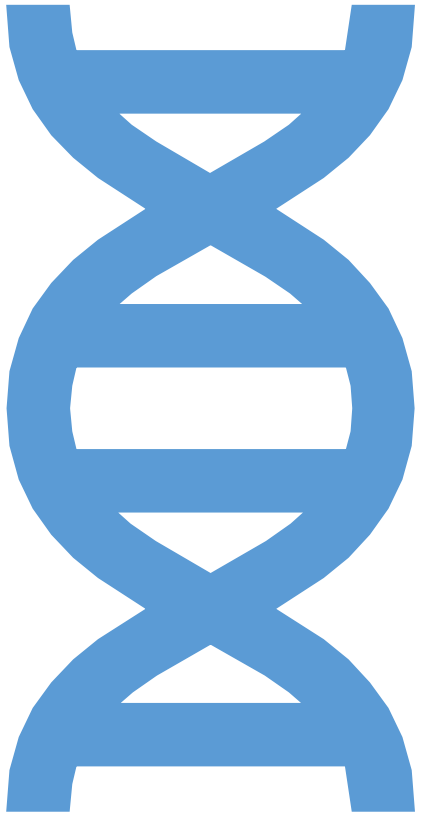
CHESAPEAKE BAY JOURNAL

‘eDNA’ reveals what’s swimming in the water



Environmental DNA sampling and assessment techniques for ***fish assemblages*** in the Mid-Atlantic Region.

National Rivers and Streams Assessment Fish Sampling 2018-19

How do we sample fish?

Question – is a species list determined by eDNA the same as that determined by NRSA electrofishing method.

Covariates – stream size, water chemistry, fish family, fish genus, total richness, fish density, electrofishing efficiency

Comparisons: Fish Species by site

88 wadeable NRSA sites in PA, WV, and VA, and MD.
~8 of these sites will be revisited as part of NRSA. eDNA and fish will be resampled at this time.

Negative Controls – Every site will include a field blank and if filtered, an equipment blank.

Positive Controls – At the Revisit sites, a positive control of water from the fish sampling bucket will be analyzed for eDNA

1L of water collected at bottom of reach and filtered within 8 hours.

DNA is extracted is lab and amplified using PCR.

Two mitochondrial gene loci – 12S and 18S – will be used to identify species.

Sequencing on MiSeq, generating several million sequences which are subsequently multiplexed for each gene loci.

Bioinformatics will be matched from GenBank

Data uses

If eDNA proves to be a useful tool to estimate presence of rare or sensitive species (this might also work the other way by signaling the presence of an invasive species) data will be used in :

- NRSA Condition estimates – IBI metrics or models
- States can adopt the methods to supplement IBI
- EPA and States can adopt methods to help develop Biological Condition Gradient models
- eDNA may help monitor the spread of invasive fish that are vagile and cryptic

Relating the effectiveness of eDNA to covariates will help us improve eDNA sampling in the future and will help us design future studies.

