Chesapeake Bay River Herring Habitat Use Study

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NFWF

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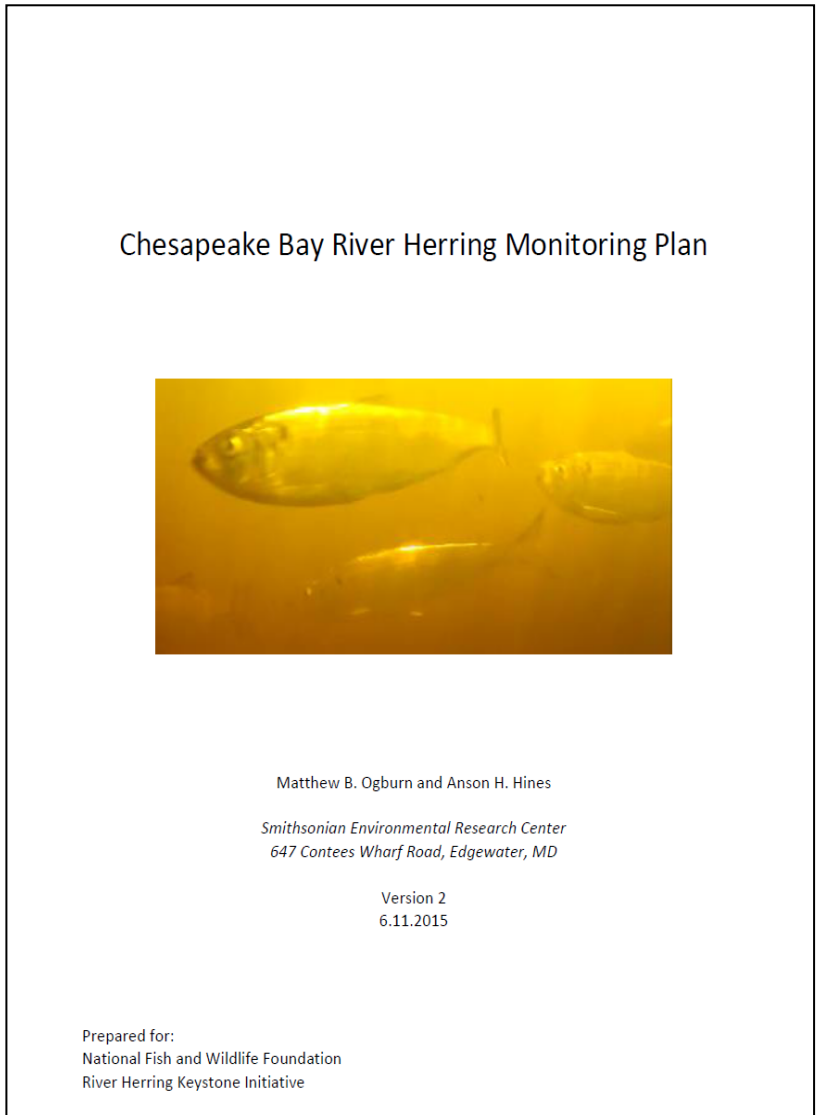
Baywide monitoring goals

