

Assessing stream health through the lens of fish eDNA

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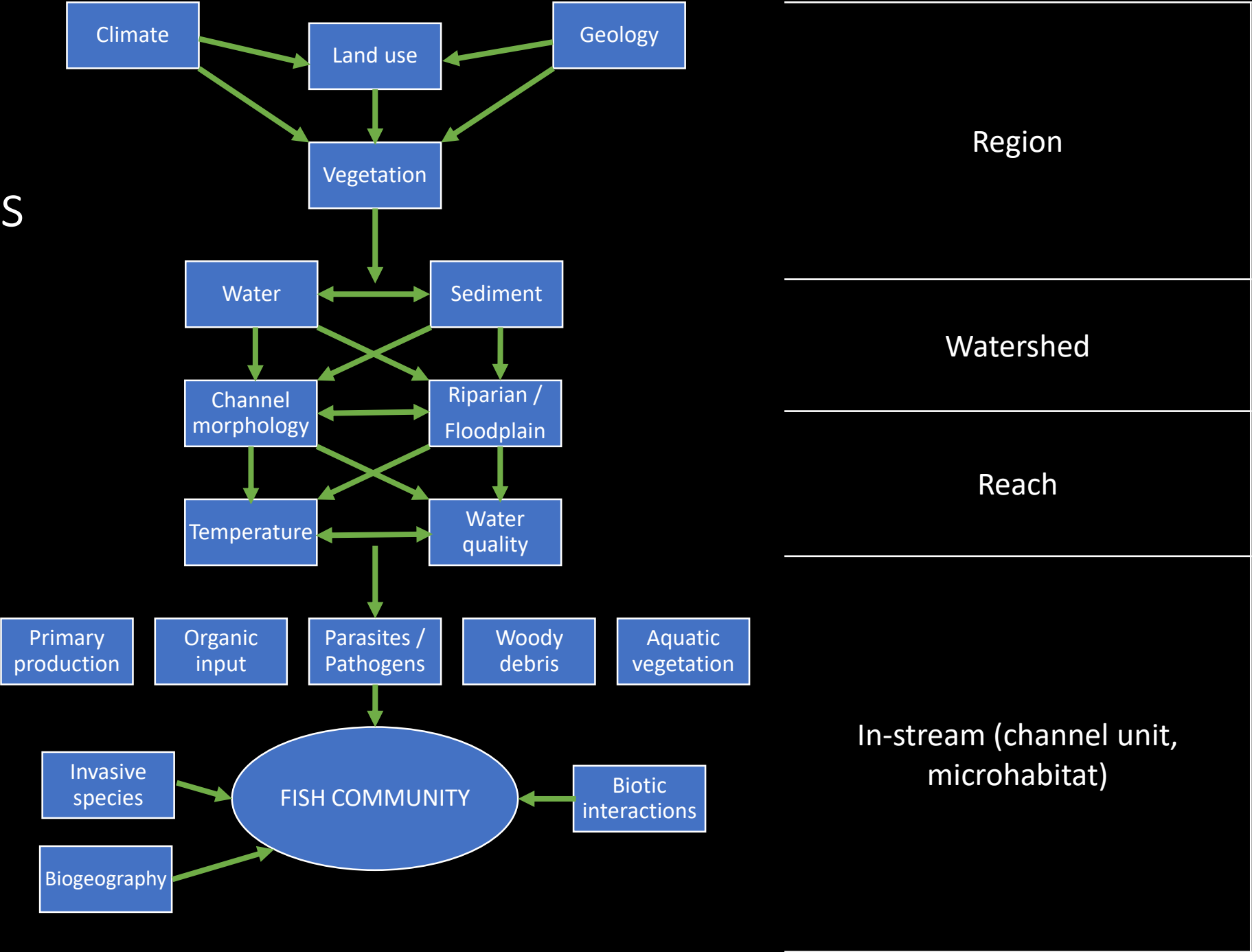
Outline

1. Biomonitoring
2. Sampling fish communities
 - CES - Conventional electrofishing & seining
 - eDNA - Environmental DNA metabarcoding
3. Delmarva case study

