Environmental DNA

Method Development and Applications in Region 3

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Clean Water Act

Sec. 101. Declaration of Goals and Policy

(a) The <u>*objective*</u> of this Act is to *restore and maintain the chemical, physical, and biological integrity* of the Nation's waters. In order to achieve this objective…

(b) it is the <u>*national goal*</u> that wherever attainable, an interim *goal of water quality which provides for the protection and propagation of fish, shellfish, and wildlife and provides for recreation* in and on the water be achieved by July 1, 1983…

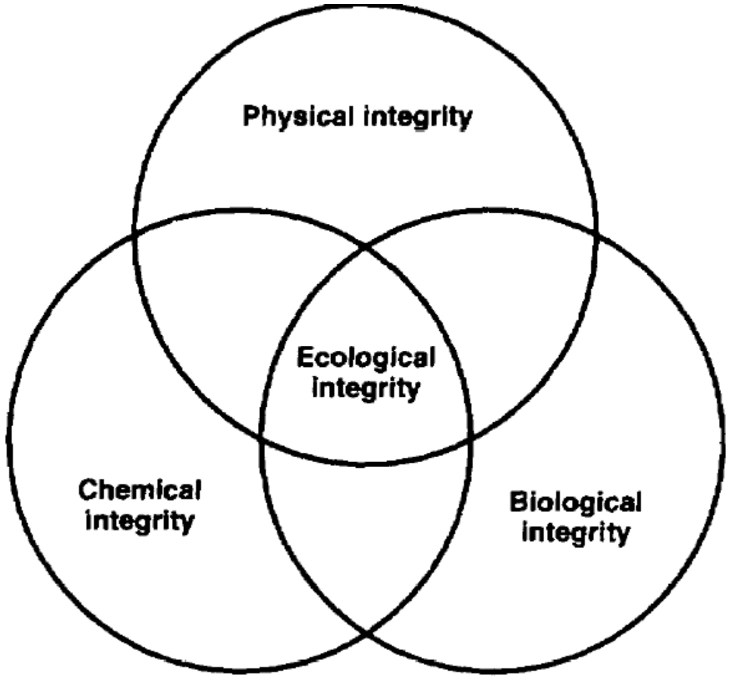
Integrated approaches for water protection (technical support document, 1991)

Water Quality Standards, Independent Application

- Chemical
- Toxics
- Biological Criteria

Integrated approaches for water protection (technical support document, 1991)

Water Quality Standards – elements of Ecological Integrity



- Biological communities reflect overall ecological integrity
- Provide meaningful goal and useful measure of environmental status
- Relates directly to overall integrity of Nation's waters
- To better protect biological integrity of aquatic communities, EPA recommends States develop and implement biological criteria or "biocritiera"
- Biocriteria are numerical values or narrative statements that describe the biological integrity of aquatic communities inhabiting waters of a given designated aquatic life use

Freshwater mussels

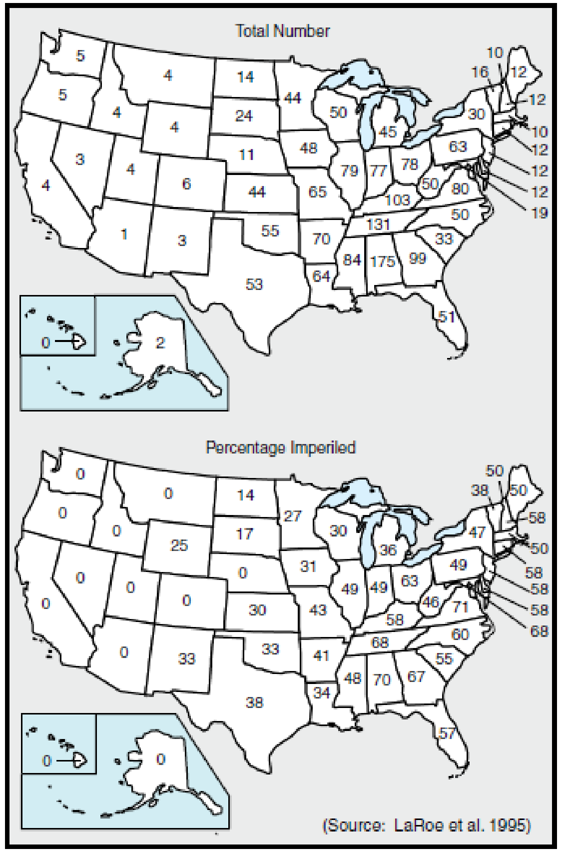
Regional Biodiversity

298 species

Presence of mussels, with recruitment, indicative of functioning river system

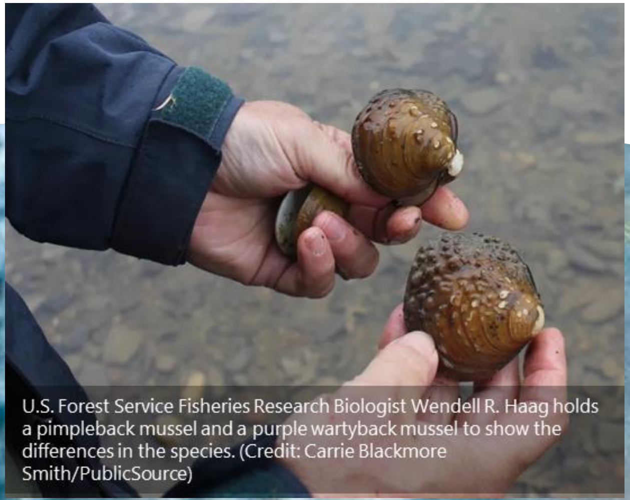
North America 225 species (most in the world)

130 species just within the Ohio River watershed



(Source: LaRoe et al. 1995)


Freshwater mussel assemblage from the Licking River, Photo by Monte McGregror

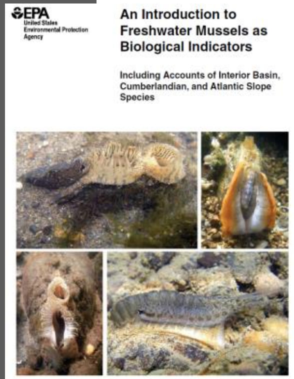

U.S. Forest Service Fisheries Research Biologist Wendell R. Haag holds a pimpleback mussel and a purple wartyback mussel to show the differences in the species. (Credit: Carrie Blackmore Smith/PublicSource)

Freshwater mussels

Suitable organism to use as an indicator species

Attributes

- Long lived
  - up to 70+ years
- Sedentary
  - move little during their lifetime but they do move
- Burrowers
  - species dependent, upwards of 6+ inches in some riverbeds
- Filter feeders
  - they obtain food and oxygen from water and via interstitial flow
- Fairly large
  - ample soft tissue for chemical analysis
- Spent valves
  - dead mussels leave historical record



A pile of dead freshwater mussels collected by biologists from the Clinch River at Sycamore Island, Virginia. *Visual:* [Meagan Racey / United States Fish and Wildlife Service / flickr]

Attributes allow individuals or assemblages to function as "**environmental logbooks**" recording changes in water and habitat quality over time

Freshwater mussels

Stressors and threats

A dead fanshell mussel in Kyle's Ford along the Clinch River. Visual: Rose Agbalog / United States Fish and Wildlife Service / flickr

- **Impoundments, dams excess sedimentation,**
  which covers their siphons and suffocates them;
- **Physical barriers**
  isolate populations and separate them from host fishes;
- **Increased pollutants**
  metals, ions, and others
- **Fluctuations in water flow**
  decreases nutrient and oxygen availability and prohibits toxic waste removal.
- **Activities that reduce and destroy habitat & entire beds**
  commercial sand and gravel dredging, navigation maintenance dredging, and barge handling associated with permanent loading and unloading facilities
- **Long Life span, up to 70+ years**
- **Species sensitivity, sensitive life stages**
  Standardized Test Methods?