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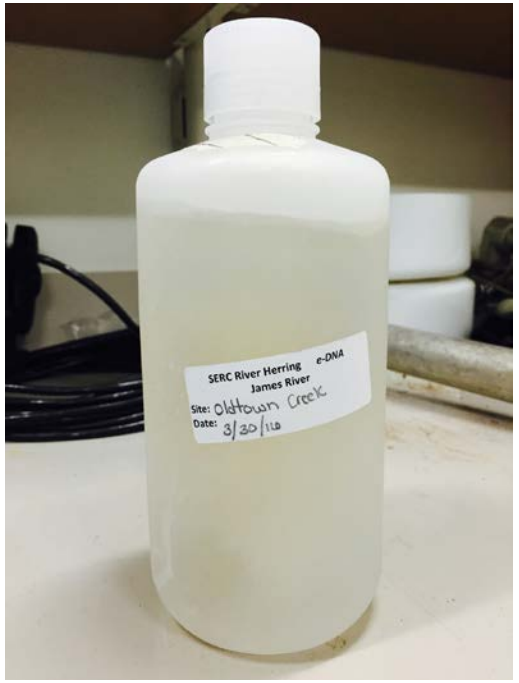
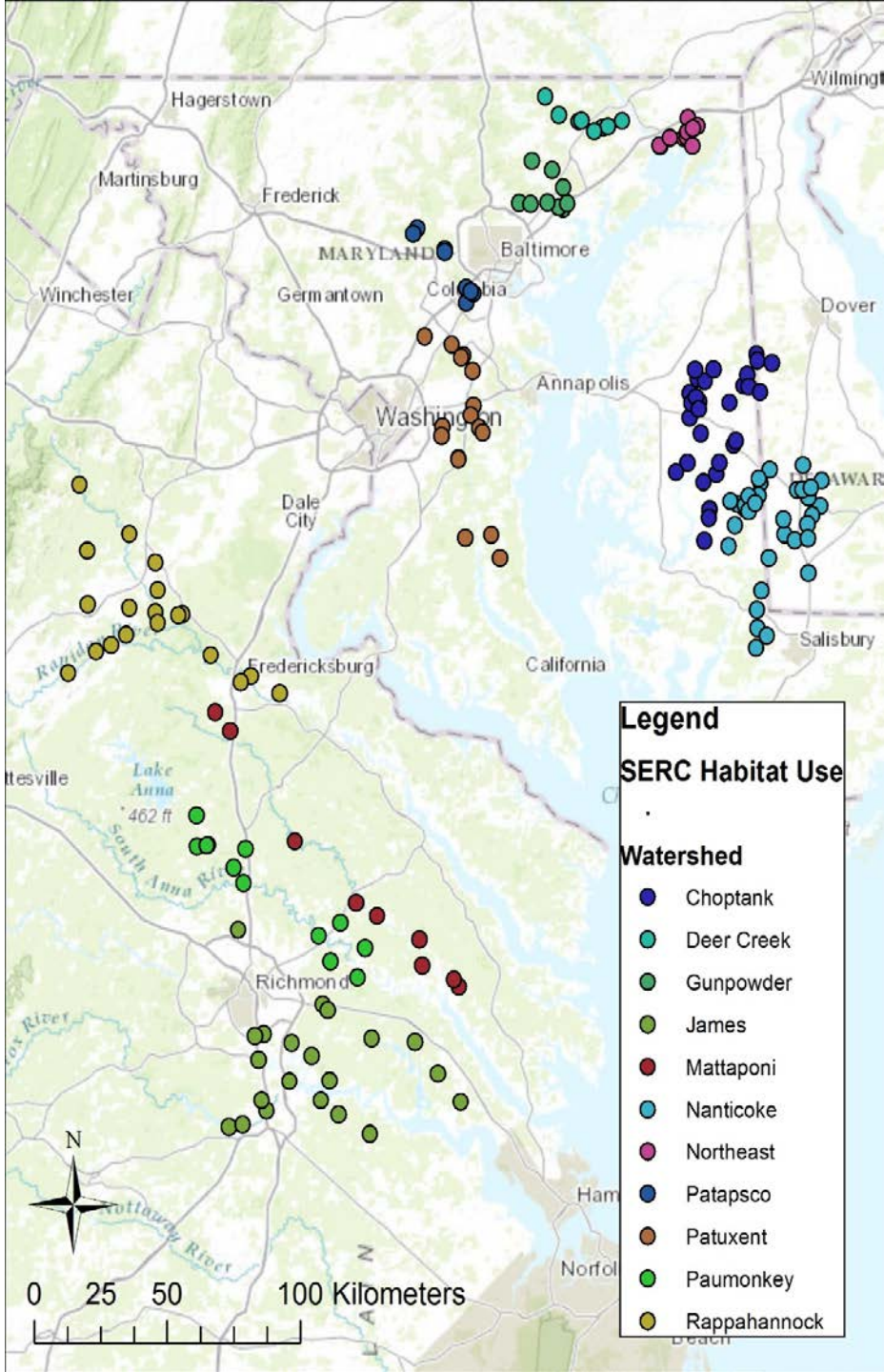
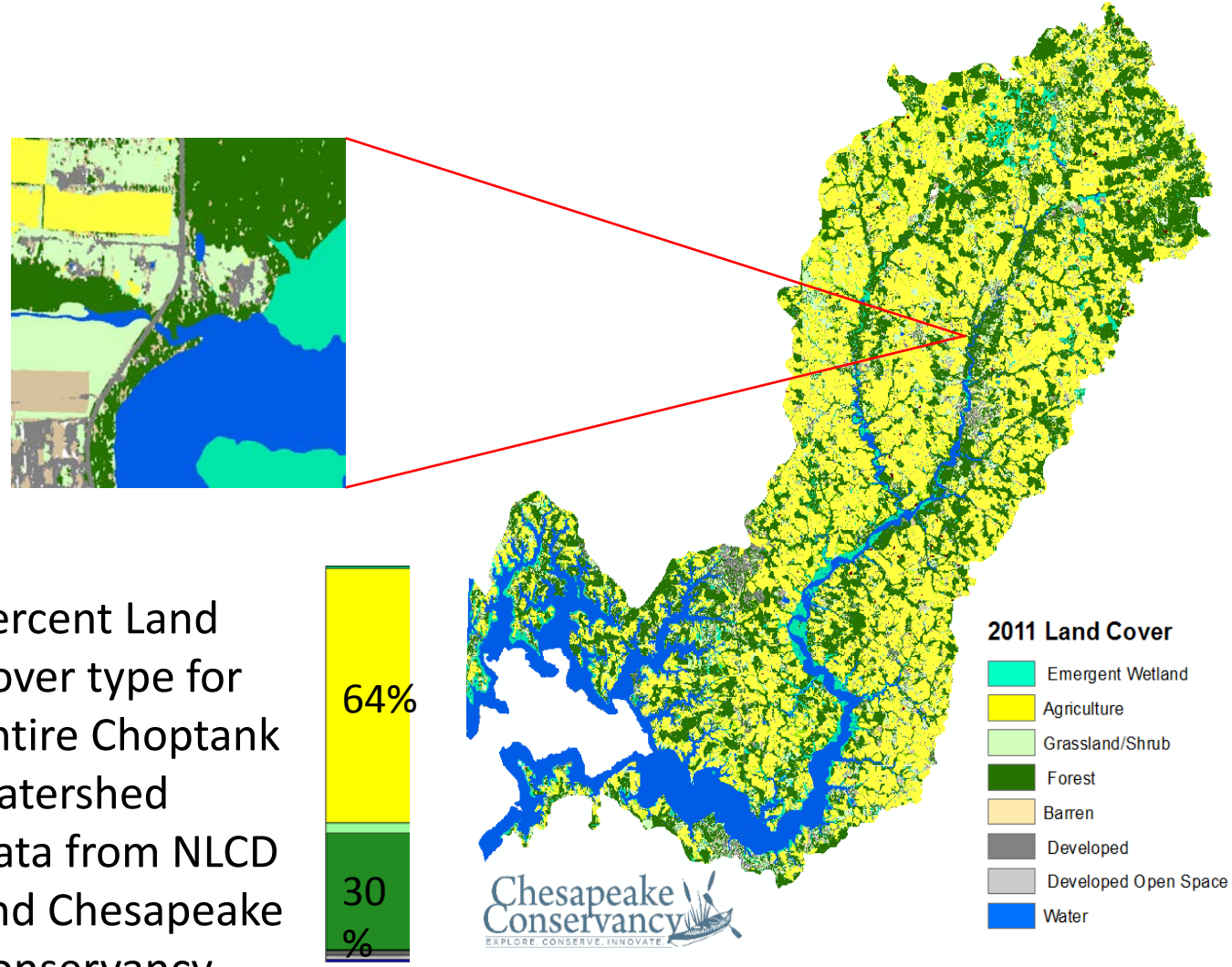
Prepared for:
National Fish and Wildlife Foundation
River Herring Keystone Initiative

Habitat use sampling

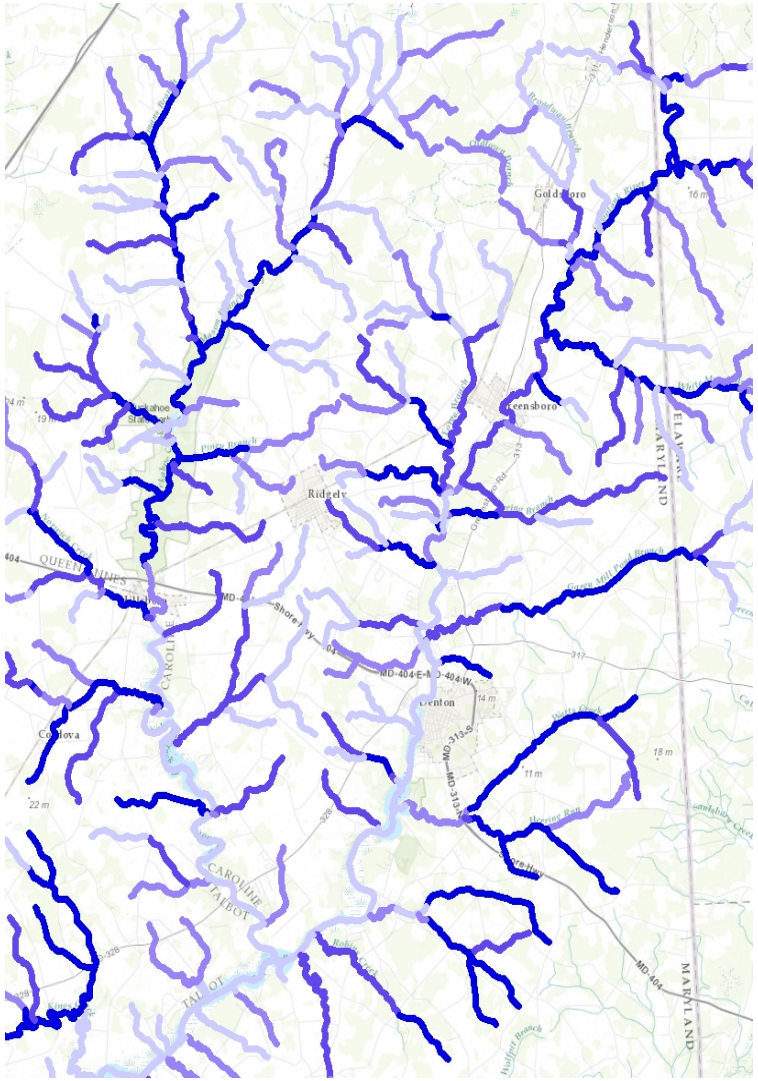




Habitat modeling for restoration prioritization at the sub-watershed or stream-reach scale