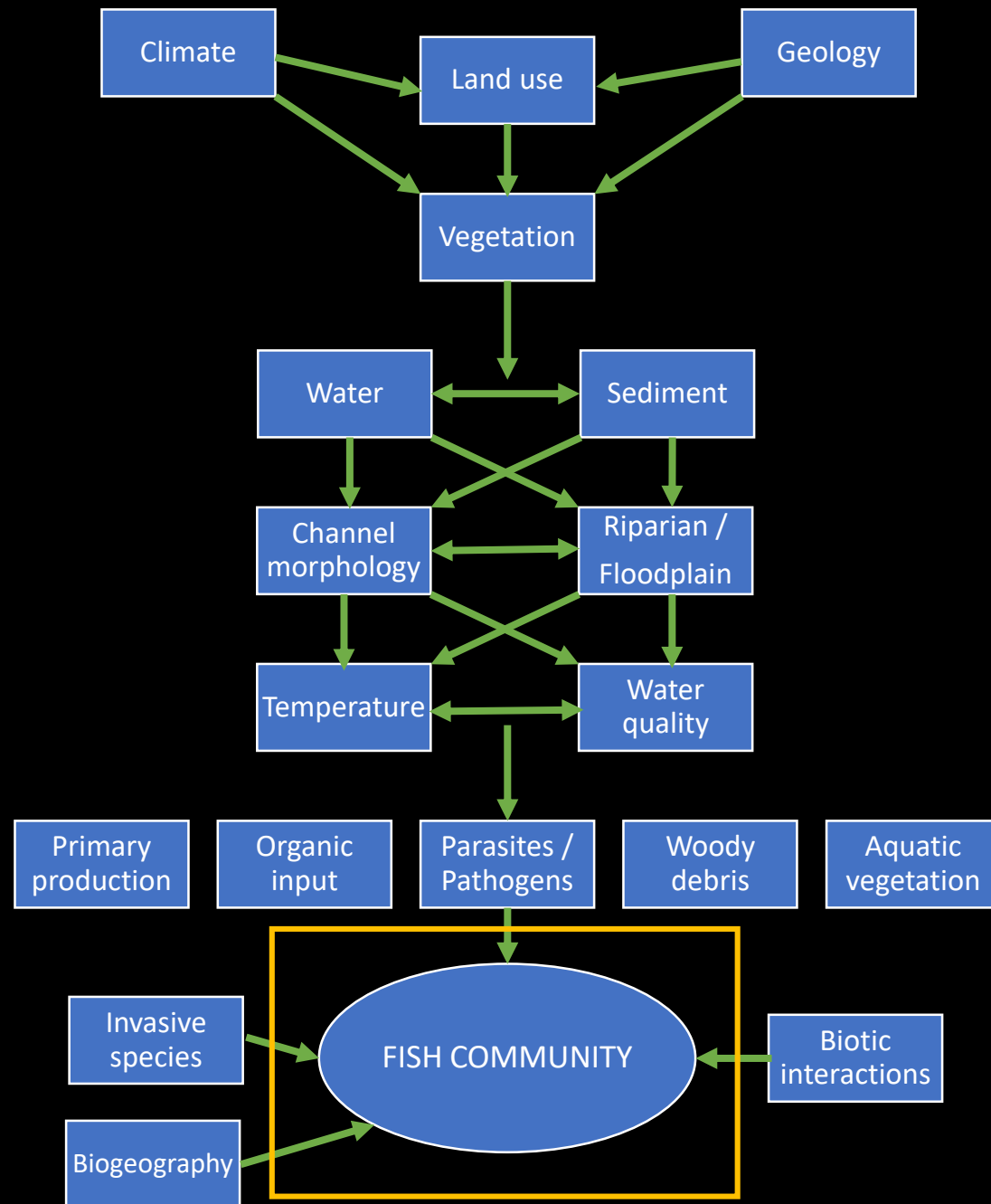




Nested processes control aquatic organisms' habitat







Which indicator organisms?

- Species diversity
- Objective?
 - Local site / point source → bugs
 - Watershed / regional conditions → fish

Macroinvertebrates (all states)

