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# Developing Environmental DNA Tools for Freshwater Mussel Conservation

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U.S.G.S.

ORDA Science Webinar

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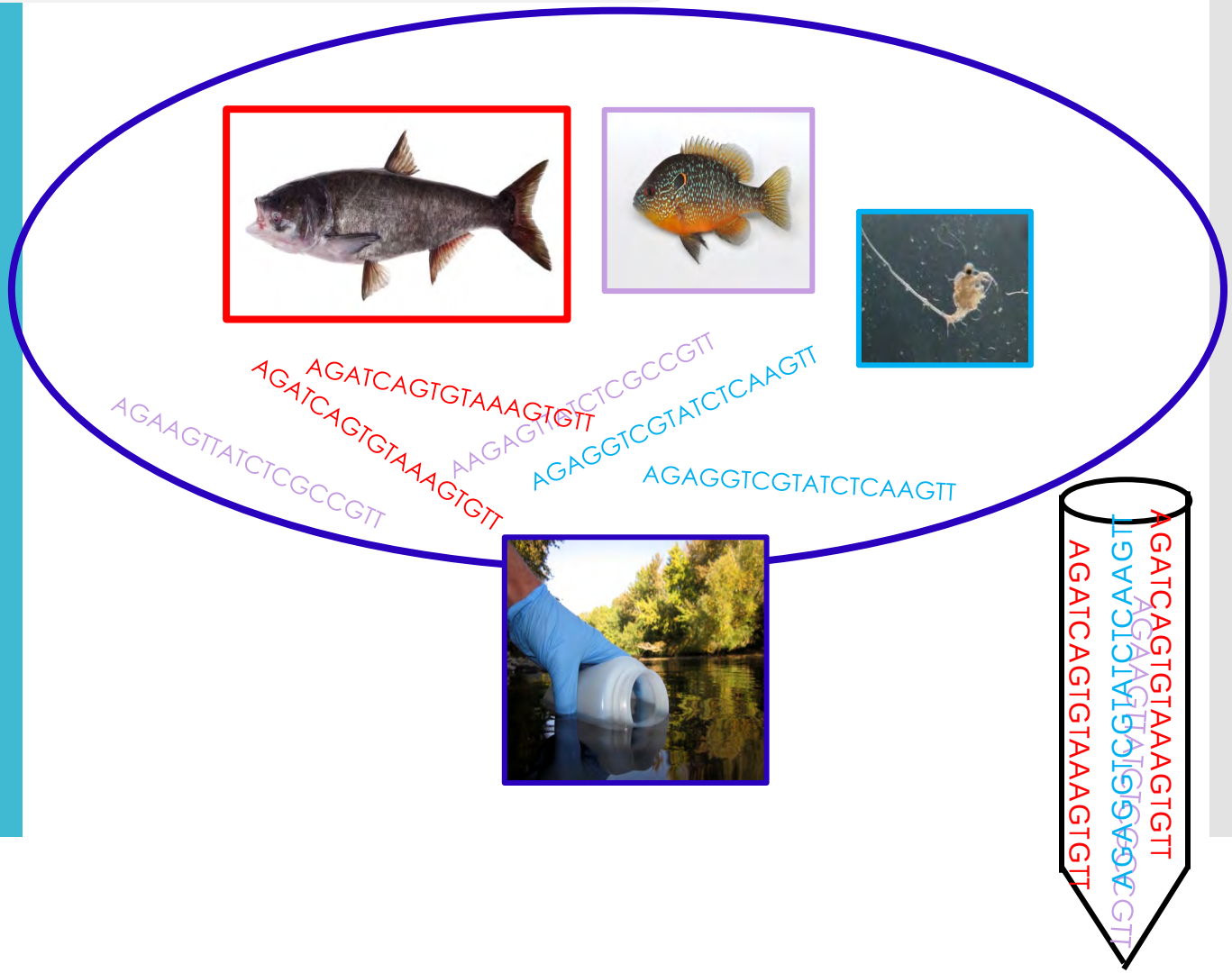
**U.S. Department of the Interior**
**U.S. Geological Survey**

# What is eDNA ?

Pawlowski et al. (2020)– "The total pool of DNA isolated from environmental samples."

A non-invasive genetic method for surveying biotic diversity

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# How is it being used?

## 1. Species Monitoring and Surveying

Species with low population sizes

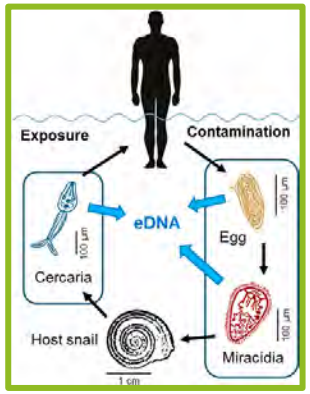
- Threatened/ Endangered


Great Crested Newt
(Biggs et al. 2015)

- Early detection of invasive species


Burmese Pythons – Everglades
(Hunter et al. 2016)

- Infectious Disease




Schistosomiasis
(Sengupta et al. 2019)

# How is it being used?

## 2. Ecological Questions

- Community Composition Changes

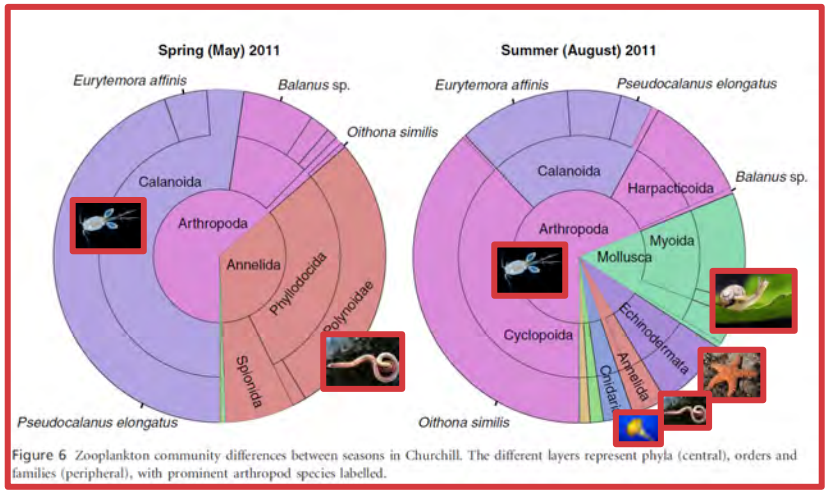
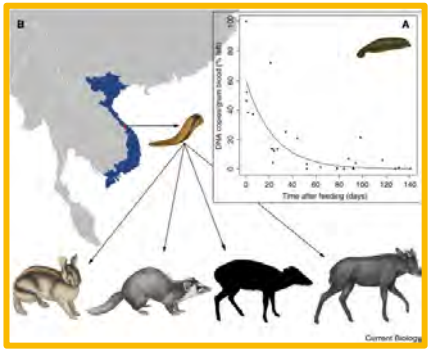


Figure 6 Zooplankton community differences between seasons in Churchill. The different layers represent phyla (central), orders and families (peripheral), with prominent arthropod species labelled.

