Using eDNA metabarcoding to assess fish assemblages in the rivers and streams of Texas

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Texas Chapter of the American Fisheries Society
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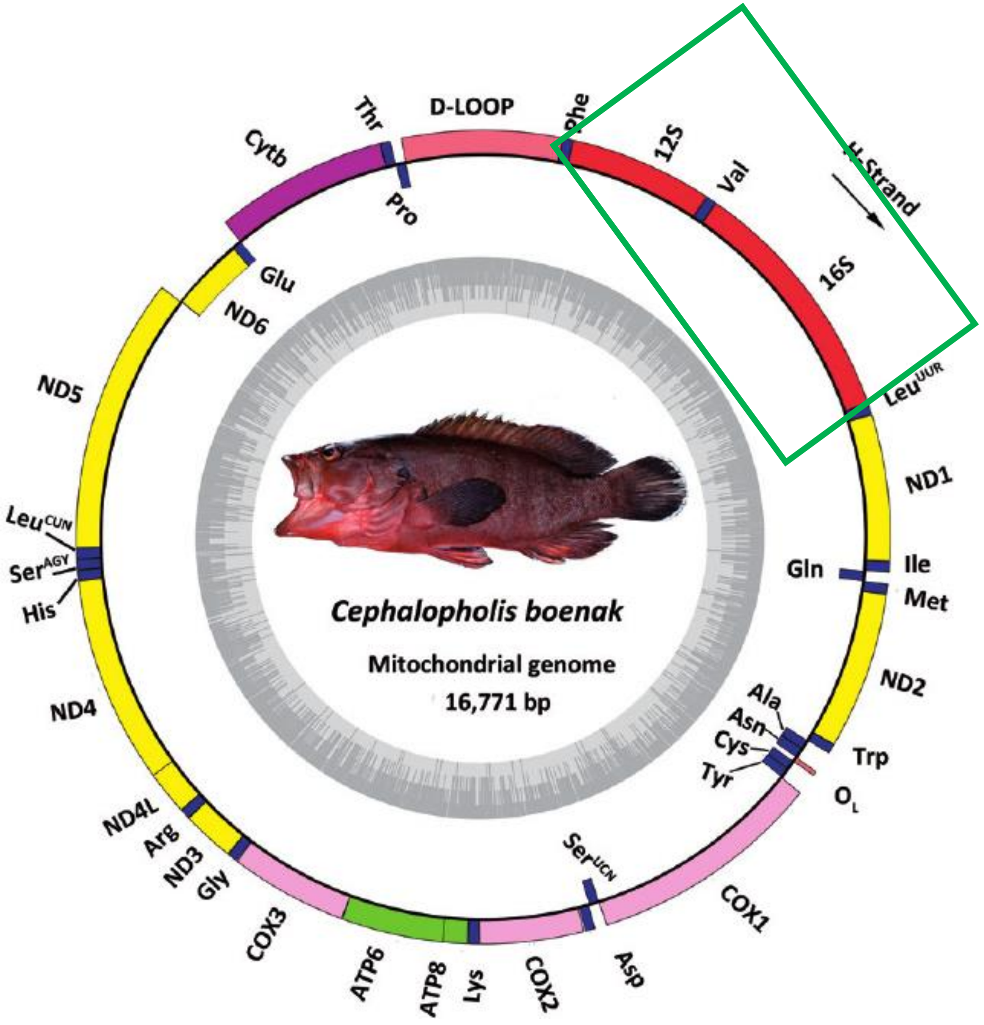
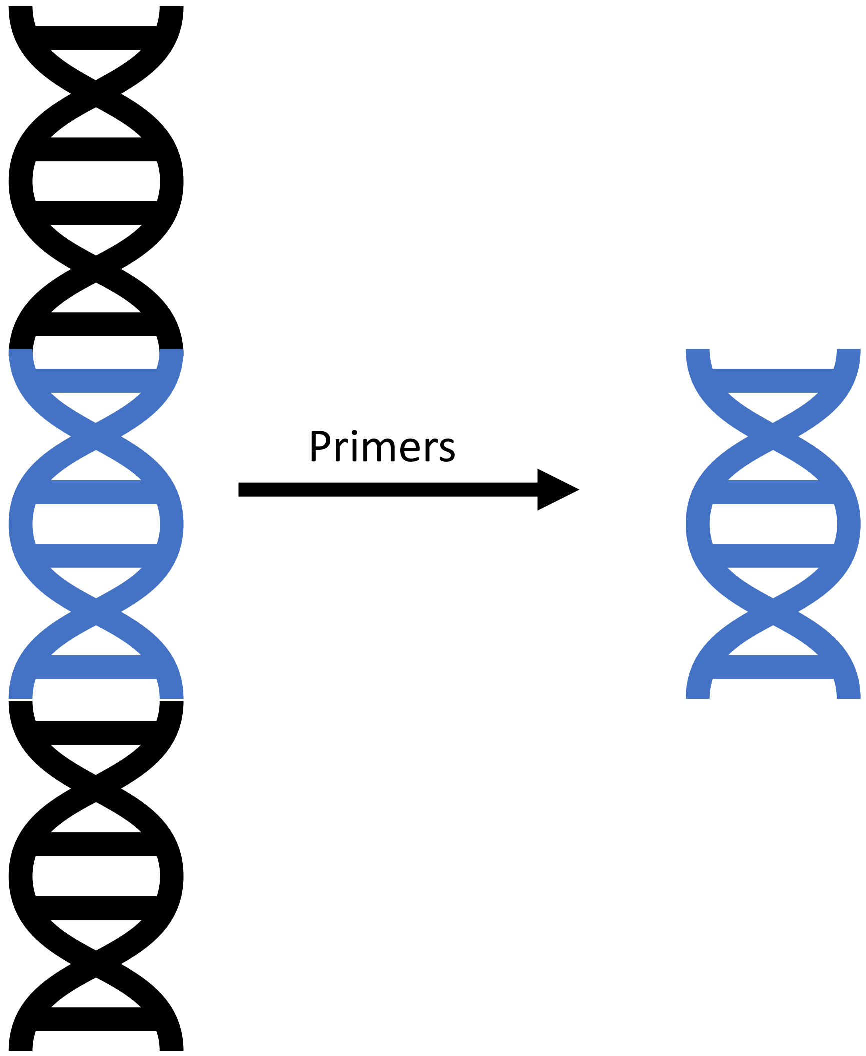
UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