Agreement between eDNA samples and Electrofishing

2018 Presence Absense Data for Brook Trout

Agreement between eDNA samples and Electrofishing

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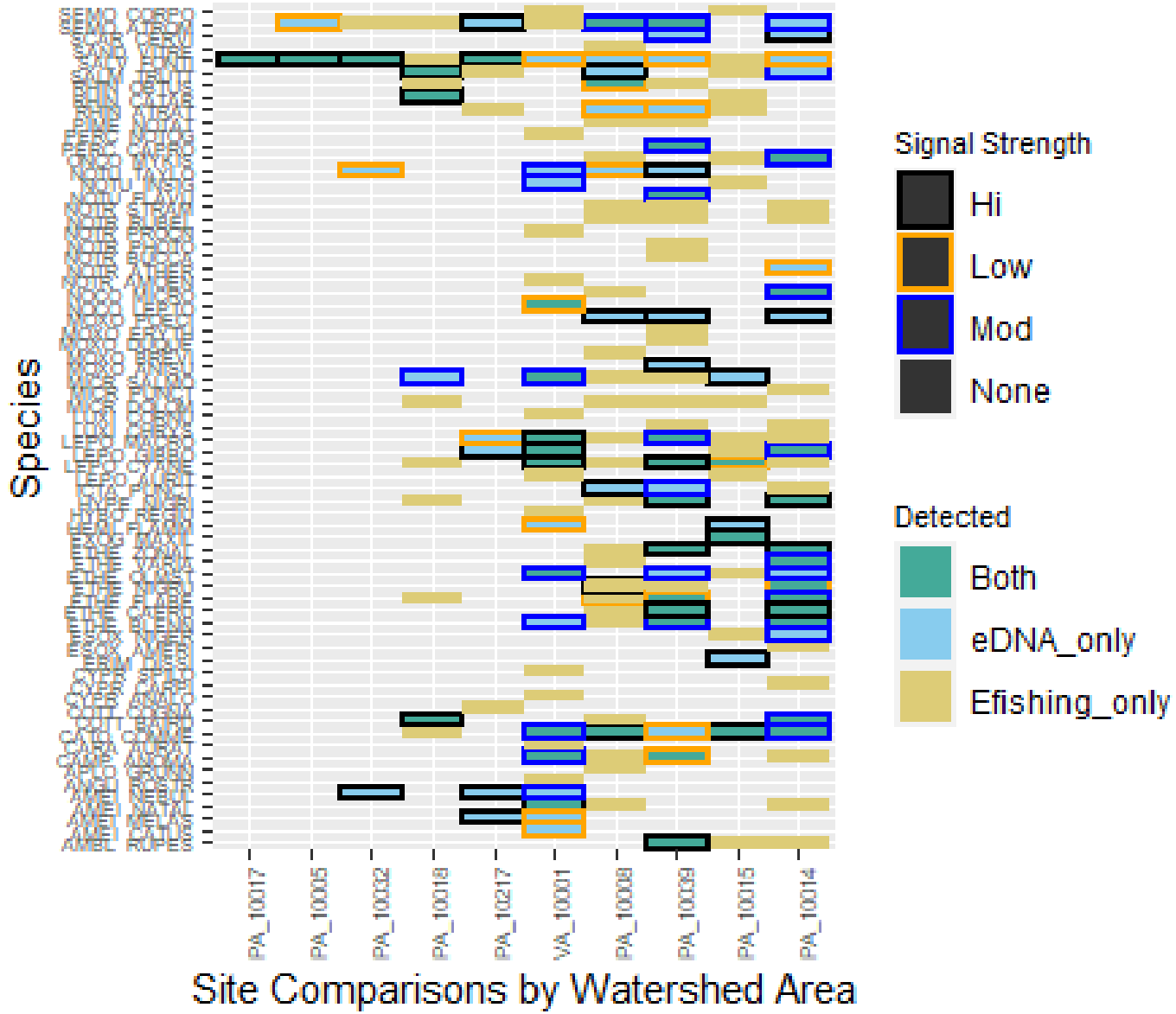
