Molecular characterization of the gut contents of Speckled trout in the Pontchartrain and Barataria River Basins, Louisiana.

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By

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Abstract:

Recent advancements in molecular techniques, like DNA metabarcoding, allow for a more accurate understanding of fish diets. Speckled trout (*Cynoscion nebulosus*) (n=54) were collected from the Pontchartrain and Barataria River Basins (Pontchartrain, n = 22, Barataria, n = 32). Tissue was collected from the stomachs of Speckled trout and was stored in 1.5 mL microcentrifuge tubes with 90% ethanol. DNA was extracted using DNeasy Blood and Tissue kits (Qiagen®, Hilden, Germany). DNA was PCR amplified using MiFish primers for the 12S rRNA gene. Mifish primers are specific for the identification of all fish species. Results revealed the presence of prey species from 12 families. Notably, four samples of Speckled trout contained species from the Clupidae family, including Gulf Menhaden (Brevoortia patronus). This analysis provides valuable insights into the diet diversity of Speckled trout , aiding in understanding predator-prey selectivity for management with the Louisiana Wildlife and Fisheries.

Introduction:

At the beginning of 2023, Speckled trout or (Cynoscion nebulosus) reached its lowest recorded population levels. Officials from the Louisiana Department of Wildlife and Fisheries presented significant data to the Senate and House natural resources committee showing that Speckled trout has fallen 57% below its mean level (Muller 2023). State fishing regulations allow anglers to catch 25 Speckled trout that are 12 inches long a day. Studies show that 83% of the Speckled trout caught by anglers are females because they develop faster than males (Muller 2023). Only 6% of females are spawning in Louisiana. Still, other states surrounding the Gulf of Mexico see up to 20% of female Speckled trout. Allowing the female Speckled trout to grow to 13.5 inches would allow survival to spawn. Speckled trout are known to have a 10% mortality rate after being caught by anglers. Researchers know that the Speckled trout population cannot sustain the number of removals (Muller 2023).

To continue the recreational and economic benefits of Speckled trout hunting, there has to be a plan set for the future. To protect high-quality fish habitats, improving and reconnecting stream systems and degraded mainstreams. During drought and flood, helping restore the delivery and storage of water supplies. Being competent in our restoration efforts, aiming to protect ecosystems composed of native species. Ensuring energy resources are being used efficiently will ensure that the energy development does not affect high-impact fish (State of the Trout, 2020). An increase in natural gas and oil has impacted renewable energy development. A significant effect is to increase a learner plan for the angler's view on restoration efforts so that they can be mindful of the significance of monitoring the policies that affect fishery resources.

Coastal Louisiana is divided into five Louisiana Department of Wildlife and Fisheries coastal study areas (CSA). CSA 1 includes the Mississippi state line to the south pass of the Mississippi River. CSA 3 consists of the south pass of the Mississippi River to Bayou Lafourche. CSA 5 includes Bayou Lafourche to the eastern shore to the Atchafalaya Bay. CSA 6 is the east shore of the Atchafalaya Bay to the western shore of the Freshwater Bayou canal. CSA 7 western shore of freshwater bayou canal to Texas to the state line (*Updated Assessment of Spotted Seatrout*). LDWF conducts routine, standardized sampling within each CSA as a monitoring program that collects the life history information and measures the size distribution of significant species (*Update assessment of spotted seatrout*). The five Gulf states regularly assess the spotted seatrout stock within their state waters. The sampling methods and coverage vary upon inconsistent data sets.

Studies have shown that environmental DNA (eDNA) analysis is rapidly evolving as a tool for monitoring the distributions of aquatic species. The detection of species' populations in streams is challenging due to persistent times for the intact DNA fragments is unknown (Baldigo et al 2016). The analysis of food webs is more significant when determining functional components of the ecosystem. Fish gut content of Speckled trout fish is used to determine prey selectivity of fish in the Gulf. A study compared three methods of rainbow trout gut contents using traditional morphology of prey items, genetic sequencing of individual prey items, and next-generation sequencing of homogenized gut contents. The prey analysis of invertebrates by morphological identification allowed for order-level classification and produced mass data (Baldigo et al 2016). Study shows that sequencing individual specimens provides a greater taxonomic resolution and that next-generation sequencing of stomach content revealed more

prey diversity in the diets (Baldigo et al 2016). The study showed and demonstrated an efficient way for prey analysis as well as molecular techniques that complement traditional taxonomy.

This study is pursued so that the Speckled trout population remains strong around the states of the Gulf of Mexico. Since Speckled trout has reached its lowest population numbers, scientists are trying to find better ways to preserve it while allowing anglers to still hunt. The Louisiana Department of Wildlife and Fisheries proposal reduced the limit of 25 to 15 fish and increased the size limit to 13.5 inches. The Louisiana Department of Wildlife and Fisheries surveyed 8000 people to understand opinions of the new amount of fish to catch a day and the size, and they have yet to figure out the best solution.

