDM results

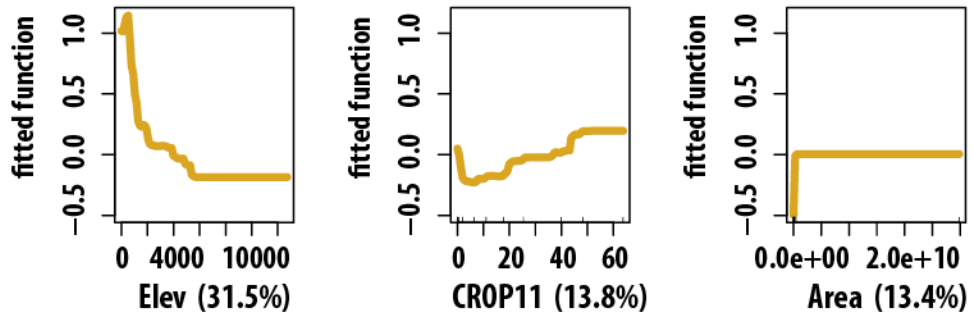


Variable	Full name (units)
%Crop	Percent landcover designated as Agriculture
%Dev	Percent landcover designated as Development
PercentWet	Percent landcover designated as Agriculture
Elev	Elevation (meters)
Area	Watershed Area (hectares)
DSArea	Downstream Watershed Area (hectares)
MeanDO	Mean dissolved oxygen
Mean Salinity	Mean Salinity
Mean conductivity	Mean Conductivity

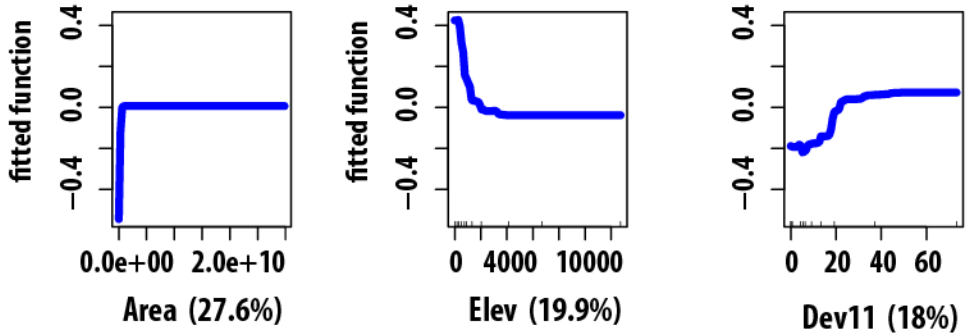
- Elevation significant for all analyses
  - Area too
- % Crop important for Alewife
- % Dev. important for blue back
- DO often top 4 of all variables, but never above.

Partial Importance plots…

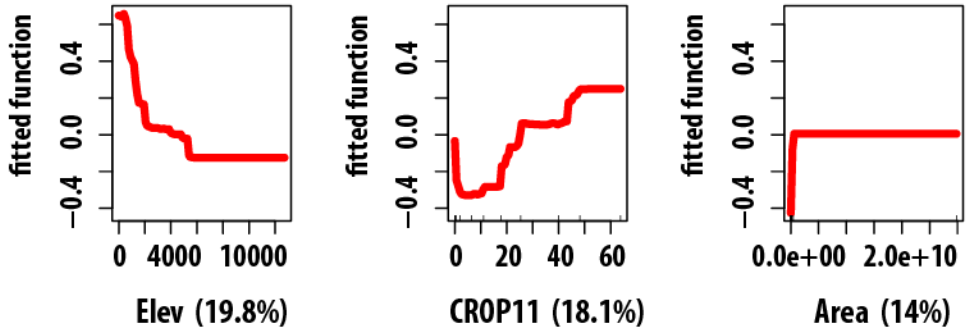
River herring



Blue back herring



Alewife



Automated and remote sampling of eDNA

Have…

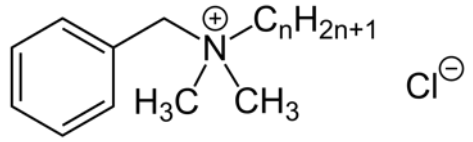
(Courtesy of Ian Enochs, NOAA)

SUBSURFACE AUTOMATIC SAMPLERS (SAS)

Getting soon!