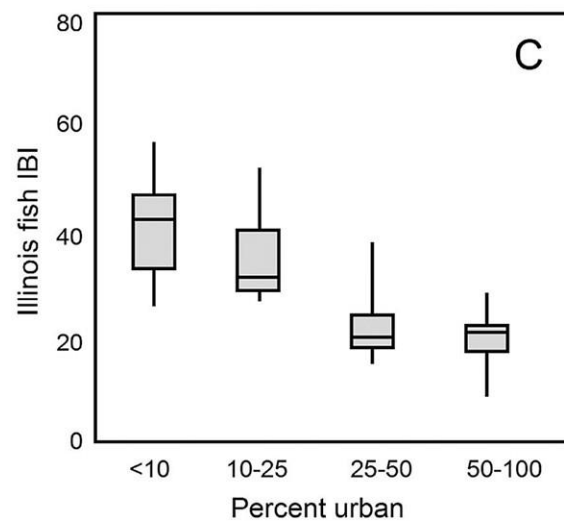
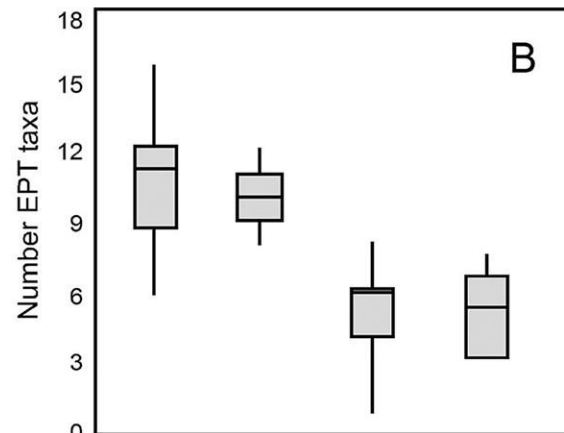
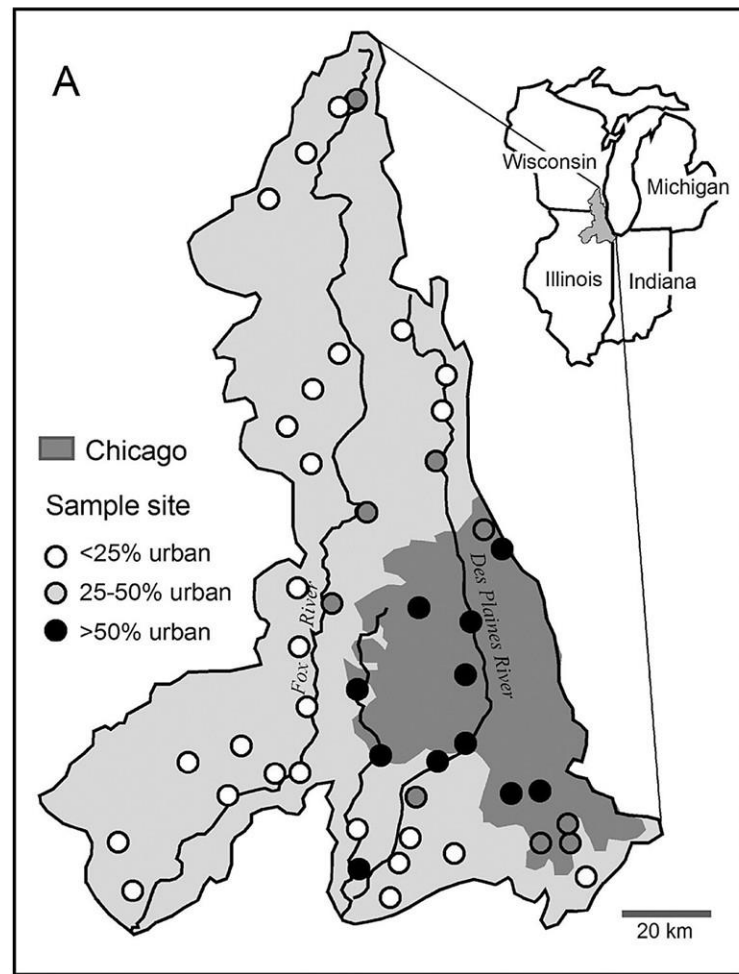
Fish (2/3 of states)

Algae (1/3 of states)



Fish Index of Biotic Integrity (generic)

Category and Metric		Scoring Criteria		
		5	3	1
Occurrence-based	Species diversity	Expectation varies with stream size, region, basin		
	Number of species			
	Number of darter species			
	Number of sunfish species			
	Number of sucker species			
	Number of intolerant species			
Abundance-based	% individuals as intolerant	<5%	5-20%	>20%
	Trophic composition			
	% individuals as omnivores	>20%	20-45%	>45%
	% individuals as insectivorous cyprinids	>45%	20-45%	<20%
	% individuals as piscivores	>5%	1-5%	<1%
	Fish abundance and condition	Expectation varies with stream size, region, basin		
	Number of individuals			
	% individuals as hybrids			
		% individuals with anomalies	0-2%	>2-5%
Sum (IBI score)		60	←————→ 12	
Integrity class		Excellent ----- Good ----- Fair ----- Poor ----- Very poor		



Invertebrates

Fish

Harris et al. 2005. USGS Sci. Invest. Rpt. 2005-5218

How to sample fish?

CES—Conventional electrofishing and seining

Advantages:

1. Standard methods
2. Provides occurrence- and abundance-based IBI metrics
3. Clearly delineated sample unit



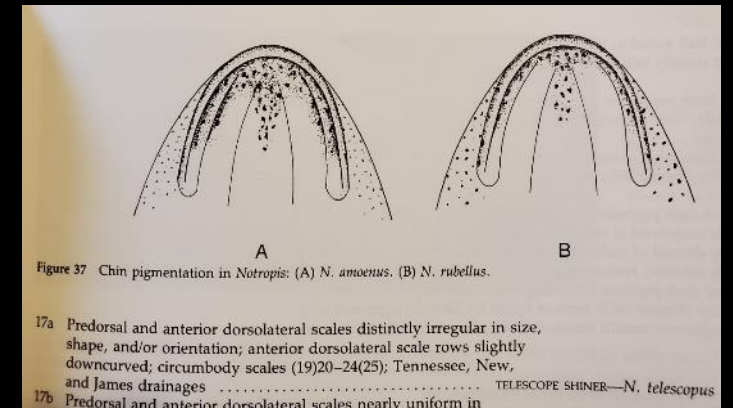
CES—advantages (cont.)