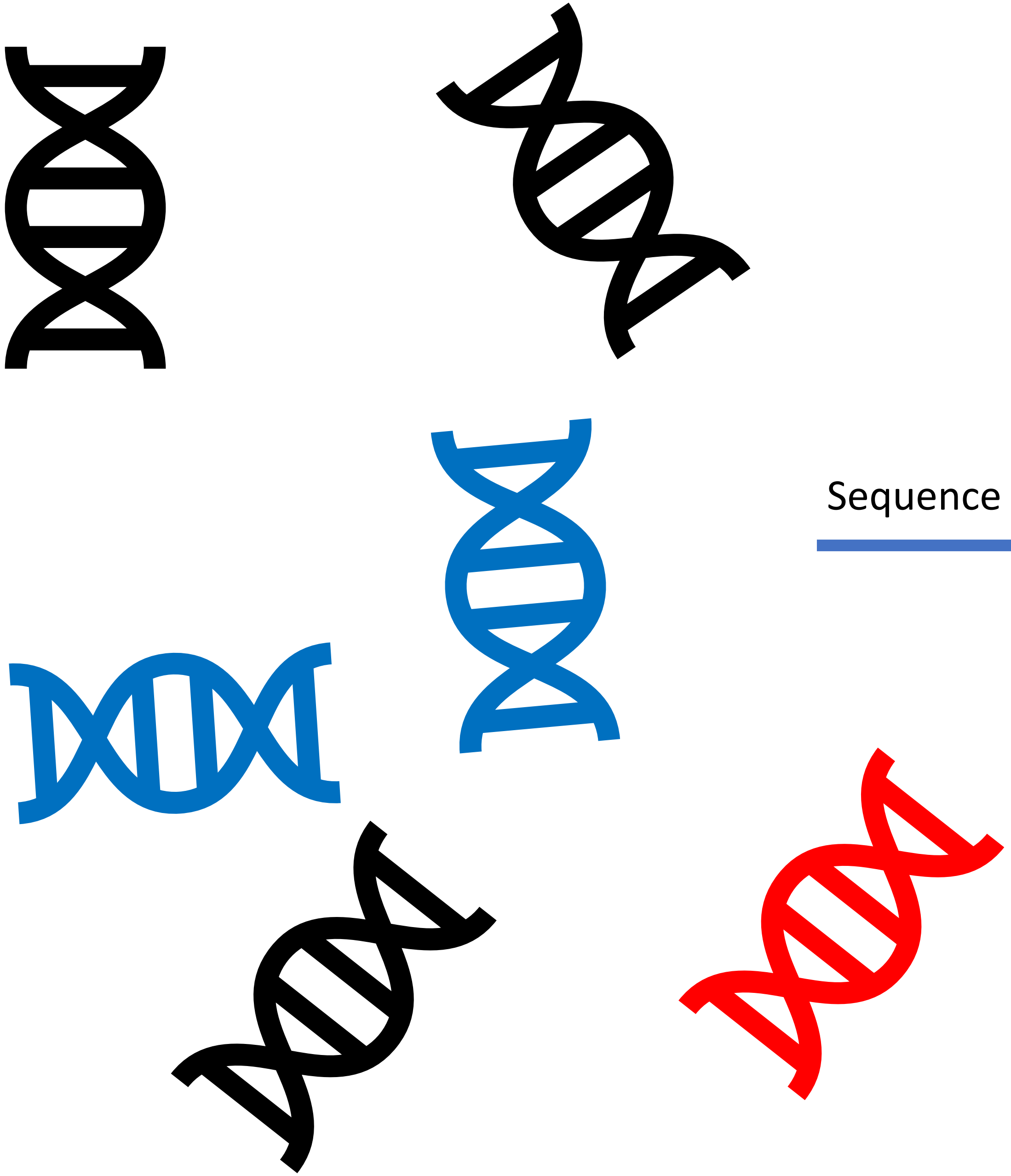
eDNA – What is it?

eDNA Metabarcoding





Li, J. et.al(2014). Complete mitochondrial genome of the chocolate hind Cephalopholis boenak (Pisces: Perciformes).