- 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)

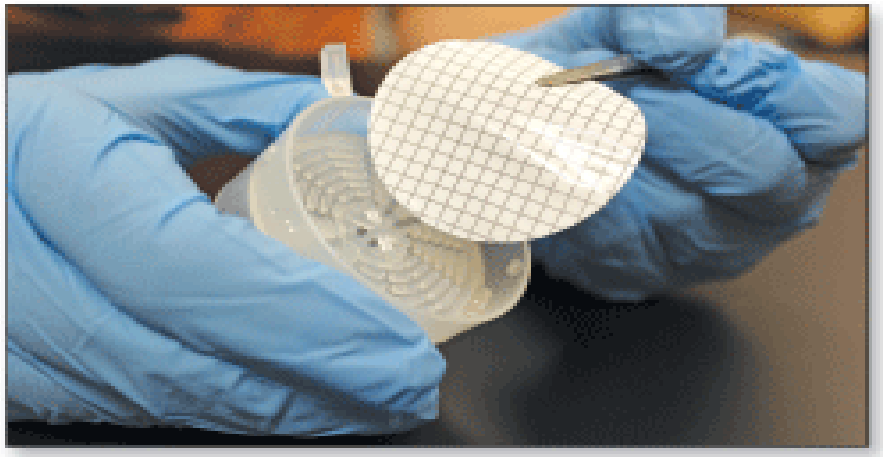
eDNA development/sampling

<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

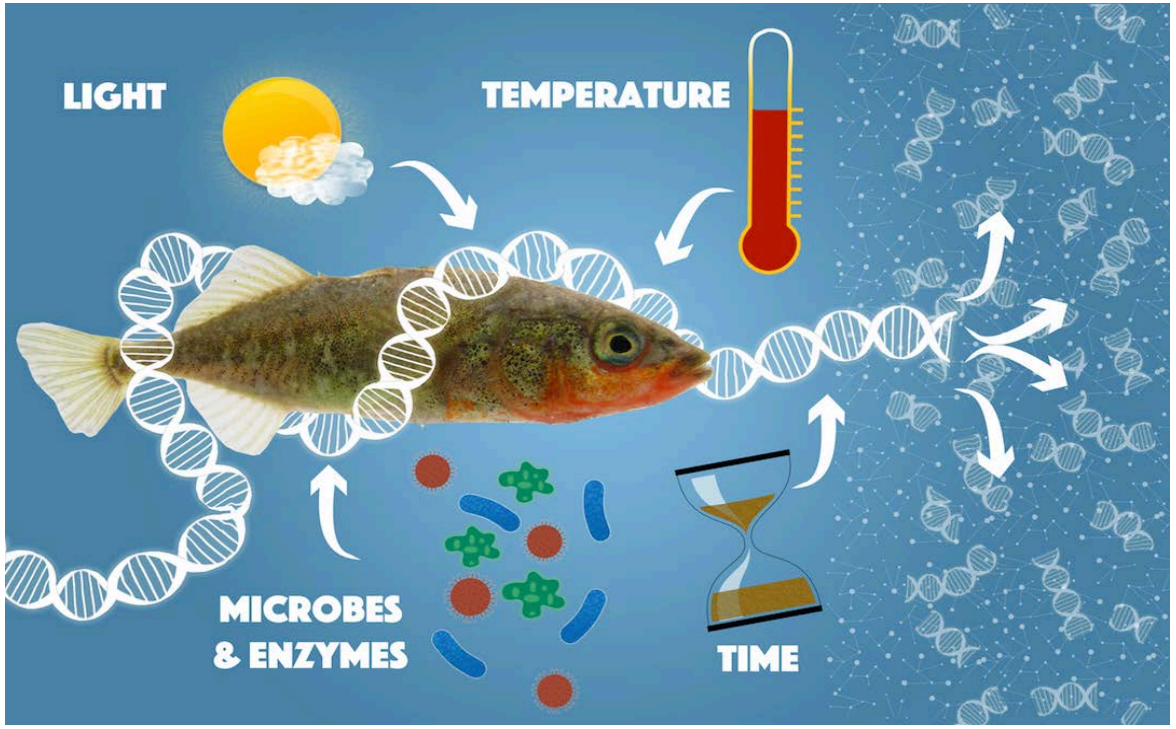
- Possible to sample many sites, cheaply
  - Citizen-science potential



Photos courtesy USGS

eDNA -- challenges

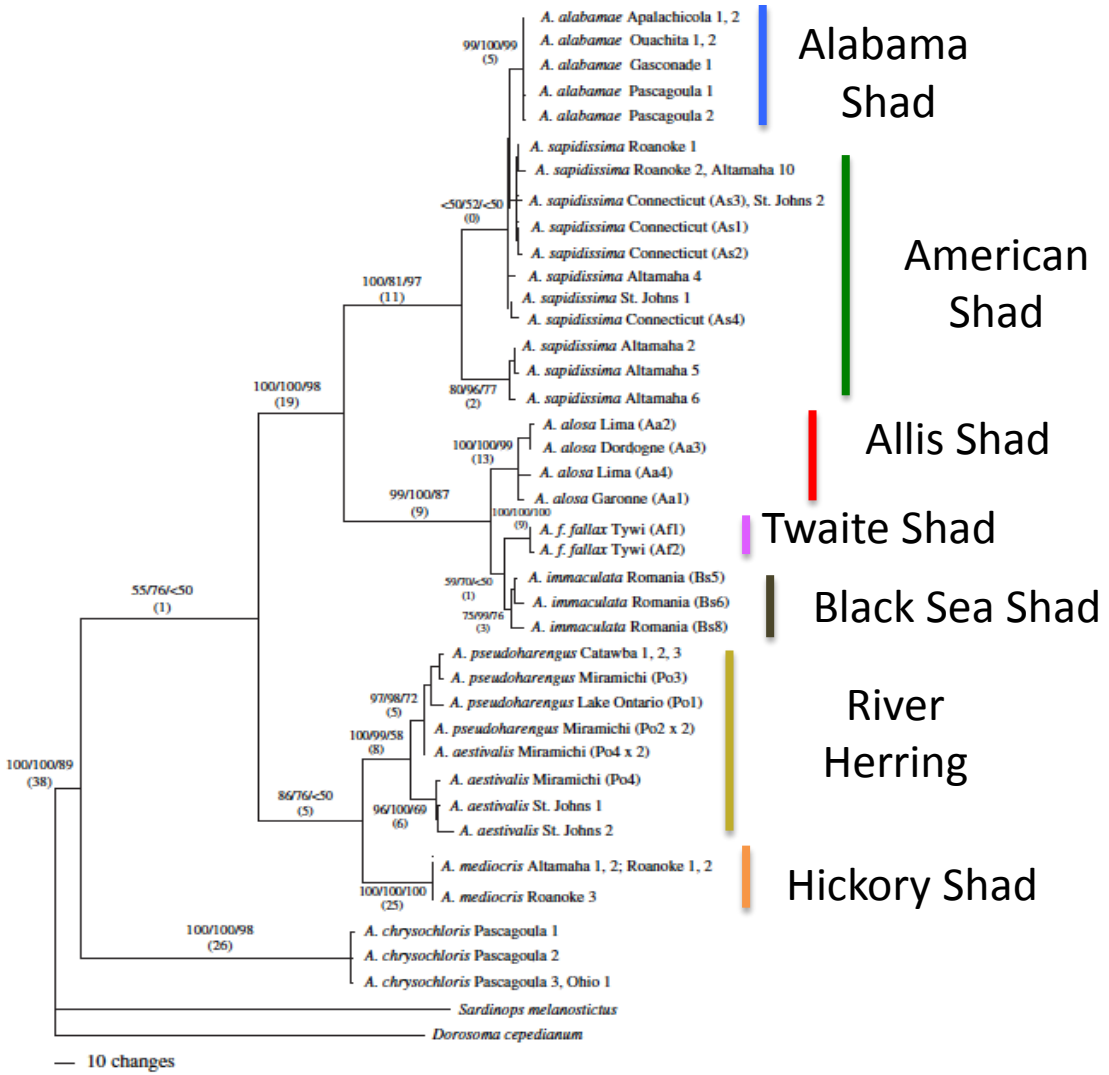
- Filtering of water in sites of variable water quality
  - **Degradation/inhibition**
- Assay development – requires molecular data and testing
- Relationship between molecular abundance and true abundance?
  - Quantitative (q)PCR