4. Fish in hand !



CES—limitations

1. Laborious!
2. Small sample unit
3. Hazardous
4. Invasive
5. Expensive equipment
6. Imperfect detection
7. Taxonomist required
8. Some habitats not accessible



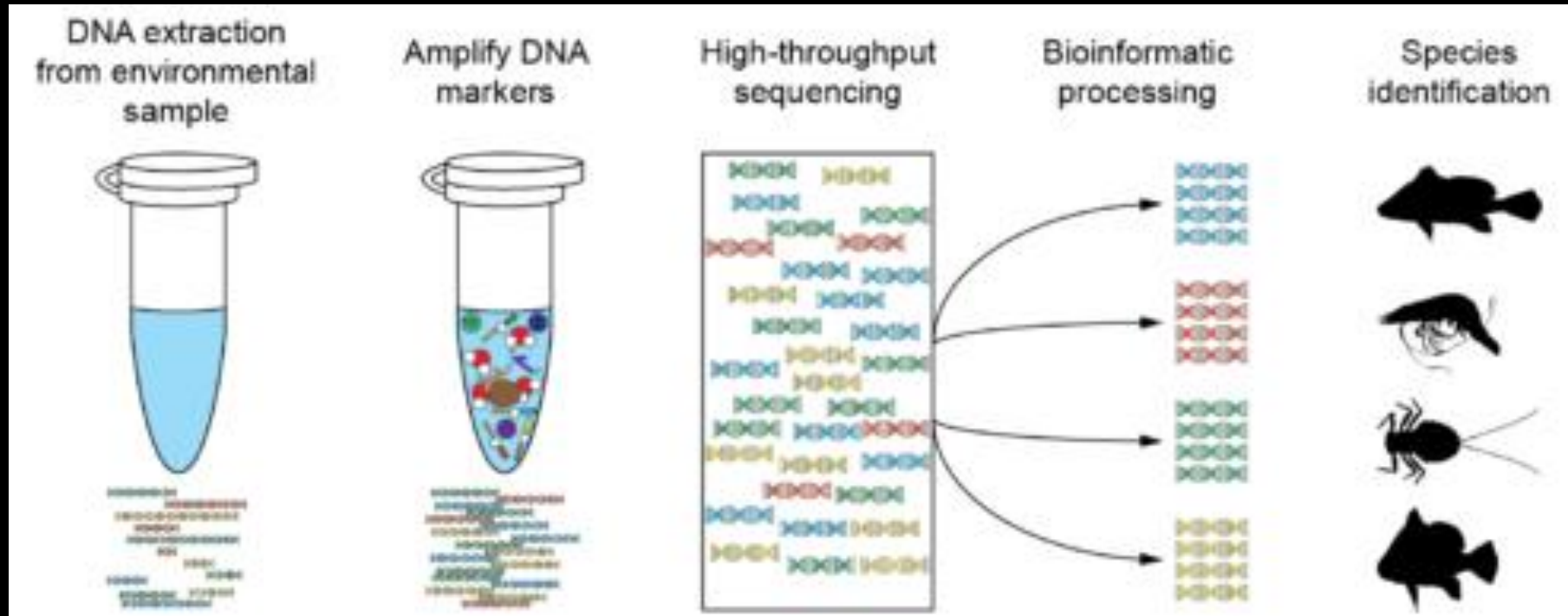
eDNA metabarcoding

A new way to sample fishes

Filter some water...



eDNA metabarcoding—lab workflow



<http://www.naturemetrics.co.uk/>

eDNA metabarcoding—deliverable

Summary data

Exact Sequence Variant unique identifier

Consensus Taxonomy

Taxonomic ranking from the library matched to each sequence.

The % of base pairs in the sequence that match to the species in the library.

Number of species matching the sequence at a given level.

The detected sequence.

Sample identifiers
Numbers after the decimal point represent lab replicates of the same sample.

ESV	Family	Genus	Species	% match	# Spp	Sequence	S10001.1	S10002.1	S10003.1	S10003.2
ESV_000031	Catostomidae	Catostomus	Catostomus commersonii	100	1	AAAAAG...	3066	1340	5780	3462
ESV_009176	Moronidae	Morone	Morone americana	100	1	AAAAAG...	0	2582	249	0
ESV_009177	Ictaluridae	Ictalurus	Ictalurus furcatus	100	1	GAAAAG...	584	101	0	1039
ESV_007836	Clupeidae	Alosa	NA	100	2	GAAAAG...	1328	0	0	0
ESV_009179	Clupeidae	Dorosoma	Dorosoma cepedianum	99	1	AAAAAG...	902	0	0	278
ESV_009181	Catostomidae	Erimyzon	Erimyzon oblongus	100	1	ATAAAG...	341	124	282	381
ESV_009182	Moronidae	Morone	Morone saxatilis	100	1	AAAAAG...	126	0	0	994

"NA" at a taxonomic ranks means multiple species present in the sample differ in that taxonomic rank

The number in each cell is the absolute number of times a given sequence was read by the sequencer.

eDNA metabarcoding—advantages

1. Easy sampling, little effort
2. Cost effective → collect more samples
3. Easy to standardize
4. Safe
5. Non-invasive
6. More sensitive (detects more species)
7. Accurate IDs—no taxonomist needed
8. Can sample any habitat
9. Larger sample unit (1-100 km)*

* But see disadvantages!



eDNA metabarcoding—limitations

1. DNA carried downstream → Fuzzy sample unit

- a. Upstream extent?
- b. Temporal extent?
- c. Depends on enviro & species?