Speckled trout are the most hunted fish across the Gulf, so they are easily tracked. Fishing reduces the Speckled trout population, and when recruitment is low, it makes it even more inadequate. The limit of Speckled trout hunted has been declining yearly since scientists have noticed the population decrease. Texas Parks and Wildlife monitors the health of species every year until the population is consistent.

This topic specifically will be focusing on the Pontchartrain and barataria river basins. With a total of 54 samples, n=22 from Pontchartrain river basin and n=32 from Baratria river basin. Looking at the gut contents of Speckled trout is significant to understand their usual diet. Speckled trout as a whole eat small fish as well as small crustaceans as crab and shrimp. Speckled trout diet changes with life cycle when they are young they eat more small fish and shrimp. Older Speckled trout eat larger fish and shrimp. Speckled trout as a whole favorite prey is the Mullet, Pinfish, and Atlantic croaker. Understanding gut contents is significant to predator-prey relationships as well as what Speckled trout eat and prefer.

Materials/Tools/Supplies:

Pipette, sieve, big microfuge tube, small microfuge tubes, medium microfuge tubes, Buffer Al (cell lysis buffer), Ethanol, vortex machine, Centrifuge, Buffer AW1, DI water, Buffer AW2, Incubator, Buffer AE, Manifest file, Mdat file, Qiime2,

Methodology:

First, start with dissections of the Speckled trout, Red Drum, Atlantic croaker, Spanish Mackerel, Sand trout, Bull shark, Hardhead Catfish, Southern Kingfish, Gafftopsail Catfish, and Norfolk spot. Fish that were caught from the different CSA 1, CSA2, CSA 3, CSA4, and CSA 5 were thawed out throughout the day and were ready for dissections at 2 pm. Collect data on the fish length in mm, gender, what was found in the stomach, the date, and which CSA they came from. Collect data on every fish so that they can distinguish it from every other fish that was also dissected. After collecting data, cut open the fish to cut the stomach line out. Between cutting the fish from the anus to the gaw it is necessary to change gloves before cutting open the stomach line. Bleach and spray DI water on the tools that are being used before and after cutting the fish and the stomach line to prevent contamination. Then proceed to cut open the stomach line of the fish. If the fish has shrimp, other fish, or crabs; collect the specimen and put it in labeled tubes. If the fish has no prey in it, swab the stomach lining and collect the fecal matter. After collecting gut contents, add EtOH (ethanol) to every tube to preserve the specimen's DNA.

After collecting the samples perform DNA extraction. DNA extraction means purifying DNA by using physical or chemical methods from a sample separating DNA from cell membranes, proteins, and other cellular components. Pour out the samples that were collected one by one into a glass bowl. Then DI water was sprayed on the sample to get all the DNA off of it into the bowl. Continue to homogenize the mixture by pipetting the DI water mixed with DNA in and out of the bowl continuously. A sample of 1000 ul was collected from the mixture labeled

based on the fish given number and transferred into a microcentrifuge tube. Continue DNA extractions until you have up to 20 samples prepped, then centrifuged at max speed for 60 seconds and poured off the supernatant. The microcentrifuge tubes were placed into the centrifuge at 37 °C, with the lids off, to dry the pellet. Dry the sample for 24 hours because the protocol of drying it for 3 minutes wasn't effective. The dried pellets served as the "tissue sample" for DNeasy Blood & Tissue extractions.

The next step is binding the DNA reagents mixtures. These mixtures were pipetted into a mini spin column that had a filter that was able to capture the DNA and were placed into the 2 mL collection tubes. The tubes were then placed into the centrifuge and spun for 1 minute at ≥ 6000 x g (8000 rpm). The collection tube as well as the flow through was discarded and the spin column was placed into new collection tubes.

The next step is washing, you pipette 500 ul of Buffer AW1 into each spin column because it works with clearing any other biomolecular material off of the DNA. Then the tubes were centrifuged for 1 minute at ≥ 6000 x g (8000 rpm). The flow through was collected, the tubes were discarded and the spin columns were placed in new collection tubes. Next, we pipette 500 ul of AW2 into each sample and were centrifuged for 3 minutes at 20000 x g (14,000rpm). The collection tubes were removed and the spin columns

were placed in 2 mL microcentrifuge tubes. The next step is Elution, 200 uL of Buffer AE was added to each of the spin columns. Buffer AE works by allowing the DNA to flow through the spin column into the microcentrifuge tube. Incubate the DNA at room temperature for 1 minute and then centrifuged it for 1 minute at ≥ 6000 x g (8000 rpm). All the spin columns were discarded. Finally, the microcentrifuge tubes were stored in the −20°C freezer ready for library preparations.