Sequence + Bioinformatics

Read count	Fish
1	Red fish
2	Blue Fish
3	Black Fish

Pros and Cons of eDNA metabarcoding

Pros	Cons
Minimally-invasive	Many sensitive steps
Cheaper than traditional methods – equipment, labor, time	Cross contamination of samples
Detecting of rare or cryptic species	Unknowns with persistence of DNA and environment
May reduce bias with other methods: mesh size, net size, fish physiology, habitat	Contamination from other sources
	*Need a complete genetic database

Motivation

Habitats are degrading and changing quickly.

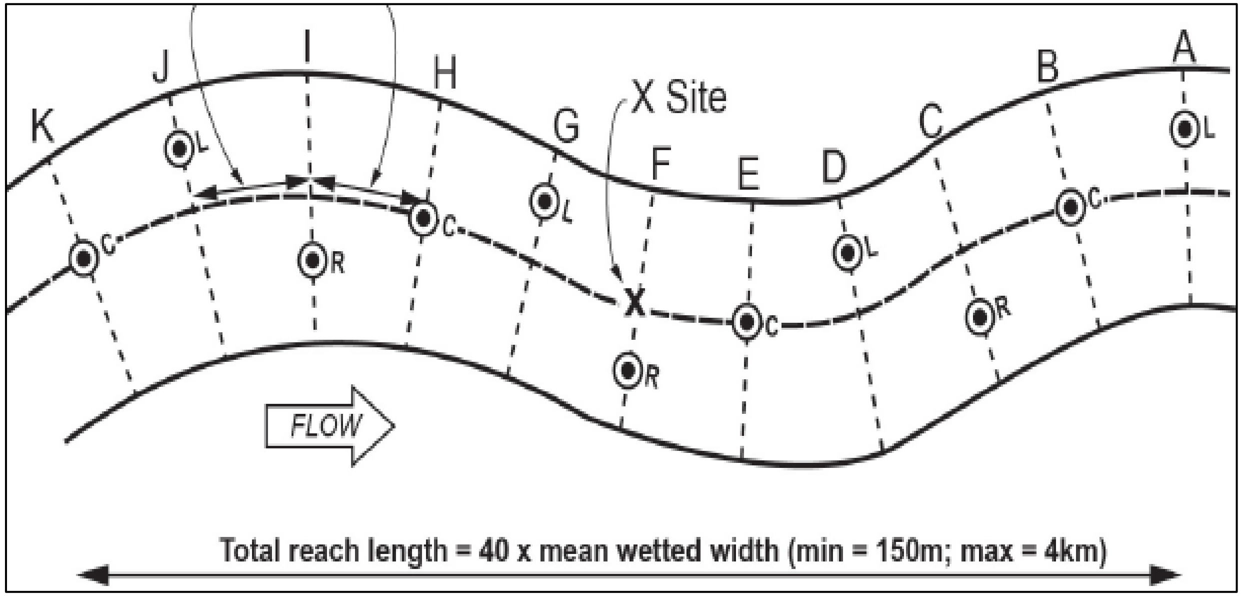
Tracking widespread species assemblages regularly, and easily could play an important role in future management decisions.

eDNA could help in those processes.

Study Design

Follows the EPA's National Rivers and Streams Assessment standards.

- Rapid assessment
  - Involves collection of data for environmental conditions, habitat within stream and riparian zones, and general stream measurements
- Random sites (80+)
  - Small Streams
  - Large Streams
  - Rivers



United States Environmental Protection Agency (USEPA). 2022c. National Rivers and Streams Assessment 2023/24: Field Operations Manual – Wadeable. EPA-841-B-22-006. U.S. Environmental Protection Agency, Office of Water Washington, DC