Freshwater mussels

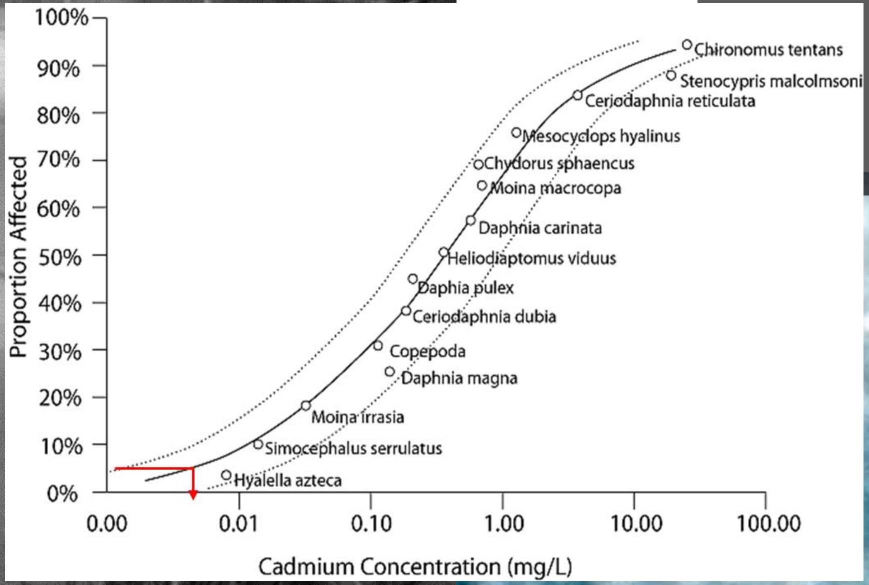
& Water Quality Standards

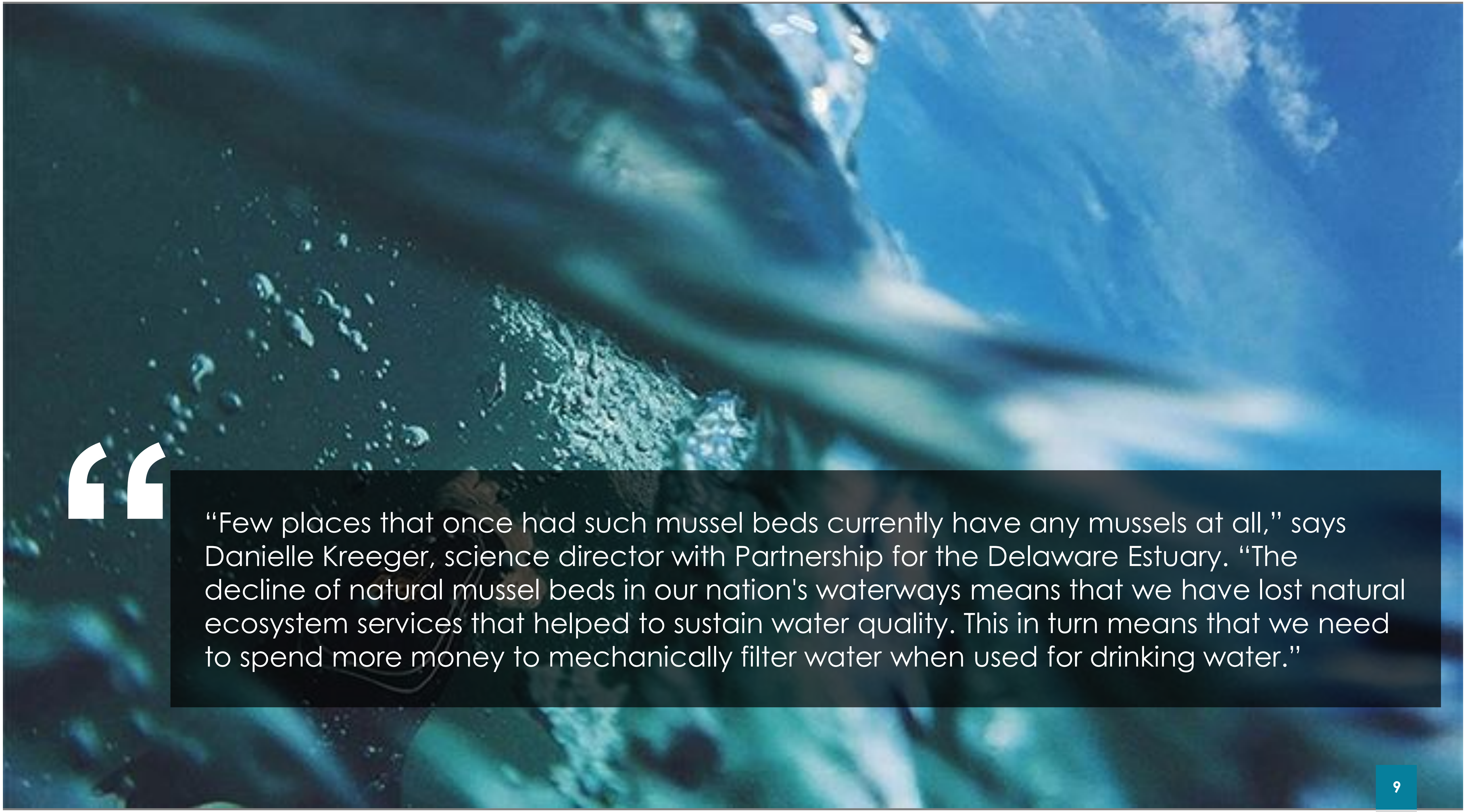
- Indicators of stream quality
- Filter water
- Remove pollutants
- Reduce suspended sediment and algae
- Provides structural habitat
- Provides food for other organisms
- Reduces nutrient overload, often caused by farm fertilizer run-off and water treatment practices
    - Mussels can naturally recycle and store some of these nutrients
- Long Life span, up to 70+ years

Typical method used to derive water quality standards

1. Collect laboratory test data of species response to chemical exposure
2. Use response data to plot a species sensitivity distribution
3. Identify the 5th percentile on the plot
    - At this level of chemical exposure we expect to protect 95% of tested species