Library preparation is the first step of next-generation sequencing. Before any DNA can be sequenced, the nucleic acids must be isolated, fragmented, end-repaired, and covalently linked to adapters using ligation or tagmentation methods. Next-generation sequencing allows for the samples of the DNA to maximize complexity. Our samples are sent off to be sequenced using Next-generation sequencing *on the illumina HiSeq*®. DNA extraction samples for PCR were prepared using the 96 well plates. A total of 8 library preparations will be analyzed at a time with 8 wells per column on the 96-well plate.

Project	Name	Primer	Bases
REKN/RESTORE	MiFish-U-F-PCR1_TS	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTCGGTAAAACTCGTGCCAGC	54
	MiFish-U-R-PCR1_TS	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCATAGTGGGGTATCTAATCCCAGTTTG	61

REKN/RESTORE	MiFish-U-F-PCR1_NX	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTCGGTAAAACTCGTCCAGC	54
	MiFish-U-R-PCR1-NX	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCATAGTGGGGTATCTAATCCCAGTTTG	61

Table 1: Mifish primers for PCR

DNA amplification is known to produce multiple copies of DNA. The goal of PCR is to make a lot of the target DNA so that the region that is being analyzed can be amplified. After amplification, the product can be seen by using agarose gel electrophoresis with ethidium bromide. Agarose gel electrophoresis is efficient in separating nucleic acids to detect a target fragment. Gel is specifically used to make sure there is DNA present in the samples.

Library prep protocol: The first step is to make a master mix of -5x Platinum SuperFi Buffer(5ul), 10mM dNTPs(0.5 ul), 2.5 uM F primer(1.25 ul), 2.5 uM R primer (1.25 ul), Platinum SuperFi DNA polymerase (.25 ul), and Nuclease Free Water (14.75 ul) for each sample. Then add 23 ul of the master mix to 0.2 mL polypropylene PCR tubes. DNA at 1-10 ng/ul is added and then run in the thermocycler. MiFish Primer Thermocycler Conditions: 94C/3 mins (1 cycle) followed by 94C/30s, 55C/30s, 72C/30s (35 cycles), followed by 72C/7 mins (1 cycle), held at 4C.

Reagent	Per reaction volume	65 reactions	Final Concentration
5x Platinum SuperFi Buffer	5 ul	325 ul	1 x

10 mM dNTPs	0.5 ul	32.5 ul	0.2 mM each
2.5 uM F primer	1.25 ul	81.25 ul	0.125 uM
2.5 uM R primer	1.25ul	81.25 ul	0.125 uM
Platinum SuperFi DNA polymerase	0.2 ul	16.25 ul	0.02 ul
Nuclease free water	14.75 ul	955.50 ul	

Table 2: Conversion Factors/ Master mix Final Concentration

PCR prep protocol:

1. The first PCR was carried out with 35 cycles of a 12 µl reaction volume containing 6.0 µl 2 × KAPA HiFi HotStart ReadyMix (including DNA polymerase, reaction buffer, dNTPs and MgCl2 (at a final concentration of 2.5 mM) (KAPA Biosystems, Wilmington, MA, USA), 0.7 µl of each primer (5 µM), 2.6 µl sterile distilled H2O and 2.0 µl template.
2. When the first PCR was multiplexed (simultaneous use of multiple primer pairs), the final concentration of each primer was 0.3 µM and sterile distilled H2O was added up to the total reaction volume of 12.0 µl.
3. The thermal cycle profile after an initial 3 min denaturation at 95◦C was as follows: denaturation at 98◦C for 20 s; annealing at 65◦C for 15 s; and extension at 72◦C for 15 s with the final extension at the same temperature for 5 min.
4. The second-round PCR (second PCR; figure 2) used the first PCR products as a template and amplified the region using primers (5-AATGATACGGCGACCACCGAGATCTACAXXXXXXXXACACTCTTTCCC

TACACGACGCTCTTCCGATCT-3) (forward) and (5-CAAGCAGAAGACGGCATACGAGATXXXXXXXXGTGACTGGAGTTCAGACGTGTCTCTTCCGATCT-3) (reverse).

5. The 5-end sequences are adapters that allow the final product to bind or hybridize to short oligos on the surface of the Illumina flow cell; and the 3-end sequences are priming sit for the MiSeq sequencing.
6. The first PCR product was diluted 10 times using Milli-Q water and used as a template for the second PCR.
7. The second PCR was carried out with 12 cycles of a 12 µl reaction volume containing 6.0 µl 2× KAPA HiFi HotStart ReadyMix, 0.7 µl each primer (5 µM), 3.6 µl sterile distilled H2O and 1.0 µl template.
8. Different combinations of indices (chosen from A/D501–508 for forward primers and A/D701–712 for reverse primers) were used for different templates for a massively parallel sequencing using the MiSeq platform.
9. The thermal cycle profile after an initial 3 min denaturation at 95◦C was as follows:denaturation at 98◦C for 20 s; annealing and extension combined at 72◦C (shuttle PCR) for 15 s with the final extension at the same temperature for 5 min.
10. The indexed second PCR products were pooled in equal volumes and the pooled libraries (total100 µl) were subjected to agarose gel electrophoresis using 2% L03 (Takara).
11. A target size of the libraries (ca 370 bp) was excised from the gel and purified using a MinElute Gel Extraction kit (Qiagen) with an elution volume of 12 µl.
12. The library concentration was estimated using a Qubit dsDNA HS assay kit and a Qubit fluorometer (Life Technologies).