Zooplankton communities (Chain et al. 2016)

- Predator/Prey/Parasite Interactions



Schnell et al. 2012

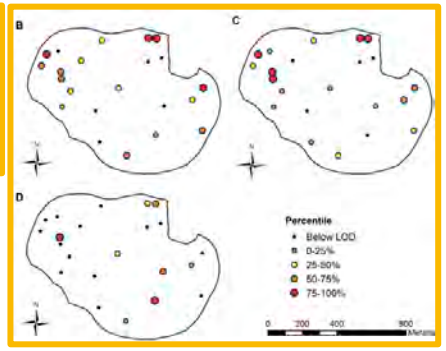
# How is it being used?

## 3. Estimate Population Location and Size

- Population spatial distribution and size/ abundance

Tagged carp


eDNA detection of carp




Common Carp
(Eichmiller et al. 2014)

- Population genetics, census and Ne

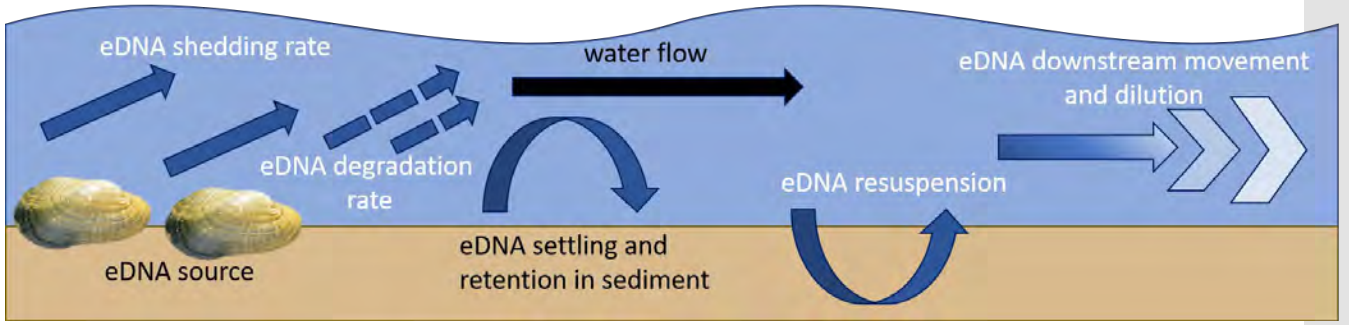
QATAR WHALE SHARK RESEARCH

Whale Sharks
(Sigsgaard et al. 2016)

# How is it being used?

4. "Ecology of eDNA" – what affects the physical state and detection of eDNA

- Origin (Production/ Shedding)
- Fate (Degradation)
- State (Particle size; sediment bound; intracellular vs extracellular)
- Transport



# Targeted Species Specific

AGATCAGTTAAAGTGTT

AGAAGTTATCTCGCCGTT

AGACGCCTTCAGGTTT

AGATCAGTGTAAAGTGTT

AGAGGTCGTATCTCAAGTT

AGAAGTTATCTCGCCGTT

AGATCAGTGTAAAGTGTT

AGATCAGTGTAAAGTGTT

AAGAGTTATCTCGCCGTT

AGACGCCTTCAGTGTT

AGACGCCTTCAGTGTT

AGAGGTCGTATCTCAAGTT

AGAGGTCGTATCTCAAGTT

## Extraction

AGACGCCTTCAGTGTT AGAGGTCGTATCTCAAGTT

AGATCAGTGTAAAGTGTT

AGAGGTCGTATCTCAAGTG

AGAAGTTATCTCGCCGTT AGATCAGTGTAAAGTGTT

## PCR

AGACGCCTTCAGTGTT

AGAGGTCGTATCTCAAGTT

AGTC

AGA**TCAGTGTAAAGT**GTT

AGAGGTCGTATCTCAAGTT

TTCA

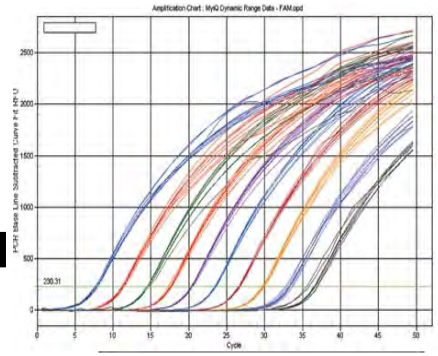
AGTC

AGA**TCAGTGTAAAGT**GTT

AGAAGTTATCTCGCCGTG

TTCA

## Quantitation



## Sanger Sequence

**TCAGTGTAAAGT**

**TCAGTGTAAAGT** **TCAGTGTAAAGT**

**TCAGTGTAAAGT** **TCAGTGTAAAGT**

**TCAGTGTAAAGT** **TCAGTGTAAAGT**

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# Community Profile (Metabarcoding)

AGAAGTTATCTCGCCGTT AGATCAGTTAAAGTGTT AGATCAGTGTAAAGTGTT AGACGCCTTCAGGTTT AGAGGTCGTATCTCAAGTT AGATCAGTGTAAAGTGTT AGAGGTCGTATCTCAAGTT