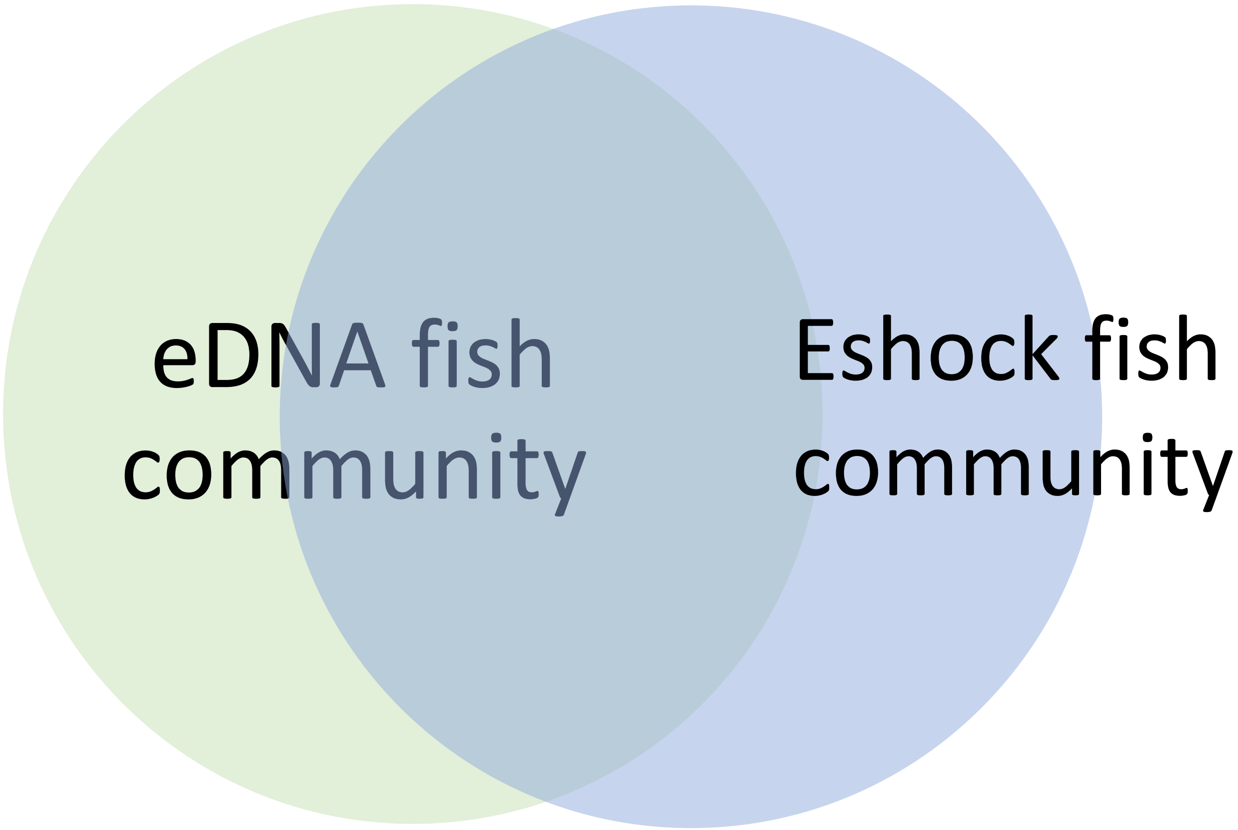
Traditional Methods

- Electrofishing

Pros	Cons
Compare data	Time intensive
Data on individual fish	Invasive to the ecosystem
Abundance and diversity data	Highly skilled crew

Study Snapshot

1. Take water samples for eDNA
2. Collect fish data via electroshocking
3. Compare the results



Objectives

- Compare fish assemblage from eDNA metabarcoding to fish assemblage from electroshocking
- Discover efficacy of using eDNA across a wide variety of stream/river sizes and classes, watersheds, substrate types, and other environmental variables
- Compare methods of of eDNA collection and processing to retrieve the most DNA

Sites

Completed:

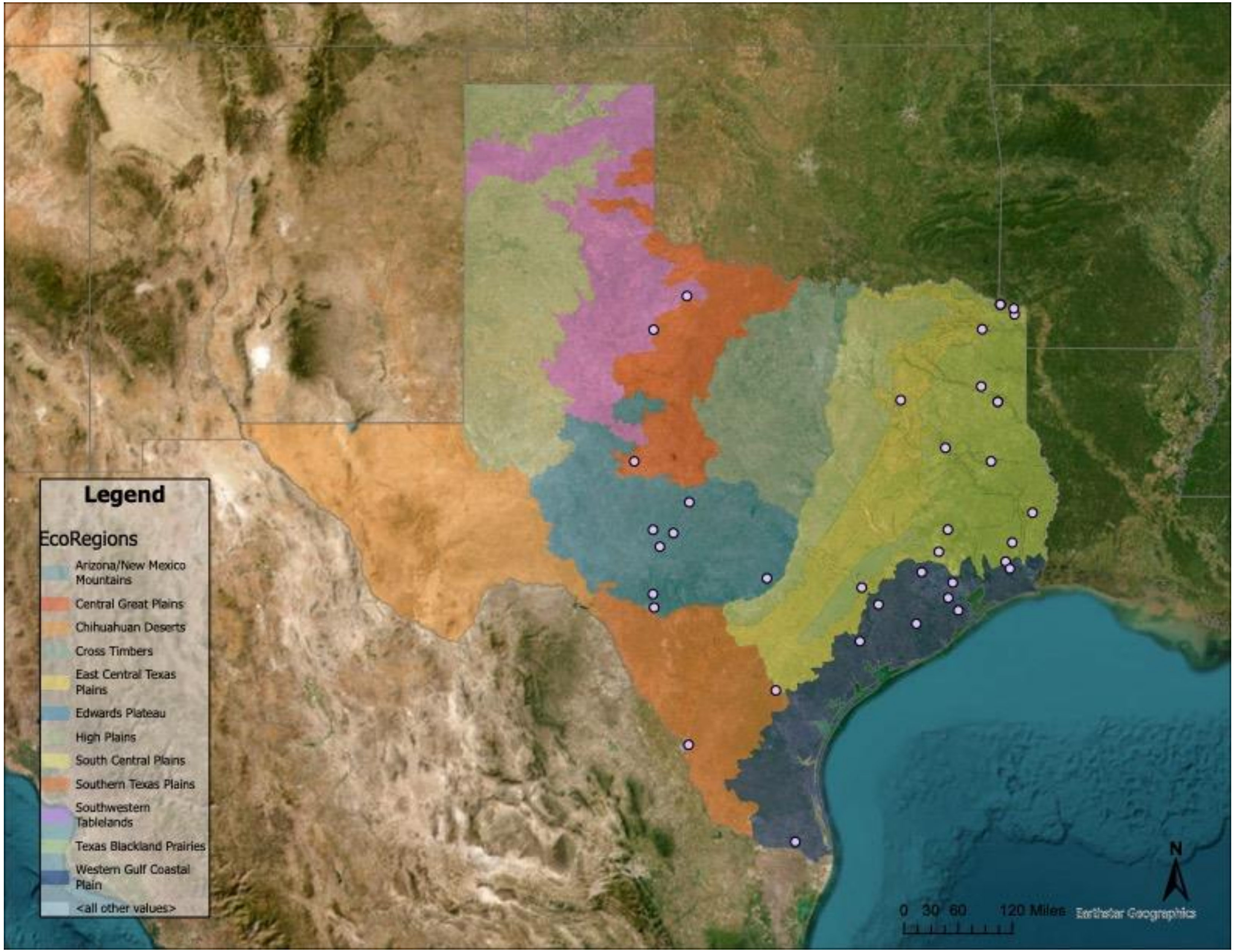
38 Sampling events

at 36 Sites (43%)

(5 SS, 10 LS, 22 R)

To sample:

49 sampling events
at 47 sites

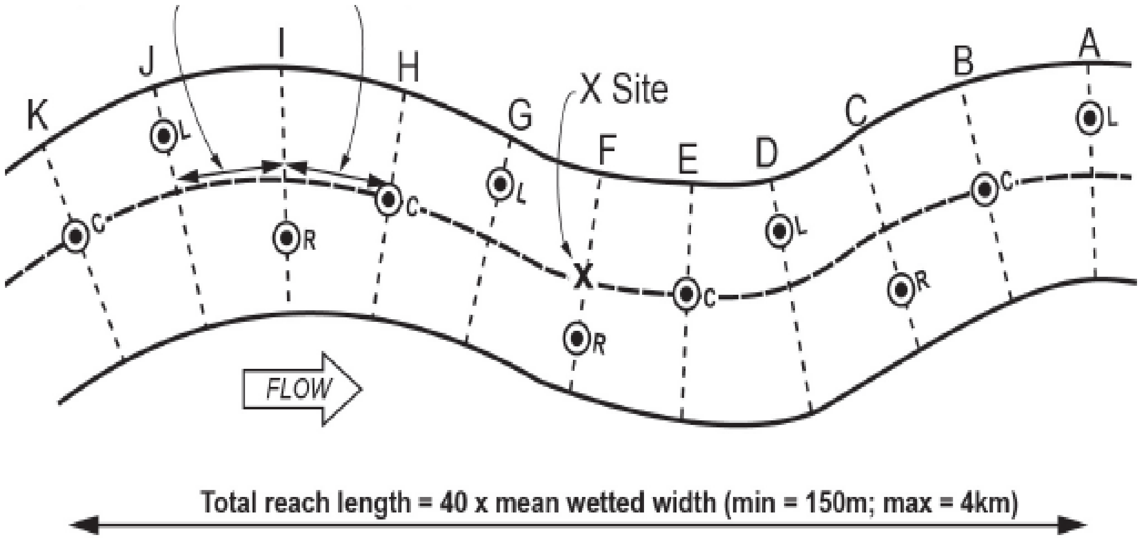


eDNA collection

1- A 1 L water sample at designated location

2- A 1 L composite sample. ~100 mL taken at 11 evenly spaces transects along reach

3- A control sample taken from a live well with species, and size recorded for every individual. (select sites)



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Fishing

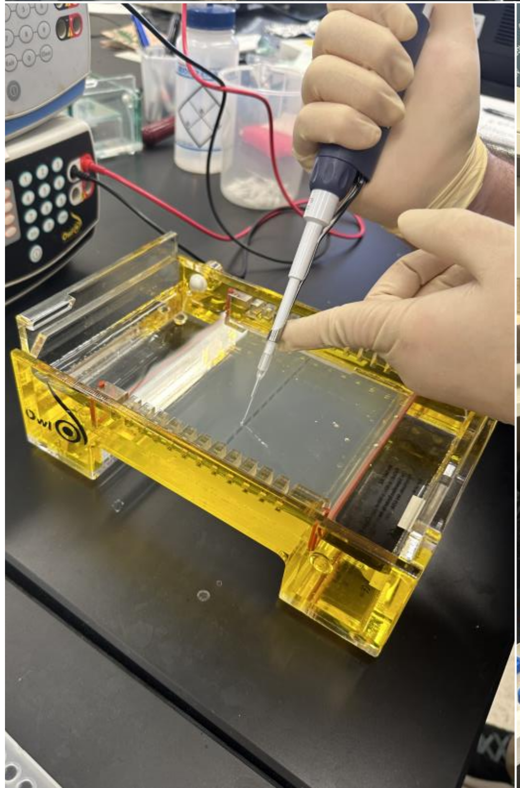
- Electrofishing was the only traditional method used for this survey
  - Every fish caught was ID’d and counted by size class
- If width < 12m = fish entire reach
- If width > 12m = fish until at least 500 fish were caught and 50% fished