2. Lack of consensus on standard methods

- a. How many liters to filter, when, how, from where?
- b. Sample preservation?
- c. Primers
- d. Sequencing & processing

3. Incomplete / inaccurate DNA ref. libraries

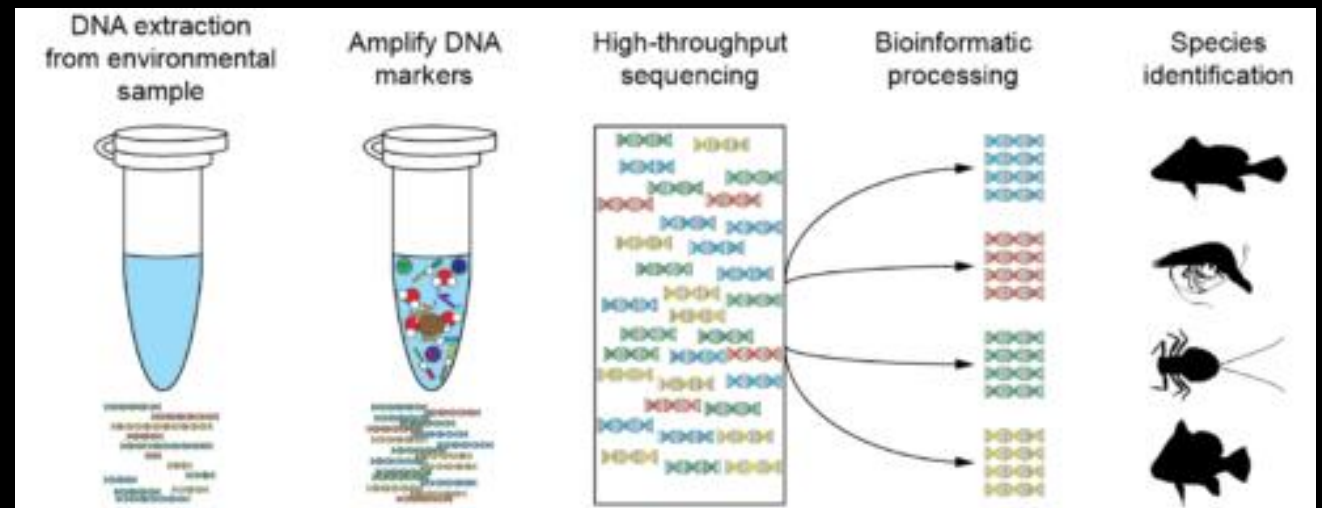
- a. Regional library is best

4. Imperfect DNA barcodes

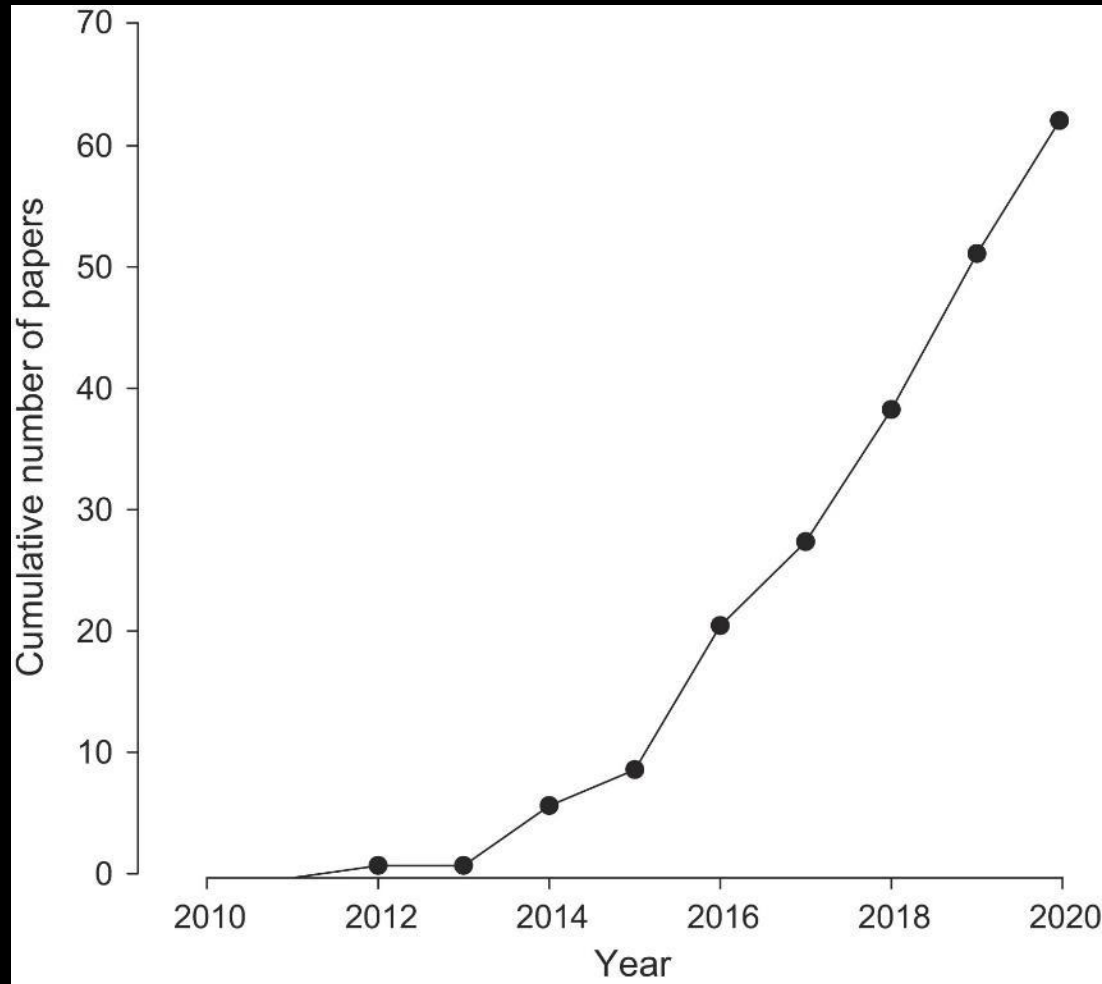
- a. Some congeners have same sequence

5. Biased/noisy abundance metrics

- a. Primer bias—some sequences preferentially amplified
- b. Species traits and behavior + environ. = $\uparrow\downarrow$ eDNA counts



Articles assessing eDNA for estimating fish abundance



Rourke et al. 2021. Environ. DNA 4:9-33

- 90% found a positive correlation between eDNA read counts and abundance and/or biomass

Delmarva case study

Objective: to compare fish communities detected by CES and eDNA.

- Hypothesis 1: eDNA will detect more fish species than CES.
- Hypothesis 2: eDNA read counts by site and species will be positively correlated with CES fish counts.

Delmarva case study—Methods