13. Double-stranded DNA concentration of the pooled library was adjusted to 4 nM (assuming 1 bp equals 660 g mol−1) using Milli-Q water and 5 µl of the 4 nM library was denatured with 5 µl of fresh 0.1 N NaOH. Including HT1 buffer (provided by the Illumina MiSeq v2 Reagent kit for 2 × 150 bp PE), the denatured library (10 µl; 2 nM) was diluted to the final concentration of 12 pM for sequencing on the MiSeq platform.

After PCR we did gel electrophoresis, which denatures and shows the stains of DNA visually.

Gel electrophoresis protocol:

First, add 0.4g of the agarose gel and 1X TBE to a flask and microwave for 30 seconds. After heating, you have to shake the flask to make sure the agarose is dissolved appropriately. Once the flask is cool enough to sit on our arm, you add 2 u of ethidium bromide. Then you pour into the gel and allow the gel to cool for 20 minutes. The next step is to load the gel with 1.5 ul of loading dye + 5 ul of PCR product into the wells. Then load 1-2 of the DNA ladder into the first well. Run the electrophoresis with 120 volts for 40 minutes. After the gel finishes, place the gel in the Biorad Chemidoc Imager and check the bands. Lastly, take photos of the results.

To understand the family taxonomy of the samples identified use a bioinformatic microbiome platform called Qiime2 which is a database that allows for an accurate identification. First make a manifest file/mdat.file used by the program Qiime2 to find all of your sequence files and bring them into Qiime2 that has a required sample id (4 letter code and the number with no spaces), absolute-file path (is the exact location where the sequence file can be found), and direction.

Qiime2 Commands used:

Running Qiime2 commands - Qiime plugin action\

--i-inputs foo\ ## input arguments start with --i

--p-parameters bar\ ## parameters start with --p

--m-metadata mdat\ ## metadata options start with --m

--o-outputs out ## and output starts with --o

Qiime tools import\

--type 'SampleData[PairedEndSequencesWithQuality]'\

--input-path manifest.csv\

--output-path demux\

--input-format PairedEndFastqManifestPhred33

The correct extension is automatically added for the output by Qiime.

This next part is used to determine how much we should truncate the reads before the paired ends are joined

Qiime demux summarize\

--i-data demux.qza\

--o-visualization demux

- Through Qiime we will be using the program dada2, to make imperfect We merge multiple runs together after sequenced reads and recover the real sequence composition of the sample that went into the sequencer. dada2. During this process dada2 also merges paired end reads, and checks for chimeric sequences.

Qiime dada2 denoise-paired\

--i-demultiplexed-seqs demux.qza\

--p-trim-left-f 20 --p-trim-left-r 17\

```
        --p-trunc-len-f 295 --p-trunc-len-r 275\
        --p-n-threads 18\
        --o-denoising-stats dns\
        --o-table table\
        --o-representative-sequences rep-seqs
    ## Metadata on denoising
Qiime metadata tabulate\
        --m-input-file dns.qza\
        --o-visualization dns
    ## Unique sequences across all samples
Qiime feature-table tabulate-seqs\
        --i-data rep-seqs.qza\
        --o-visualization rep-seqs
    ## Table of per-sample sequence counts
Qiime feature-table summarize\
        --i-table table.qza\
        --m-sample-metadata-file mdat.tsv\
        --o-visualization table
Qiime feature-classifier classify-consensus-vsearch\
        --i-query rep-seqs.qza\
        --i-reference-reads /home/share/databases/SILVA_databases/silva-138-99-seqs.qza\
        --i-reference-taxonomy /home/share/databases/SILVA_databases/silva-138-99-tax.qza\
        --p-maxaccepts 5 --p-query-cov 0.4\
```

```
        --p-perc-identity 0.7\
        --o-classification taxonomy\
        --p-threads 72
Qiime metadata tabulate\
        --m-input-file taxonomy.qza\
        --o-visualization taxonomy.qzv
Qiime taxa barplot --i-table table.qza\
        --i-taxonomy taxonomy.qza\
        --o-visualization taxa-barplot\
       --m-metadata-file mdat.tsv
```

Taxonomic assignment:

After using Qiime2, it was found that it only relates samples to taxonomic families and not Genus/species. Using Taxonomic assignment to categorize (OTUs) operational taxonomic units which are clustered sequences that are related by groups of closely related species. Use a database called Blast N where we manually go in and blast 10 OTUs at a time. With each OTU there are 3 species and genus identified for one. With a total of 203 OTUs manually blast 10 sets of OTUs 20 times with one set of 3. Then find each OTU on another sequence file and relate them to the 203 OTUs and then you will be able to identify each sample with the sample number and CSA. Pick the top Genus and species for one OTU and put it in an excel file with the common name and separate each OTU by basin. Separating by basin allows you to determine the different species for both and to see which basins have the highest presence of species and the highest presence of species overall. The species that were determined to be the most popular is found in the results Graph 1.