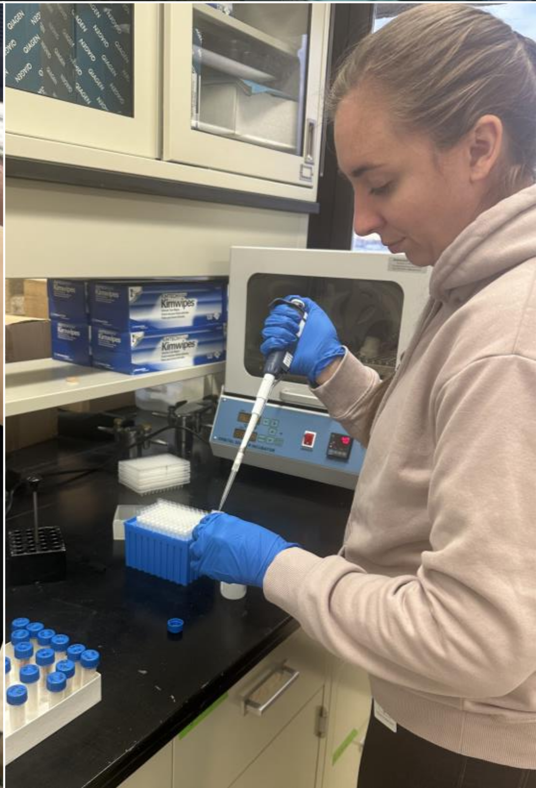
eDNA processing

- Water was filtered same day through a 3 micron, ISOPORE™ Membrane Filter using a peristaltic pump.

- At the lab, DNA then went through an extraction and purification process before being amplified with PCR and checking for DNA with gel electrophoresis.

- Currently on final rounds of PCR to then be sequenced.









Outcomes:

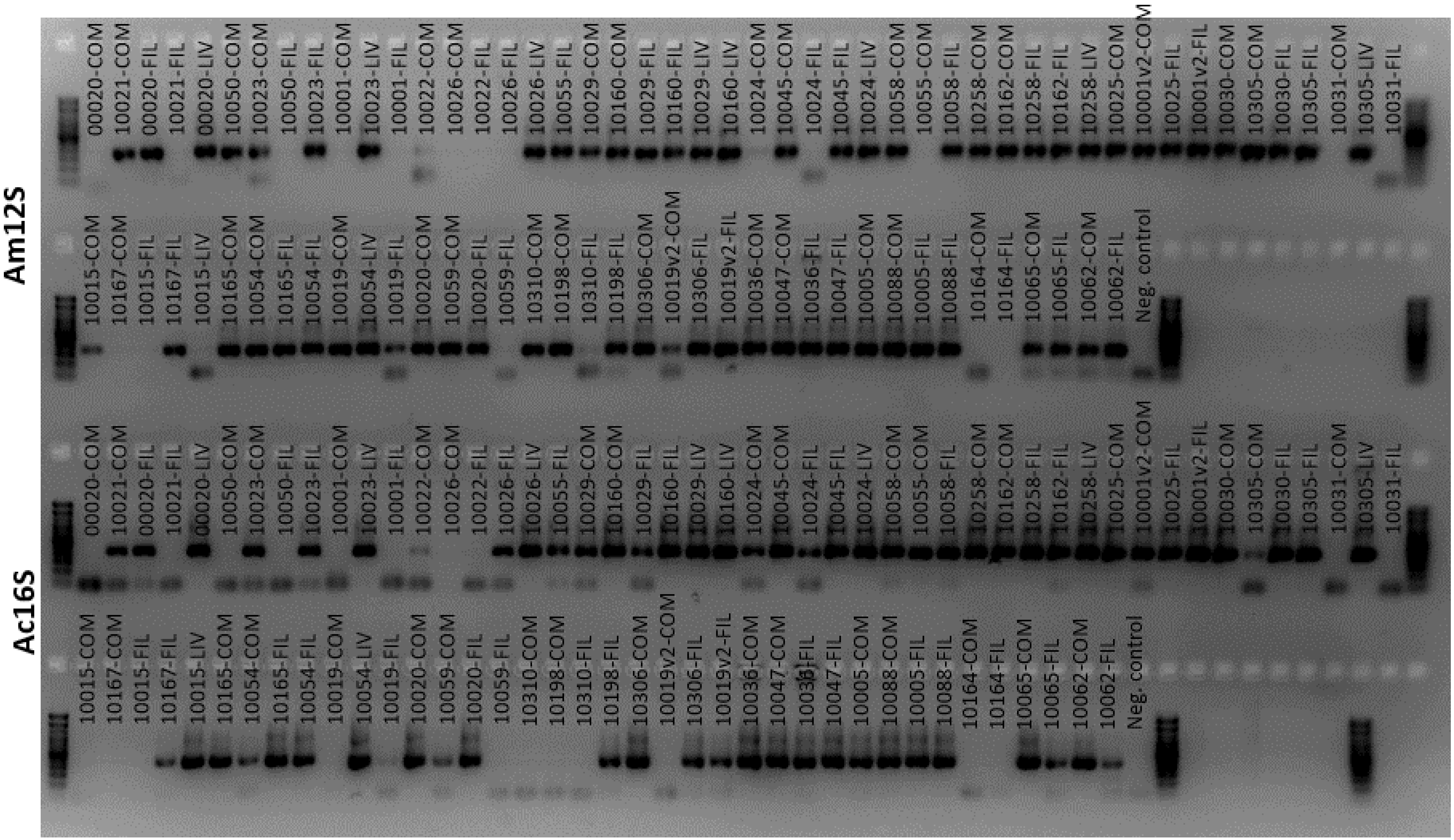
From electrofishing we caught a total of