29 Delmarva streams, June 2022, 10-50 km² watersheds

Methods—CES:

- Electrofished two 20-CW reaches (~80 m), two passes each.
- Two side-by-side shockers.
- Seined pools

Methods—eDNA metabarcoding:

- Two 25 m reaches
 - upstream and downstream of CES reaches)
- Filtered 1-3 L of water (5 micron).
- MiFishU primers
 - mtDNA 12S rRNA gene
- Jonah Ventures eDNA lab
- Regional DNA barcode library
 - Chesapeake Bay Barcode Initiative (CBBi, Smithsonian)

Results (preliminary)—CBBI Barcode Library

- 503 distinct fish sequences

Barcode library	Sequences matched...	
	to species	to genus
GenBank	361 (72%)	440 (87%)
CBBI	457 (91%)	503 (100%)

- GenBank misidentified...
 - Mud Sunfish as Rock Bass
 - Redear Sunfish as Redspotted Sunfish

Results

H1.—eDNA will detect more species than CES

	Approach	
	CES	eDNA
Taxa detected	40 spp	36 spp + 2 genera ^a
Spp. detected by only one approach	3 spp.	3 spp.
Spp. per site ^b	15.2	16.9
Sites with more spp. detected	5	18

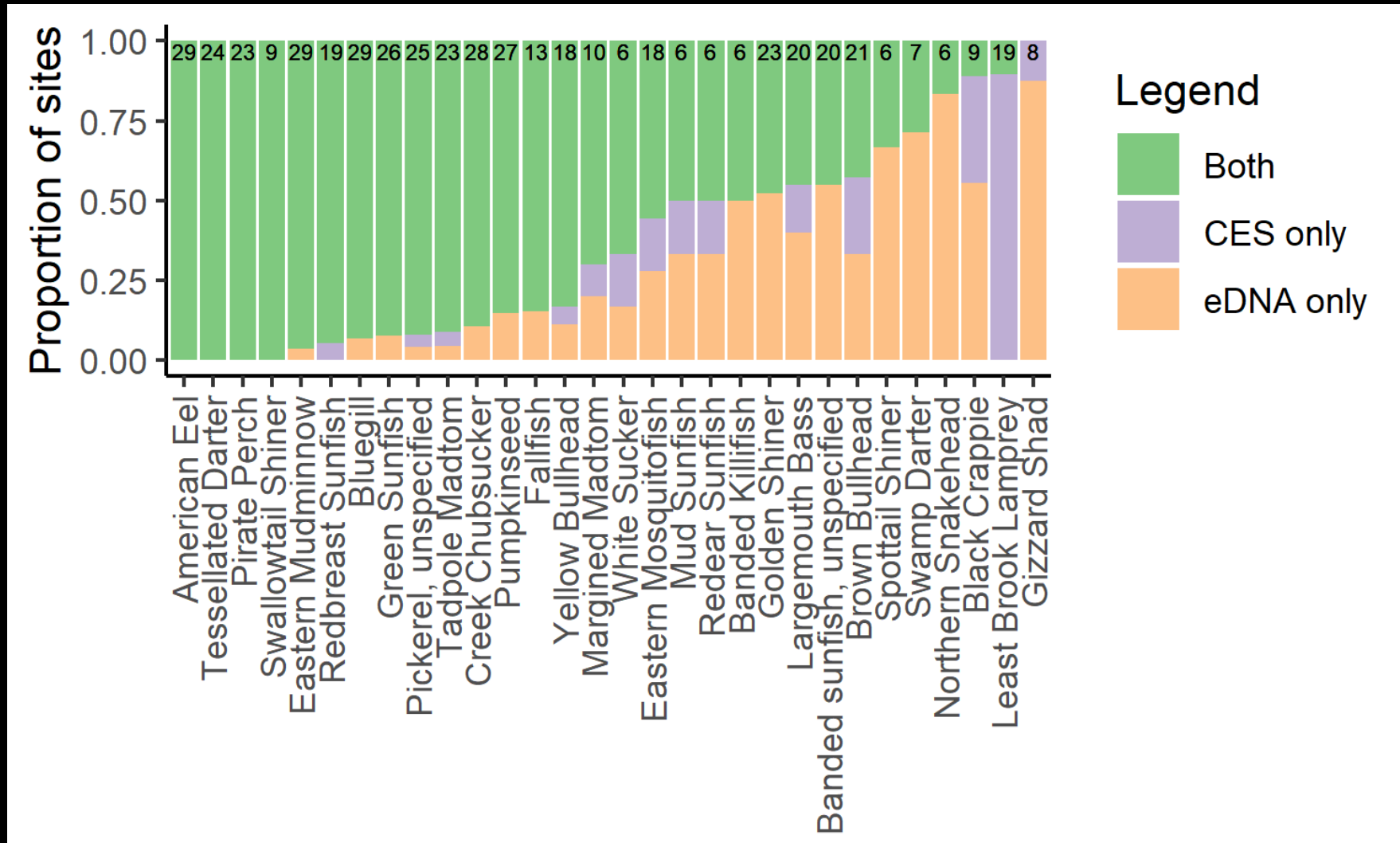
^a MiFish barcode unable to resolve 2 congener pairs:

1. Chain vs Redfin pickerel
2. Bluespotted vs Banded sunfish

^b paired t-test, $t(28) = 3.548$, $p = 0.0007$

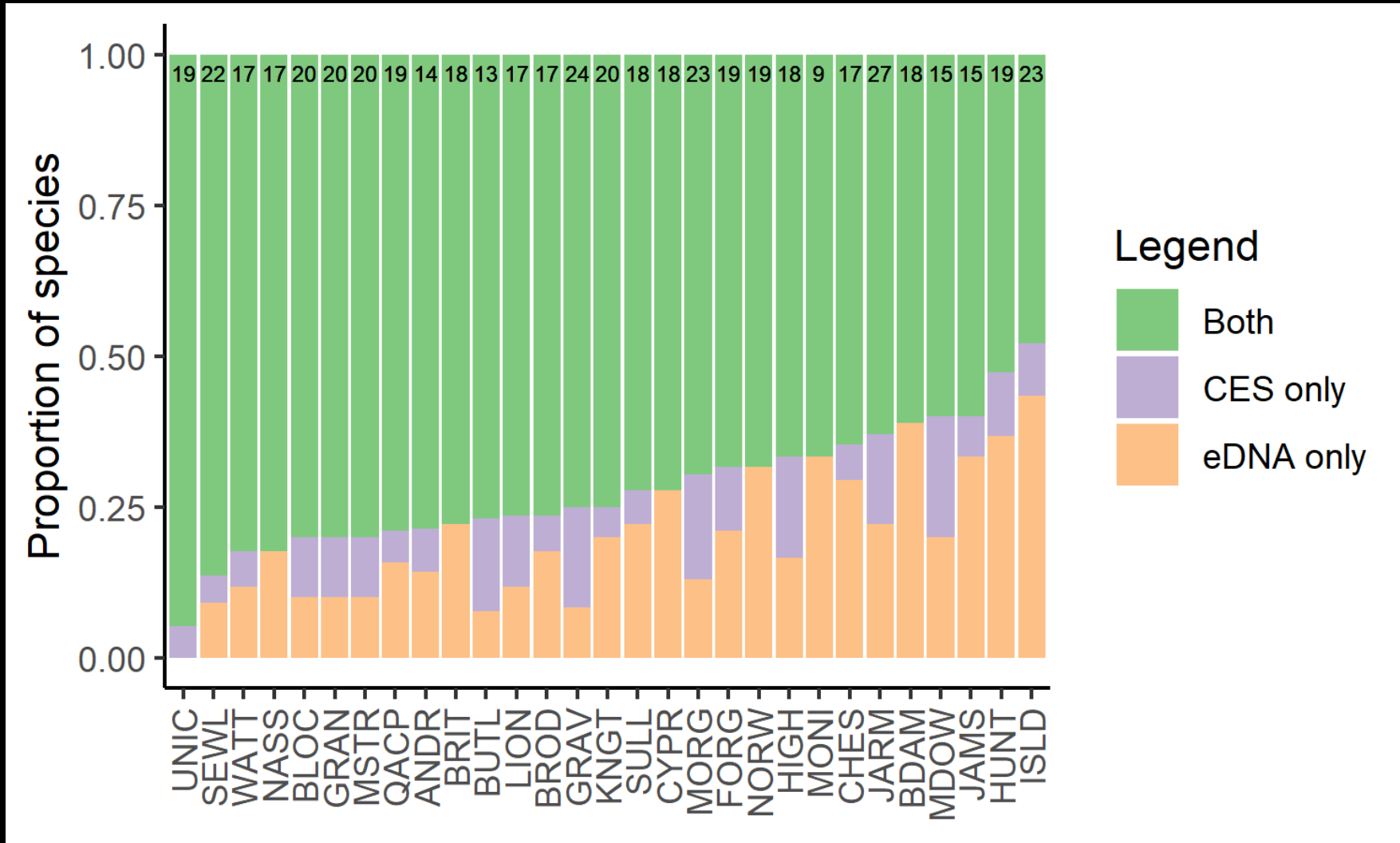
Results H1.-(cont.)

