COMPARING EDNA METABARCODING AND STANDARDIZED ELECTROSHOCKING TO ASSESS FISH ASSEMBLAGES IN TEXAS RIVERS AND STREAMS

by

Kylie Jo Perkins, B.S.

THESIS PROJECT

Presented to the Faculty of

The University of Houston-Clear Lake

In Partial Fulfillment

Of the Requirements

For the Degree

MASTER OF SCIENCE

in Biological Science

THE UNIVERSITY OF HOUSTON-CLEAR LAKE

MAY, 2025

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Acknowledgements

This project was only possible due to the countless hours of effort from many people.

Thanks to my advisor, Jenny, for bringing me in and trusting me to get the work done, guiding me through the process, mentoring me through everything, and Mandi for imparting all your knowledge, being a sounding board when I needed to vent and giving advice as I worked on my thesis.

Thanks to Erik for showing me the ropes and giving up your time and effort to make this project happen and meet with me whenever I needed it.

Thanks to everyone who worked on NRSA and carried the extra pounds of water for my samples, helped filter, or cleaned things so I could filter late at the hotel. They are all champs for sticking to it through one or even two seasons of fieldwork. I am forever grateful to all the people at EIH who called and coordinated with landowners to make anything happen, because it was not easy to find and access sites.

Thanks to the Noah's for being the fish taxonomists and collecting all the electrofishing data and assisting with the extra tasks to collect fin clips and measuring every fish for some samples.

This was all only possible with funding from TCEQ and the EPA for providing the funding to complete fieldwork for 84 sites, the EPA for providing the funding and effort for processing the lab work, and to EIH funding my work down to helping me ship samples.

Finally, thanks to my husband, Jason, for being the calm in this otherwise chaotic thing that is life.

ABSTRACT

COMPARING EDNA METABARCODING AND STANDARDIZED ELECTROSHOCKING TO ASSESS FISH ASSEMBLAGES IN TEXAS RIVERS AND STREAMS

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University of Houston-Clear Lake, 2025

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Long-term and large spatial-scale studies on fish distribution and community assemblage are important in understanding the health of aquatic systems. Traditional methods for monitoring fish in riverine environments (e.g., electrofishing) are widely accepted. However, they are time and resource consuming, require skilled taxonomists, and can underrepresent the diversity and the presence of rare and cryptic species. Environmental DNA (eDNA) metabarcoding has emerged as a rapid and non-invasive tool for inventorying fish communities in diverse freshwater systems. I sought to evaluate the efficacy and limitations of eDNA metabarcoding in the diverse river systems of Texas. A total of 38 sites were sampled in the summer (May-September) of 2023 using paired electroshocking and eDNA metabarcoding sampling. Two types of eDNA samples were collected: a 1L grab sample at the centroid of the flow (FIL) and a composite sample of

eleven 100mL aliquots collected near alternating banks at evenly spaced transects throughout the survey reach (COM). Metabarcoding of eDNA using the 12S and 16S mitochondrial genes was completed for each sample. Among the analyzed sites, a total of 120 species of fish were detected throughout the study; 91 species detected using eDNA and 90 species detected using electrofishing. A total of 61 species of fish (51%) were detected with both methods, 30 species were detected with eDNA only (25%), and 29 species were detected with electrofishing only (24%). I failed to detect a significant difference in the number of fish species detected between the two eDNA collection methods (FIL and COM). While using two primers in studies is not always possible due to funding constraints, the fish community was found to be significantly different between the 12S and 16S eDNA primers used. This study found the use of eDNA to not be as effective in larger rivers, and depending on the specific goals of a study, a combination of both eDNA and traditional methods such as electrofishing can provide the most robust representation of the fish species present within a waterbody.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION:

Fish Community Assemblage

Aquatic ecosystems are under pressure from climatic changes and other anthropogenic influences on watersheds. Due to this pressure, it is important to monitor the health of this system. One metric used to measure ecosystem health is the species assemblage of major taxa such as fish community assemblages. Tracking this metric is helpful because species composition provides an indication of the health of a system on account of the variance in tolerance levels of different species to poor-quality water (Linam et al., 2002). Fishes are considered good bioindicators of river water quality and habitat at the watershed level because they are highly mobile and can often move within a system to other areas when the water conditions deteriorate or are unsuitable (USEPA, 2013; Fausch et al., 1990; Welcomme et al., 2006). As anthropogenic pressures increases and climate changes continue to occur, it is critical to understand the current state of our freshwater systems, including which species are present, to track changes as they occur, to identify stressors, and assess what management or intervention steps may be appropriate. Consistent monitoring over time is one way to ensure that water resources are clean, support the normal assemblage of aquatic life, and are sufficient and stable across climatic variance to enable smart, data-driven conservation efforts.

Traditional methods to evaluate freshwater fish assemblage include seining, traps, gill nets, and electrofishing. One of the most widely used methods to sample fish assemblages in rivers is electrofishing. (Oberdorff et al., 2001). Electrofishing involves creating an electrical field in the water; when active, this can have the ability to stun or impair the swimming of fish in range of the field. The impaired fish can then be netted, examined, and identified. Traditional methods, including electrofishing, provide valuable information about fish populations including species composition, richness, diversity,

individual size, and growth, but it can be expensive and labor intensive (Lapointe et al., 2006). Additionally, traditional methods have bias and shortcomings depending on site conditions such as turbidity, vegetation density, conductivity, and flow velocity (Harmelin-Vivien and Fancour, 1992; Mercado-Silva and Escandón-Sandoval, 2008). As additional methods and tools become available, especially with technological advancements, improvements can be made to how fish are studied and monitored.

eDNA

The use of Environmental DNA (eDNA) is becoming increasingly common as the costs of sequencing technology has declined (Huver et al., 2015, Granqvist et al., 2025). Environmental DNA consists of any DNA that persists in the environment apart from an organism (Ficetola et al., 2008). This has included DNA present in soil, water, air, and ice. The use of eDNA is a helpful way to study aquatic systems with minimal disturbance and, depending on the goals of the study, can save time and money compared to traditional methods (Meulenbroek et al., 2022). Initial applications of eDNA included the characterization of microbial communities in habitats such as soil or water by taking samples and extracting DNA from those samples directly (Venter et al., 2004). Ficetola et al. (2008) was the first documented use of eDNA to characterize contemporary and current macroorganisms and was utilized to determine the spread of an invasive species. This area of research has continued to assist in efforts to detect a single or a few species of interest to determine spatial presence in a large system or across systems (McColl-Gausden et al., 2024, Meulenbroek et al., 2022). The use of eDNA has proven useful in detecting small amounts of DNA in a body of water. This has increased the confidence to employ its use in tracking the spread of invasive species and use as a tool to understand the distribution of species that are endangered, threatened, rare, cryptic, or invasive (McColl-Gausden et al., 2024, Simpfendorfer et al., 2016, Blackman et al., 2020).

Many studies since Ficetola (2008) have focused on how to best collect eDNA to detect macroorganisms. There are also questions on how DNA degrades over time, how the DNA moves through a system or precipitates, and how environmental conditions effect quality of DNA (Seymour et al., 2018; Strickler et al., 2015). For detection, DNA must be intact with minimal degradation, and depending on environmental conditions the DNA could have come from another area upstream, and it may have come from an organism that has since died. Dejean et al. (2011) found that DNA degradation occurs easily and rapidly depending on environmental variables, including radiation, temperature, wind, water flow, microbial breakdown, and time. However, the rate of DNA degradation will also vary depending on the system, location, and time of day and can vary from hours to several weeks (Strickler et al., 2015). To have the best chance at detecting organisms the process uses small segments of mitochondrial DNA, which gives a greater likelihood of having intact DNA for sequencing, due to the increased copies of DNA compared to nuclear DNA (Jo et al., 2022)

Further questions remain, to understand the best methodology when it comes to sampling procedure and processing. Studies have tested what primers to use, how much water to filter, where to collect water, preservation method, and filter size (Zhang et al., 2020; Kumar et al., 2022a; Hunter et al., 2019; Bessey et al., 2020; Kumar et al., 2022b; Sales et al., 2019). Studies are carried out most frequently in freshwater systems, but also include estuarine, marine, and terrestrial environments, all requiring related but subtly different procedures. While still a relatively new branch of science, the use of eDNA has become very important in detecting rare and cryptic species that often evade detection through traditional methods (Feng et al., 2023). Detecting rare species makes it ideal for early detection of invasive species before populations can be established or give indications on what areas to prioritize for better management to protect endangered

species (Jerde, 2021; Ficetola et al., 2008; Thomas et al. 2020; De Ventura et al., 2017; Meulenbroek et al., 2022; Feng et al., 2023).

With improved technique and more affordable access to sequencing technology, utilizing eDNA to target single species has become even more widely used, and has also given rise to eDNA metabarcoding to characterize whole communities (Taberlet et al., 2012). Metabarcoding is accomplished by matching specific conserved regions of a gene of DNA to a database or library of known genomes. Pont et al., (2021) tested eDNA metabarcoding against standard river assessment methods used by the European Union and found that eDNA is more effective than other methods when it comes to species detection in large rivers. Additionally, the collection of eDNA does not put environmental stress on a system compared to electroshocking or dragging a net (Deiner et al., 2016). This technique has been used in locations across the globe to compare traditional methods of community sampling to utilizing eDNA metabarcoding. Among the various sampling methods, eDNA metabarcoding has largely resulted in higher number of species detections (Goutte et al., 2020; Shaw et al., 2016; Shen et al., 2022). However, there are limitations to using eDNA to characterize a community. Limitations include remaining questions of determining population size using read counts, and the inability of data to define community structure, age, sex, or growth. In addition, metabarcoding requires a complete taxonomic genetic database to be established for any analysis to be done confidently to the species level (Taberlet et al., 2012; Ruppert et al., 2019).

The efficacy of eDNA metabarcoding has since been repeatedly tested and is considered a valid tool for monitoring the distribution of macroorganisms (Milhou et al., 2021). Studies on eDNA metabarcoding have been conducted for over a decade, with most aimed at validating the methods and techniques of processing or collection, proving

their effectiveness, exploring the weaknesses, and comparing results to traditional approaches (Fujii et al., 2019; Shen et al., 2022; Shaw et al., 2016). This validation is still a part of eDNA metabarcoding because the relationship of many variables and eDNA are still not fully understood and standardized. The ability to optimize eDNA sampling and define its limitations will only be understood after widespread application and testing.

Objectives

Most eDNA metabarcoding studies have all focused on a single or a few rivers, river basins, or watersheds. In this study, the question being tested is whether eDNA metabarcoding performs equally across watersheds, stream size, ecoregion, and other environmental variables in freshwater streams. This study will compare statewide fish community results obtained from electrofishing and eDNA metabarcoding to determine if there are conditions that strongly influence the efficacy and quality of the resulting data for river and stream sampling in Texas. This project will also contribute to future methods for management and conservation projects. This will be done by:

1.Describing the fish assemblages in rivers and streams of Texas

2.Comparing fish assemblage results from electrofishing and eDNA metabarcoding

3.Comparing the species detections from the 12S and 16S primer

4.Comparing two methods of eDNA collection

5.Evaluating how environmental variables impact the applicability of eDNA metabarcoding.

METHODS:

Site Selection and Prep

This study involved 38 sampling events in 36 randomly selected rivers or streams across Texas between May and September of 2023 as part of the National Rivers and Streams Assessment (NRSA)(USEPA, 2022a). Desktop reconnaissance was used to determine the accessibility of the site. If it appeared that the sites were reasonable to access and had water, landowner permission was sought out. If permission was received, sampling would occur as long as at least 50% of the reach had water following the US EPA Field Operations Manual site evaluation guidelines (USEPA, 2022a). Additionally, sites were shifted as needed to avoid confluence with a stream of a higher Strahler order (USEPA, 2022a).