- 104 species
- 16,927 individuals

Collected:

Paired data for 36 sites

1st PCR


Am12S
Ac16S
00020-COM
10021-COM
00020-FIL
10021-FIL
00020-LIV
10050-COM
10023-COM
10050-FIL
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10001-COM
10023-LIV
10001-FIL
10022-COM
10026-COM
10022-FIL
10026-FIL
10026-LIV
10055-FIL
10029-COM
10160-COM
10029-FIL
10160-FIL
10029-LIV
10160-LIV
10024-COM
10045-COM
10024-FIL
10045-FIL
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10058-COM
10055-COM
10058-FIL
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10162-FIL
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10025-COM
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10025-FIL
10001v2-FIL
10030-COM
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10030-FIL
10305-FIL
10031-COM
10305-LIV
10031-FIL
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10019-FIL
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10059-COM
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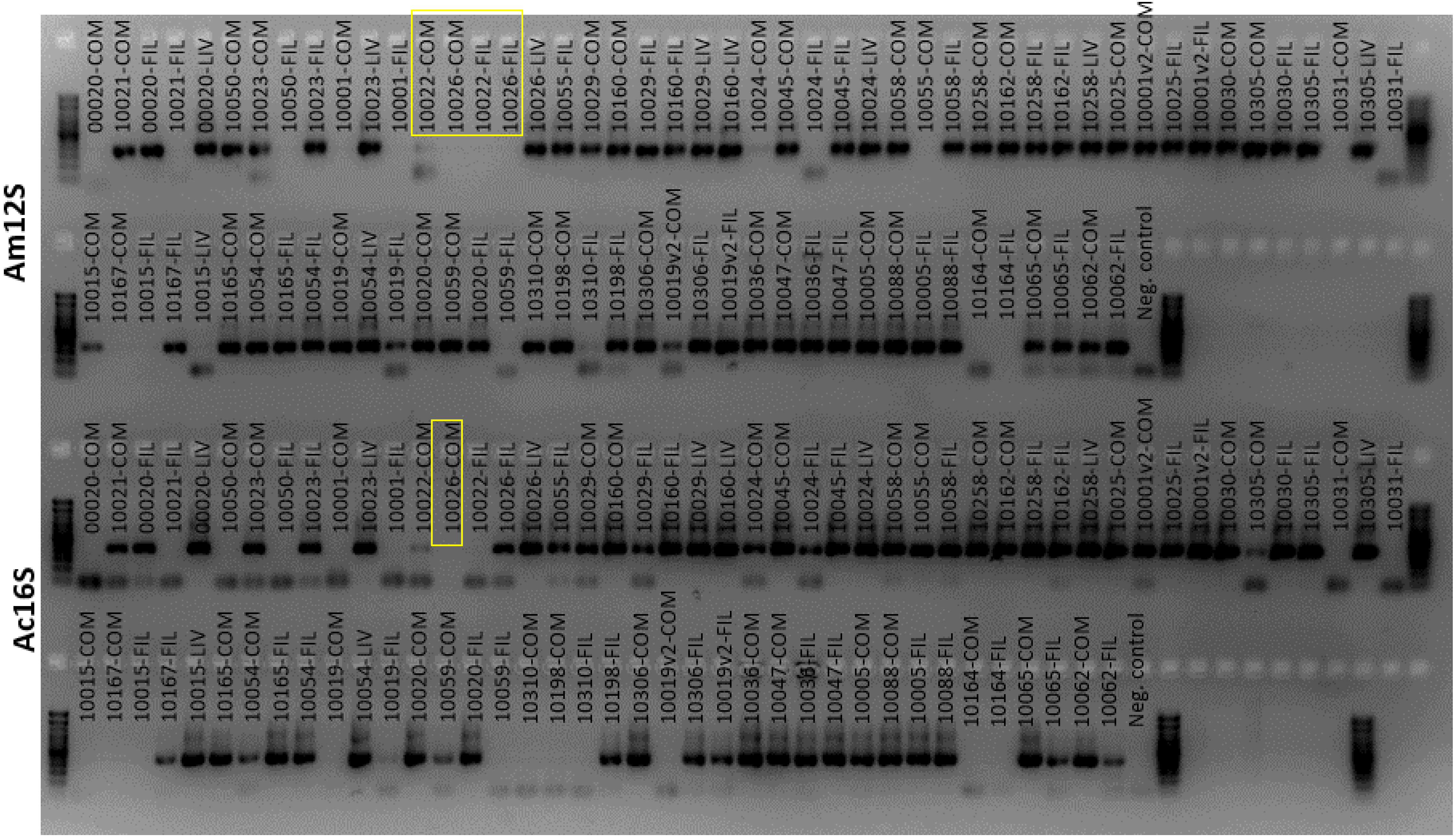
10310-FIL
10198-FIL
10306-COM
10019v2-COM
10306-FIL
10019v2-FIL
10036-COM
10047-COM
10036-FIL
10047-FIL
10005-COM
10088-COM
10005-FIL
10088-FIL
10164-COM
10164-FIL
10065-COM
10065-FIL
10062-COM
10062-FIL
Neg. control