AGATCAGTGTAAAGTGTT AAGAGTTATCTCGCCGTT AGACGCCTTCAGTGTT AGACGCCTTCAGTGTT AGAGGTCGTATCTCAAGTT

## Extraction

AGACGCCTTCAGTGTT AGAGGTCGTATCTCAAGTT

AGATCAGTGTAAAGTGTT

AGAGGTCGTATCTCAAGTG

AGAAGTTATCTCGCCGTT AGATCAGTGTAAAGTGTT

## PCR

TCT AGACGCCTTCAGTGTT CAA

TCT AGAGGTCGTATCTCAAGTT CAA

TCT AGATCAGTGTAAAGTGTT CAA

TCT AGAGGTCGTATCTCAAGTT CAA

TCT AGAAGTTATCTCGCCGTG CAA

TCT AGATCAGTGTAAAGTGTT CAA

## High-Throughput Sequencing

AGACGCCTTCAGTGTT AGAGGTCGTATCTCAAGTT

AGATCAGTGTAAAGTGTT AGAGGTCGTATCTCAAGTT

AGAAGTTATCTCGCCGTG AGATCAGTGTAAAGTGTT

AGACGCCTTCAGTGTT AGAGGTCGTATCTCAAGTT

AGACGCCTTCAGTGTT AGAGGTCGTATCTCAAGTT

AGATCAGTGTAAAGTGTT AGAGGTCGTATCTCAAGTT

AGATCAGTGTAAAGTGTT AGAGGTCGTATCTCAAGTT

AGATCAGTGTAAAGTGTT AGAAGTTATCTCGCCGTG AGATCAGTGTAAAGTGTT

AGAAGTTATCTCGCCGTG AGATCAGTGTAAAGTGTT

AGAAGTTATCTCGCCGTG AGATCAGTGTAAAGTGTT

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# Current eDNA FWM projects

**Metabarcoding assays for the detection of freshwater mussels with environmental DNA**

Katy Klymus, Catherine Richter, Nathan Thompson, Jo Ellen Hinck, and Jess Jones

Funding: ORDA

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# Objectives

- **Develop metabarcoding assays (universal primers) that can identify to species level, unionid mussel eDNA from water samples in the Clinch River.**
- **Test assays with field samples collected near well characterized mussel beds in the Clinch River.**

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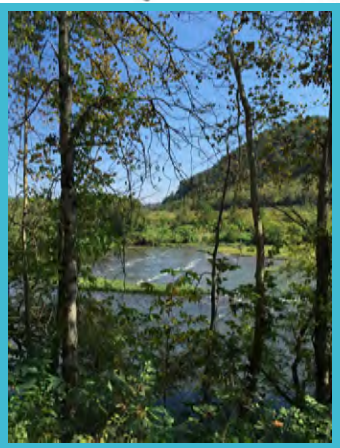
# Background



## FWMs in the Clinch River

- Appalachian Valley, VA and TN
- High species richness in freshwater mussels and fishes
- The highest concentration of extant federally listed aquatic species but chemical spills and damming of the river has led to major population declines
- 1998 a chemical spill led to a restoration involving reintroductions of mussels to depleted populations
- eDNA metabarcoding might aid monitoring restoration of populations

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# Methods

- Utilized public genetic databases (GenBank):
  - for primer development
  -to identify what species our sequence data belong to
- Developed and tested two different Metabarcoding assays (amplify different regions of the genome)
- Sampled 6 sites in the Clinch River, August 2017
- Took 8 - 16, 50 ml water samples at each site plus field blanks at selected sites

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# Results

## Assay Development

- Primers were developed based off of sequences from 55 NA FWM spp. across 29 genera
- The genetic database had sequence data for 50 of the 56 historically known species in the Clinch River.
- Primers tested against genomic DNA from 30 FWM spp. as well as against 2 non-target species (*Corbicula* spp. and silver carp *Hypophthalmichthys molitrix*)
- Primers amplified all FWM tissue samples and did not amplify the non-targets
  - -> assays appear to be unionid specific, increases assays' sensitivity to detect FWMs as the primers are not amplifying non-target DNA

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# Results

**Field eDNA Samples**

- ★ eDNA detected 19 different FWM species including 8 Federally Endangered Species
- One assay appears to amplify more species, but the two assays appear to differ in their ability to amplify the same species, recommend use of both assays for further research
- Increased replicate samples or sampled volume should improve detections

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| | COI – Percentage of Reads | | | | | | ND1- Percentage of Reads | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Indian Creek (5) | Bennett Island (14) | Cleveland Island (5) | Pendleton Island (16) | Wallens Bend (7) | Kyles Ford (8) | Indian Creek (5) | Bennett Island (14) | Cleveland Island (5) | Pendleton Island (16) | Wallens Bend (7) | Kyles Ford (8) |
| *Actinonaias ligamentina* | | | | 0.0941 | 7.6191 | 3.4348 | | | | | | |
| *Actinonaias pecterosa* | 100.0000 | 0.0616 | 56.3297 | 1.2451 | 48.7767 | 74.0827 | 0.0876 | 51.1741 | 23.8576 | 0.5621 | 54.6858 | 68.6961 |
| *Alasmidonta marginata* | | | | | | | | | | | | 0.0011 |
| *Cyclonaias tuberculata* | | | | | 0.3933 | 1.7724 | | | | 0.0018 | 1.4778 | 0.0001 |
| ***Epioblasma brevidens*** | | | | | | 5.4842 | | | | | | |
| ***Epioblasma capsaeformis*** | | | | 0.0528 | 0.2010 | 2.5128 | 0.0004 | | | 0.0018 | | 1.2129 |
| *Epioblasma OTU ?** | | | | | 0.0034 | 0.0155 | | | | | | |
| ***Epioblasma triquetra*** | | 0.0003 | | | | 1.3299 | | | | | | |
| *Eurynia dilatata* | | | | 0.0191 | 6.4847 | 3.5386 | 0.0105 | 0.0090 | 76.0898 | 0.0526 | 10.6724 | 2.2094 |
| ***Fusconaia cor*** | | | | | 0.0152 | 1.6235 | | | | | | |
| ***Fusconaia cuneolus*** | | | | 0.0294 | 4.7913 | 0.1103 | 0.0009 | 0.0035 | 0.0013 | 0.0241 | 7.6055 | 0.0002 |
| ***Hemistena lata*** | | | | | 3.8246 | | 0.0013 | 0.0022 | | 0.0170 | 8.8719 | |
| *Lampsilis fasciola* | | | | | 18.7808 | 2.1093 | 0.0135 | 1.8369 | 0.0306 | 0.0553 | 6.8119 | 15.1183 |
| *Lasmigona costata* | | 10.9570 | | | | | 0.0004 | 4.2120 | | 0.0054 | 3.2225 | |
| *Medionidus conradicus* | | 0.0023 | 43.6703 | | | 0.2119 | 0.0009 | 26.1427 | | 0.0054 | 0.0006 | 0.0005 |
| ***Pleurobema plenum*** | | | | | | 0.0098 | | | | | | |
| *Pleuronaia barnesiana* | | 30.9767 | | | | | | 0.6532 | | | | |
| *Ptychobranchus fasciolaris* | | | | | 9.1099 | 2.6242 | 0.0028 | 0.0051 | | 0.0562 | 2.5971 | 9.8679 |
| ***Ptychobranchus subtenus*** | | | | | | | 0.0007 | 0.0045 | 0.0013 | 0.0054 | 4.0484 | 2.8535 |
| *Villosa iris* | | 58.0021 | | 98.5595 | | 1.1400 | 99.8811 | 15.9568 | 0.0193 | 99.2131 | 0.0060 | 0.0401 |
| Number of Species | 1 | 6 | 2 | 6 | 11 | 15 | 11 | 11 | 6 | 12 | 11 | 11 |
| Total # Reads | 5900 | 915545 | 1177 | 329541 | 1102988 | 1431197 | 459149 | 312324 | 300433 | 112079 | 1460724 | 1948970 |