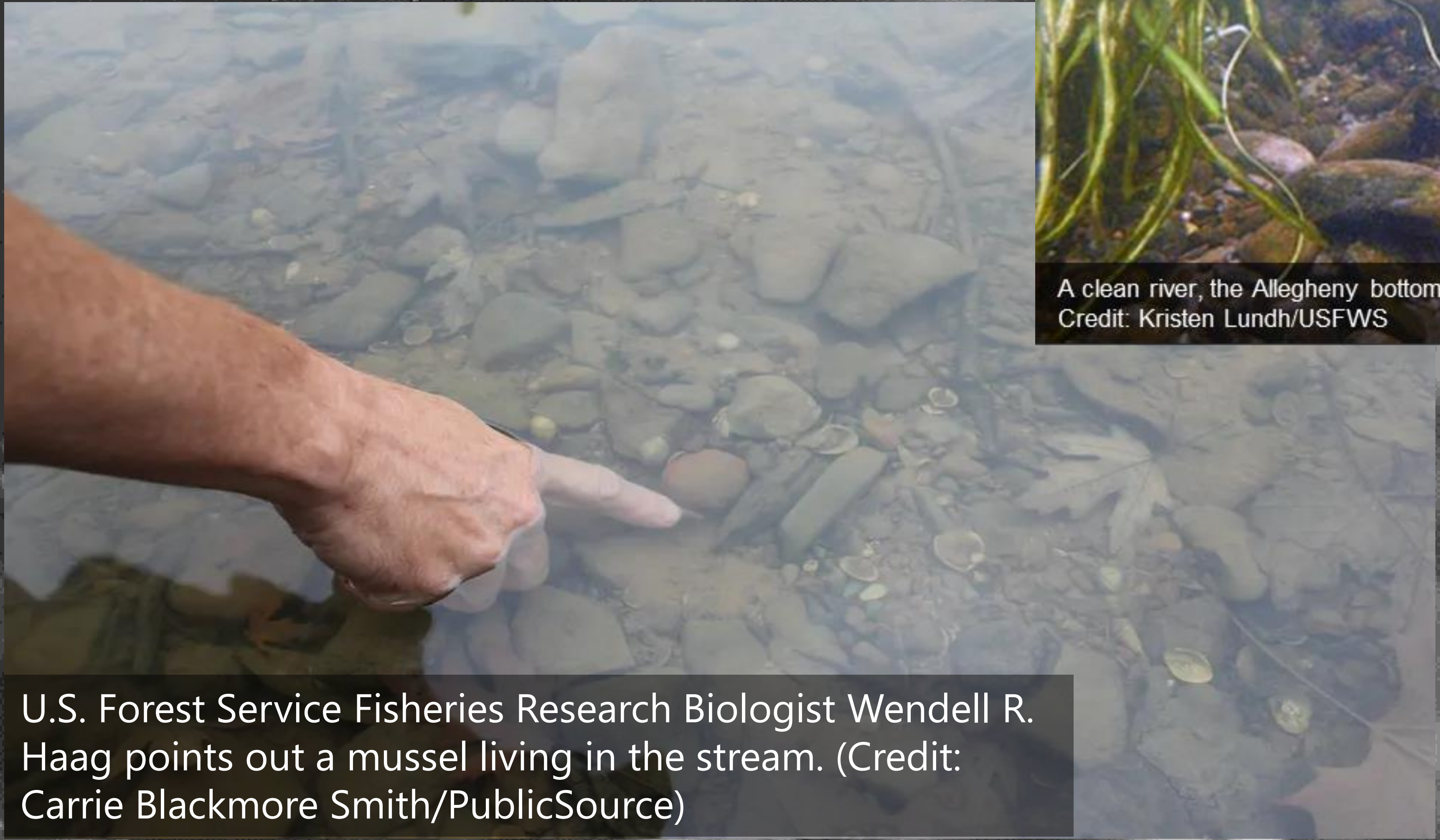
    The most sensitive native taxa may not be protected





"Few places that once had such mussel beds currently have any mussels at all," says Danielle Kreeger, science director with Partnership for the Delaware Estuary. "The decline of natural mussel beds in our nation's waterways means that we have lost natural ecosystem services that helped to sustain water quality. This in turn means that we need to spend more money to mechanically filter water when used for drinking water."

A clean river, the Allegheny bottom is rocky with stands of eelgrass.
Credit: Kristen Lundh/USFWS



U.S. Forest Service Fisheries Research Biologist Wendell R. Haag points out a mussel living in the stream. (Credit: Carrie Blackmore Smith/PublicSource)

Environmental DNA

"The development and validation of the eDNA methodology will profoundly change how aquatic populations are monitored and significantly improve the ability to conserve and recover rare aquatic species. West Virginia looks forward to assisting with this project." -Janet Clayton, WV Dept. of Natural Resources

Species detection via Environmental DNA (eDNA)

Environmental DNA (eDNA)

- Extra-organismal DNA suspended in the environment
- Sloughed cells, any excretions from living organisms, carcasses

Species Detection via eDNA

- Non-invasive
- Highly sensitive
- Efficient, cost-effective
- High resolution spatiotemporal sampling
- Low effort once molecular assays are developed
- Detect individual species or community
- eDNA archive

Species detection via eDNA

Develop a more cost effective, sustainable assessment method for sensitive species (T&E)

Advance molecular techniques for taxonomic ID of species based on DNA in environmental samples

Northern riffleshell, *Epioblasma torolosa rangiana*

Snuff box, *Epioblasma triquetra*

Dwarfwedge mussel, *Alasmidonta heterondon*

1. Determine the metrics needed to calibrate models of species distribution using eDNA.
2. Develop models of target species based on eDNA detection
3. Validate models in natural systems

Species detection

Assessment Method Challenges


Divers sort mussels collected in the Allegheny River under the Hunter Station bridge. Credit: Jim Carroll/PennDOT

Freshwater mussel Inventory:

- *Where do imperiled species occur?*
- Difficult to detect

Field Surveys

- Invasive
- Resource/Time intensive
  - Limited spatial scale
- Inherently biased –
  - Systematic underestimation of occupancy

Genetics:

Abundance

- Presence/absence
- Positive correlation b/n eDNA signal and species abundance?

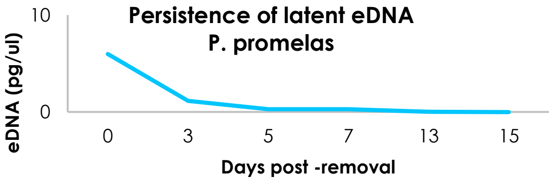
[eDNA]

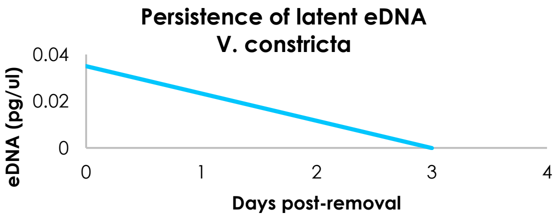
- *Production*: density, health, reproductive status, metabolism
- *Degradation*: UVB exposure, water temperature, pH
- *Environment*: water volume, flow, habitat, temperature