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

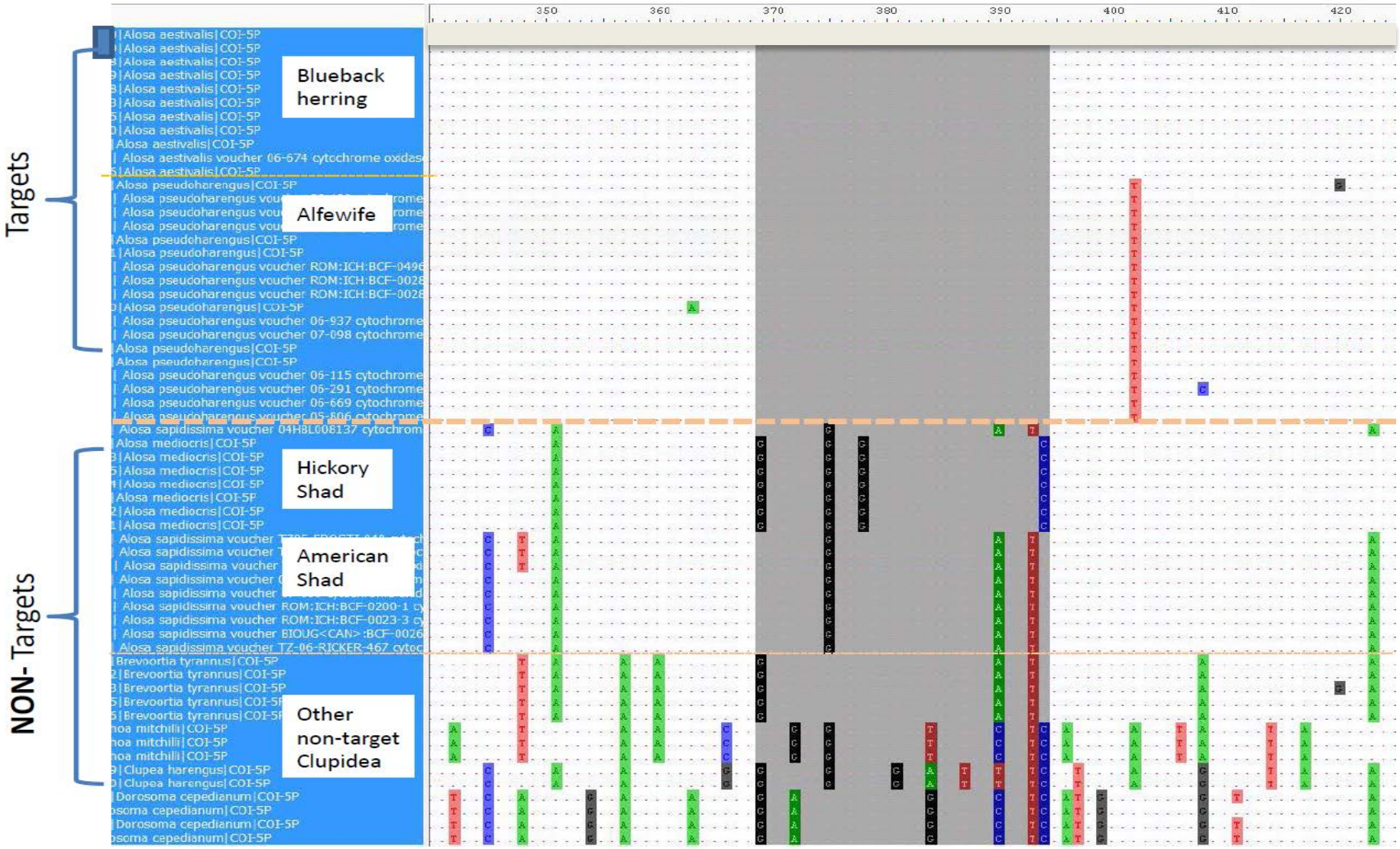
Design of assay

- Mitochondrial data publicly available for Alosids and Clupeidae
- River herring (Alewife and Blueback) not monophyletic
- **River herring have high sequence similarity to Hickory Shad**



Bowen et al 2008 J. Fish Bio

Sequence alignment for the assay



Cytochrome Oxidase subunit 1 (CO1)