Klymus, K.E., Richter, C. A., Thompson, N., Hinck, Jo Ellen, & Jones, J. W. "Metabarcoding assays for the detection of freshwater mussels (Unionida) with environmental DNA." Environmental DNA, doi.org/10.1002/edn3.166

Federally endangered

## Cleveland Island

| | 2017 Number of mussels Visual | 2017 COI Reads eDNA | 2017 ND1 Reads eDNA |
|---|---|---|---|
| *Actinonaias pectorosa* | 13 | 663 | 71677 |
| *Eurynia dilatate* | 4 | | 228599 |
| *Epioblasma capsaeformis* | 3 | | |
| *Pleuronaia barnesiana* | 3 | | |
| *Ptychobranchus fasciolaris* | 3 | | |
| *Fusconaia cor* | 2 | | |
| *Lampsilis fasciola* | 2 | | 92 |
| *Medionidus conradicus* | 2 | 514 | |
| *Amblema plicata* | 1 | | |
| *Cyclonaias tuberculata* | 1 | | |
| *Fusconaia subrotunda* | 1 | | |
| *Villosa iris* | 1 | | 58 |
| *Fusconaia cuneolus* | | | 4 |
| *Ptychobranchus subtentus* | | | 4 |
| *Actinonaias ligamentina* | | | |
| *Alasmidonta marginata* | | | |
| *Cyprogenia stegaria* | | | |
| *Dromus dromas* | | | |
| *Epioblasma brevidens* | | | |
| *Epioblamsa OTU ? ** | | | |
| *Epioblasma triquetra* | | | |
| *Hemistena lata* | | | |
| *Lampsilis abrupta* | | | |
| *Lampsilis ovata* | | | |
| *Lasmigona costata* | | | |
| *Lemiox rimosus* | | | |
| *Ligumia recta* | | | |
| *Plethobasus cyphyus* | | | |
| *Pleurobema plenum* | | | |
| *Strophitus undulatus* | | | |
| *Theliderma cylindrica* | | | |
| *Villosa vanuxemensis* | | | |

## Pendleton Island

| | 2016 Number of mussels Visual | 2017 COI Reads eDNA | 2017 ND1 Reads eDNA |
|---|---|---|---|
| *Actinonaias ligamentina* | 214 | 310 | |
| *Actinonaias pectorosa* | 131 | 4103 | 630 |
| *Amblema plicata* | 66 | | |
| *Ptychobranchus fasciolaris* | 66 | | 63 |
| *Cyclonaias tuberculata* | 36 | | 2 |
| *Eurynia dilatata* | 31 | 63 | 59 |
| *Villosa iris* | 17 | 324794 | 111197 |
| *Fusconaia subrotunda* | 7 | | |
| *Ptychobranchus subtentus* | 5 | | 6 |
| *Lampsilis fasciola* | 4 | | 62 |
| *Epioblasma brevidens* | 2 | | |
| *Fusconaia cuneolus* | 2 | 97 | 27 |
| *Lasmigona costata* | 2 | | 6 |
| *Medionidus conradicus* | 2 | | 6 |
| *Epioblasma triquetra* | 1 | | |
| *Fusconaia cor* | 1 | | |
| *Ligumia recta* | 1 | | |
| *Theliderma cylindrica* | 1 | | |
| *Villosa vanuxemensis* | 1 | | |
| *Epioblasma capsaeformis* | | 174 | 2 |
| *Hemistena lata* | | | 19 |
| *Alasmidonta marginata* | | | |
| *Cyprogenia stegaria* | | | |
| *Dromus dromas* | | | |
| *Epioblamsa OTU ? ** | | | |
| *Lampsilis abrupta* | | | |
| *Lampsilis ovata* | | | |
| *Lemiox rimosus* | | | |
| *Plethobasus cyphyus* | | | |
| *Pleurobema plenum* | | | |
| *Pleuronaia barnesiana* | | | |
| *Strophitus undulatus* | | | |