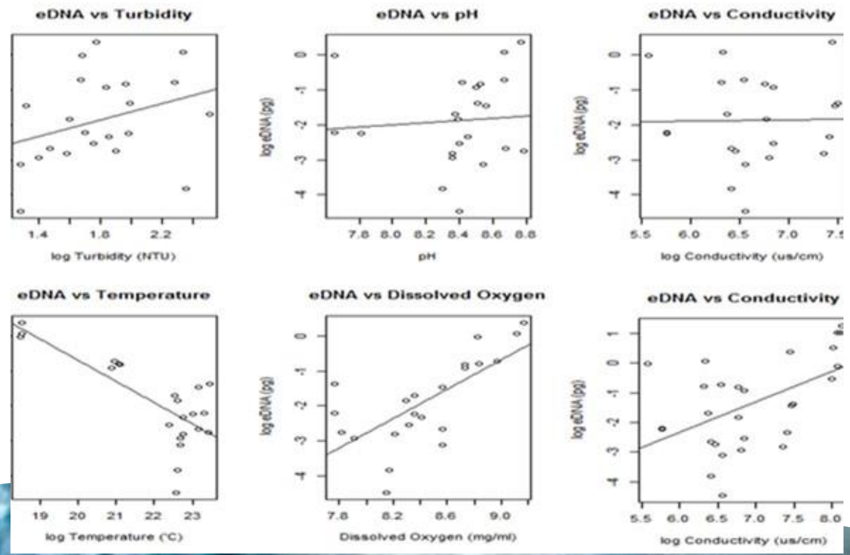
Mesocosm Pilot Study

eDNA method development

- Deployed fish, amphibians, mussels
- Collected Temporal mesocosm samples for eDNA
- Developed eDNA probes for
  - –*Pimephales promelas* (fathead minnow)
  - – *Eurycea cirrigera* (two-lined salamander)
  - –*Villosa constricta* (notched rainbow)
- What influences probability of target detection?
  - –Time, Distance, Physicochemical parameters, Abundance
    - How persistent is latent eDNA?



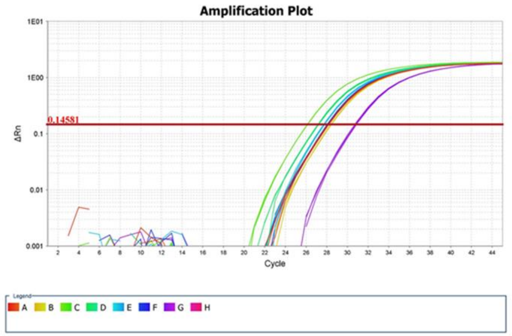




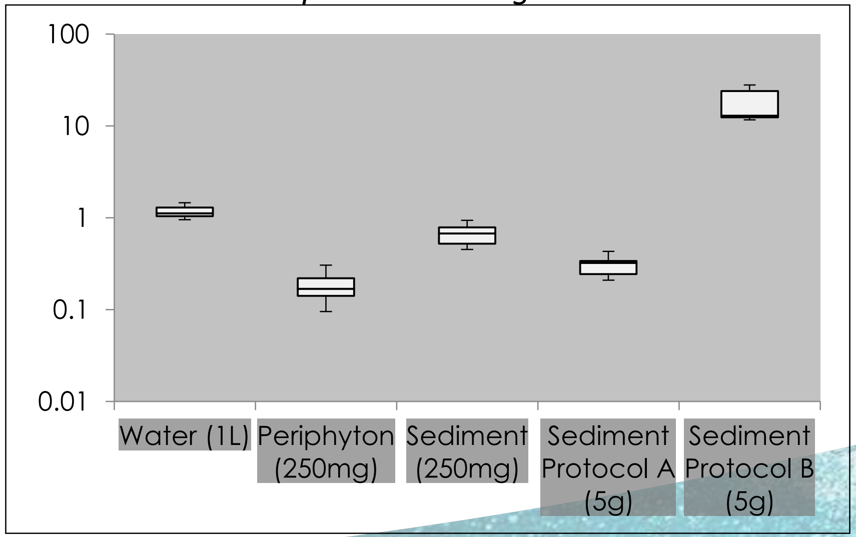
Investigated physiochemical parameters that may influence the probability of detecting targets in water samples.