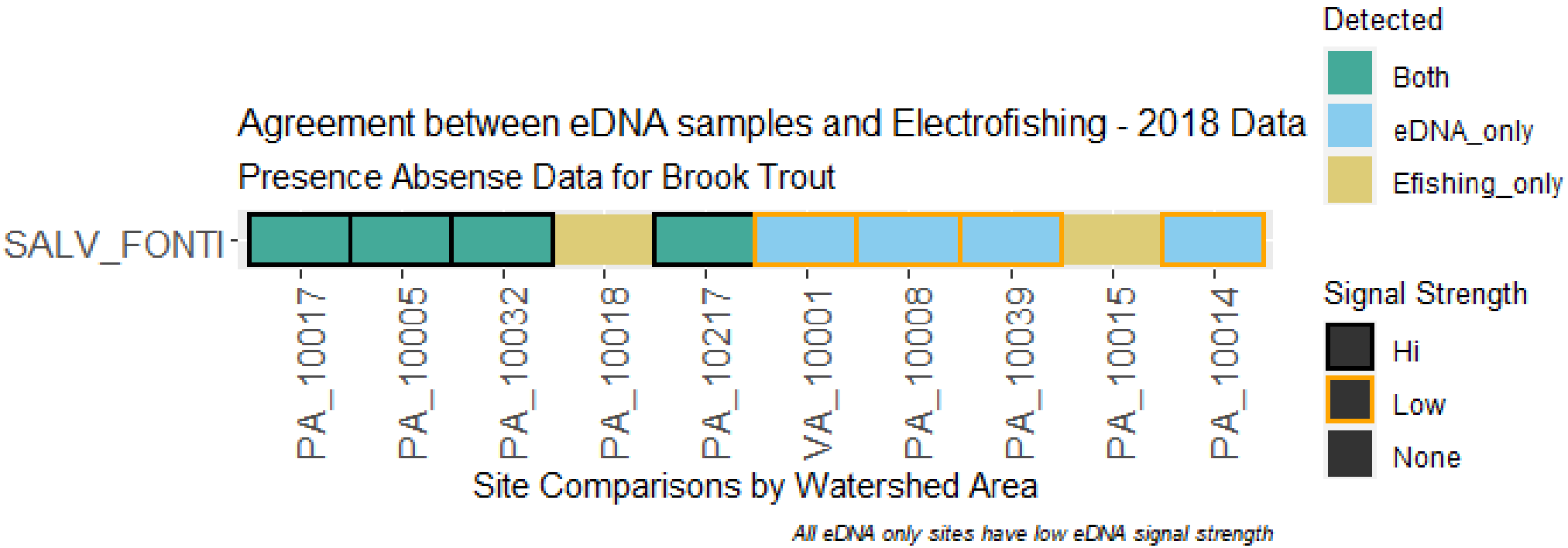
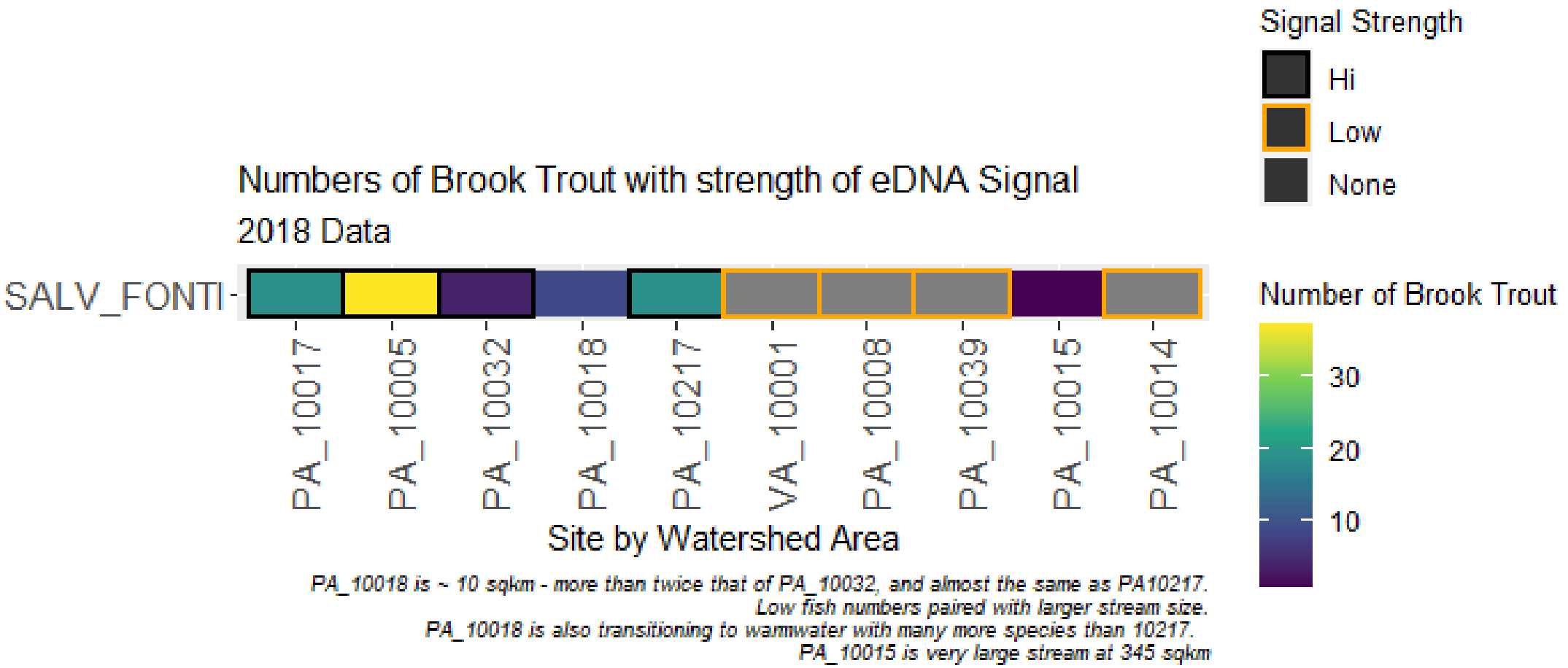
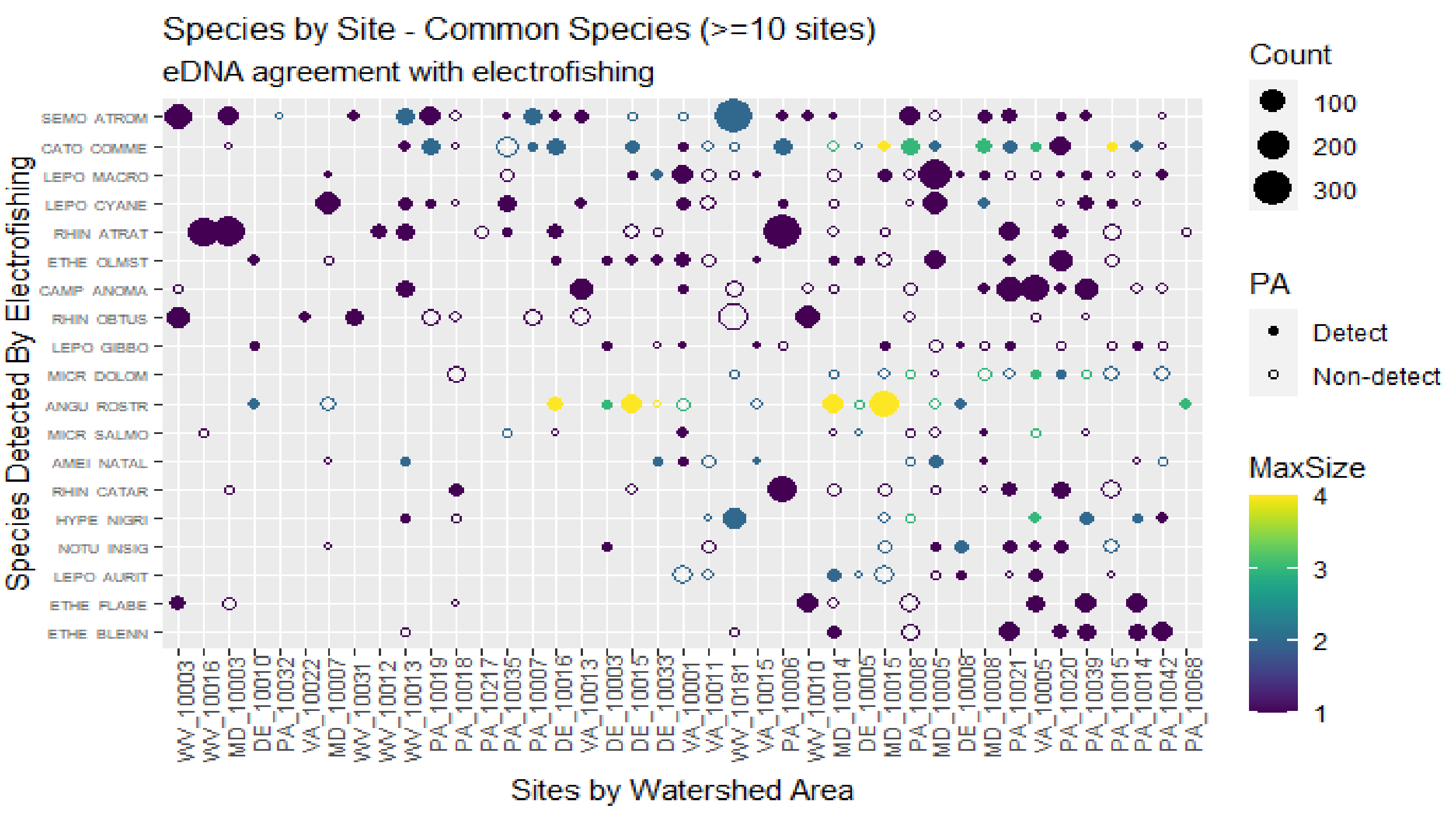