## Kyles Ford

| | 2017 Number of mussels Visual | 2017 COI Reads eDNA | 2017 ND1 Reads eDNA |
|---|---|---|---|
| *Medionidus conradicus* | 169 | 3033 | 10 |
| *Ptychobranchus subtentus* | 106 | | 55613 |
| *Actinonaias pectorosa* | 103 | 1060270 | 1338867 |
| *Actinonaias ligamentina* | 88 | 49159 | |
| *Epioblasma capsaeformis* | 80 | 35963 | 23640 |
| *Eurynia dilatata* | 63 | 50644 | 43060 |
| *Ptychobranchus fasciolaris* | 49 | 37558 | 192322 |
| *Villosa iris* | 19 | 16316 | 781 |
| *Lemiox rimosus* | 11 | | |
| *Fusconaia cor* | 9 | 23236 | 3 |
| *Cyclonaias tuberculata* | 8 | 25367 | 2 |
| *Epioblasma brevidens* | 7 | 78490 | |
| *Lampsilis fasciola* | 7 | 30188 | 294651 |
| *Cyprogenia stegaria* | 5 | | |
| *Hemistena lata* | 5 | | |
| *Dromus dromas* | 4 | | |
| *Fusconaia cuneolus* | 4 | 1578 | |
| *Alasmidonta marginata* | 2 | | 21 |
| *Epioblasma triquetra* | 2 | 19033 | |
| *Lampsilis ovata* | 2 | | |
| *Lasmigona costata* | 2 | | |
| *Plethobasus cyphyus* | 2 | | |
| *Fusconaia subrotunda* | 1 | | |
| *Lampsilis abrupta* | 1 | | |
| *Strophitus undulatus* | 1 | | |
| *Theliderma cylindrica* | 1 | | |
| *Epioblamsa OTU ? ** | | 222 | |
| *Pleurobema plenum* | | 140 | |
| *Amblema plicata* | | | |
| *Ligumia recta* | | | |
| *Pleuronaia barnesiana* | | | |
| *Villosa vanuxemensis* | | | |

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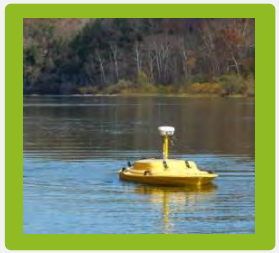
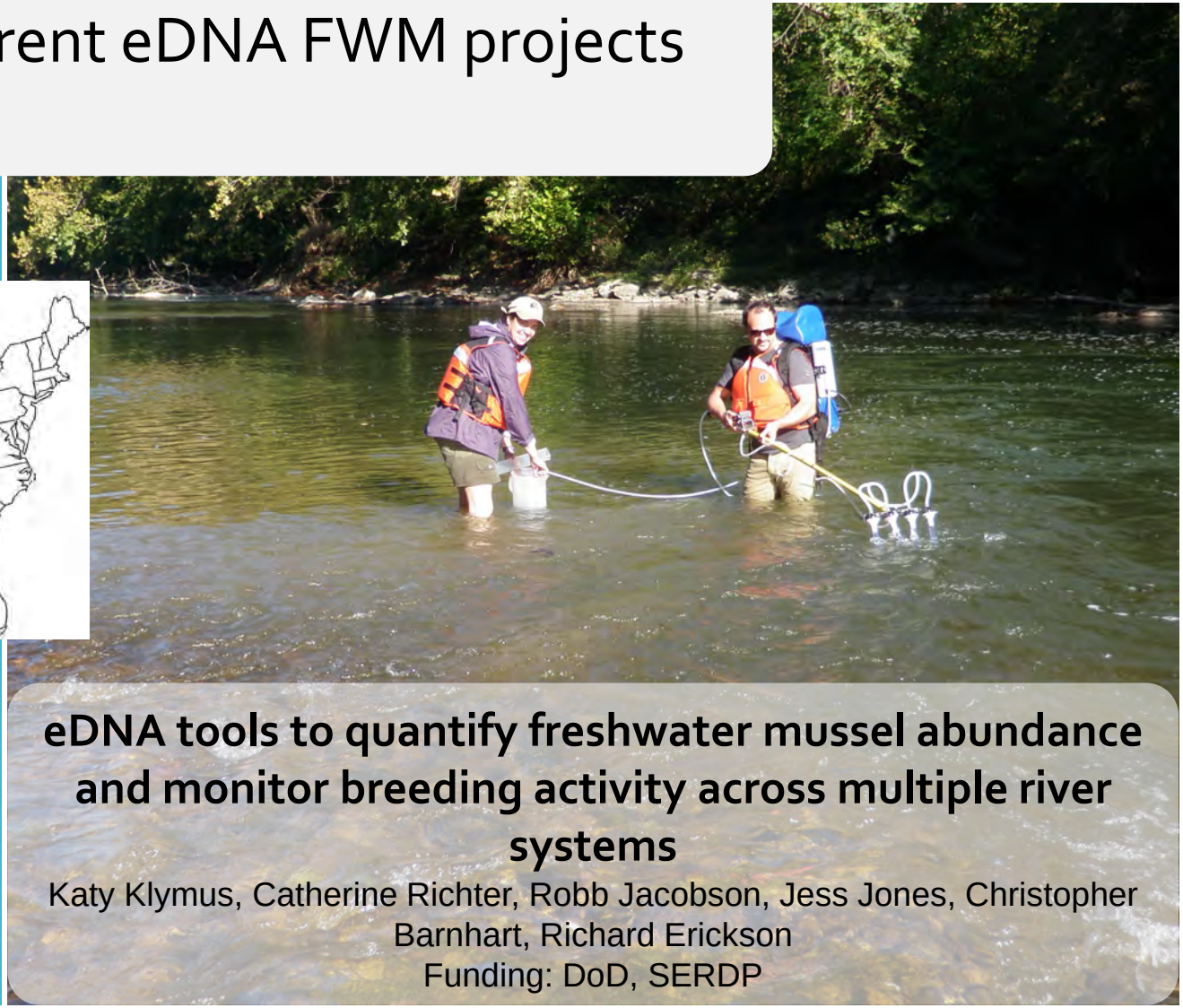


# Current eDNA FWM projects



## eDNA tools to quantify freshwater mussel abundance and monitor breeding activity across multiple river systems

Katy Klymus, Catherine Richter, Robb Jacobson, Jess Jones, Christopher Barnhart, Richard Erickson

Funding: DoD, SERDP

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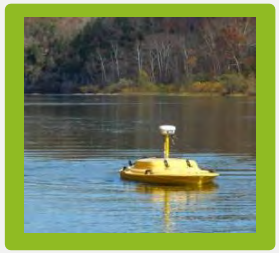
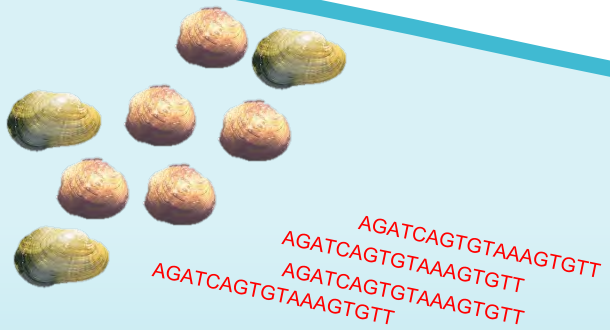