Species detection via eDNA

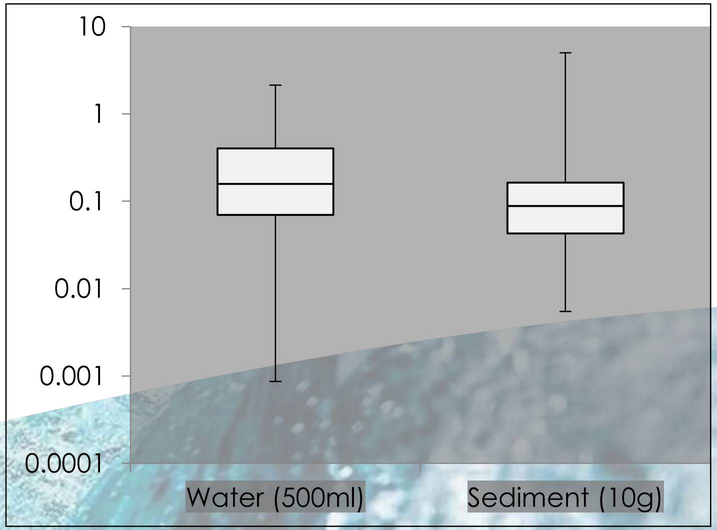
Developing Sampling Protocols

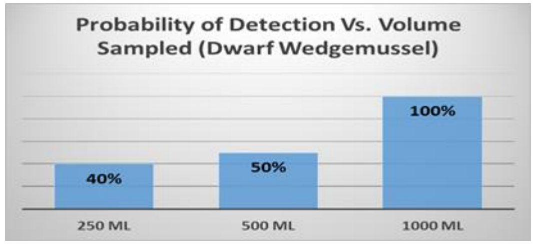


Epioblasma rangiana



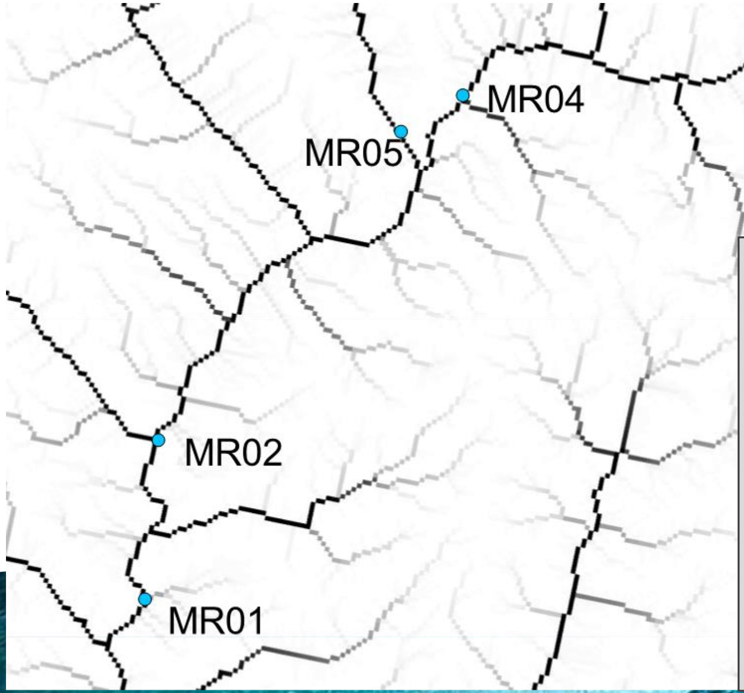
Alasmidonta heterodon





Dwarfwedge mussel in Maryland

eDNA method development



MR07, nondetect

MR04, detect

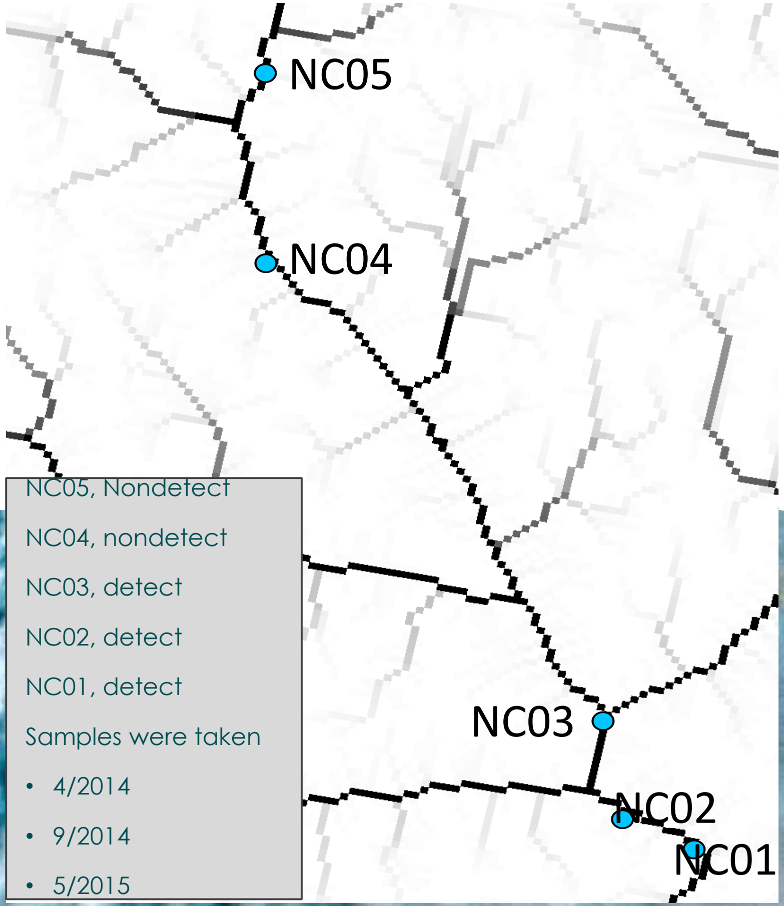
MR05, nondetect

MR02, detect

MR01, detect

samples were taken

- 4/2014
- 10/2014
- 5/2016



NC05, Nondetect

NC04, nondetect

NC03, detect

NC02, detect

NC01, detect

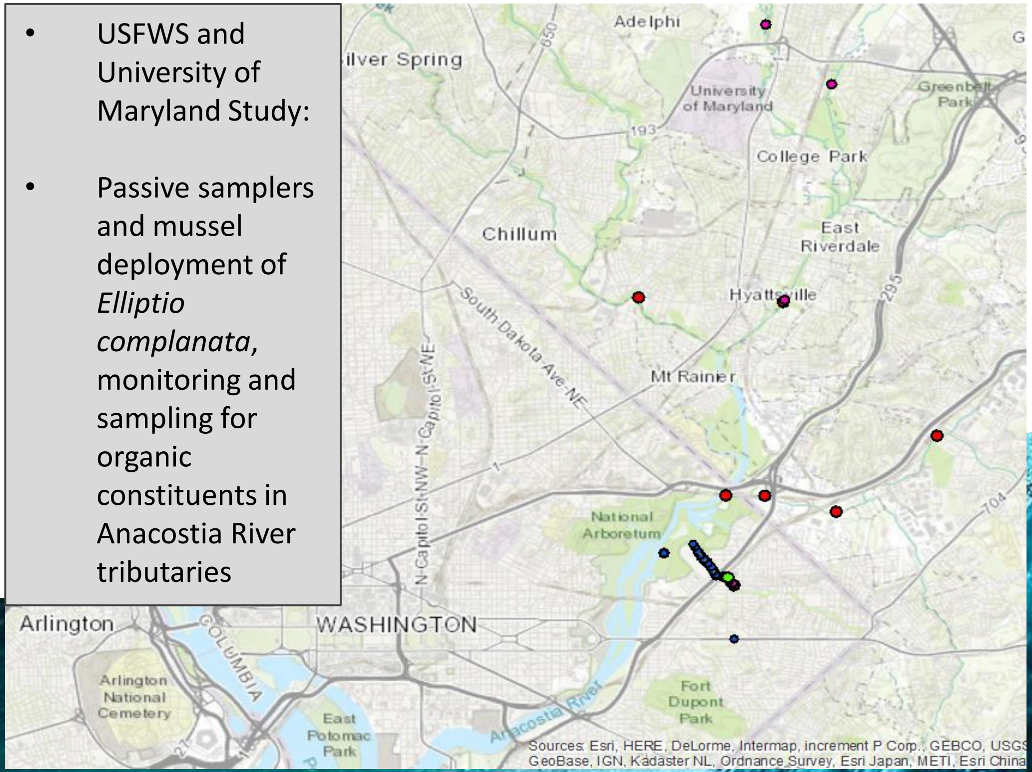
Samples were taken

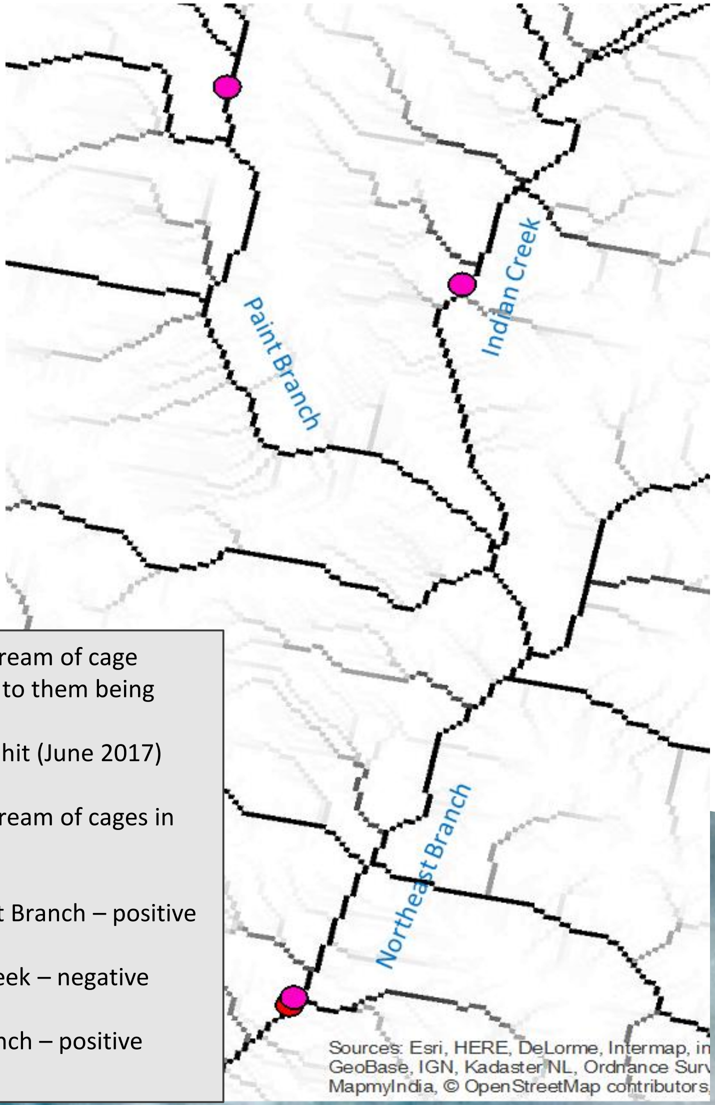
- 4/2014
- 9/2014
- 5/2015

Anacostia River Project

Deployed cages with known number of freshwater mussels…how far can we detect eDNA downstream?