Agreement between eDNA samples and Electrofishing - 2018 Data
Presence Absense Data for Brook Trout
SALV_FONTI
PA_10017
PA_10005
PA_10032
PA_10018
PA_10217
VA_10001
PA_10008
PA_10039
PA_10015
PA_10014
Site Comparisons by Watershed Area
All eDNA only sites have low eDNA signal strength
Detected
Both
eDNA_only
Efishing_only
Signal Strength
Hi
Low
None

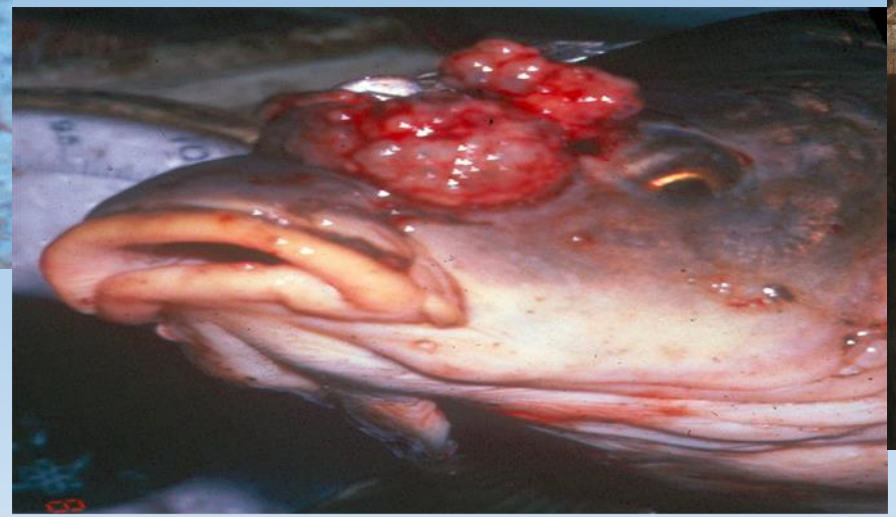


PA_10018 is ~ 10 sqkm - more than twice that of PA_10032, and almost the same as PA10217.
Low fish numbers paired with larger stream size.
PA_10018 is also transitioning to warmwater with many more species than 10217.
PA_10015 is very large stream at 345 sqkm


Species by Site - Common Species (>=10 sites)
eDNA agreement with electrofishing
Species Detected By Electrofishing
SEMO ATROM
CATO COMME
LEPO MACRO
LEPO CYANE
RHIN ATRAT
ETHE OLMST
CAMP ANOMA
RHIN OBTUS
LEPO GIBBO
MICR DOLOM
ANGU ROSTR
MICR SALMO
AMEI NATAL
RHIN CATAR
HYPE NIGRI
NOTU INSIG
LEPO AURIT
ETHE FLABE
ETHE BLENN
WV_10003
WV_10016
MD_10003
DE_10010
PA_10032
VA_10022
MD_10007
WV_10031
WV_10012
WV_10013
PA_10019
PA_10018
PA_10217
PA_10035
PA_10007
DE_10016
VA_10013
DE_10003
DE_10015
DE_10033
VA_10001
VA_10011
WV_10181
VA_10015
PA_10006
WV_10010
MD_10014
DE_10005
MD_10015
PA_10008
MD_10005
DE_10008
MD_10008
PA_10021
VA_10005
PA_10020
PA_10039
PA_10015
PA_10014
PA_10042
PA_10068
Sites by Watershed Area
Count
100
200
300
PA
Detect
Non-detect
MaxSize
4
3
2
1

eDNA should be used within a context of a directed question .

There are some things eDNA won’t answer for us -
Relative abundance of fish.
Presence of Diseases, Fin Erosions, Lesions, or Tumors on fish.
Fish tissue contaminant samples can be collected from actual fish.
We can ensure the quality of fish identifications in the sample of fish collected.