# Objectives

- **Gain better understanding of how eDNA moves in a system in order to inform about a species presence, abundance and breeding behavior**

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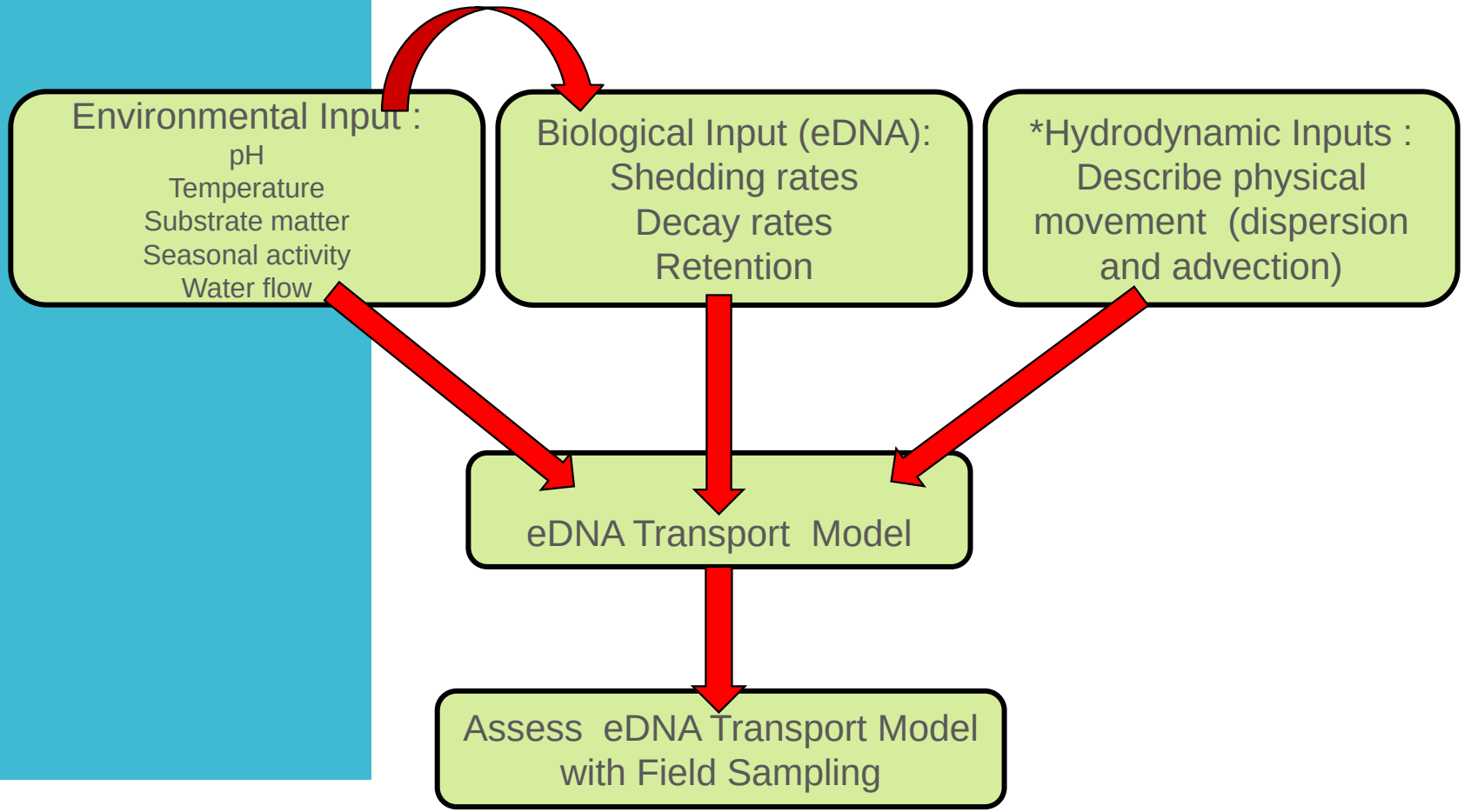
# Objective 1 – Develop an eDNA Transport Model to Infer Distance and Biomass of Mussel Bed