- USFWS and University of Maryland Study:
- Passive samplers and mussel deployment of *Elliptio complanata*, monitoring and sampling for organic constituents in Anacostia River tributaries





Sampled upstream of cage location prior to them being deployed

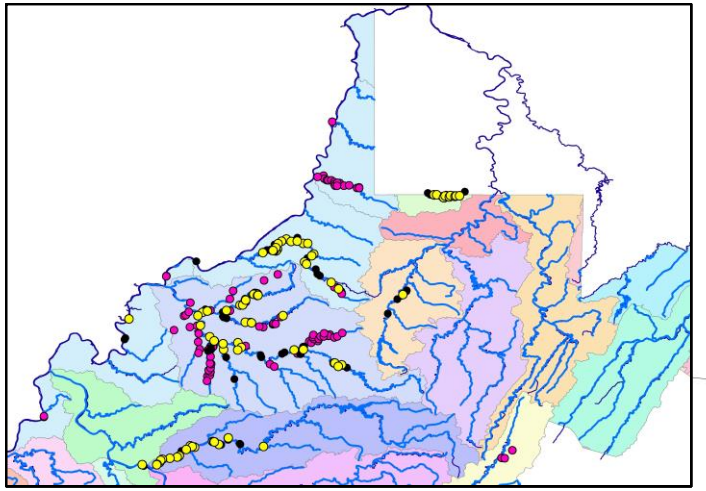
- positive hit (June 2017)

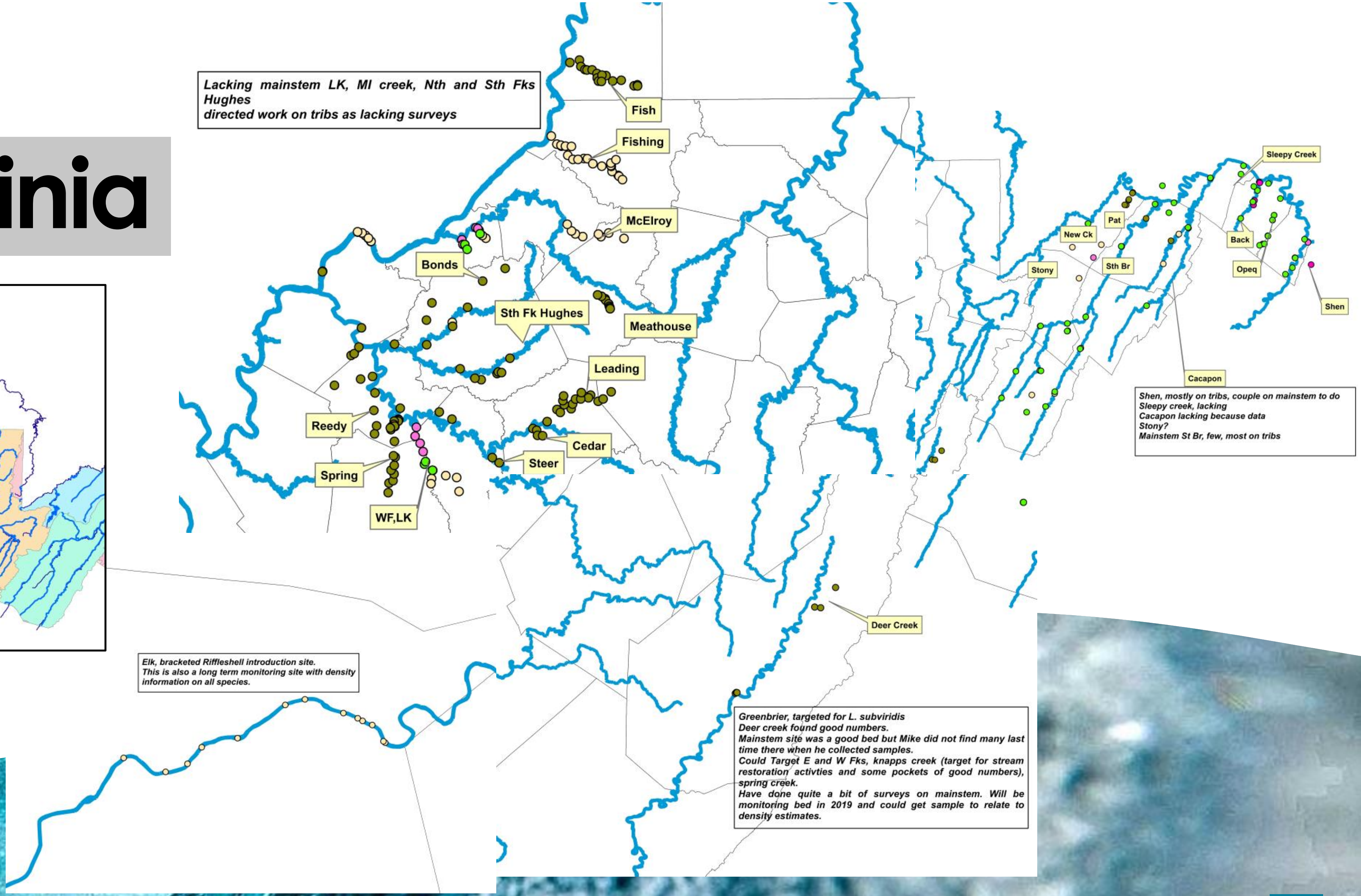
Sampled upstream of cages in August

- Northeast Branch – positive
- Indian Creek – negative
- Paint Branch – positive