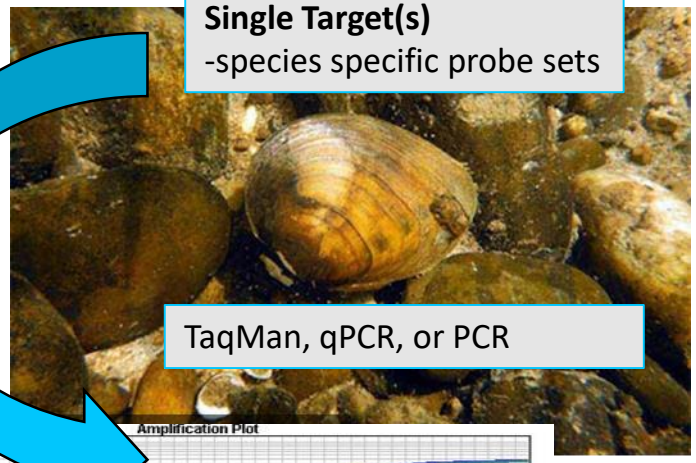
eDNA samples have no impact on fish in the stream. Electrofishing and handling fish is very stressful to organisms and can result in high mortality.
eDNA can be particularly useful in places where the presence of threatened or endangered species preclude the use of electrofishing.

An eDNA sample can’t give you the same notion of a site and sample. Six hours fishing a stream forces a thorough examination of the fish in their habitat – an experience for a fish biologist that deepens their knowledge of fish and the streams they inhabit.

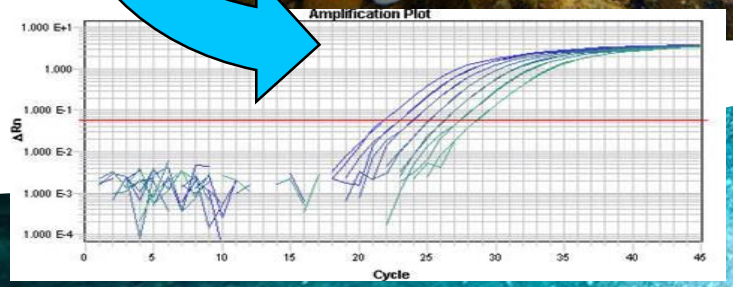
eDNA Method Development

Single species specific probe and General probe libraries

Single Target(s)
-species specific probe sets

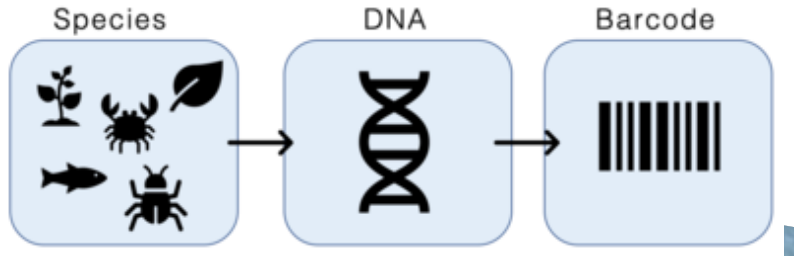


TaqMan, qPCR, or PCR

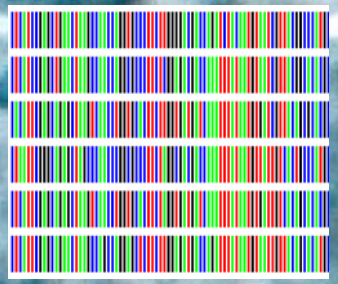


Community/Biodiversity
-generally non-specific probes

Pyrosequencing



Epioblasma torulosa
Epioblasma triquetra
Alasmidonta heterodon
Lasmigona subviridis
Alasmidonta varicosa
Pleurobema clava



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)