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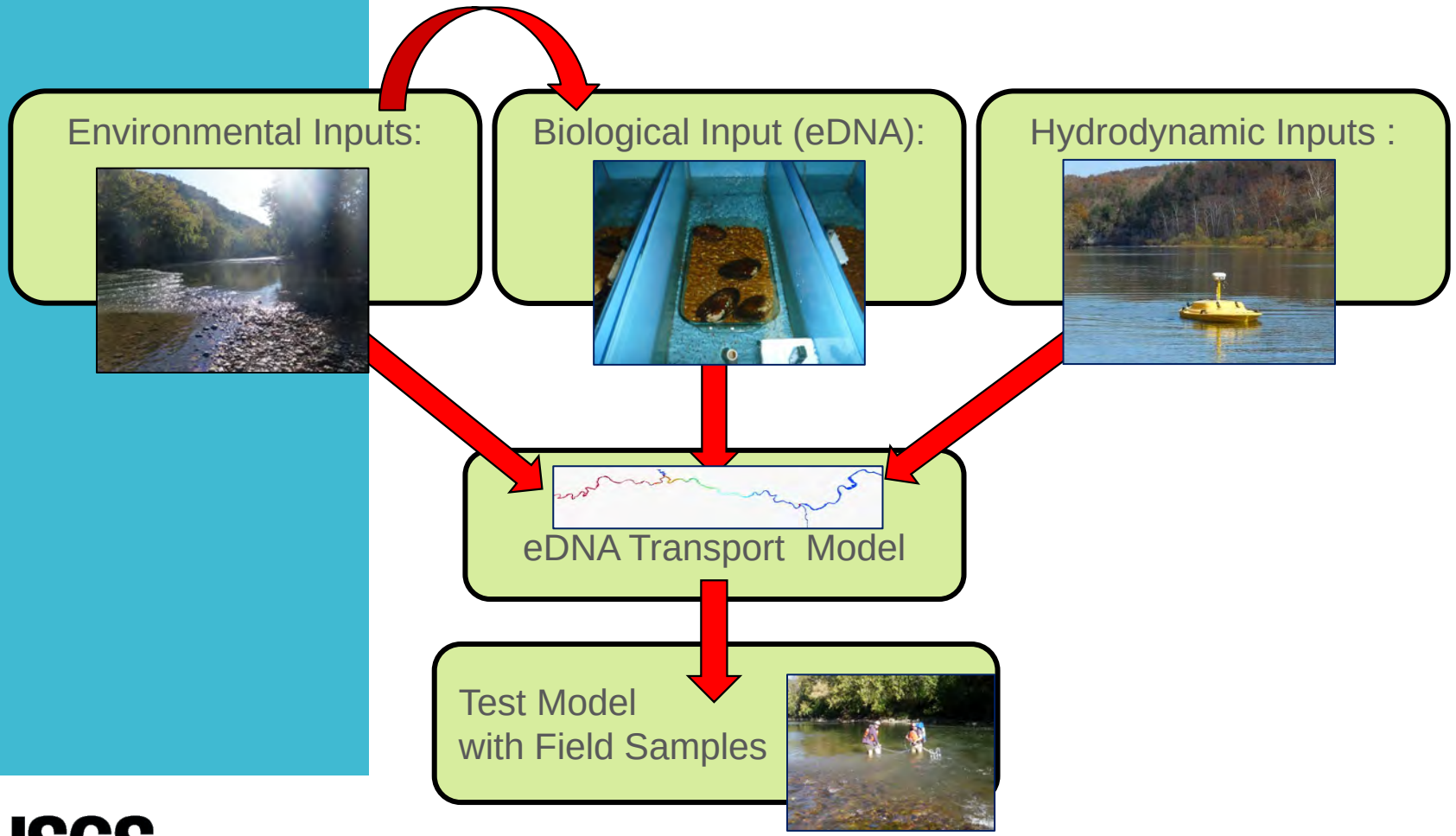
*Hydrodynamic – hydraulic, hydrologic and geomorphologic variables