1st PCR


Am12S
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Ac16S
00020-COM 10021-COM 00020-FIL 10021-FIL 00020-LIV 10050-COM 10023-COM 10050-FIL 10023-FIL 10001-COM 10023-LIV 10001-FIL 10022-COM 10026-COM 10022-FIL 10026-FIL 10026-LIV 10055-FIL 10029-COM 10160-COM 10029-FIL 10160-FIL 10029-LIV 10160-LIV 10024-COM 10045-COM 10024-FIL 10045-FIL 10024-LIV 10058-COM 10055-COM 10058-FIL 10258-COM 10162-COM 10258-FIL 10162-FIL 10258-LIV 10025-COM 10001v2-COM 10025-FIL 10001v2-FIL 10030-COM 10305-COM 10030-FIL 10305-FIL 10031-COM 10305-LIV 10031-FIL
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	***Need a genetic library**

Genetic Library

<u>2023</u>

Texas FW Fish count: 242

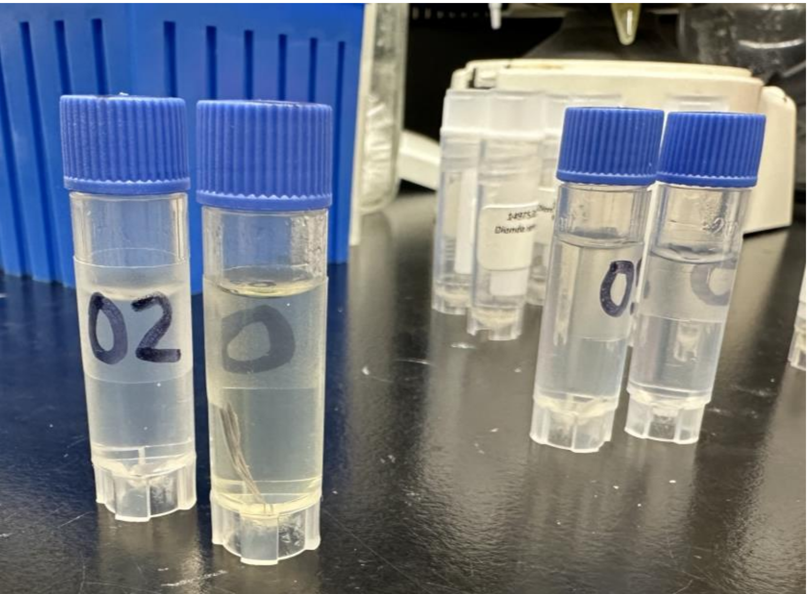
Genetic data available: 152

<u>Completed:</u>

Field Collected: 76 clips

Biodiversity Research and Teaching collections at Texas A&M: 199 fin clips

For a total of 59 additional species



<u>2024</u>

Still no data or fin clips for 37 species

Acknowledgements

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- Noah Daun, Angelica Castillo, Mandi Gordon, Gabrielle Hammerbach, Heather Hinchliffe, Aurora Alvarez, Kaylei Chau, Danielle DeChellis, Ashlyn Sak, Erica Underwood, Jenny Oakley

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Species with no genetic data

- **Amistad gambusia** - *Gambusia amistadensis*
- **Big Bend gambusia** - *Gambusia gaigei*
- **Blotched gambusia** - *Gambusia senilis*
- **Bluehead shiner** - *Pteronotropis hubbsi*
- **Chestnut lamprey** - *Ichthyomyzon castaneus*
- **Clear Creek gambusia** - *Gambusia heterochir*
- **Clown goby** - *Microgobius gulosus*
- **Cypress minnow** - *Hybognathus hayi*
- **Darter goby** - *Ctenogobius boleosoma*
- **Fountain darter** - *Etheostoma fonticola*
- **Freshwater goby** - *Ctenogobius shufeldti*
- **Golden redhorse** - *Moxostoma erythrurum*
- **Goldstripe darter** - *Etheostoma parvipinne*
- **Highfin goby** - *Gobionellus oceanicus*
- **Leon Springs pupfish** - *Cyprinodon bovinus*
- **Lyre goby** - *Evorthodus lyricus*
- **Mexican goby** - *Ctenogobius claytonii*
- **Mud darter** - *Etheostoma asprigene*
- **Pecos bluntnose shiner** - *Notropis simus pecosensis*
- **Peppered chub** - *Macrhybopsis tetranema*
- **Phantom shiner** - *Notropis orca*
- **Redfin darter** - *Etheostoma whipplei*
- **Rio Grande bluntnose shiner** - *Notropis simus simus*
- **Rio Grande chub** - *Gila pandora*
- **Rio Grande silvery minnow** - *Hybognathus amarus*
- **River goby** - *Awaous tajasica*
- **San Marcos gambusia** - *Gambusia georgei*
- **Skipjack herring** - *Alosa chrysochloris*
- **Spinycheek sleeper** - *Eleotris pisonis*
- **Swamp darter** - *Etheostoma fusiforme*
- **Tex-Mex gambusia** - *Gambusia speciosa*
- **Texas silverside** - *Menidia clarkhubbsi*
- **Toothless blindcat** - *Trogloglanis pattersoni*
- **West Mexican redhorse** - *Moxostoma austrinium*
- **Western creek chubsucker** - *Erimyzon claviformis*
- **Western sand darter** - *Ammocrypta clara*
- **Western starhead topminnow** - *Fundulus blairae*
- **Widemouth blindcat** - *Satan eurystomus*