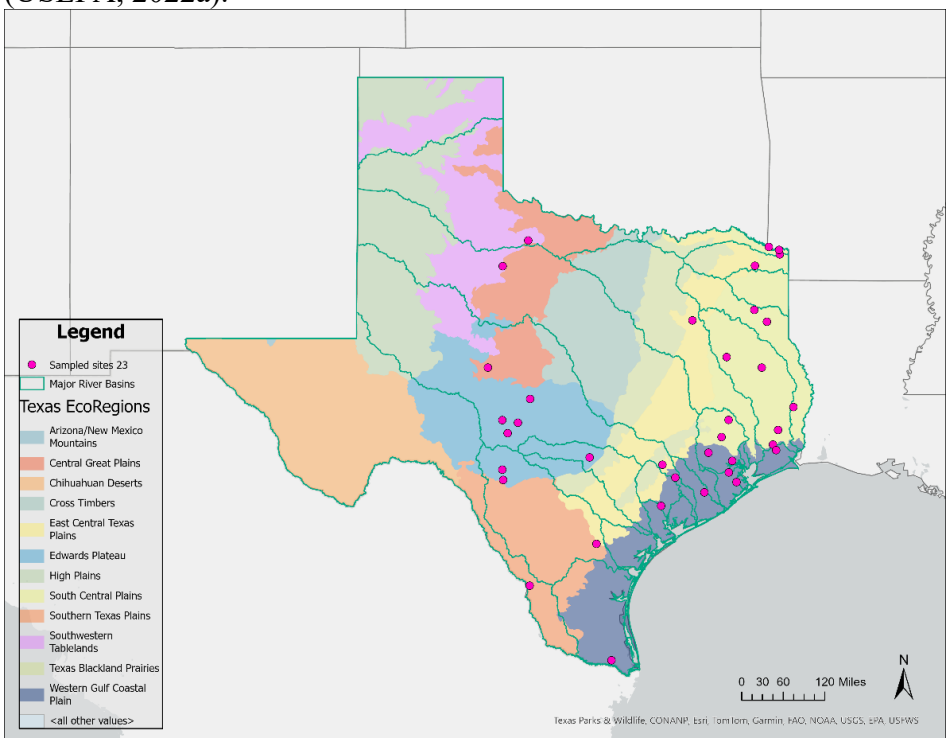

Figure 1: *Map of sites sampled in 2023, including ecoregions and major river basin boundaries.*

Table 1: *Site and metadata for the sites that had all fish data and were used for analysis*

Site ID	Site Name	County	River Basin[s]	Category
10001_2	Martin Creek	Panola	Sabine	Small Stream
10005_1	Lavaca River	Jackson	Lavaca	Small Stream
10019_1	Red River	Bowie	Red	River
10019_2	Red River	Bowie	Red	River
10020_1	Village Creek	Hardin	Neches	River
10023_1	Nueces River	Real	Nueces	River
10025_1	Llano River	Kimble	Colorado	River
10029_1	Colorado River	Colorado	Colorado	River
10030_1	Concho River	Tom Green	Colorado	River
10036_1	Unnamed Creek	Galveston	San Jacinto-Brazos	Small Stream
10045_1	Everette Creek	Jasper	Sabine	Large Stream
10047_1	Jones Creek	Bowie	Red	Large Stream
10054_1	Pine Island Bayou	Jefferson	Neches	River
10055_1	South Llano River	Kimble	Colorado	River
10058_1	Winters Bayou	San Jacinto	San Jacinto	River
10062_1	Nueces River	Live Oak	Nueces	River
10065_1	Colorado River	Colorado	Colorado	River
10088_1	Chacon Creek	Webb	Rio Grande	Small Stream
10160_1	Finley Branch	Nacogdoches	Neches	Large Stream
10162_1	East Copperas Creek	Kimble	Colorado	Large Stream
10165_1	Cypress Creek	Harris	San Jacinto	Large Stream
10198_1	San Bernard River	Brazoria	Brazos-Colorado	River
10258_1	Grace Creek	Gregg	Sabine	Large Stream
10305_1	San Jacinto River	Montgomery	San Jacinto	River
10310_1	White Oak Creek	Morris	Sulphur	River

In preparation for taking eDNA samples, all equipment and tools that would come into contact with the water sample or assist in filtering samples were decontaminated. Decontamination was completed by submerging all bottles and filter apparatus pieces in a 10% bleach solution for at least 10 minutes, followed by thorough rinsing with DI water. All pieces were laid out to air dry on a clean surface. After drying, bottles were capped, and filters were packed and placed in sterile bags until use. The filtering apparatus pieces

used were recycled Smith-Root eDNA Filter Packs sterilized using the same procedure as the bottles and refitted with 3.0 μm pore size Isopore™ polycarbonate membrane filters (EMD Millipore Corporation, Billerica,MA, USA) with a Polyester Drain Disc (Sterlitech Corporation, Washington, USA) filter backer and placed in a sterile bag until use (Gordon et al., 2023, Kumar et al., 2022b).

Sampling Design

Water samples for eDNA were taken at every site. Electrofishing was utilized at 36 sites with one not fished due to permit restrictions and another due to equipment failure. Sampling reach length was verified at each site by locating the randomized coordinates (x site) taking the average stream width around that point and multiplying it by 40. The x site was the middle of the sampling reach, with the reach extending in both directions. For wadeable sites, sampling moved from downstream to upstream (Figure 2). For boatable sites, sampling moved with the flow, moving from upstream to downstream. The minimum for a reach was 150 m and the maximum reach size was 4 km (Figure 3).

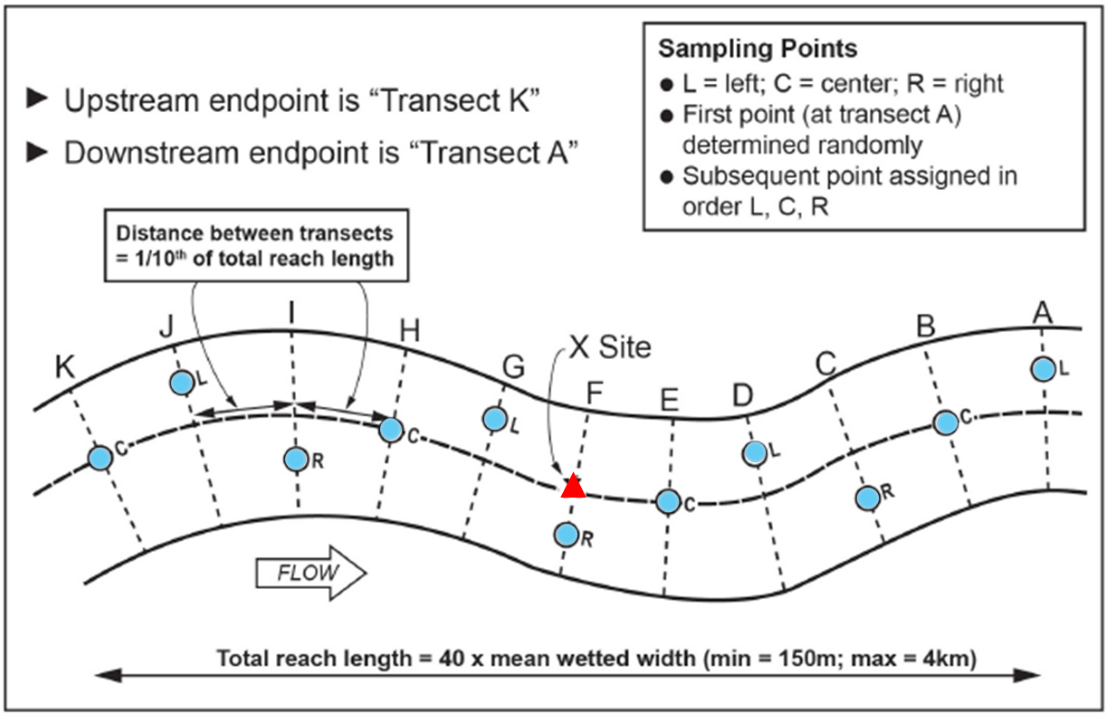

Figure 2: *Figure modified from USEPA (2022b). Representative of sampling in wadeable streams. Blue circles represent where the 100 mL aliquots were taken for the COM sample at all 11 of the evenly spaced transects, and the red triangle represents where the FIL sample was taken at the x-site (usually the center of reach).*

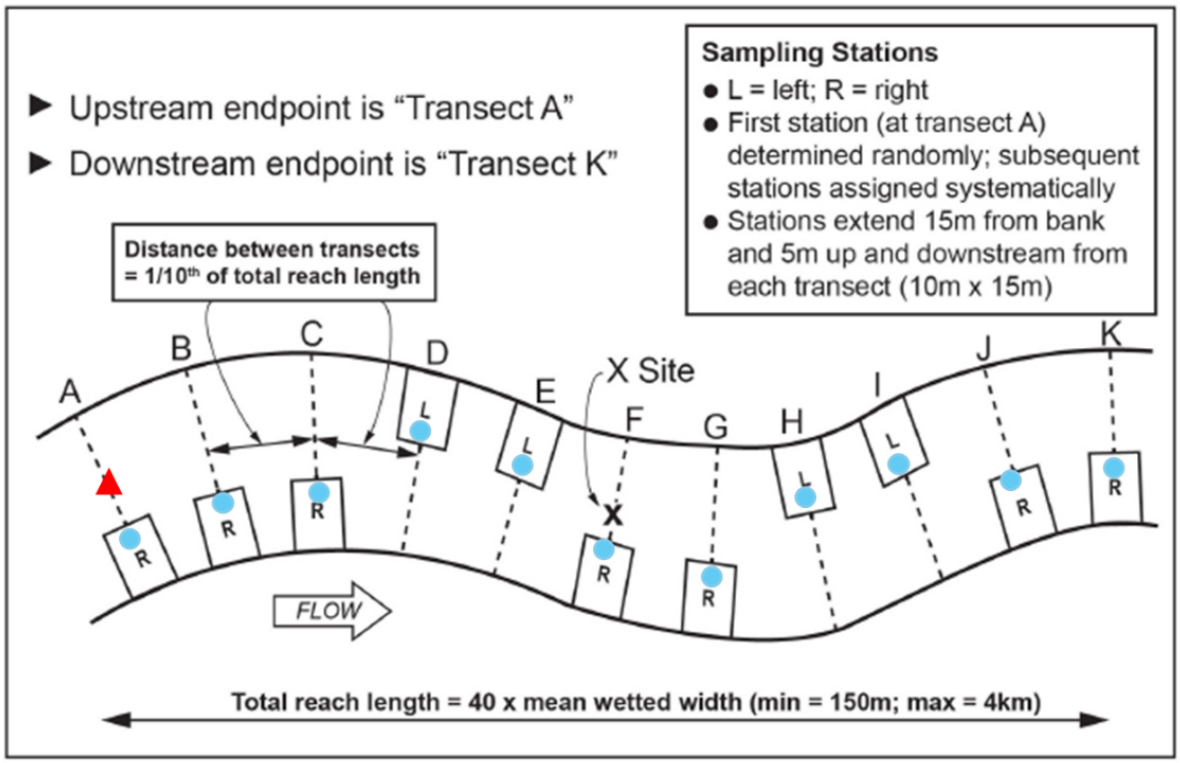

Figure 3: *Figure modified from USEPA (2023). Representative of boatable stream sampling. Blue circles represent where the 100 mL aliquots were taken for the COM sample at all 11 of the evenly spaced transects, and the red triangle represents where the FIL sample was taken at the most upstream (A) transect..*

eDNA Collection

Collecting eDNA began with rinsing each collection bottle three times with site water. The eDNA was then collected at a depth of 0.3 m, or half the depth if less than 0.5 m in depth by submerging the bottle mouth down underwater and inverting the bottle. Two samples each of 1 L were collected at each site. The first sample (FIL) was a one liter collected directly into a 1000 mL Nalgene bottle, at the x-site (typically the center of reach) for wadeable sites and most upstream transect (A) for boatable sites, taken at the centroid of the flow (Figure 2; Figure 3). A second liter (COM) was a composite sample, collected using a 125mL Nalgene bottle collecting ~100mL of water at 11 evenly spaces

transects and added to a 1000mL Nalgene bottle (Figure 2; Figure 3). Samples were kept in a cooler bag with ice packs or on ice until filtered.

Before other sampling began, the FIL sample was collected at the X site for wadeable sites, and the A transect for boatable sites. For large wadeable sites, with difficult access, the FIL sample was often taken as soon as the x transect was reached during sampling. After eDNA sample collection, water parameters were taken on a calibrated YSI ProDSS sonde at the same location. Parameters recorded were temperature, dissolved oxygen (DO), pH, and conductivity.

Electroshocking

Electroshocking used a variety of methods depending on depth, size, and access. Gear included a SmithRoot electroshock backpack, a 2.5 GPP barge/boat, or 5.0 GPP boat. Electroshocking was performed using the methodology as described in USEPA (2022b) and USEPA (2023), with sampling occurring in the same direction as eDNA sampling. For rivers with an average width >12m the area shocked was along the bank to 8 m into the channel. This sampling involved alternating banks every two transects with shocking continued until 500 fish were caught and at least half of the transects had been sampled or until the entire reach was sampled (Figure 4). For rivers with an average width <12m the whole width of the stream was shocked throughout the entire reach. Regular stops were made as needed to identify and count each fish netted.