Results:

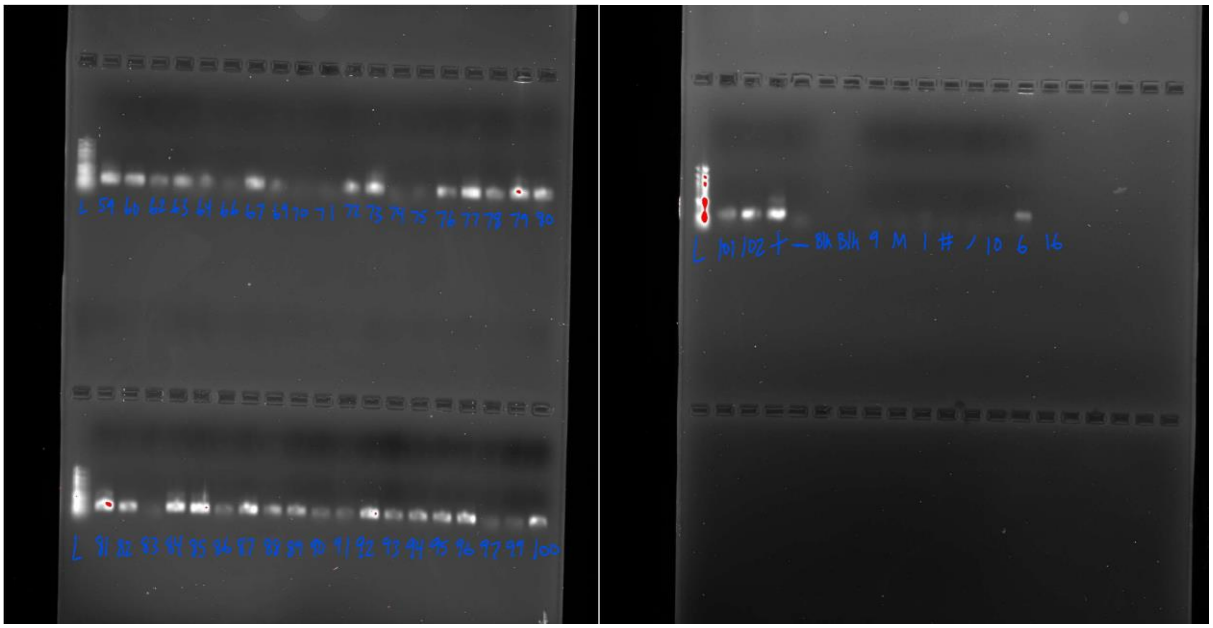