Need for more than just T&E probes

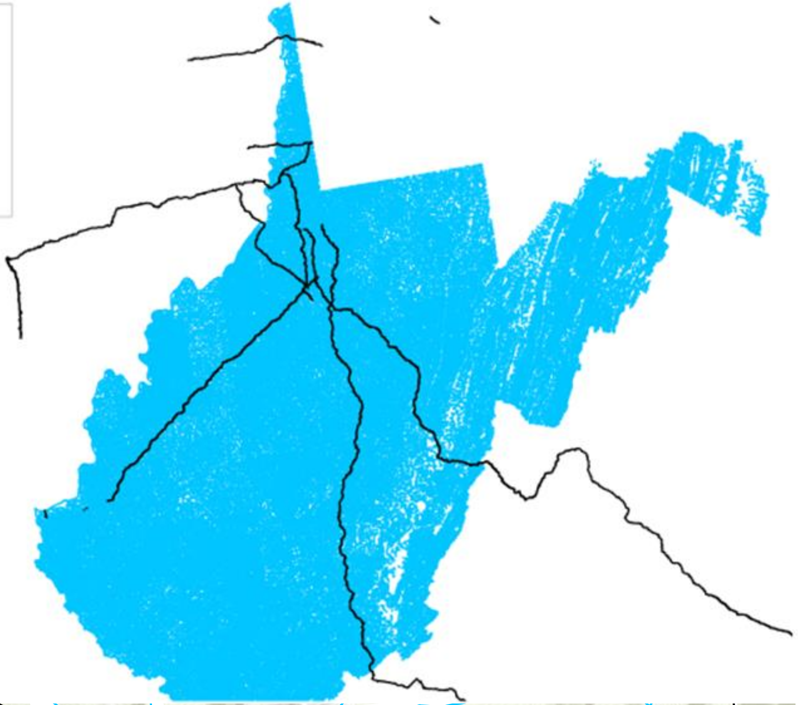
West Virginia

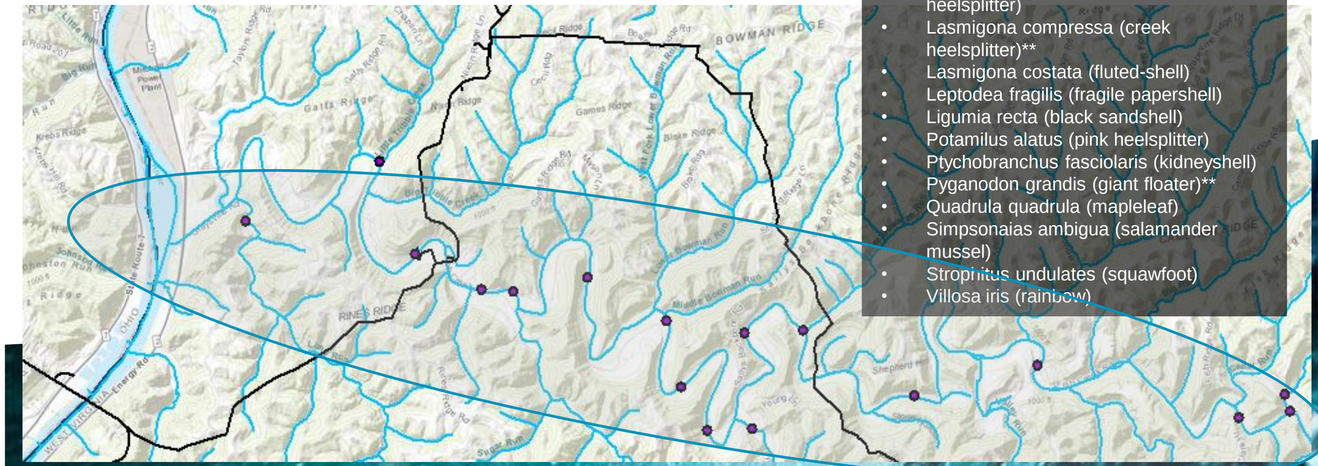




West Virginia

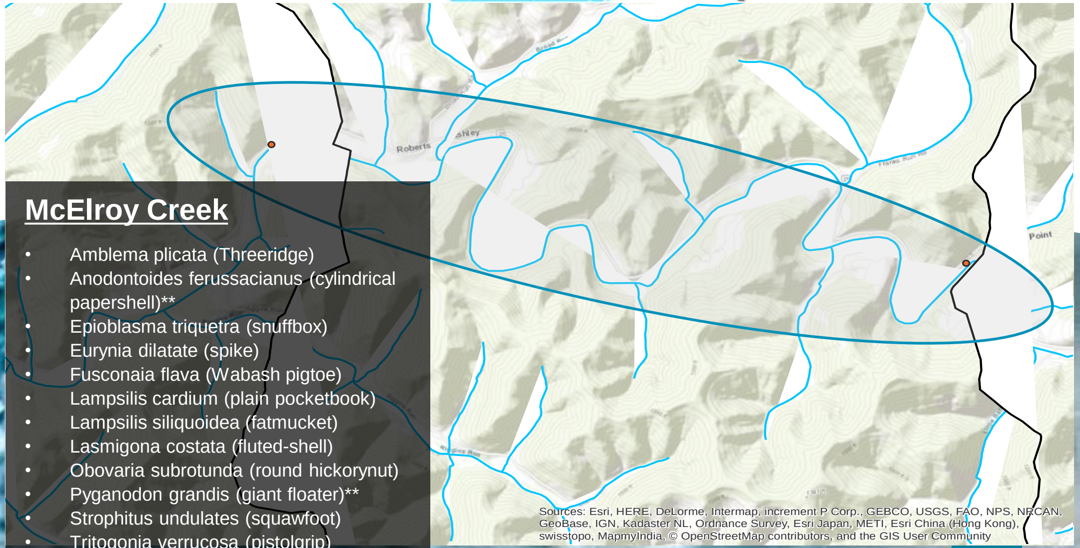
Need for more than just T&E probes





Fish Creek - 2016

- Cyclonaias pustulosa (pimpleback)
- Fusconaia flava (Wasbash pigtoe)
- Fusconaia subrotunda (long solid)
- Lampsilis cardium (plain pocketbook)
- Lampsilis ovata (pocketbook)
- Lampsilis siliquoidea (fatmucket)
- Lasmigona complanata (white heelsplitter)
- Lasmigona compressa (creek heelsplitter)**
- Lasmigona costata (fluted-shell)
- Leptodea fragilis (fragile papershell)
- Ligumia recta (black sandshell)
- Potamilus alatus (pink heelsplitter)
- Ptychobranchus fasciolaris (kidneyshell)
- Pyganodon grandis (giant floater)**
- Quadrula quadrula (mapleleaf)
- Simpsonaias ambigua (salamander mussel)
- Strophitus undulates (squawfoot)
- Villosa iris (rainbow)



McElroy Creek

- Amblema plicata (Threeridge)
- Anodontoides ferussacianus (cylindrical papershell)**
- Epioblasma triquetra (snuffbox)
- Eurynia dilatate (spike)
- Fusconaia flava (Wabash pigtoe)
- Lampsilis cardium (plain pocketbook)
- Lampsilis siliquoidea (fatmucket)
- Lasmigona costata (fluted-shell)
- Obovaria subrotunda (round hickorynut)
- Pyganodon grandis (giant floater)**
- Strophitus undulates (squawfoot)
- Tritogonia verrucosa (pistolgrip)
- Utterbackia imbecillis (paper pondshell)**
- Villosa iris (rainbow)

Developing General Mussel Probe

Need for more than just T&E probes

Targeting small fragment of mitochondrial genome (i.e., 12S, COI, etc) ***patent pending*

- designed to discriminate *all* Unionid species

Cladogram of freshwater mussels (WV DNR, FWS, MD DNR)