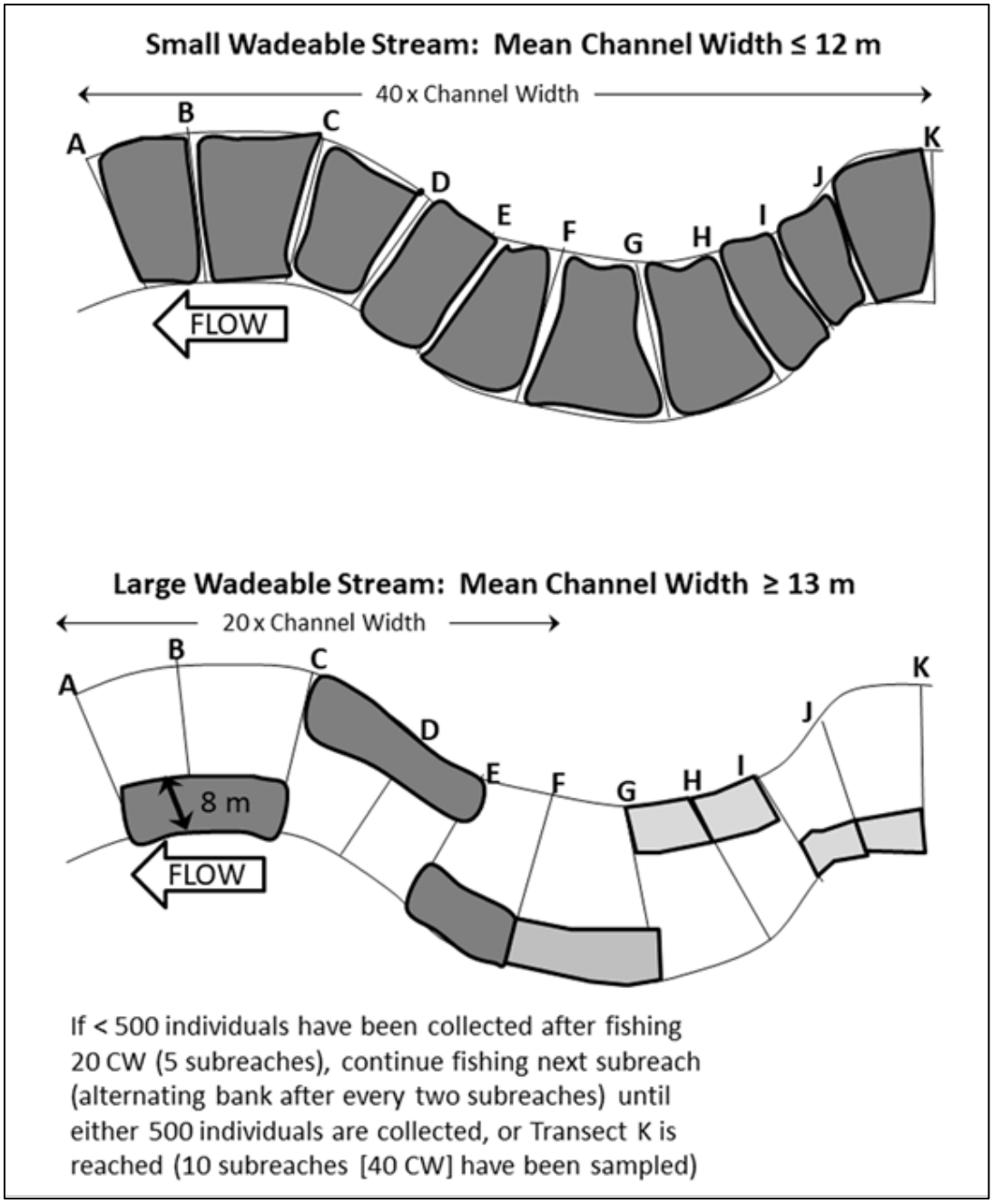

Figure 4: *Figure taken from USEPA, 2022b. Represents electrofishing sampling area for wadeable rivers and streams.*

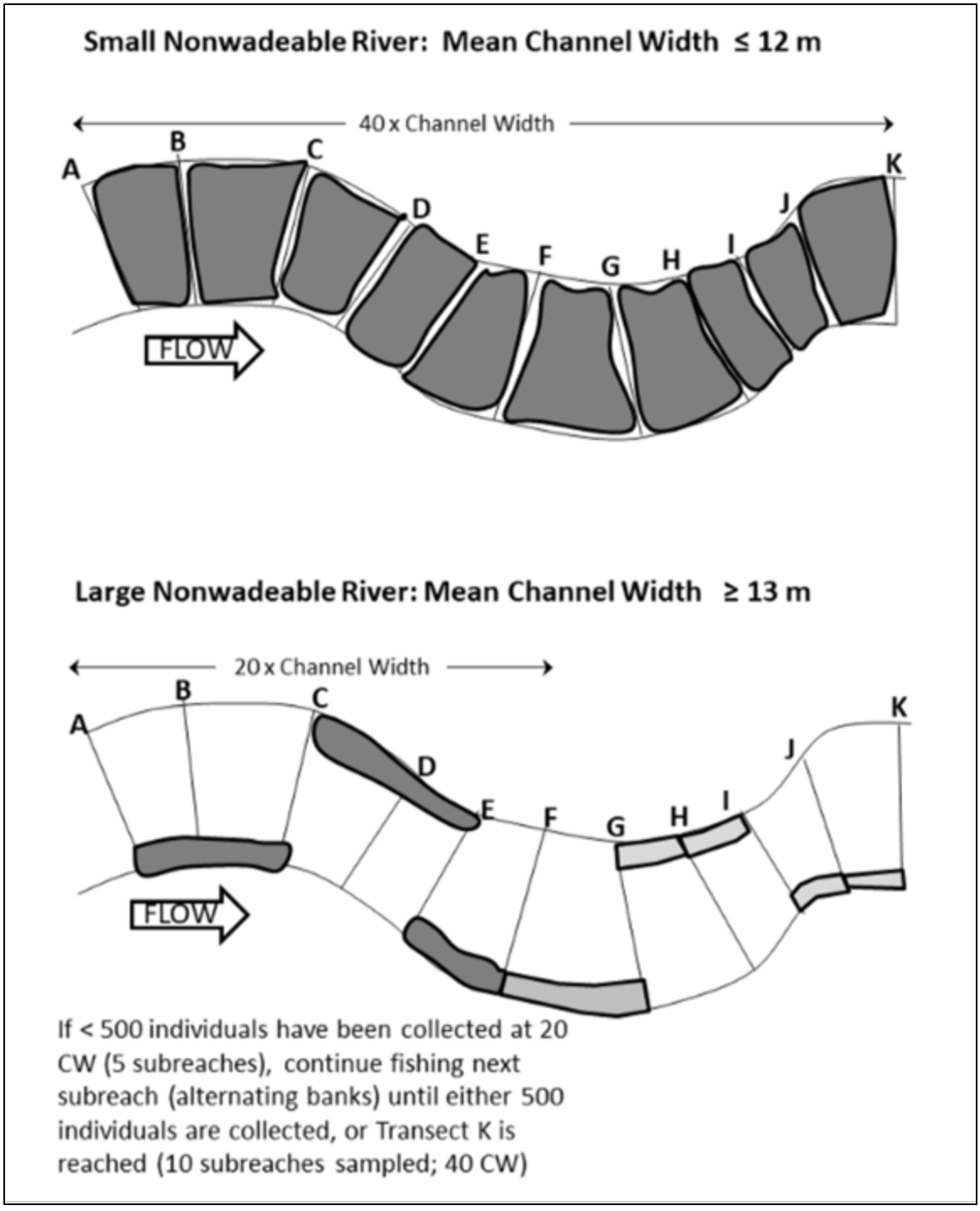

Figure 5: *Figure taken from USEPA, 2023. Represents the electrofishing sampling area for boatable rivers and streams.*

eDNA processing and sequencing

Filtering took place as soon as possible after sampling either in the field or upon returning to lodging. Water samples were homogenized by inverting several times or until settled substrate was all suspended, and 1000 mL was poured into a sterilized Nalgene beaker. Filtration was performed with an Alexis Peristaltic pump until the entire volume was filtered or until saturation and the amount filtered was recorded. Filters were placed in sterile 50 mL polypropylene centrifuge tubes labeled with the site, sample type, and date collected. Filters were kept on dry ice until a freezer was available; batches of up to 8 samples were shipped on dry ice to the EPA Office of Research and Development genomics lab in Cincinnati, Ohio and kept frozen until processing.

Extraction of DNA was completed using DNeasy PowerWater kits from QIAGEN. The PCR was performed in triplicate with a PCRmax machine using the Am12S vertebrate primers and the Ac16S primer (Evans et.al. 2016; Shu et al., 2021). Gel electrophoresis was used at each replication to ensure the presence of successful amplification prior to sequencing. Sequencing was done using the Illumina MiSeq system. Before assigning taxonomy, the raw reads were trimmed for quality using Fastp (v0.23.4), primers and sequences under 200 base pairs were removed (CutAdapt v4.9). Remaining sequences were processed using QIIME2 (with a DADA2 plugin v 2021.11.0) where sequencing and reads were separated and chimeras removed, and quality was ensured (Bolyen et al., 2019; Callahan et al., 2016). Taxonomy was assigned using the BLASTn function of the National Center for Biotechnology Information (NCBI) website and extracting the best hit for each sequence. For positive identification, this usually meant a 99-100% match. With many species not having a reference genome, or lower percentage matching closely related species, further considerations were made according to species native to the area or that were detected with an alternate method.

Species with a read count under 10 were not used as a species detection (Drake et al., 2022; Shu et al., 2021). Other detections immediately removed were the non-fish detections with the 12S vertebrate specific primer.

Computer processing and database organization

Initial electroshocking data contained five individual hybrid sunfish at three different sites, for consideration of species detections, all hybrid individuals were tallied as the parent species that was most dominantly captured with electrofishing at the site. Further changes were made to the eDNA data using corrections based on taxonomy reclassification, genome similarity, and spatial likelihood. Two species that had no close reference genome, indication from species caught electrofishing, and low read counts (under 20) were removed from the count. Species placeholders were used for species that had no positive match in the NCBI database (Table 2). Placeholders were determined based on the family match, species detected using the alternate genetic loci, species that were physically caught electroshocking, and species that are known to be present in the area.

Table 2: *Top match for sequences from NCBI database and changes that were made to my database for analysis.*

Original			Placeholder species or change made		
Family	**Scientific Name**	**Common Name**	**Family**	**Scientific Name**	**Common Name**
Catostomidae	*Moxostoma anisurum*	Silver redhorse	Catostomidae	*Moxostoma erythrurum*	Golden redhorse
Catostomidae	*Thoburnia rhothoeca*	Torrent sucker	Catostomidae	*Moxostoma congestum*	Gray redhorse
Centrarchidae	*Lepomis gibbosus*	Pumpkinseed	Centrarchidae	*Lepomis gulosus*	Warmouth
Centrarchidae	*NA*	Florida Bass	Centrarchidae	*Micropterus salmoides*	Largemouth Bass
Cichlidae	*Parachromis managuensis*	Jaguar guapote	Cichlidae	*Herichthys cyanoguttatus*	Rio Grande cichlid
Cichlidae	*Sarotherodon lohbergeri*	Keppi	Cichlidae	*Oreochromis aureus*	Blue tilapia
Cyprinidae	*Cyprinella formosa*	Beautiful shiner	Cyprinidae	*Notropis potteri*	Chub shiner
Cyprinidae	*Luxilus cornutus*	Common shiner	Cyprinidae	*Lythrurus fumeus*	Ribbon shiner
Cyprinidae	*Notropis baileyi*	Rough shiner	Cyprinidae	*Notropis sabinae*	Sabine shiner
Cyprinidae	*Notropis stilbius*	Silverstripe shiner	Cyprinidae	*Notropis amabilis*	Texas shiner
Cyprinodontidae	*Cyprinodon tularosa*	White Sands pupfish	Cyprinodontidae	*Cyprinodon variegatus*	Sheepshead Minnow
Gobiidae	*Gobiosoma ginsburgi*	Seaboard goby	Gobiidae	*Gobiosoma bosc*	Naked Goby
Gobiidae	*Neogobius gymnotrachelus*	Racer goby	Gobiidae	*Neogobius melanostomus*	Round Goby
Lepisosteidae	*Lepisosteus platyrhincus*	Florida Gar	Lepisosteidae	*Lepisosteus oculatus*	Spotted Gar
Percidae	*Etheostoma caeruleum*	Rainbow darter	Percidae	*Etheostoma asprigene*	Mud darter
Percidae	*Etheostoma cragini*	Arkansas darter	Percidae	*Etheostoma lepidum*	Greenthroat darter
Percidae	*Etheostoma exile*	Iowa darter	Deleted		
Percidae	*Etheostoma jessiae*	Blueside darter	Percidae	*Percina sciera*	Dusky darter
Percidae	*Etheostoma olmstedi*	Tessellated darter	Percidae	*Etheostoma gracile*	Slough darter
Percidae	*Etheostoma trisella*	Trispot darter	Deleted		
Percidae	*Percina rex*	Roanoke logperch	Percidae	*Percina caprodes*	Common logperch
Poeciliidae	*Gambusia holbrooki*	Eastern mosquitofish	Poeciliidae	*Gambusia affinis*	Western mosquitofish

Analysis

Fish data for electrofishing and eDNA were compared only using the sites with complete data. Data were deemed "complete" if all the following were collected and had results: electrofishing data, eDNA data for 12S and 16S from both the FIL and COM samples, and 12S and 16S data. This resulted in 25 sites for comparison of methods, and 50 eDNA samples (Table 1). Analyses looking at richness of eDNA used combined data from both primers and both the FIL and COM samples. Linear regression analysis was performed using Microsoft Excel (version 2501). RStudio (version 4.3.2) with the stats package (version 4.3.2) was used for all paired T-tests and the VennDiagram package (version 1.7.3) helped to create accurate visuals on Richness in each method. SAS on demand was used for the One-way MANOVA analysis of sites. The ANOSIM and Pearson-correlations were performed on Primer7 (Clark and Gorley, 2015). Results are considered statistically significant at the 95% confidence level ($\alpha = 0.05$).