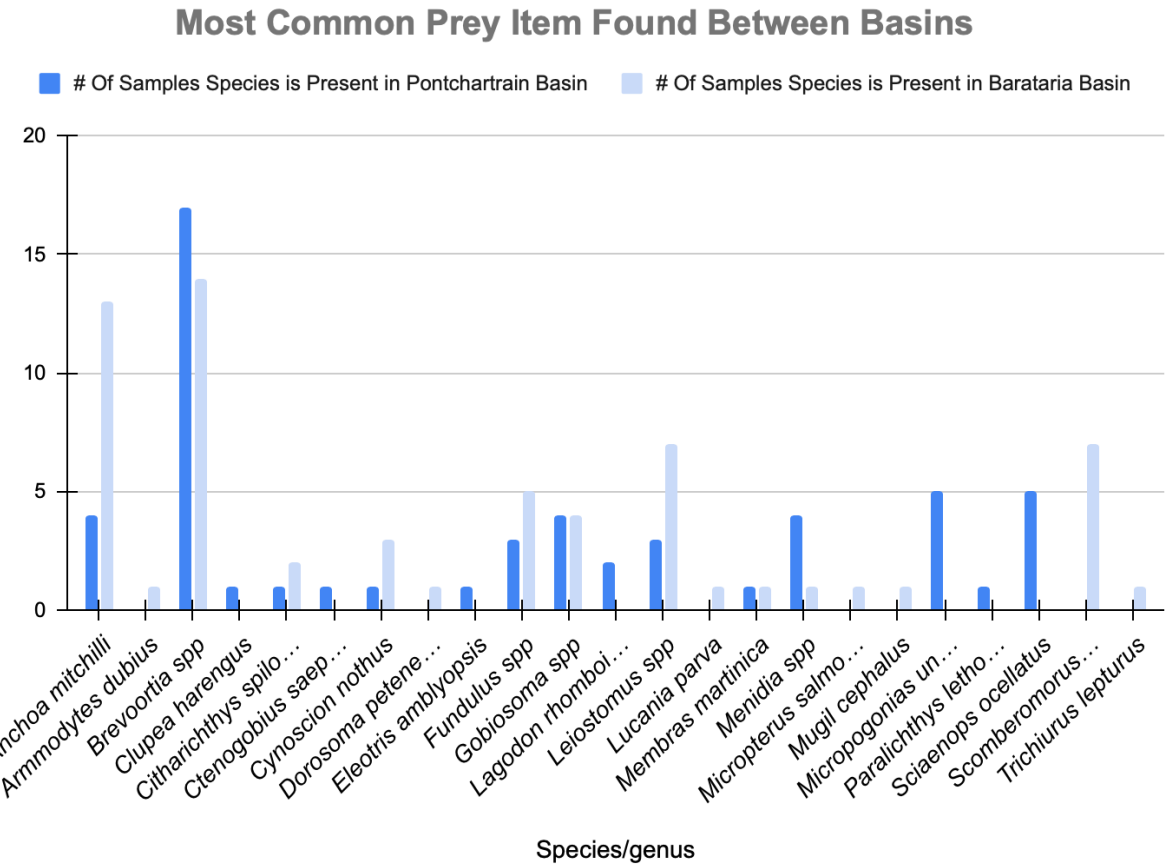
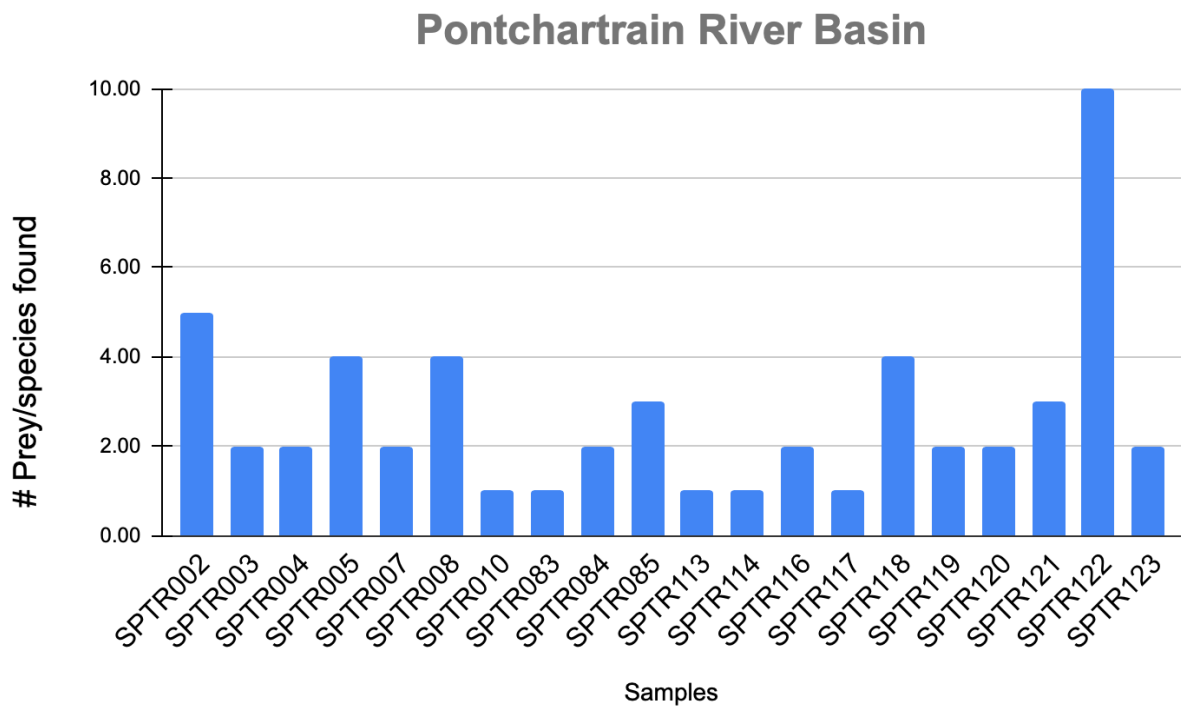