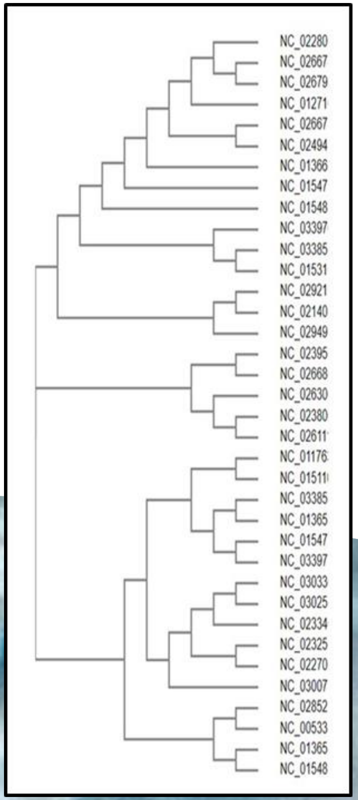
4th version of UnionID general freshwater mussel probe

2016 – WV DNR 32 species

2017 – WV DNR 33 species

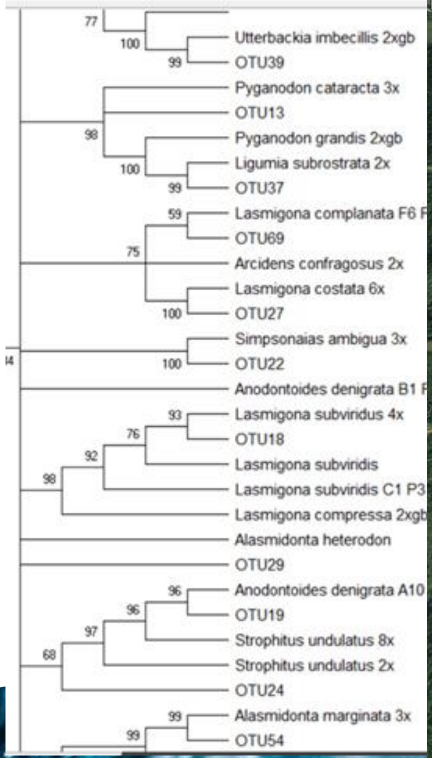
2018 – KY DFWR 34 species

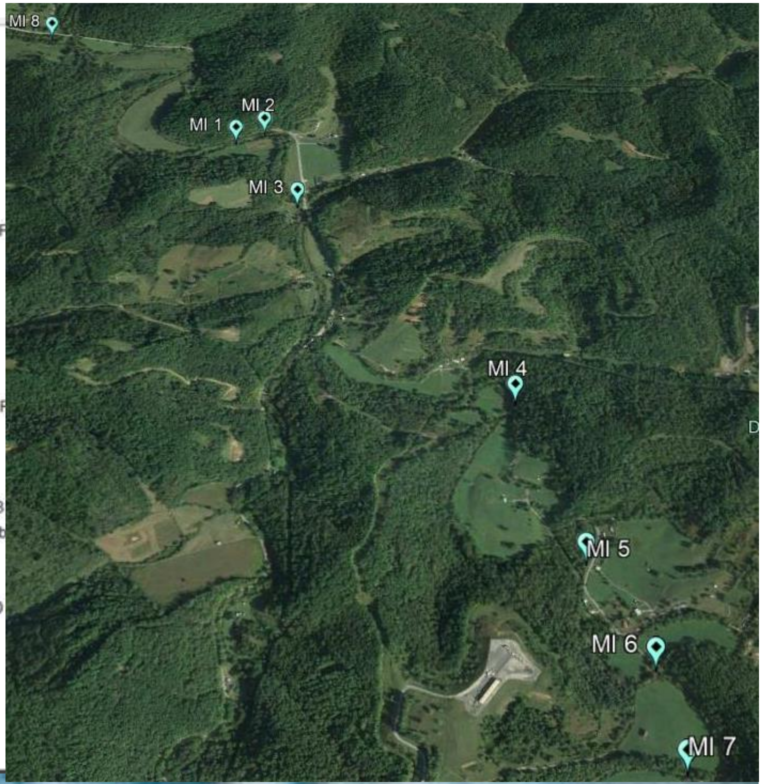
2020 – 137 species in library (>500 individuals)



Introducing General Mussel Probe

Example: Threeridge, *Amblema plicata*





#OTU ID	Primary Subject Hit	% ID	LdCK1	LdCK10	LdCK12	LdCK13	ELKDS10	OR500LB	OR500LT
			WV161061	WV161123	WV161212	WV161222	WV171071	WV17892	WV17901
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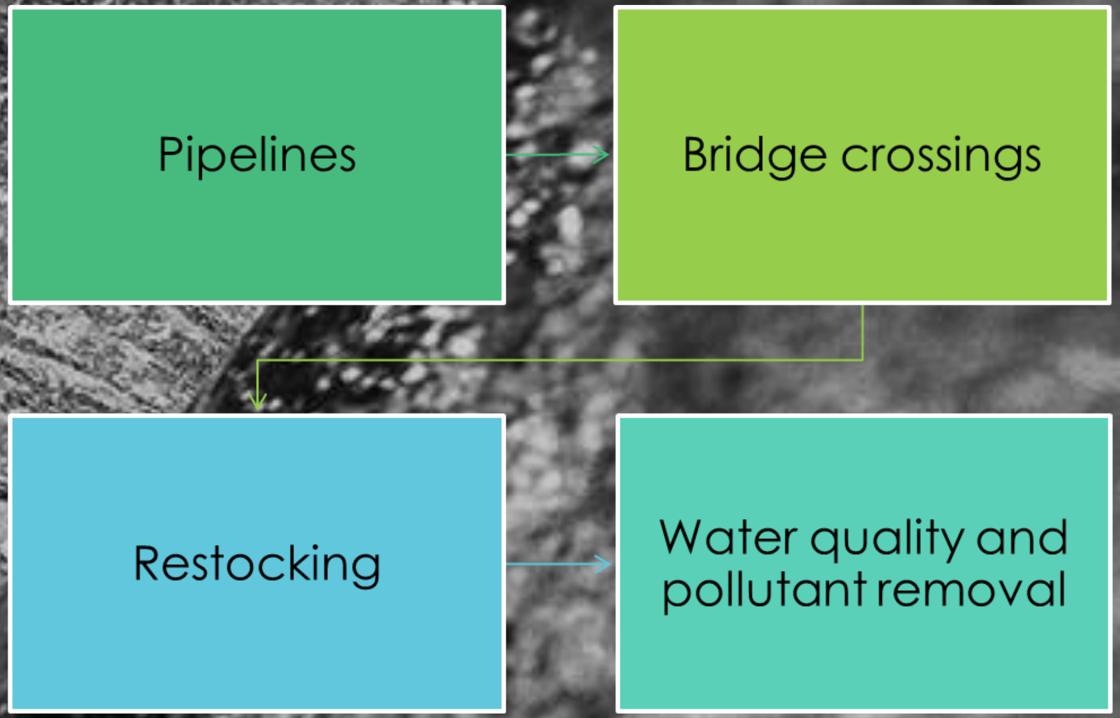

UnionID eDNA probe

Applications in Region 3