RESULTS:

General

A total of 76 eDNA samples were collected, 38 COM samples and 38 FIL samples. Each of those samples was amplified using the 12S and 16S primers for a total of 152 samples. Of the samples, 119 had successful fish detections (78% success), while 33 samples (22%) did not produce any fish detections. Of the 76 12S samples, 60 succeeded in producing fish detections, and 59 of the 76 16S samples succeeded. Looking at all the eDNA data for the sites sampled, 10 or 26% of sites had partial success (1-3 of the samples succeeded), 25 or 66% of sites had all samples yield fish reads and 3 of the 38 sites or 8% of sites sampled had all samples fail to produce any fish sequences. Of those 3 sites not yielding any viable data, 2 of them were the sites not fished with the use of electroshocking or another traditional method.

FIL vs COM

In the 25 sites analyzed, 13 sites yielded more species with the COM sample, 12 detected more species with the FIL sample (Figure 6), with no significant differences in terms of the size of the stream between the categories: small stream, large stream, and river. This resulted in no detected significance between richness alone at each site (paired t-test p-value = 0.6727). One of the sampled sites (10019) analyzed was a revisit of the same site and did not show consistency here between the first and second visit. The first visit had a higher number of species detections with COM (8 vs 5), the second visit had higher species detection with the FIL sample (4 vs 12).

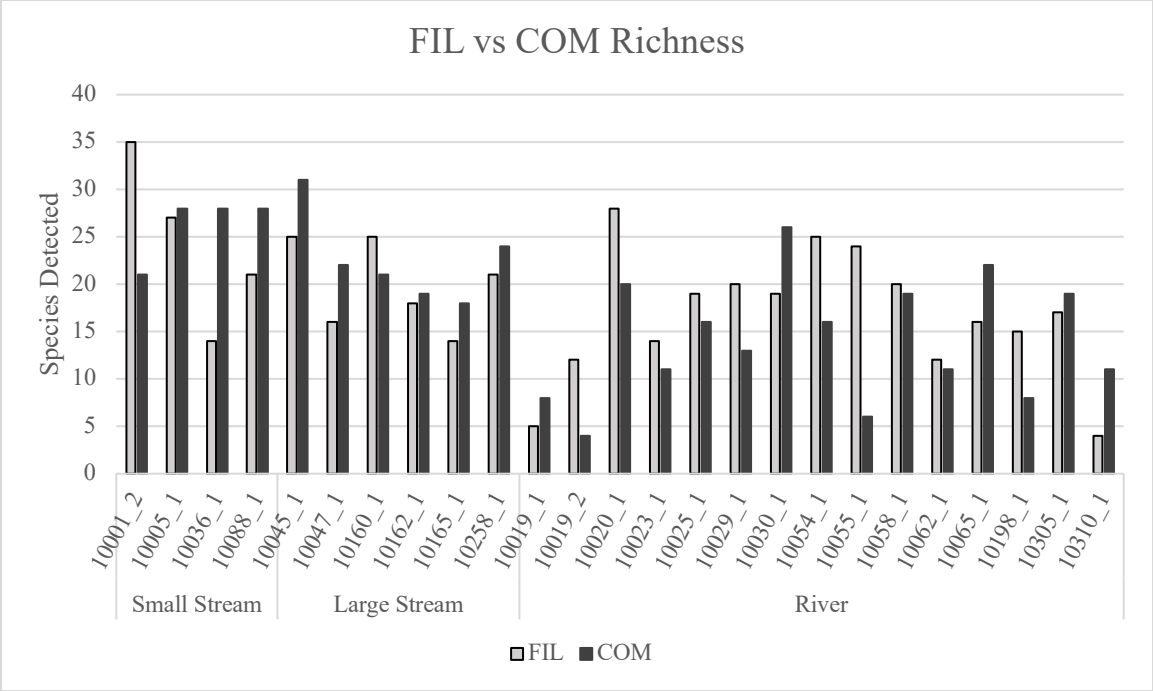

Figure 6: *Species detected from the two samples collection methods (FIL and COM), by stream category. The COM sample had 13 sites with higher richness detected and the FIL samples had 12 sites more species detections.*

A One-Way ANOSIM failed to detect statistically significant dissimilarities between fish communities by eDNA collection type (FIL vs COM) (One-way ANOSIM, R = -0.033, p-value = 0.964). This can also be demonstrated with the use of a Venn diagram for species detected by sampling type. Across all sites, 15 (16%) species were detected with the FIL sample only, and six (7%) were detected with the COM sample only. Seventy (77%) species were found using both sample types(Figure 7a; Appendix A). Dividing the species caught between small streams, large streams, and rivers we can see that the communities are very similar between all the groups. Sites designated as "rivers", which are the highest Strahler order sites, showed more variation between the number of species detected with only FIL vs COM (Figure 7b-d).

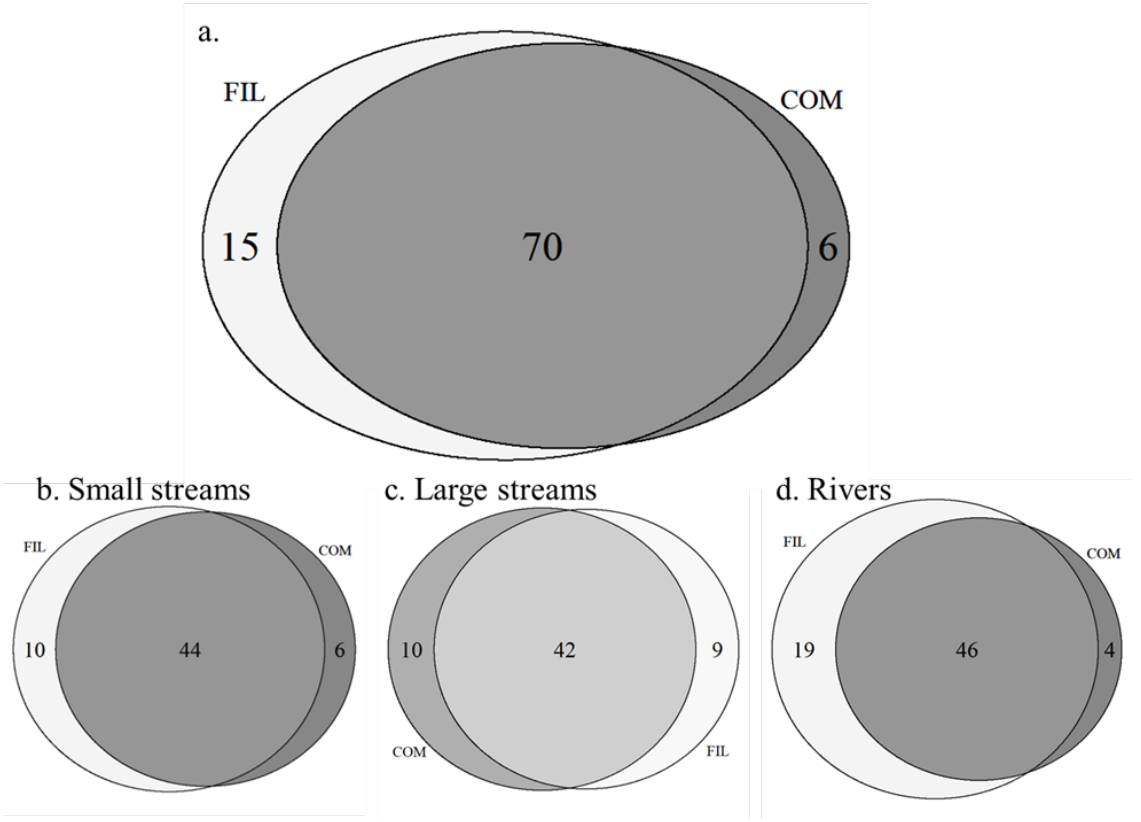

Figure 7: *a) Venn Diagram of all species detected between FIL and COM samples. 15 species or 16% were detected in FIL samples only; 6 species or 7% were only detected in COM samples; 70 species or 77% were detected in both the FIL and COM sample. Venn diagrams for species caught in FIL and COM sample by site category b) small streams, c) large streams, d) river*

12S vs 16S

Comparing the use of primer used for PCR, I failed to detect a statistically significant difference in species richness detected between 12S and 16S primers (paired t-test, p-value = 0.672). There was no observed consistent trend as to one primer detecting a greater number of species than the other (Figure 8).

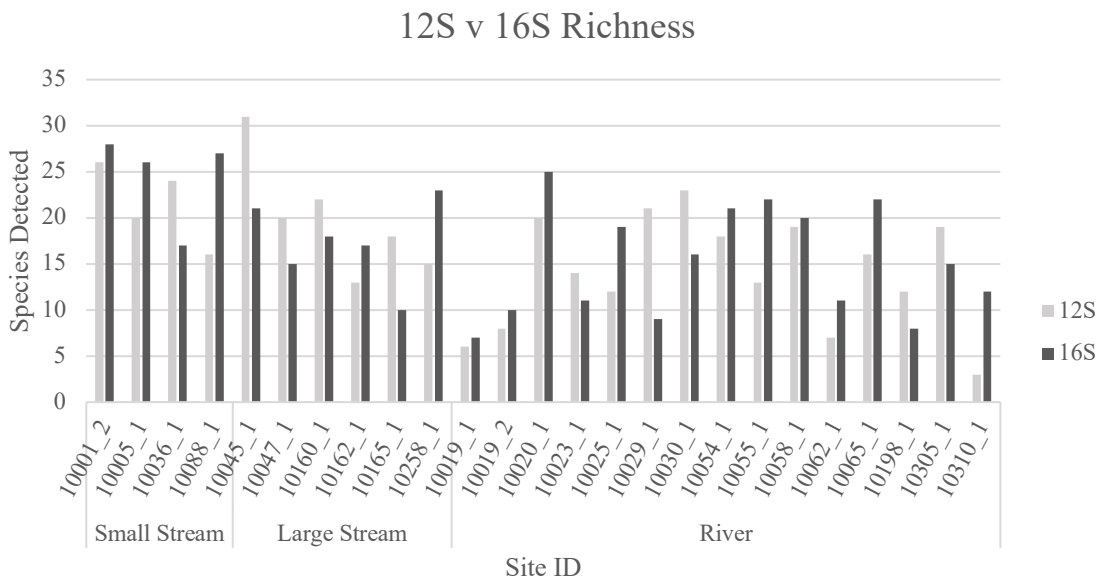

Figure 8: *Species detected with the two primers used in eDNA processing (12S and 16S), by stream category.*

A statistically dissimilar community composition was detected between the two primer types (One-way ANOSIM, R = 0.32, p-value = 0.001). An nMDS plot displays this well by showing communities detected with each primer with each triangle representing a different site. It shows a visually defined separation between the 12S (blue triangles pointing up) community and the 16S (red triangle pointing down) communities, demonstrating that the communities detected with the 12S and 16S primers different (Figure 9). A Pearson correlation of 0.4 along the MDS2 ordination identified seven species that contributed to the dissimilarities in community structure with 3 species contributing strongly to the 12S community differences: *Lempomis gulosus*, *Lepomis megalotis,* and *Lepisosteus platosotmus* and 4 species heavily contributing to the downward vertical shift of the 16S communities: *Cyprinella venusta*, *Lepisosteus osseus*, *Lepomis marginatus*, and *Pimephales vigilax* (Figure 9).