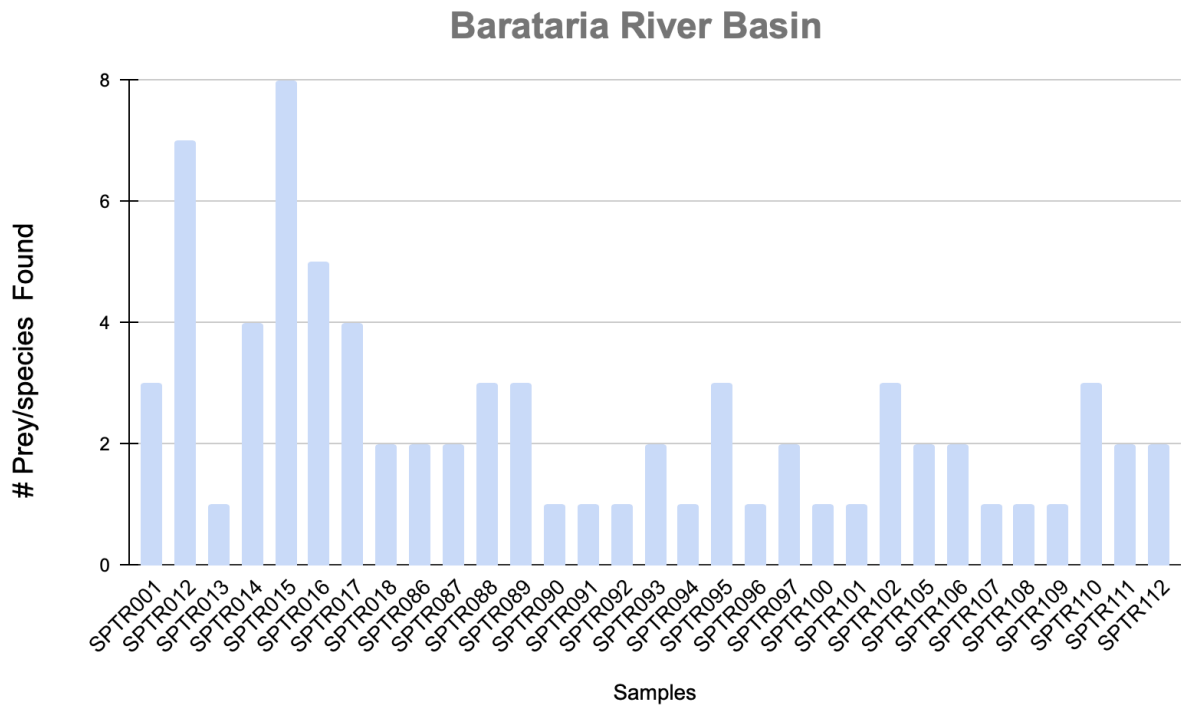
Figure 1&2: Shows gel done after PCR. Gel was done to make sure DNA is present. Darker bands on a gel have more DNA and lighter bands have DNA but just not as much.



Graph 1: Shows most common prey found between basins. There are a total of 22 genus and species identified with 54 samples with *Brevoortia spp* and *Anchoa mitchilli* in Barataria being the most popular. Some species were not present in one basin but were present in the other but it is important when comparing them.



Graph 2: Shows how much prey is found within each sample in the Pontchartrain River. Prey being the different species that's found in a sample. Pontchartrain river basin has a total of 10 different species found in one sample but the rest of the samples are not consistent with that one sample.



Graph 3: Shows how much prey is found in all the samples within the Brarratria river basin. Prey being the different species found within each sample. The Bararia river basin has a total of 8 different species found in one sample and the rest of the samples are consistent with the one sample.

Discussion:

The most common species in the gut contents of fish collected from Lake Pontchartrain and the baratria Basin is *Brevoortia spp*. The Pontchartrain river consists of inflowing water and sediments and the *Brevoortia spp* like all salinities and drift with the currents this species is common in CSA 1. *Brevoortia spp* was also found in 14 fish out of the Bartratria Basin.The *Anchoa mitchilli* is the second most common species found only in the Barataria river basin at a total of 13 samples. This is common because *Anchoa mitchilli* are tolerant of any salinity and temperature and they live in muddy waters. Baratria river basin is south west of New Orleans and is known to not have any inflowing water as well as no sediments besides only inflowing

rain water. Being that the Baratria river basin has no inflowing water it is known to be very muddy so *Anchoa mitchilli* are common to be present in the Baratria river basin. Speckled trout eat a variety of species like crustaceans, shrimp and crabs. All Speckled trout that was dissected had at least 2 different species found in the gut contents with some fish ranging from 8-10 different species identified. Speckled trout with the most identified gut contents were larger in size. The Speckled trout that were smaller in size had less gut contents. This shows the bigger the trout the more they eat and the smaller the trout the less they eat. With a Speckled trout diverse diet it was surprising to see that they were not eating one of their favorite prey, the *mullet*. *Mullet* species were not found in any of the Speckled trout in the barataria or pontchartrain basin. This is possible because Speckled trout do have a diverse diet. Although this paper focused on just the Pontchartrain and Barataria river basins, other research projects are focusing on other basins which could mean Mullet species can be present in other basins. This could mean that different basins have a select amount of species present.