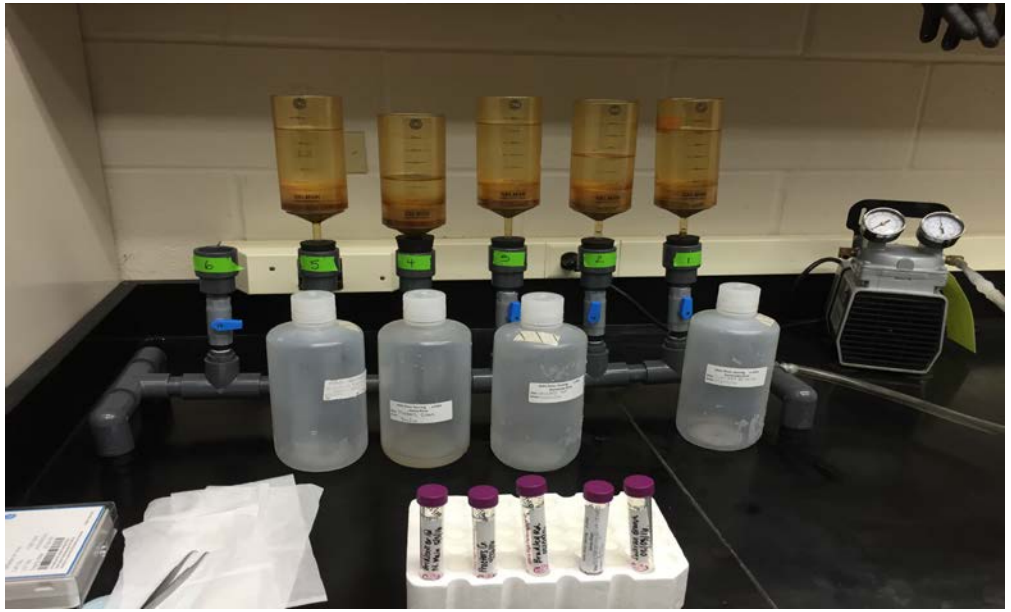
Progress to date…

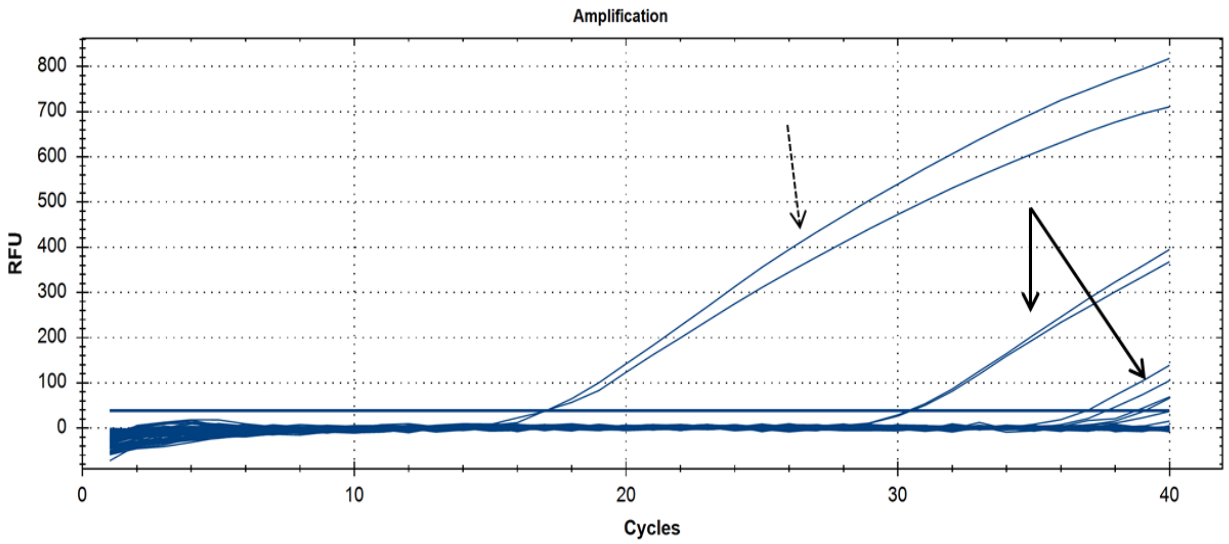
1) Assay development ✔

2) In vitro testing ✔

- PCR with Herring, American Shad, Hickory shad samples, Bay anchovy, Gizzard shad
- Amplification only of Herring targets < 45 cycles*

3) Field testing ✔





Assay details

Extraction/Filtering

- 1µm CN filter
- Omega EZWater kit
  - Bead beating, large volume with spin column finish
  - Up to ~70ng/ul
- All pre-pcr work done in dedicated tissue room

qPCR assay

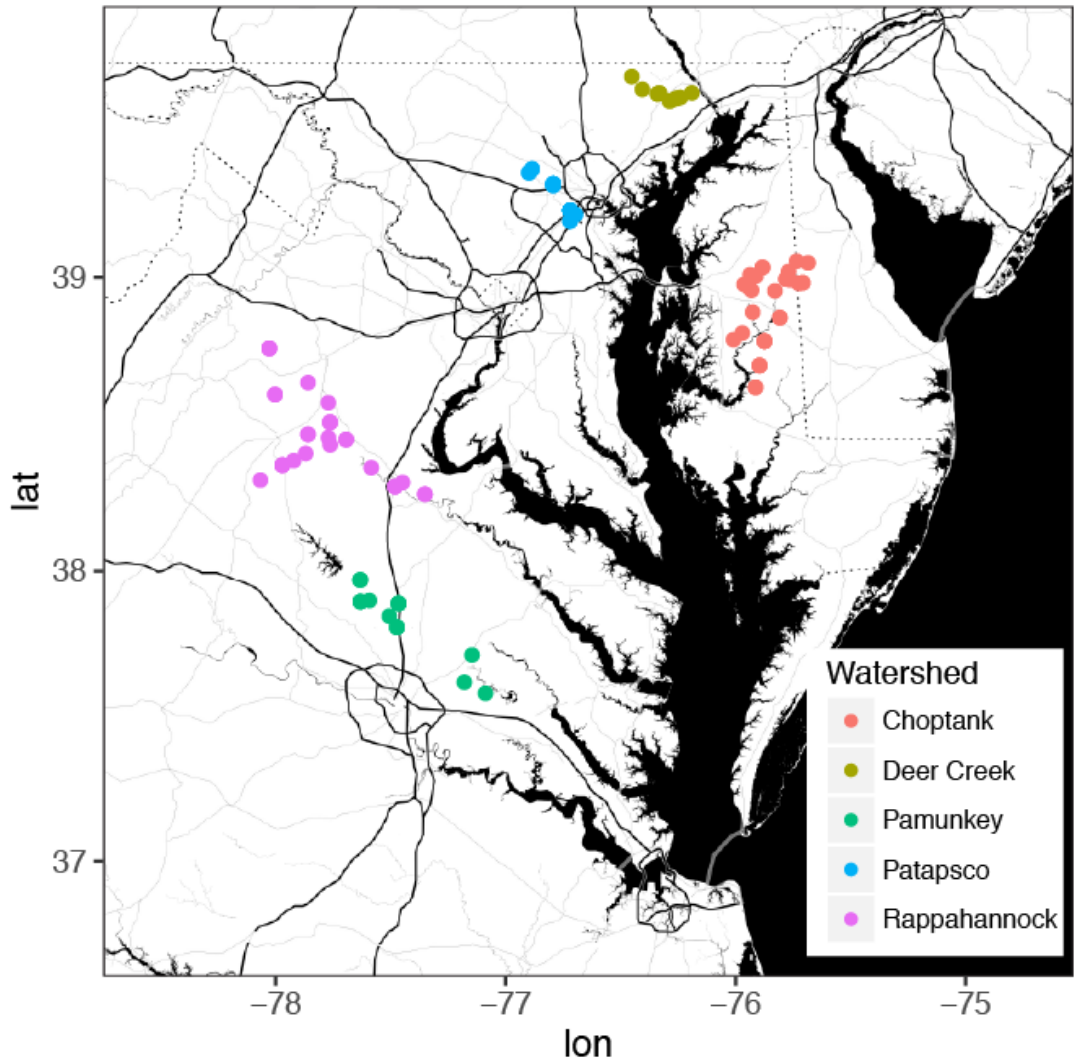
- Molecular beacon assay (120bp amplicon)
- Each sample run in triplicate
  - Positive control spiked with sample
  - Negative controls
  - 5 order of magnitude dilution/standard curve
- Cq value converted to concentration
- Positive hits re-amplified and Sanger sequenced