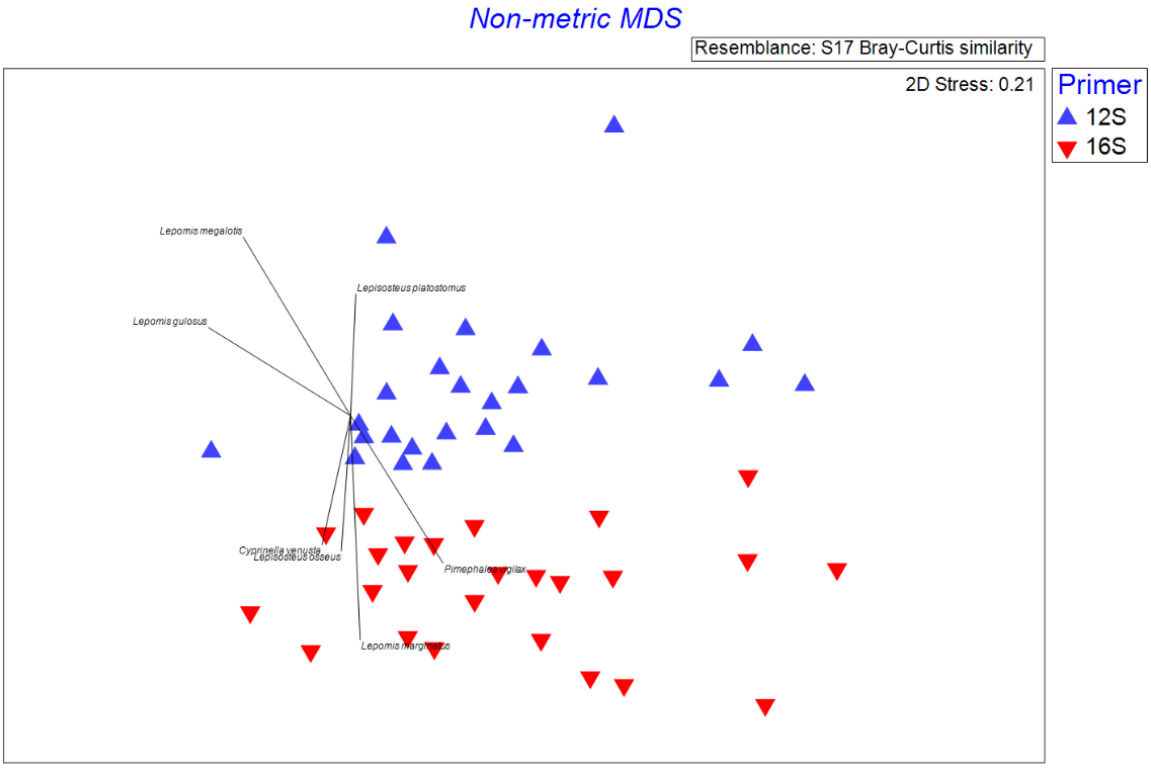

Figure 9: *nMDS plot with Pearson Correlations over 0.40 for MDS2 ordination for the 12S and 16S Primer communities. Shows a separation in the MSD2 ordination between the communities detected in 12S and 16S samples. Species that contributed to this separation in the direction of the 12S samples and the species that contributed in the direction of the 16S samples are listed in upper corners.*

Table 3: *Species with a Pearson Correlation over 0.40 from the 12S and 16S communities in either ordination of the nMDS plot.*

Family	Species	Common Name	MDS1	MDS2
Ictaluridae	*Ameiurus natalis*	Yellow bullhead	**-0.678184**	-0.15471
Lepisosteidae	*Atractosteus spatula*	Alligator gar	**0.406530**	0.007062
Cyprinidae	*Cyprinella venusta*	Blacktail shiner	-0.102837	**-0.46473**
Dorosomatidae	*Dorosoma petenense*	Threadfin shad	**0.562095**	0.087419
Percidae	*Etheostoma asprigene*	Mud darter	**-0.401097**	-0.28748
Percidae	*Etheostoma gracile*	Slough darter	**-0.510409**	-0.07897
Catostomidae	*Ictiobus cyprinellus*	Bigmouth buffalo	**0.590727**	-0.08972
Lepisosteidae	*Lepisosteus osseus*	Longnose gar	-0.033057	**-0.48694**
Lepisosteidae	*Lepisosteus platostomus*	Shortnose gar	0.018578	**0.438229**
Centrarchidae	*Lepomis auritus*	Redbreast sunfish	**-0.415908**	-0.04351
Centrarchidae	*Lepomis cyanellus*	Green sunfish	**-0.559821**	-0.22903
Centrarchidae	*Lepomis gulosus*	Warmouth	**-0.512523**	0.317756
Centrarchidae	*Lepomis macrochirus*	Bluegill	**-0.545706**	-0.13167
Centrarchidae	*Lepomis marginatus*	Dollar sunfish	0.034192	**-0.80729**
Centrarchidae	*Lepomis megalotis*	Longear sunfish	-0.386426	**0.643223**
Centrarchidae	*Lepomis miniatus*	Redspotted sunfish	**-0.429810**	-0.187
Centrarchidae	*Micropterus salmoides*	Largemouth bass	**-0.692652**	-0.10583
Catostomidae	*Minytrema melanops*	Spotted sucker	**-0.462477**	0.037476
Ictaluridae	*Noturus gyrinus*	Tadpole madtom	**-0.405870**	-0.14411
Cyprinidae	*Pimephales vigilax*	Bullhead minnow	0.332388	**-0.53046**

From eDNA 67 species were detected using the 12S primer and 70 species were detected using the 16S primer. From those, 21 (23%) species were detected only when amplified with the 12S primer, 24 (26%) species were detected only with the 16S primer, and 46 (51%) species were detected with both. Nearly half (49%) of the species detected were only detected with one of the primers (Figure 10, Appendix B).

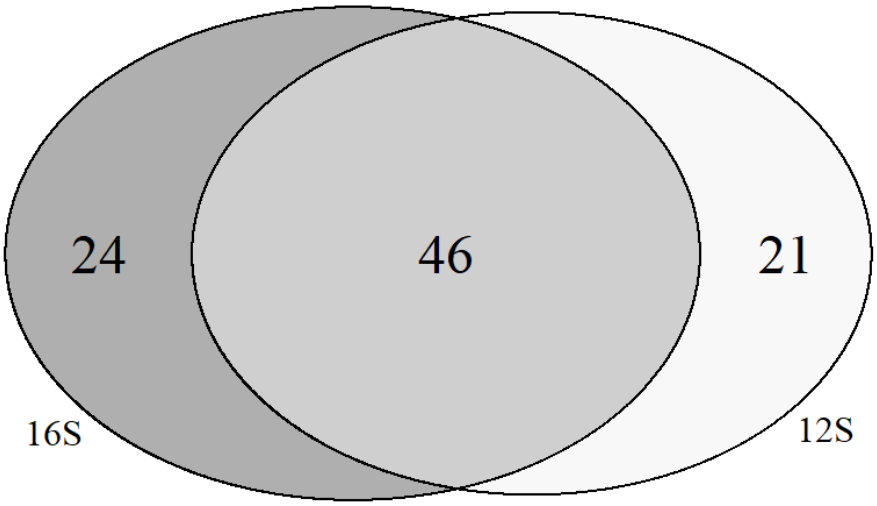

Figure 10: *Venn Diagram of all species detected using eDNA and with what primer they were detected with between the 16S and 12S primers. 24 species or 26% were detected in the 16S samples only; 21 species or 23% were detected in the 12S samples only; 46 species were detected in both the 12S and 16S samples.*

eDNA vs eShock

From the 25 sites analyzed, there was a total of 120 species caught/detected; 90 species were collected via electrofishing and 91 species were detected with the use of eDNA. Between methods, 30 (25%) species were only detected with eDNA, 29 (24%) species were only detected with electrofishing, and 61 (51%) species were detected with both methods. Again showing that nearly half (49%) of species were only detected in one method (Figure 11, Appendix C).

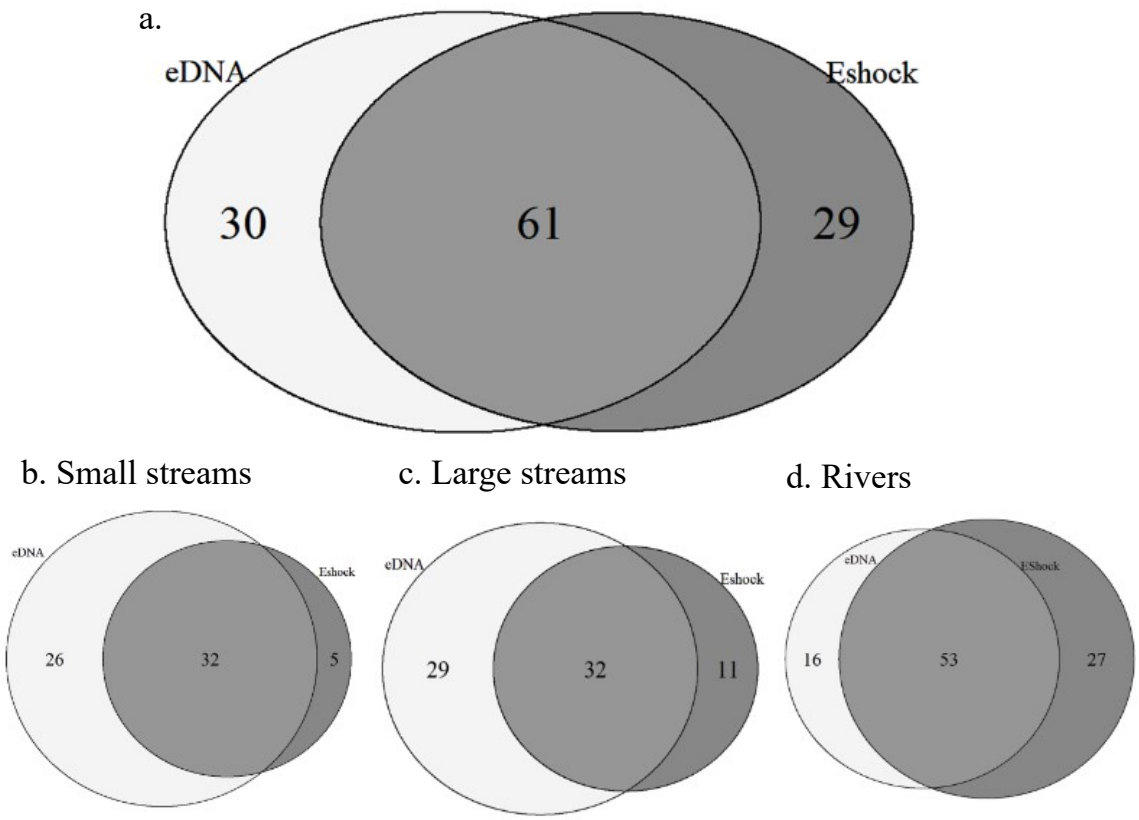

Figure 11: *a) Venn Diagram of all species detected/caught in the 25 analyzed sites between eDNA and Electroshocking. 30 species or 25% of species were detected only with eDNA; 29 species or 24% of species were detected only through electroshocking; 61 species were detected using both methods. Venn diagrams for species caught between methods by site category b) small streams, c) large streams, and d) rivers..*

Most sites sampled had a higher species detection with eDNA metabarcoding over electroshocking. This is particularly evident with the small streams sites, and the obvious increase in species detections for eDNA when compared to electroshocking at each site (paired t-test, p-value = 0.00096) (Figure 12).

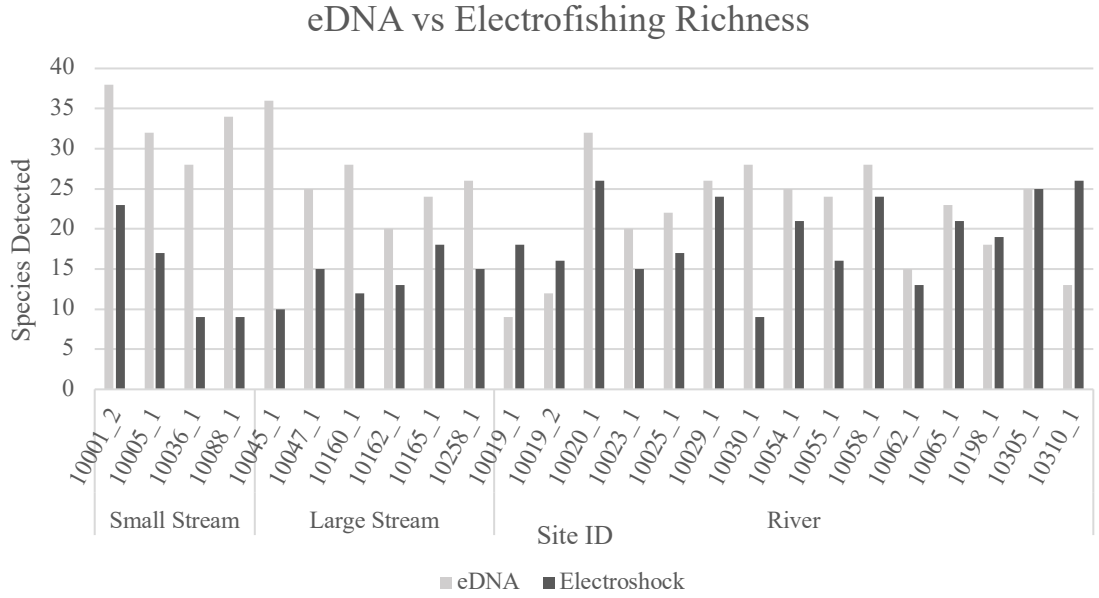

Figure 12: *Species detected from the two fish sampling methods (eDNA and electroshocking), by stream category. The eDNA samples consistently and usually detected a higher number of species when compared to electroshocking at each site.*

There was also a statistically dissimilar community between the electroshocking and eDNA sample types (One-way ANOSIM, R=0.275, p-value = 0.001). An nMDS plot of Bray-Curtis similarity provides distinct groupings between the community that is detected with eDNA (D or the blue upward pointing triangles) and the community detected with electroshocking (E or the red downward pointing triangles) at each site (Figure 13). A Pearson Correlation identified 16 species are strongly contributing (over 0.40) to this dissimilarity in community between the eDNA and electroshocking and the directionality of the groupings (Table 4).