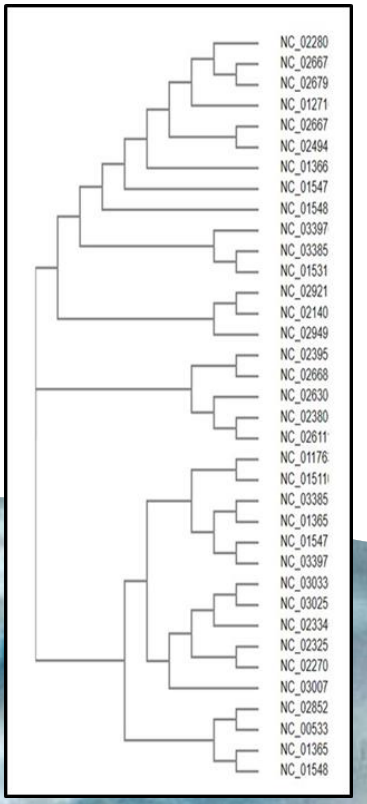
4th version of UnionID general freshwater mussel probe

2016 – WV DNR 32 species

2017 – WV DNR 33 species

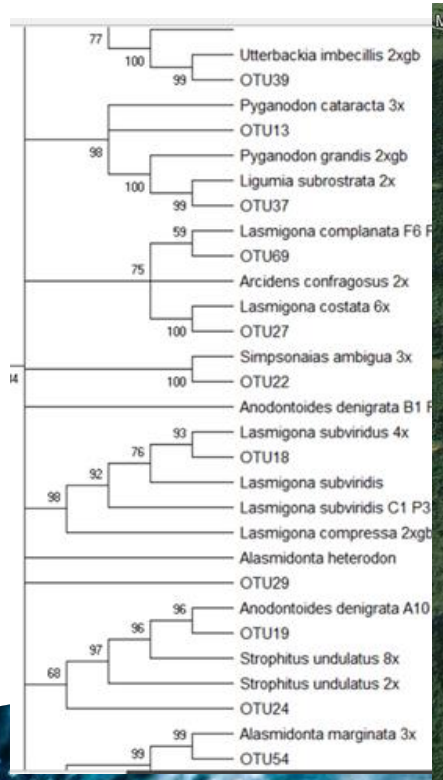
2018 – KY DFWR 34 species

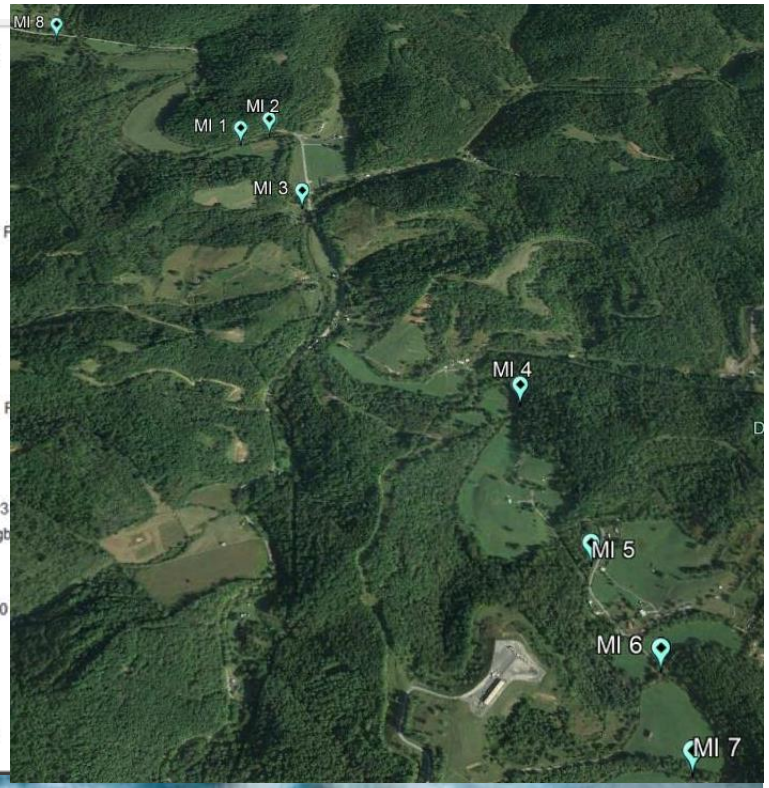
2020 – 137 species in library (>500 individuals)



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
242OTU11	Amblema_plicata_9x	100	1009	1	1	245	263	2076	