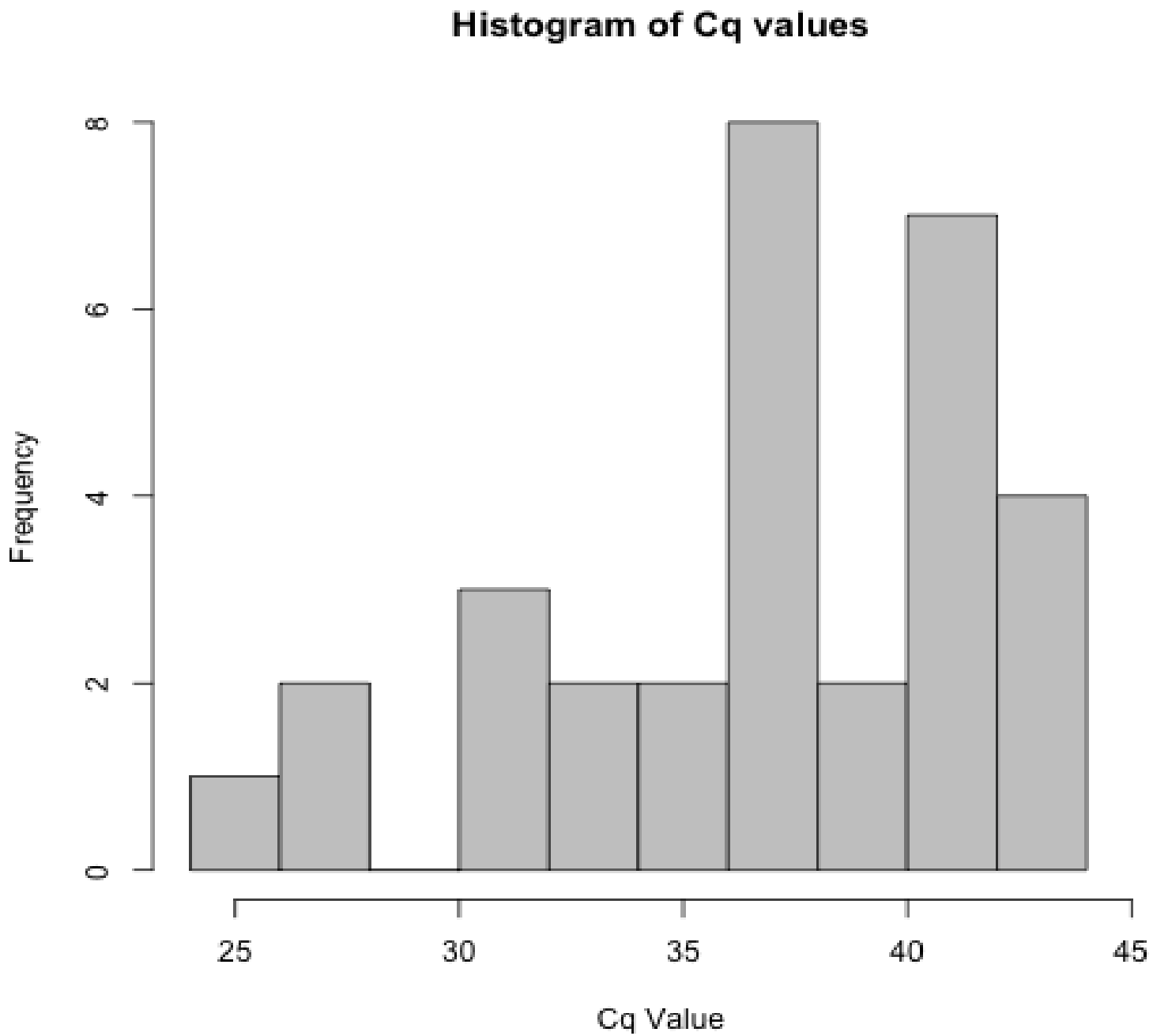
By the numbers…

- 170 samples processed from 2015
- 109/170 produced viable eDNA data
  - Inhibition!
- Positive hits:

eDNA	Ichthyo.
31/109	25/109

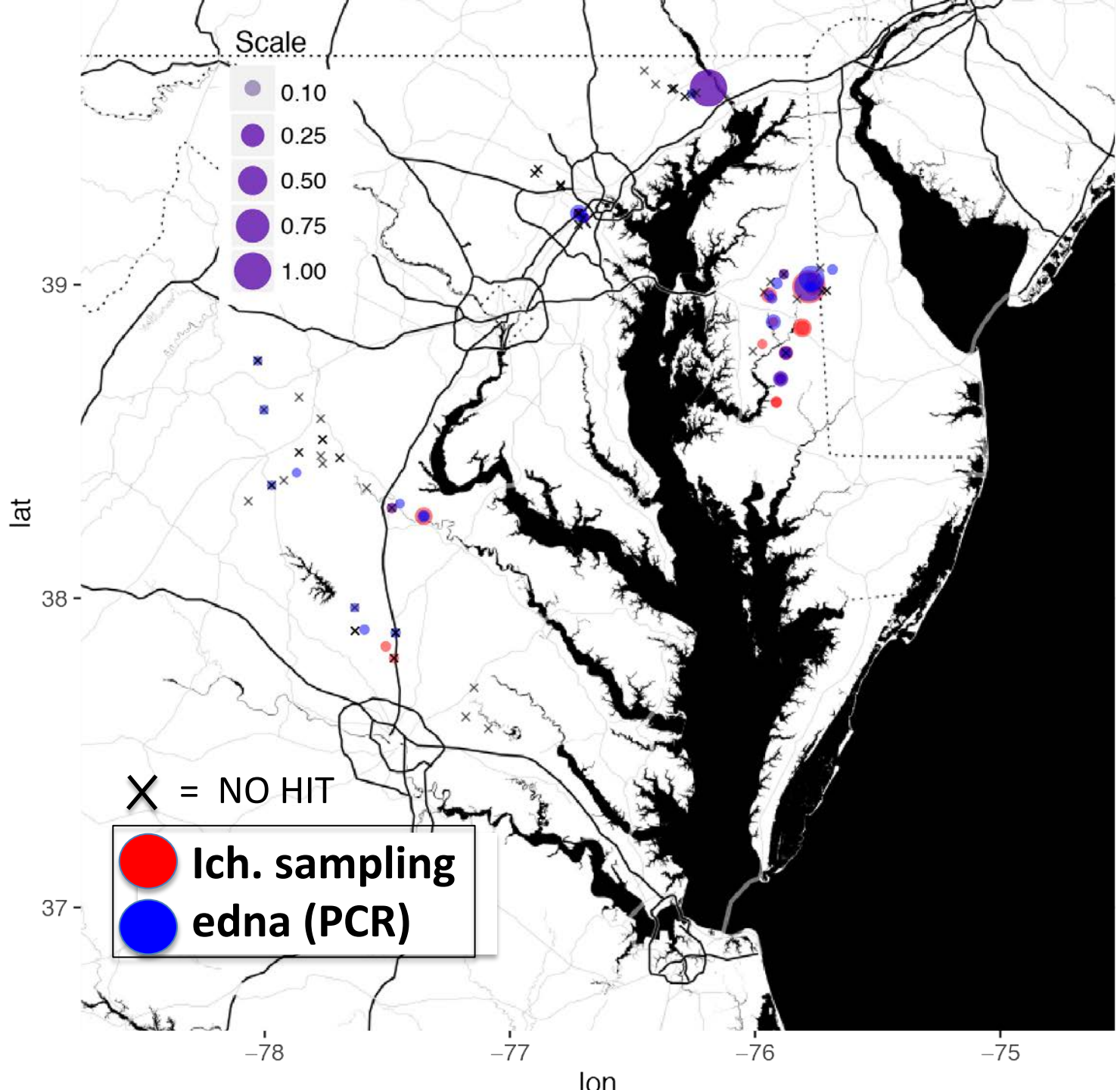
  - **Cq values from 24 to 42**
  - Same top 2 hits (Deer Creek and Choptank,Gravelly)
  - Spearmans Rho correlation, 0.53 P<0.00001
  - Probe hits confirmed via Sanger Seq