# Objective 1 – Develop an eDNA Transport Model to Infer Distance and Biomass of Mussel Bed

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# Clinch River

**Oyster Mussel**
***Epioblasma capsaeformis***

**Kidneyshell**
***Ptychobranchus fasciolaris***

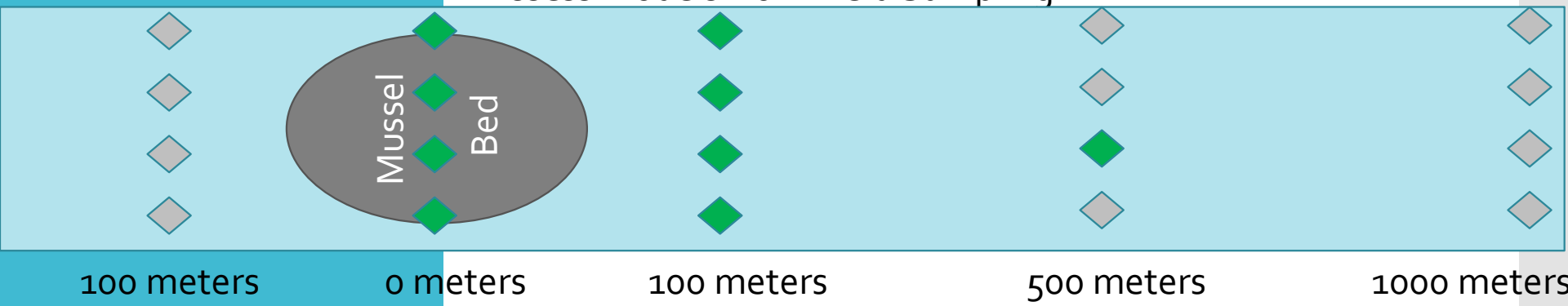
# Big Piney River

**Spectaclecase**
***Cumberlandia monodonta***

**Mucket**
***Actinonaias ligamentina***

# Objective 1 – Develop an eDNA Transport Model to Infer Distance and Biomass of Mussel Bed

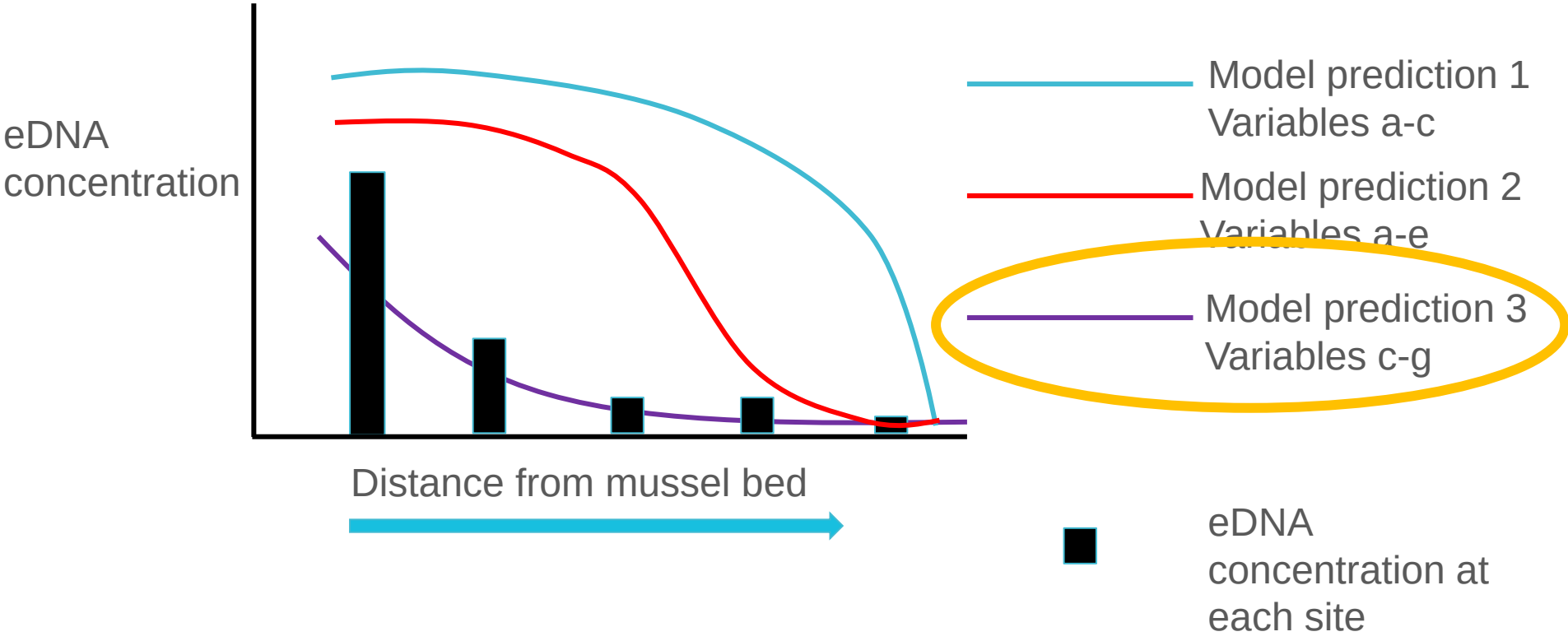
Assess Models from Field Sampling




No eDNA detection

eDNA detection

# Objective 1 – Develop an eDNA Transport Model to Infer Distance and Biomass of Mussel Bed

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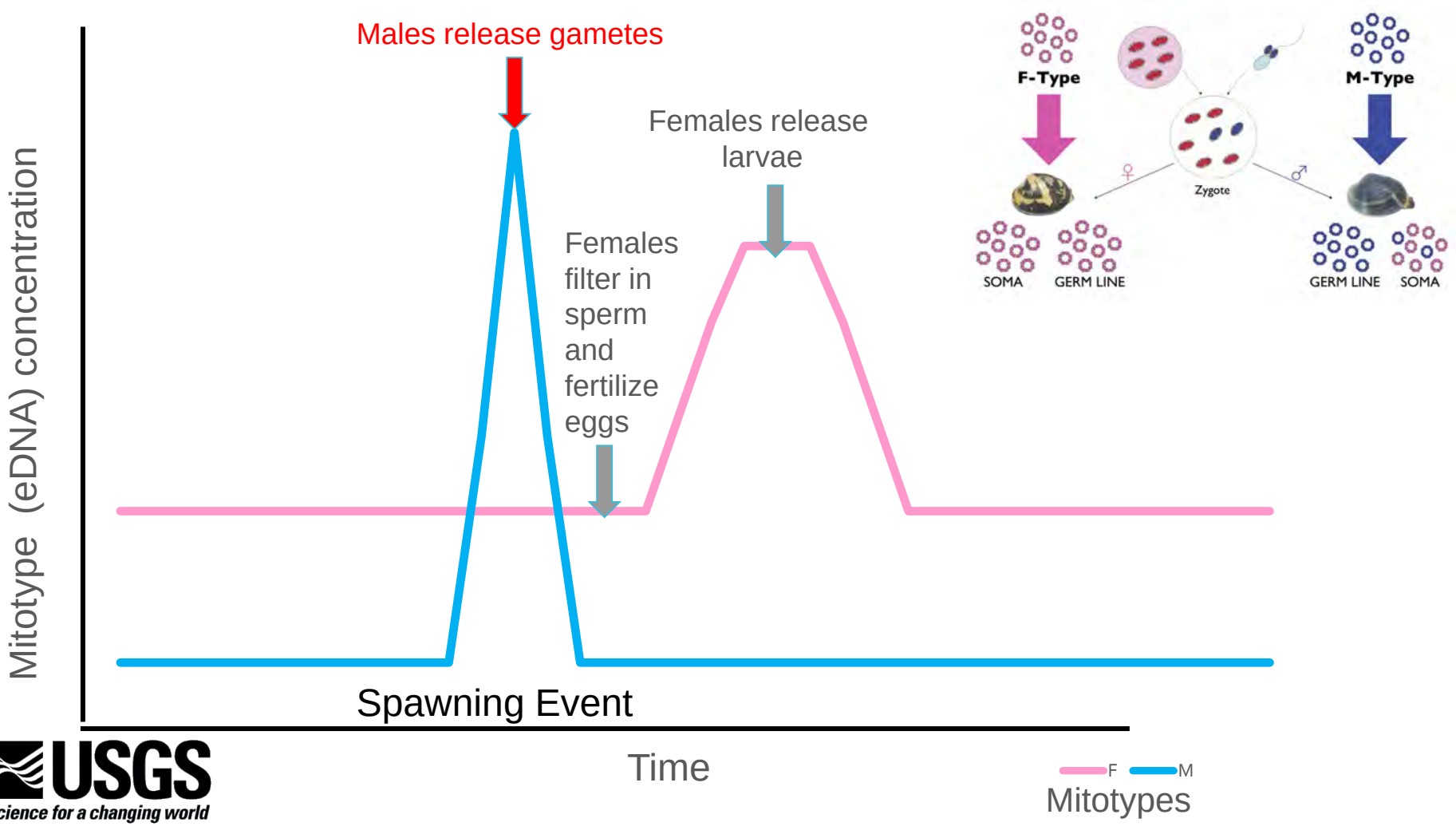
# Objective 2 – Infer Reproductive Behavior with eDNA sampling

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# Future Directions

- Use the samples from the eDNA transport project and run with the metabarcoding assays to look at seasonal changes of FWM assemblages
- Compare eDNA data with the current FWM visual surveys to better assess how well the eDNA metabarcoding can identifying species composition of FWM assemblages
- Continue to increase the genetic database for FWM species with both the female and male mitotypes to improve FWM eDNA tools

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# Questions?

Acknowledgements:

Cathy Richter, Nathan Thompson, Dannise Ruiz, Trudi Frost, Thea Edwards, Jo Hinck, Susannah Erwin, Brian Anderson, Robb Jacobson, Brandon Sansom, Maura Roberts, Ty Helmuth, Jess Jones, Katie Ortiz, Chris Barnhart, Richard Erickson, Andy Roberts, Scott Faiman, James Candrl, James Kunz, Rachel Claunch

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