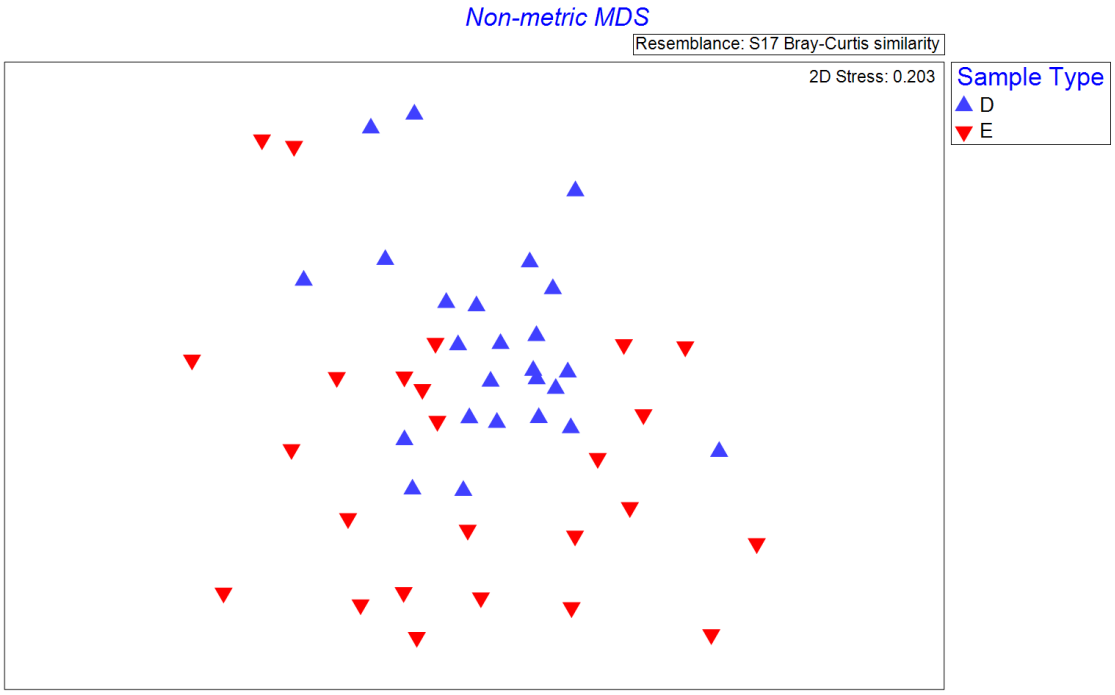

Figure 13: *nMDS plot for eDNA (D or Blue) and electroshocking (E or Red) communities. Communities showed grouping based on sampling type.*

Table 4: *Species with a Pearson Correlation over 0.40 for eDNA and electroshocking communities and the direction on the nMDS plot.*

Family	Species	Common Name	MDS1	MDS2
Aphredoderidae	*Aphredoderus sayanus*	Pirate perch	**0.44059**	**0.46852**
Catostomidae	*Moxostoma poecilurum*	Blacktail redhorse	0.022847	**0.44706**
Centrarchidae	*Lepomis marginatus*	Dollar sunfish	-0.13978	**0.43616**
Centrarchidae	*Lepomis miniatus*	Redspotted sunfish	0.263241	**0.47758**
Centrarchidae	*Micropterus salmoides*	Largemouth bass	**0.54848**	-0.15604
Centrarchidae	*Micropterus treculi*	Guadalupe bass	0.124335	**-0.474**
Cichlidae	*Herichthys cyanoguttatus*	Rio Grande cichlid	0.107003	**-0.5816**
Cyprinidae	*Cyprinella lutrensis*	Red shiner	**-0.4987**	0.07207
Cyprinidae	*Lythrurus fumeus*	Ribbon shiner	0.31075	**0.40229**
Cyprinidae	*Notropis amabilis*	Texas shiner	0.111004	**-0.4346**
Dorosomatidae	*Dorosoma cepedianum*	Gizzard shad	**-0.57**	0.198576
Dorosomatidae	*Dorosoma petenense*	Threadfin shad	**-0.6631**	0.060389
Fundulidae	*Fundulus olivaceus*	Blackspotted topminnow	0.304143	**0.45479**
Ictaluridae	*Ameiurus natalis*	Yellow bullhead	**0.51583**	0.189741
Ictaluridae	*Ictalurus furcatus*	Blue catfish	**-0.5272**	-0.22006
Lepisosteidae	*Lepisosteus platostomus*	Shortnose gar	**-0.4115**	0.266601

Linear regression was used to evaluate the relationship of species richness versus stream size (average width). I failed to detect a significant linear relationship between species richness from electroshocking effort and stream size ($p = 0.435969$), but I do see a slight positive trend. Richness from eDNA samples had a significant negative linear correlation to average width ($p = 0.000636$) (Figure 14). There could also be a strong influence from the one site visited twice that was significantly larger due to the large gap in the widths for mid-width streams.

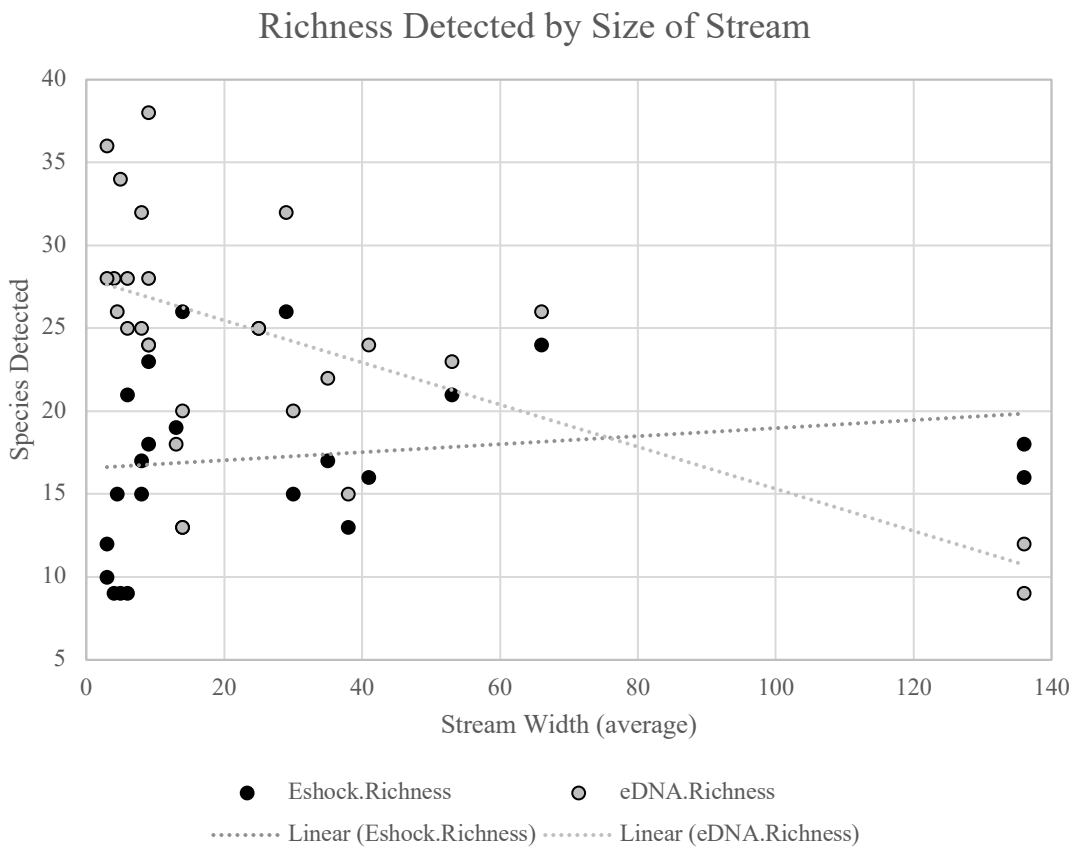

Figure 14: *Linear Regression (best fit line) between the Species Richness and Stream Width by sampling method. Electroshocking did not show a statistically significant linear relationship (p = 0.435); eDNA did show a statistically significant linear relationship (p = 0.0006).*

Site differences

To evaluate site conditions that may have negatively impacted my ability to detect DNA, a One-way MANOVA was used to compare the sites. Sites were divided by the successful detection of eDNA. The groupings were: sites that all samples yield results (Y) (n = 25), some of the samples yielded results (P) (n = 10), or no samples yielded DNA (N) (n = 3). Environmental variables analyzed in the One-way MANOVA included: amount filtered, average stream width, DO%, pH, water temperature, and conductivity. I failed to detect any statistically significant correlation between the site variables selected and the detection of DNA (Table 5).

Table 5: *Variables used in the One-Way MANOVA and the corresponding p-values, F values, and degrees of freedom (DF).*

Variable	p-value	F value	DF
amount filtered	0.6135	0.50	2
average width	0.8246	0.19	2
DO%	0.8844	0.12	2
pH	0.7455	0.30	2
water temperature	0.1130	2.32	2
conductivity	0.1158	2.29	2

DISCUSSION:

Composite sampling of eDNA did not affect the number of species or the species composition being detected, when compared to a single 1L grab. It has been shown that composite eDNA samples across a range of space is unnecessary for smaller streams due to the prolific transport of DNA in flowing aquatic systems (Perry et al., 2024; Sakata et al., 2021). However, in a study on eukaryotic organisms, it was recommended to use a composite sample for better sampling as well as greater representation of species abundance (Cornman et al., 2018). When sampling very long stretches of rivers with extensive branching, or when trying to locate higher concentrations of individuals or populations, as well as looking at drought or ephemeral conditions with isolated pools, composite or multiple eDNA samples within a stream are beneficial (Meulenbroek et al., 2022; Van Driessche et al., 2024).

The composite sample did not contribute much in detecting more species, however, it did contribute to increasing the quantity of eDNA filtered for each site. While space and manpower were limited for this project alongside NRSA sampling, replicate samples for the purpose of retrieving more DNA are important, in the case that there are sequencing or sample failures, contaminations, safeguard against false positives and negatives, and have a better chance at detecting more species (Pont et al., 2018; Fujii et al., 2019). For this study the COM sample did not provide different or better results when it came to richness, even though it represents higher Strahler order, and typically covers a larger range in distance and in habitat types. However, a second sample and filter, assisted by providing a more robust picture of the species present. Increasing the volume filtered and the number of samples being taken has been the solution for most studies for maximizing detections and having a higher likelihood of detecting rare or cryptic species (Capo et al., 2020; Hunter et al. 2019; Sepulveda et al., 2019).

Based on the significant variance in communities detected with the 12S and 16S data, it is recommended to continue using two genetic loci to capture a more complete profile of species present (Evans et al., 2016). Although this is heavily dependent on the types of fish being targeted and the budget, to best capture the community, having multiple primers reduces inherent bias with primers. Bias typically is shown partially due to the completeness and breadth of the genetic library, or in PCR stochasticity, and increases likelihood of detection (Miya et al., 2015; Kumar et al., 2022a; Zhang et al., 2020, Elbrecht and Leese, 2015), and although there is no obvious divisions in what primers detected certain species, the 16S primer was responsible for detecting more of the small cyprinids.

When determining which fish sampling methods to use, careful consideration for the goals of the study should be considered. If the aim of the study is to evaluate species richness or presence/absence alone, eDNA appears to be sufficient, particularly in smaller streams with the sampling methodology used in this study (McColl-Gausden et al., 2021). While the species richness here was similar between eDNA and electroshocking, the difference in community demonstrates that combining efforts maximizes detections. A combination of eDNA with electroshocking, especially in larger rivers provides a more complete estimate of the species assemblage. While eDNA is becoming more commonly utilized across study systems, it is important to note the methodology involved.

For this study a one method approach was used for all sites. Where I did not see as much success in detections with larger rivers, other studies showed significant improvements over traditional methods (Pont et al., 2018; Goutte et al., 2020; Meulenbroek et al., 2022; Zhang et al., 2023). Methodology between this and those studies largely differed in the volume being filtered at many of the sites for large river studies. Filter amount ranges from 3 – 30 L of water instead of the 1 L used here (Goutte

et al.,2020; Meulenbroek et al., 2022). It is apparent that one size does not fit all for sampling. One size does not fit all for traditional methods either.

I failed to detect a significant correlation between species richness as measured by electroshocking effort and the average width of the river. This was surprising as the number of species present is expected to increase with the size of waterbody (Rosenzweig, 1995). The lower species detections, when compared to eDNA results, indicates that electrofishing may not be sufficient on its own to describe the species richness in a large river. However, due to our small sample size in large rivers, further sampling would be important to fill in the gaps on the larger stream sizes. Other studies have found that using eDNA metabarcoding is sufficient alone for characterizing species richness in large rivers, by increasing the volume filtered to increase DNA intake or by increasing the number of samples taken (Hunter et al. 2019; Sepulveda et al., 2019; Meulenbroek et al., 2022; Zhang et al., 2023; Goute et al., 2023) and could complement ongoing studies.