Histogram of Cq values
Frequency
0
2
4
6
8
25
30
35
40
45
Cq Value

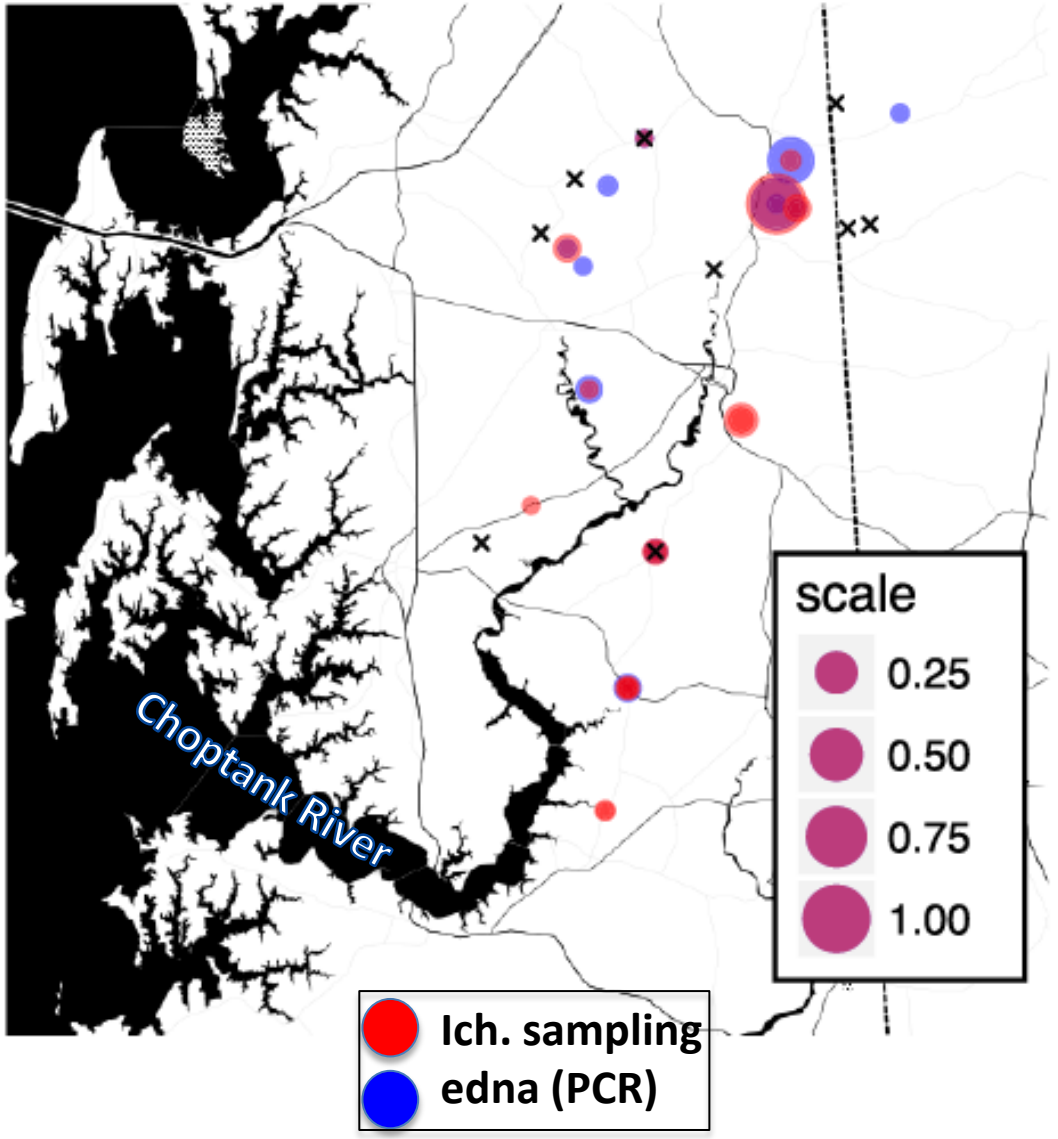
Overall....

=> Size of point scaled to count or eDNA conc (div. by max)