My strengths during my research is being able to find enough time to do everything. During the dissection/tissue preparation and the DNeasy protocol I found that I took hours alone to do all of the processes efficiently. While doing my results I struggled with time. Using Qiime2 only gave us taxonomic families but we wanted to know the genus/species of all my 54 samples. Using taxonomic assignment I was able to manually go into Blast N and blast the genus/species of all my samples. Taxonomic assignment is basically categorizing (OTUs) which are operational taxonomic units that are clustered sequences that are similar. I was able to go in and BLAST 10 OTUs at a time but I also had to separate the OTUs with a common name. It took me 2 hours alone to do 40 OTUs. It took me a total of a week to get all my results written but I also had to separate my results by basins as well as find the common name for each OTUs and I had a

total of 203 OTUs. With me determined to get it all done I spent 10 hours a week working on my results and an extra week working on putting it all together. My strengths are how much time I spend on my research to get it all done in a timely manner.

My weaknesses are during the DNeasy protocol I use to work really fast and finish 20 samples within an hour. This can be a good thing or bad when there can be contamination within our samples. While doing results 3 samples showed that the fish ate Homo sapiens. Speckled trout do not eat humans, it is just contamination when I was doing the Dneasy protocol even though I always wear gloves. Sometimes rushing can cause problems in research so you should always take your time when you are doing research preparation. Another weakness is I always update my research notebook weeks after doing the Dneasy protocols such as Gel or even PCR in the lab. This makes it very hard to remember what I did in the lab and it can cause me to miss things as I am doing my lab notebook. Another weakness is not talking to my mentor enough about what I am doing with my research. This can cause problems with my research like having to do a lot of things over again. Lastly, another weakness that I had was just understanding the background of my research. With this research not being my own it took me a lot of time to understand why we were doing this project in the first place. I did not have the basic information that I was supposed to know and this caused my research to be weak because I didn't know a lot of background information. Without this basic knowledge when people ask me what's the purpose of my project I don't know exactly what I am supposed to say, I just say what I have done research on but not what my mentor professor actually started the project.

If I was to do this research project again I would do a lot of things differently. First when starting our project after dissections and tissue preparations all of the Speckled trout samples were placed together but not in order. It was very hard to just do samples out of order because

the samples were separated between big, small, and medium microfuge tubes. If we sample SPTR122 sometimes there is another small tube that is labeled SPTR122 and all samples are put together as one when doing Dneasy protocol. So if we miss one sample we have to do the whole process over again. Before starting DNA extractions I will have all the samples in order based on small, large, and medium microfuge tubes so that doing Dneasy extractions will be easier for me if everything is ordered. I would also start working and putting my files in Qiime2 earlier so that it doesn't take me 3 weeks to do my results but instead while I'm doing my methods for my paper I can also be working on my results. Lastly, I wouldn't allow myself to get help in the lab, this throws off everything because there is something I can't explain or I do not know because I didn't actually work on it. Working in the research lab with partners can be beneficial but if you are not there with them and making sure they are doing everything right or if they are writing down what they are doing you can also miss significant things that are supposed to be in your lab report.

There are a lot of fish that are surrounding the Gulf of Mexico but knowing in detail what the fish eats can give marine scientists a better understanding of the environmental world beneath the water. Speckled trout are significant to Louisiana; it is the most popular fish here as well as the most hunted. Louisiana's eat Speckled trout and with them being so important to us it is important to understand the food web of Speckled trout and what they contribute to. With Speckled trout also being overfished they are decreasing everyday in population. It is significant to know their food web so they can increase their eating habitat as well as enforcing regulations to which anglers are no longer eating as many Speckled trout as they want but only 15 per day. In conclusion, Speckled trout are not just important to humans because of food but they are also

important to the environment. Speckled trout from the Pontractrian and Baratraia river basin eat mostly the *Brevooria spp.* and this is proven based on graph 1.

Future research will be looking at the prey predator selectivity of more species besides just Speckled trout. We will be able to provide (LDWF) Louisiana Department of Wildlife and Fisheries with scientific backing with Speckled trout and what they prefer because of the lack of information about the food web in the Gulf of Mexico. Usually when you cut a Speckled trout open you get empty stomach contents or possibly fish contents but it's hard to identify what exactly the species is. It is hard to identify taxonomy once food degrades so understanding gut contents is another way to identify what exactly those species are. We found Brevoortia spp and Anchovy prey items but there are a more diverse amount of fishes. Using 18s rRNA tells us the invertebrates of our samples so we can get a complete picture of not just our taxonomy of our samples but also the invertebrates species of our samples. With Speckled trout being one of the most popular saltwater fishes in Louisiana it is significant to researchers to understand their diet in terms of what they are eating and what they prefer.

Manuscript Format:
The manuscript will be completed using MLA format.

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I hereby certify aspects of this thesis document, as well as the work it reports, as my original work.