The use of more primers, more replicates, more time shocking, and more methods of looking at fish assemblage should almost always increase chances of detecting more species. While there are always limitations to what is possible for sampling, providing the dual use of eDNA and electroshocking helps account for some of the disparity no matter the number of samples or primers. Due to the physiology of some fish, their reaction to stimulation, habitat, water conditions, or even permit restrictions, the addition of eDNA gives the opportunity to detect things that would otherwise not be and will increase knowledge and efficiency.

It is still unclear what may have caused the failure to detect any fish species in certain samples, but a common problem in the rivers and streams of Texas that could cause interference is high turbidity. Unfortunately, turbidity was not measured in this

study, but it can restrict the volume filtered due to clogging. Low filtration is one of the main reasons eDNA studies have low detections (Kumar et al., 2022). Another issue with turbidity is that DNA molecules in water have been found to adhere to fine sediment in water (Brandão, 2024). Finally, turbidity could cause further complications with PCR inhibitors when processing the samples. (Kumar et al., 2022; Fujii et al., 2019). The kit we used to extract DNA specifically was designed to help remove inhibitors and extract DNA in a range of turbidity, however Kumar et al., (2022) suggests that further steps are needed.

At the beginning of this project, a list was made of 242 species of freshwater and brackish water species that could be encountered in rivers and streams in Texas. From those species, 152 already had genomes available in the NCBI database. Due to this disparity, not all the species encountered could be matched to a reference genome. While it can be determined that there are distinct species, because of the incomplete reference library, inferences were made based on the family designation and the species physically caught at sites using electrofishing and known species distributions. While this gives us a better picture, without a complete library, the data cannot be verified, creating issues in recorded data and management practices. For widespread sampling or surveys, similar to this sampling, the completion of a genetic library is a crucial component in making eDNA most effective. Collecting genetic tissue and fin clips throughout any study will help with filling these data gaps over time as funding becomes available and technology becomes more accessible.

Because of the range of procedures in sampling and processing, the work of creating complete genetic libraries can be slow and costly, particularly due to the effort that goes in to sequencing tissues for each species. Standardizing methods, particularly with lab procedures and primers, will assist in creating the genetic library, by decreasing

the work and cost that goes into either a full genome sequencing or sequencing for a range of loci. Further work should be done for the optimization of filtering and processing turbid water samples, to allow the quantity necessary for filtering and avoid DNA loss related to DNA adherence to sediment type (Kumar et al., 2022; Brandão, 2024). The standardization of protocols is not yet in place, but is ongoing work (Hebling and Hobbs, 2019; Theroux et al., 2025; Goodwin et al., 2024).

Even in the small volume samples of one liter from this study, the eDNA data resulted in more robust species lists than traditional methods alone. The need for a complete genetic library limits its current use across systems, but eDNA can be collected easily with minimal effort compared to most traditional methods and could help in monitoring fish communities and distributions more regularly and efficiently.

Further Directions

Sampling occurred at an additional 45 sites with 47 sampling events from April to October 2024. Analyzing these additional data will offer a more robust understanding of the functionality of the methods used across diverse systems and provide greater perspective into environmental factors that could affect the usage of eDNA metabarcoding.

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APPENDIX A:

SPECIES DETECTION (FIL VS COM)

Table 6: *Species that were detected in either COM or FIL samples at all analyzed sites.*

Family	Scientific Name	Common Name	COM	FIL
Amiidae	*Amia calva*	Bowfin	X	X
Aphredoderidae	*Aphredoderus sayanus*	Pirate perch	X	X
Atherinopsidae	*Labidesthes sicculus*	Brook silverside	X	X
	Menidia beryllina	Inland silverside	X	X
Catostomidae	*Carpiodes carpio*	River carpsucker	X	X
	Cycleptus elongatus	Blue sucker	X	X
	Erimyzon oblongus	Creek chubsucker	X	X
	Erimyzon sucetta	Lake chubsucker	X	
	Ictiobus cyprinellus	Bigmouth buffalo	X	X
	Minytrema melanops	Spotted sucker	X	X
	Moxostoma congestum	Gray redhorse	X	X
	Moxostoma erythrurum	Golden redhorse		X
	Moxostoma poecilurum	Blacktail redhorse	X	X
Centrarchidae	*Lepomis auritus*	Redbreast sunfish	X	X
	Lepomis cyanellus	Green sunfish	X	X
	Lepomis gulosus	Warmouth	X	X
	Lepomis humilis	Orangespotted sunfish	X	X
	Lepomis macrochirus	Bluegill	X	X
	Lepomis marginatus	Dollar sunfish	X	X
	Lepomis megalotis	Longear sunfish	X	X
	Lepomis microlophus	Redear sunfish	X	X
	Lepomis miniatus	Redspotted sunfish	X	X
	Lepomis symmetricus	Bantam sunfish	X	X
	Micropterus dolomieu	Smallmouth bass	X	X
	Micropterus punctulatus	Spotted bass		X
	Micropterus salmoides	Largemouth bass	X	X
Characidae	*Astyanax mexicanus*	Mexican tetra	X	X
Cichlidae	*Herichthys cyanoguttatus*	Rio Grande cichlid	X	X
	Oreochromis	Tilapia	X	X
	Oreochromis aureus	Blue Tilapia	X	X
Cyprinidae	*Campostoma anomalum*	Central stoneroller	X	X
	Cyprinella lutrensis	Red shiner	X	X
	Cyprinella venusta	Blacktail shiner	X	X
	Cyprinus carpio	Common carp	X	X
	Lythrurus fumeus	Ribbon shiner	X	X

Family	Scientific Name	Common Name	COM	FIL
	Notropis amabilis	Texas shiner		X
	Notropis blennius	River shiner		X
	Notropis potteri	Chub shiner	X	X
	Notropis sabinae	Sabine shiner	X	X
	Pimephales vigilax	Bullhead minnow	X	X
Cyprinodontidae	*Cyprinodon variegatus*	Sheepshead minnow		X
Dorosomatidae	*Dorosoma cepedianum*	Gizzard shad	X	X
	Dorosoma petenense	Threadfin shad	X	X
Elassomatidae	*Elassoma zonatum*	Banded pygmy sunfish	X	
Esocidae	*Esox americanus*	Redfin pickerel	X	X
	Esox niger	Chain pickerel	X	X
Fundulidae	*Fundulus grandis*	Gulf killifish		X
	Fundulus notatus	Blackstripe topminnow	X	X
	Fundulus olivaceus	Blackspotted topminnow	X	X
	Fundulus zebrinus	Plains killifish		X
Gobiidae	*Gobiosoma bosc*	Naked goby	X	X
	Neogobius melanostomus	Round goby		X
Ictaluridae	*Ameiurus melas*	Black bullhead	X	X
	Ameiurus natalis	Yellow bullhead	X	X
	Ameiurus nebulosus	Brown bullhead	X	X
	Ictalurus furcatus	Blue catfish	X	
	Ictalurus punctatus	Channel catfish	X	X
	Noturus gyrinus	Tadpole madtom	X	X
	Noturus taylori	Caddo madtom	X	X
	Pylodictis olivaris	Flathead catfish	X	X
Lepisosteidae	*Atractosteus spatula*	Alligator gar	X	X
	Lepisosteus oculatus	Spotted gar	X	X
	Lepisosteus osseus	Longnose gar	X	X
	Lepisosteus platostomus	Shortnose gar	X	X
Leuciscidae	*Notemigonus crysoleucas*	Golden shiner	X	X
Loricariidae	*Pterygoplichthys anisitsi*	Southern sailfin catfish	X	X
	Pterygoplichthys pardalis	Amazon sailfin catfish	X	
Moronidae	*Morone chrysops*	White bass		X
Mugilidae	*Mugil cephalus*	Striped mullet	X	X
	Mugil curema	White mullet		X
	Mugil liza	Lebranche mullet	X	X
Percidae	*Etheostoma asprigene*	Mud darter	X	X
	Etheostoma chlorosoma	Bluntnose darter	X	X
	Etheostoma fonticola	Fountain darter		X

Family	Scientific Name	Common Name	COM	FIL
	Etheostoma gracile	Slough darter	X	X
	Etheostoma lepidum	Greenthroat darter	X	X
	Etheostoma proeliare	Cypress darter		X
	Etheostoma radiosum	Orangebelly darter	X	X
	Etheostoma spectabile	Orangethroat darter		X
	Perca flavescens	Yellow perch		X
	Percina caprodes	Common logperch	X	X
	Percina macrolepida	Bigscale logperch	X	X
	Percina sciera	Dusky darter	X	X
Pimelodidae	*Brachyplatystoma vaillantii*	Laulao catfish	X	
Poeciliidae	*Gambusia affinis*	Western mosquitofish	X	X
	Poecilia formosa	Amazon molly	X	X
	Poecilia latipinna	Sailfin molly	X	X
Sciaenidae	*Aplodinotus grunniens*	Freshwater drum	X	X
Xenocyprididae	*Ctenopharyngodon idella*	Grass carp		X

APPENDIX B:

SPECIES DETECTION (12S VS 16S)

Table 7: *Species that were detected with either the 12S primer or the 16S primer for eDNA samples.*

Family	Scientific Name	Common Name	12S	16S
Amiidae	*Amia calva*	Bowfin	X	X
Aphredoderidae	*Aphredoderus sayanus*	Pirate perch	X	
Atherinopsidae	*Labidesthes sicculus*	Brook silverside		X
	Menidia beryllina	Inland silverside	X	X
Catostomidae	*Carpiodes carpio*	River carpsucker	X	X
	Cycleptus elongatus	Blue sucker	X	X
	Erimyzon oblongus	Creek chubsucker	X	
	Erimyzon sucetta	Lake chubsucker	X	
	Ictiobus cyprinellus	Bigmouth buffalo	X	X
	Minytrema melanops	Spotted sucker	X	X
	Moxostoma congestum	Gray redhorse	X	X
	Moxostoma erythrurum	Golden redhorse	X	
	Moxostoma poecilurum	Blacktail redhorse	X	X
Centrarchidae	*Lepomis auritus*	Redbreast sunfish	X	X
	Lepomis cyanellus	Green sunfish	X	X
	Lepomis gulosus	Warmouth	X	X
	Lepomis humilis	Orangespotted sunfish	X	X
	Lepomis macrochirus	Bluegill	X	X
	Lepomis marginatus	Dollar sunfish		X
	Lepomis megalotis	Longear sunfish	X	X
	Lepomis microlophus	Redear sunfish		X
	Lepomis miniatus	Redspotted sunfish	X	X
	Lepomis symmetricus	Bantam sunfish		X
	Micropterus dolomieu	Smallmouth bass	X	X
	Micropterus punctulatus	Spotted bass		X
	Micropterus salmoides	Largemouth bass	X	X
Characidae	*Astyanax mexicanus*	Mexican tetra	X	
Cichlidae	*Herichthys cyanoguttatus*	Rio Grande cichlid	X	X
	Oreochromis	Tilapia	X	X
	Oreochromis aureus	Blue Tilapia		X
Cyprinidae	*Campostoma anomalum*	Central stoneroller		X
	Cyprinella lutrensis	Red shiner	X	X
	Cyprinella venusta	Blacktail shiner		X
	Cyprinus carpio	Common carp	X	X

Family	Scientific Name	Common Name	12S	16S
	Lythrurus fumeus	Ribbon shiner		X
	Notropis amabilis	Texas shiner		X
	Notropis blennius	River shiner		X
	Notropis potteri	Chub shiner		X
	Notropis sabinae	Sabine shiner		X
	Pimephales vigilax	Bullhead minnow		X
Cyprinodontidae	*Cyprinodon variegatus*	Sheepshead minnow	X	
Dorosomatidae	*Dorosoma cepedianum*	Gizzard shad	X	X
	Dorosoma petenense	Threadfin shad	X	X
Elassomatidae	*Elassoma zonatum*	Banded pygmy sunfish	X	
Esocidae	*Esox americanus*	Redfin pickerel		X
	Esox niger	Chain pickerel	X	X
Fundulidae	*Fundulus grandis*	Gulf killifish	X	X
	Fundulus notatus	Blackstripe topminnow	X	X
	Fundulus olivaceus	Blackspotted topminnow	X	X
	Fundulus zebrinus	Plains killifish		X
Gobiidae	*Gobiosoma bosc*	Naked goby	X	X
	Neogobius melanostomus	Round goby		X
Ictaluridae	*Ameiurus melas*	Black bullhead	X	
	Ameiurus natalis	Yellow bullhead	X	X
	Ameiurus nebulosus	Brown bullhead		X
	Ictalurus furcatus	Blue catfish	X	X
	Ictalurus punctatus	Channel catfish	X	X
	Noturus gyrinus	Tadpole madtom	X	X
	Noturus taylori	Caddo madtom	X	
	Pylodictis olivaris	Flathead catfish	X	X
Lepisosteidae	*Atractosteus spatula*	Alligator gar	X	X
	Lepisosteus oculatus	Spotted gar	X	X
	Lepisosteus osseus	Longnose gar		X
	Lepisosteus platostomus	Shortnose gar	X	
Leuciscidae	*Notemigonus crysoleucas*	Golden shiner		X
Loricariidae	*Pterygoplichthys anisitsi*	Southern sailfin catfish		X
	Pterygoplichthys pardalis	Amazon sailfin catfish	X	
Moronidae	*Morone chrysops*	White bass	X	
Mugilidae	*Mugil cephalus*	Striped mullet	X	X
	Mugil curema	White mullet	X	
	Mugil liza	Lebranche mullet		X
Percidae	*Etheostoma asprigene*	Mud darter	X	X
	Etheostoma chlorosoma	Bluntnose darter	X	