What species would we have missed if we had only sampled with CES? With eDNA?



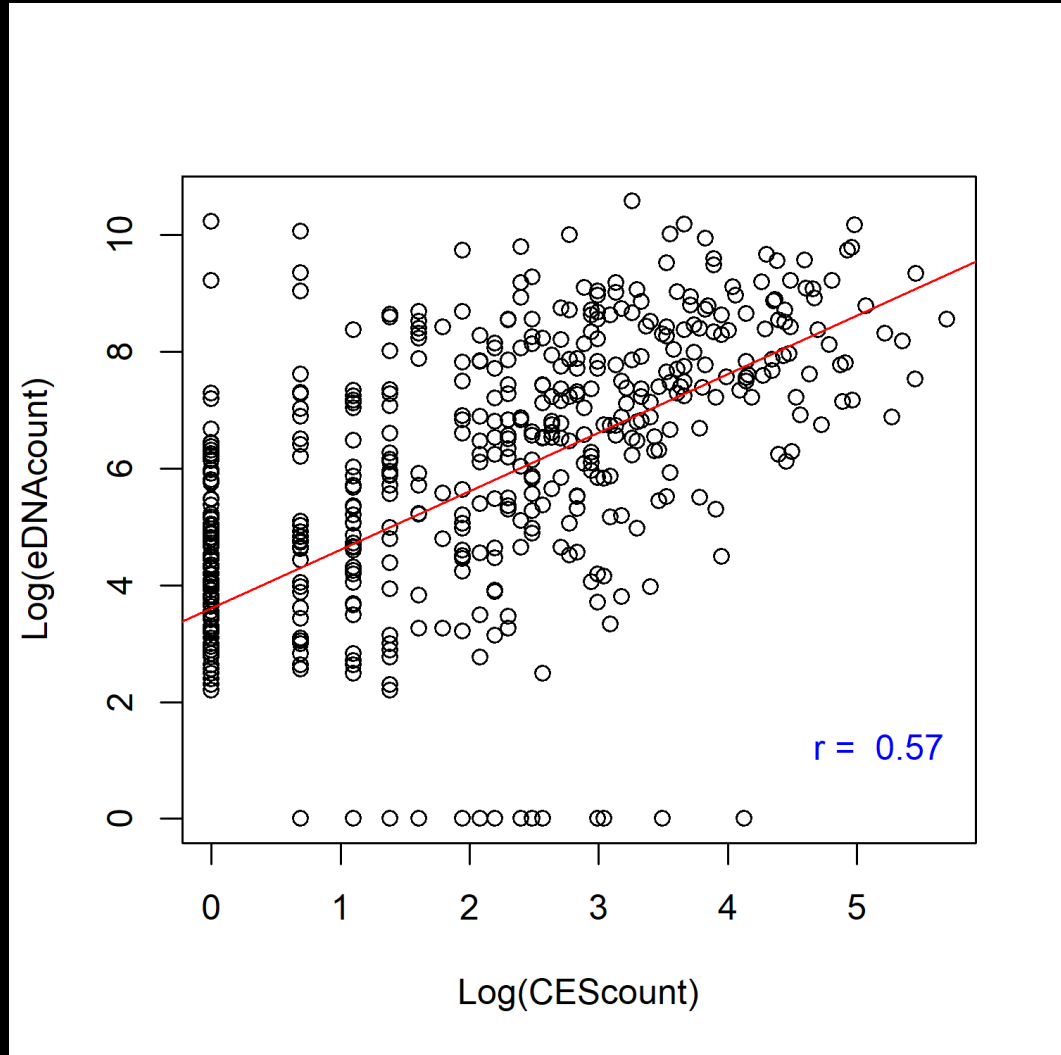
Results H1.-(cont.)

If we chose only one approach, eDNA would have yielded more species at 18 sites, CES at 5 sites.



Results

H2.-eDNA read counts by site and species will be positively correlated with CES counts.



$$r(488) = 0.579, t = 15.70, p < 0.0001$$

Next steps

- Replicate in Piedmont streams
- Biomass → eDNA count
- Explain CES-eDNA discrepancies in terms of:
 - Environmental factors (e.g., discharge, temp, pH, open canopy)
 - Species traits (e.g., body size, June spawning, habitat preference)
- Upstream-downstream eDNA samples
 - Is the diff. related to stream distance, discharge, temp, pH?
- Do watershed characteristics (e.g., land use, vegetation) explain more of the variability in eDNA or CES fish data? What about reach chars?
- Compare IBI scores from CES vs eDNA

Acknowledgements

- USGS funding
- Greg Noe's team (site selection and permissions)
- Rob Aguilar (Smithsonian CBBI barcode)
- Joe Craine (Jonah Ventures, metabarcoding)
- Aaron Maloy (USFWS, metabarcoding tips)