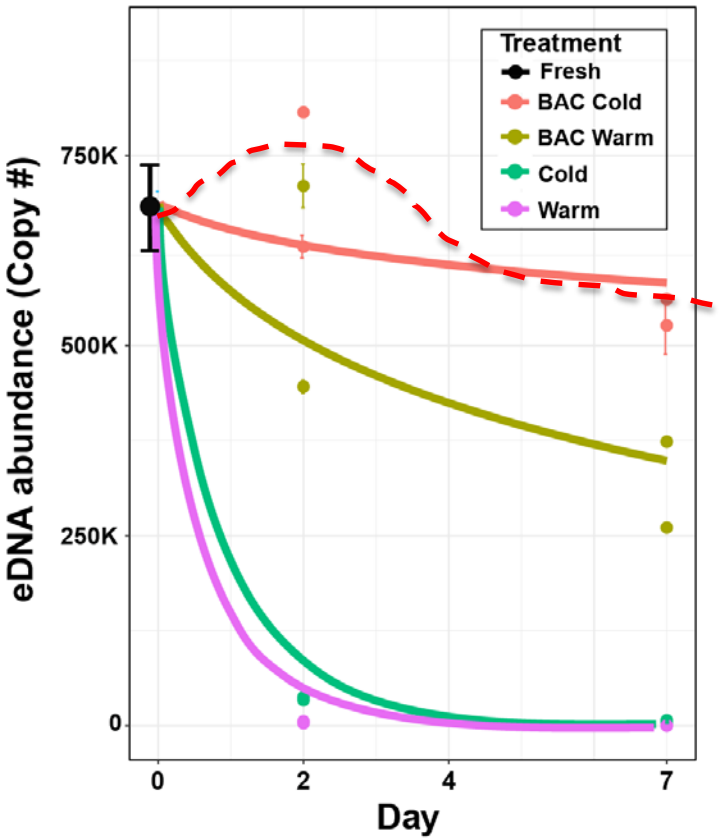
Preservation of eDNA in the field

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



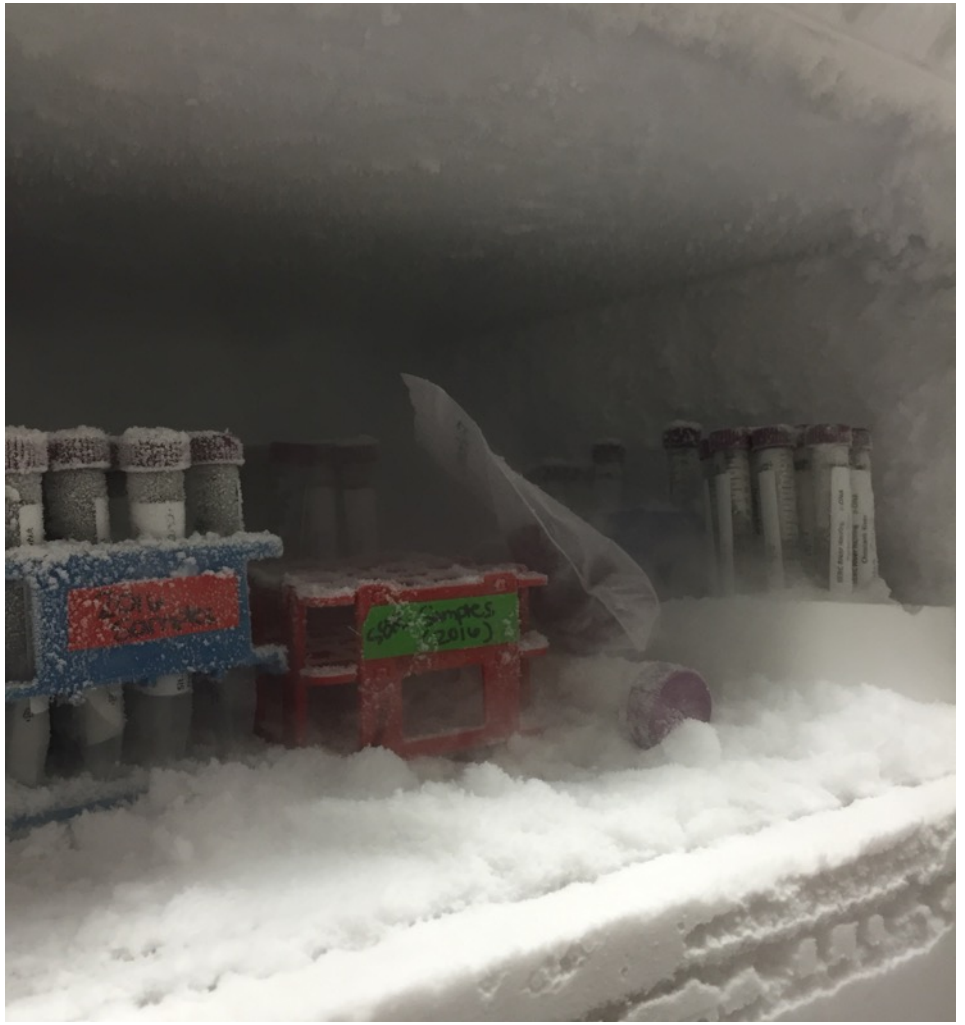
n = 8, 10, 12, 14, 16, 18

- 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***



Summary/future work…

- Completion of habitat analyses, including **fish passage***
- Planning work on high-resolution remote sampling (ISCO) of herring and shad eDNA for run counts
- eDNA analysis in other systems (New Jersey, Virginia, MD Coastal bays…)



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