Family	Scientific Name	Common Name	12S	16S
	Etheostoma fonticola	Fountain darter		X
	Etheostoma gracile	Slough darter	X	X
	Etheostoma lepidum	Greenthroat darter	X	
	Etheostoma proeliare	Cypress darter	X	
	Etheostoma radiosum	Orangebelly darter	X	X
	Etheostoma spectabile	Orangethroat darter	X	X
	Perca flavescens	Yellow perch	X	
	Percina caprodes	Common logperch	X	
	Percina macrolepida	Bigscale logperch	X	X
	Percina sciera	Dusky darter	X	X
Pimelodidae	*Brachyplatystoma vaillantii*	Laulao catfish	X	
Poeciliidae	*Gambusia affinis*	Western mosquitofish	X	X
	Poecilia formosa	Amazon molly	X	X
	Poecilia latipinna	Sailfin molly	X	X
Sciaenidae	*Aplodinotus grunniens*	Freshwater drum	X	X
Xenocyprididae	*Ctenopharyngodon idella*	Grass carp		X

APPENDIX C:

SPECIES DETECTION (EDNA VS ELECTROSHOCK)

Table 8: *Species that were detected with either eDNA (any sample) or electroshocking.*

Family	Scientific Name	Common Name	eDNA	Eshock
Achiridae	*Trinectes maculatus*	Hogchoker		X
Acipenseridae	*Scaphirhynchus platorynchus*	Shovelnose sturgeon		X
Amiidae	*Amia calva*	Bowfin	X	X
Aphredoderidae	*Aphredoderus sayanus*	Pirate perch	X	X
Atherinopsidae	*Labidesthes sicculus*	Brook silverside	X	X
	Menidia beryllina	Inland silverside	X	X
Catostomidae	*Carpiodes carpio*	River carpsucker	X	X
	Cycleptus elongatus	Blue sucker	X	X
	Erimyzon claviformis	Western creek chubsucker		X
	Erimyzon oblongus	Creek chubsucker	X	
	Erimyzon sucetta	Lake chubsucker	X	
	Ictiobus bubalus	Smallmouth buffalo		X
	Ictiobus cyprinellus	Bigmouth buffalo	X	
	Minytrema melanops	Spotted sucker	X	X
	Moxostoma congestum	Gray redhorse	X	X
	Moxostoma erythrurum	Golden redhorse	X	
	Moxostoma poecilurum	Blacktail redhorse	X	X
Centrarchidae	*Centrarchus macropterus*	Flier		X
	Lepomis auritus	Redbreast sunfish	X	X
	Lepomis cyanellus	Green sunfish	X	X
	Lepomis gulosus	Warmouth	X	X
	Lepomis humilis	Orangespotted sunfish	X	X
	Lepomis macrochirus	Bluegill	X	X
	Lepomis marginatus	Dollar sunfish	X	X
	Lepomis megalotis	Longear sunfish	X	X
	Lepomis microlophus	Redear sunfish	X	X
	Lepomis miniatus	Redspotted sunfish	X	X
	Lepomis symmetricus	Bantam sunfish	X	X
	Micropterus dolomieu	Smallmouth bass	X	
	Micropterus punctulatus	Spotted bass	X	X
	Micropterus salmoides	Largemouth bass	X	X
	Micropterus treculi	Guadalupe bass		X

Family	Scientific Name	Common Name	eDNA	Eshock
	Pomoxis annularis	White crappie		X
	Pomoxis nigromaculatus	Black crappie		X
Characidae	*Astyanax mexicanus*	Mexican tetra	X	
Cichlidae	*Herichthys cyanoguttatus*	Rio Grande cichlid	X	X
	Oreochromis	Tilapia	X	
	Oreochromis aureus	Blue Tilapia	X	X
Cyprinidae	*Campostoma anomalum*	Central stoneroller	X	X
	Cyprinella lepida	Plateau shiner		X
	Cyprinella lutrensis	Red shiner	X	X
	Cyprinella venusta	Blacktail shiner	X	X
	Cyprinus carpio	Common carp	X	X
	Hybopsis amnis	Pallid shiner		X
	Lythrurus fumeus	Ribbon shiner	X	X
	Lythrurus umbratilis	Redfin shiner		X
	Macrhybopsis hyostoma	Shoal chub		X
	Macrhybopsis storeriana	Silver chub		X
	Notropis amabilis	Texas shiner	X	X
	Notropis atherinoides	Emerald shiner		X
	Notropis atrocaudalis	Blackspot shiner		X
	Notropis blennius	River shiner	X	
	Notropis potteri	Chub shiner	X	X
	Notropis sabinae	Sabine shiner	X	X
	Notropis stramineus	Sand shiner		X
	Notropis texanus	Weed shiner		X
	Notropis volucellus	Mimic shiner		X
	Phenacobius mirabilis	Suckermouth minnow		X
	Pimephales vigilax	Bullhead minnow	X	X
	Semotilus atromaculatus	Creek chub		X
Cyprinodontidae	*Cyprinodon variegatus*	Sheepshead minnow	X	
Dorosomatidae	*Dorosoma cepedianum*	Gizzard shad	X	X
	Dorosoma petenense	Threadfin shad	X	X
Elassomatidae	*Elassoma zonatum*	Banded pygmy sunfish	X	X
Esocidae	*Esox americanus*	Redfin pickerel	X	X
	Esox niger	Chain pickerel	X	
Fundulidae	*Fundulus grandis*	Gulf killifish	X	
	Fundulus notatus	Blackstripe topminnow	X	X
	Fundulus olivaceus	Blackspotted topminnow	X	X
	Fundulus zebrinus	Plains killifish	X	
Gobiidae	*Gobiosoma bosc*	Naked goby	X	X

Family	Scientific Name	Common Name	eDNA	Eshock
	Neogobius melanostomus	Round goby	X	
Ictaluridae	*Ameiurus melas*	Black bullhead	X	
	Ameiurus natalis	Yellow bullhead	X	X
	Ameiurus nebulosus	Brown bullhead	X	
	Ictalurus furcatus	Blue catfish	X	X
Ictaluridae	*Ictalurus punctatus*	Channel catfish	X	X
	Noturus gyrinus	Tadpole madtom	X	X
	Noturus nocturnus	Freckled madtom		X
	Noturus taylori	Caddo madtom	X	
	Pylodictis olivaris	Flathead catfish	X	X
Lepisosteidae	*Atractosteus spatula*	Alligator gar	X	
	Lepisosteus oculatus	Spotted gar	X	X
	Lepisosteus osseus	Longnose gar	X	X
	Lepisosteus platostomus	Shortnose gar	X	X
Leuciscidae	*Dionda nigrotaeniata*	Guadalupe roundnose minnow		X
	Dionda serena	Nueces roundnose minnow		X
	Notemigonus crysoleucas	Golden shiner	X	
	Opsopoeodus emiliae	Pugnose minnow		X
Loricariidae	*Pterygoplichthys anisitsi*	Southern sailfin catfish	X	
	Pterygoplichthys pardalis	Amazon sailfin catfish	X	
Moronidae	*Morone chrysops*	White bass	X	X
Mugilidae	*Mugil cephalus*	Striped mullet	X	X
	Mugil curema	White mullet	X	
	Mugil liza	Lebranche mullet	X	
Percidae	*Ammocrypta vivax*	Scaly sand darter		X
	Etheostoma artesiae	Redspot darter		X
	Etheostoma asprigene	Mud darter	X	X
	Etheostoma chlorosoma	Bluntnose darter	X	X
	Etheostoma fonticola	Fountain darter	X	
	Etheostoma gracile	Slough darter	X	X
	Etheostoma histrio	Harlequin darter		X
	Etheostoma lepidum	Greenthroat darter	X	X
	Etheostoma proeliare	Cypress darter	X	X
	Etheostoma radiosum	Orangebelly darter	X	
	Etheostoma spectabile	Orangethroat darter	X	
	Perca flavescens	Yellow perch	X	
	Percina caprodes	Common logperch	X	
	Percina caprodes	Logperch		X
	Percina carbonaria	Texas logperch		X

Family	Scientific Name	Common Name	eDNA	Eshock
	Percina macrolepida	Bigscale logperch	X	X
	Percina maculata	Blackside darter		X
	Percina sciera	Dusky darter	X	X
Pimelodidae	*Brachyplatystoma vaillantii*	Laulao catfish	X	
Poeciliidae	*Gambusia affinis*	Western mosquitofish	X	X
	Poecilia formosa	Amazon molly	X	X
	Poecilia latipinna	Sailfin molly	X	X
Sciaenidae	*Aplodinotus grunniens*	Freshwater drum	X	X
Xenocyprididae	*Ctenopharyngodon idella*	Grass carp	X	

APPENDIX D:

PEARSON CORRELATION FROM EDNA VS ELECTROSHOCKING

COMMUNITIES (OVER 0.40)

Table 9: *Species with a Pearson Correlation over 0.40 for eDNA vs. Electroshocking and the ordination.*

Family	**Species**	**Common Name**	**MDS1**	**MDS2**
Aphredoderidae	*Aphredoderus sayanus*	Pirate perch	**0.44059**	**0.46852**
Catostomidae	*Moxostoma poecilurum*	Blacktail redhorse	0.022847	**0.44706**
Centrarchidae	*Lepomis marginatus*	Dollar sunfish	-0.13978	**0.43616**
Centrarchidae	*Lepomis miniatus*	Redspotted sunfish	0.263241	**0.47758**
Centrarchidae	*Micropterus salmoides*	Largemouth bass	**0.54848**	-0.15604
Centrarchidae	*Micropterus treculi*	Guadalupe bass	0.124335	**-0.474**
Cichlidae	*Herichthys cyanoguttatus*	Rio Grande cichlid	0.107003	**-0.5816**
Cyprinidae	*Cyprinella lutrensis*	Red shiner	**-0.4987**	0.07207
Cyprinidae	*Lythrurus fumeus*	Ribbon shiner	0.31075	**0.40229**
Cyprinidae	*Notropis amabilis*	Texas shiner	0.111004	**-0.4346**
Dorosomatidae	*Dorosoma cepedianum*	Gizzard shad	**-0.57**	0.198576
Dorosomatidae	*Dorosoma petenense*	Threadfin shad	**-0.6631**	0.060389
Fundulidae	*Fundulus olivaceus*	Blackspotted topminnow	0.304143	**0.45479**
Ictaluridae	*Ameiurus natalis*	Yellow bullhead	**0.51583**	0.189741
Ictaluridae	*Ictalurus furcatus*	Blue catfish	**-0.5272**	-0.22006
Lepisosteidae	*Lepisosteus platostomus*	Shortnose gar	**-0.4115**	0.266601

APPENDIX E:

SITES SAMPLED THAT DID NOT YIELD ANY EDNA DETECTIONS

Table 10: *Sites that did not yield any eDNA data.*

Site ID	Site Name	County	River Basin[s]	Category
10001_1	Martin Creek	Panola	Sabine	Small Stream
10031_1	Brazos River	Stonewall	Brazos	River
10164_1	Arroyo Colorado	Cameron	Nueces-Rio Grande	Large Stream

APPENDIX F:

SITES SAMPLED THAT YIELDED PARTIAL RESULTS FOR EDNA DETECTIONS

Table 11: *Sites that yielded partial results from eDNA samples and what samples had results for each site (marked with X). Category: R = River, LS = Large Stream, SS = Small Stream.*

Site ID	Site Name	River Basin[s]	Category	FIL		COM	
				12S	16S	12S	16S
10022_1	Nueces River	Nueces	R			X	
10015_1	North Wichita River	Red	LS			X	
10021_1	Guadalupe River	Guadalupe	R	X	X		X
10024_1	San Saba River	Colorado	R				X
10026_1	Greens Bayou	San Jacinto	R			X	
10050_1	South Twin River	Trinity	LS			X	X
10059_1	Red River	Red	R			X	X
10167_1	Green Pond Gully	Neches-Trinity	LS	X	X	X	
10306_1	Ioni Creek	Neches	R		X	X	X
00020_1	Clear Creek	San Jacinto-Brazos	SS	X	X		