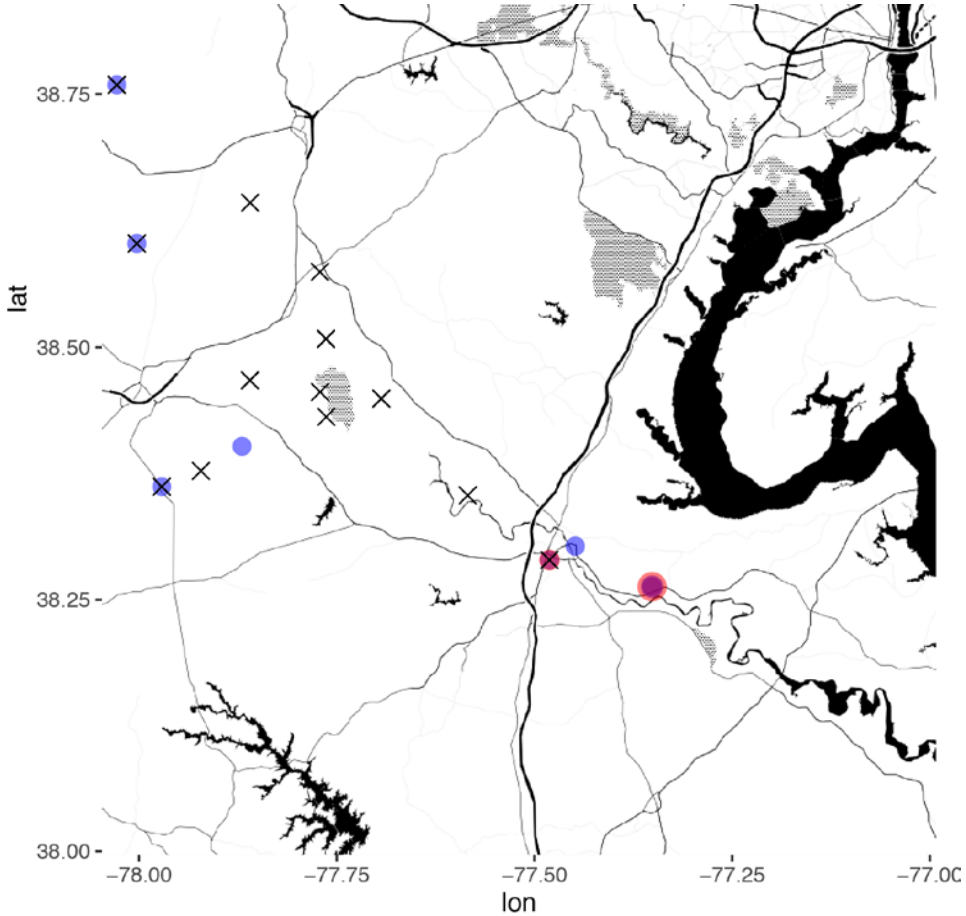


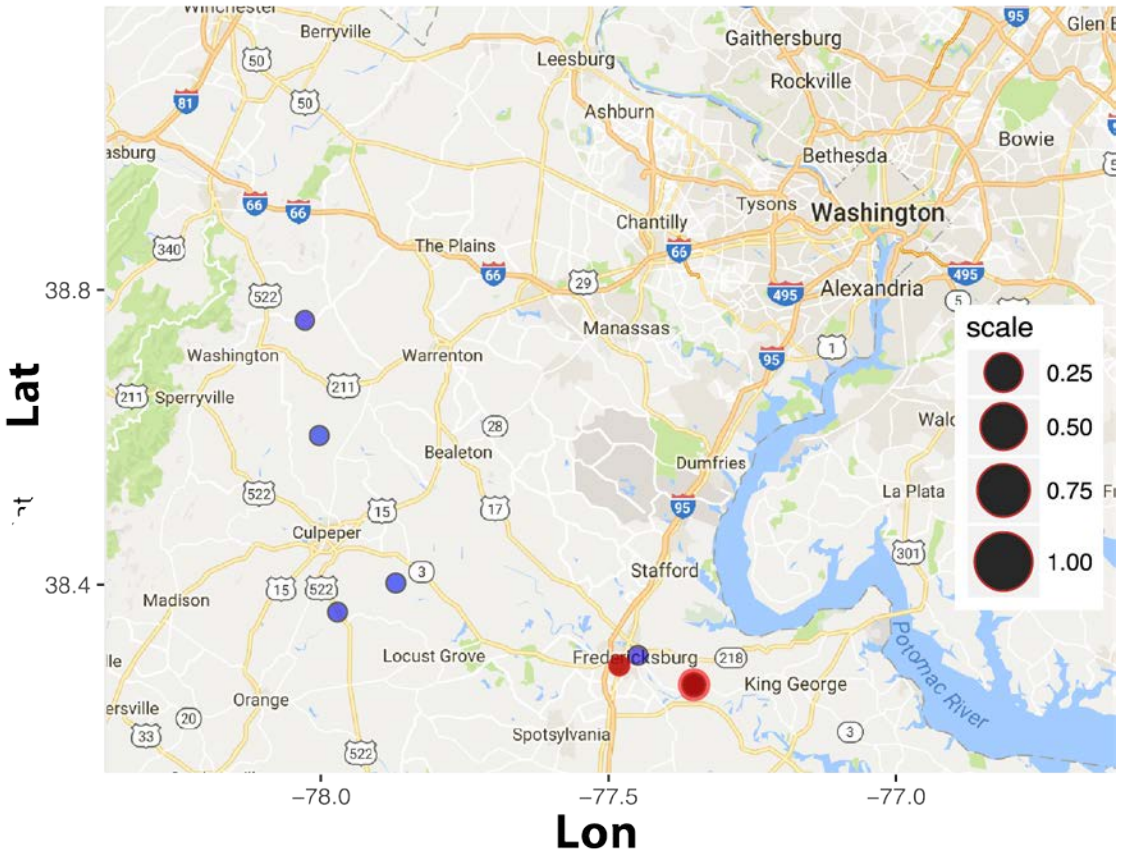
Choptank

- Positive eDNA hits up to Mason's branch
- Good agreement between methods



Rappahannock

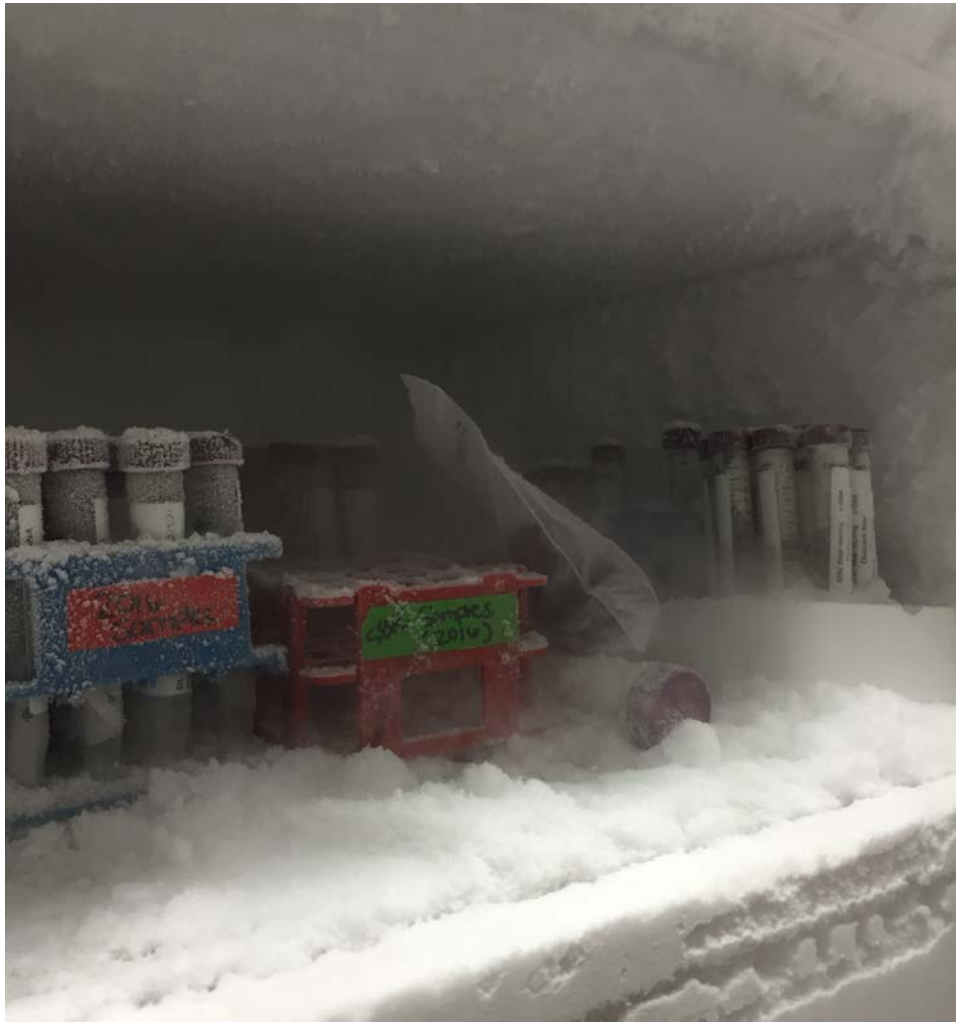




X = NO HIT

Ich. sampling
edna (PCR)

Positive hits at (e.g.) Hazel, Brooke, Cedar, Crest hill run

Summary/future work…

- eDNA detection at many upstream locations
  - Weak hits validated with Sanger
- <u>Good agreement between Ich. and eDNA methods</u>
- Test inhibition control methods
  - Patapsco.
- Process 2016 samples (on-going)