CHARACTERIZING ELASMOBRANCHS IN PENSACOLA BAY SYSTEM USING ENVIRONMENTAL DNA METABARCODING

by

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THESIS CERTIFICATION

Melissa M. Hebert defended this thesis on 28 September 2020. The members of the thesis committee were:

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The University of West Florida Graduate School verifies the names of the committee members and certifies that the thesis has been approved in accordance with University requirements.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

CHARACTERIZING ELASMOBRANCHS IN PENSACOLA BAY SYSTEM USING ENVIRONMENTAL DNA METABARCODING

Melissa M. Hebert

Estuaries are often used as foraging habitats and nursery grounds by many elasmobranch species due to the protection as well as an abundance of nutrients and available prey that estuaries provide. However, identifying essential habitats for elasmobranchs has been a challenge due to frequent migrations of elasmobranchs into estuaries and coastal waters. Therefore, essential habitats for many elasmobranchs have not been identified. Traditional survey methods make it difficult to obtain accurate results because elasmobranchs are highly mobile; thus the resolution lies with using molecular tools such as environmental DNA (eDNA) metabarcoding. Environmental DNA metabarcoding refers to the identification of multiple species from a single environmental sample using a generalist molecular marker. This molecular tool has shown to represent the biodiversity present in a collected sample and has been more efficient than traditional *in situ* sampling. Environmental DNA metabarcoding revealed 266 total fish detections from 57 different species with only three elasmobranch species (*Rostoraja eglanteria, Hypanus sabinus, Rhinoptera bonasus*) being detected. Elasmobranch DNA was primarily detected in spring, with only one detection in both winter and summer and no detections in fall. These results imply that elasmobranchs may not be utilizing the Pensacola Bay System often or they were not present during time of sampling.

CHAPTER I

INTRODUCTION

Elasmobranch Habitat and Estuary Usage

Elasmobranchs are classified as marine organisms. However, a myriad of elasmobranch species (sharks, skates, and rays) use estuaries as nurseries and foraging habitats because estuaries provide protection, as well as an abundance of nutrients and available prey (Froeschke et al. 2010b; Curtis et al. 2011; Drymon et al. 2014; Ajemian et al. 2016). Elasmobranchs travel into estuarine and/or freshwater environments with some individuals remaining in freshwater for extended periods of time. In particular, females utilize estuarine environments for pupping in spring and summer, and then they depart in the winter (Pang et al. 1977; Hopkins and Cech 2003; Bishop et al. 2016). Abiotic conditions of estuaries fluctuate due to temporal changes and anthropogenic activities; thus for effective conservation management, it is important to understand how elasmobranchs use these habitats in response to environmental changes (Drymon et al. 2014).

Essential habitats are necessary environments for breeding, spawning, feeding, or growth to maturity. Due to increases in the human population, along with coastal urbanization and development, it is important to identify these essential habitats for elasmobranchs, especially for coastal species (Heithaus et al. 2007). For many elasmobranchs, essential habitats have not been identified and environmental conditions that influence selection of these habitats are not well understood. In particular, identification of essential habitats for sharks is problematic due to their frequent migrations into estuaries and coastal waters (Froeschke et al. 2010a). Early studies hypothesized that adult sharks remain offshore and only travel into coastal waters to give birth, while young sharks born in bays and estuaries remain until reaching sexual maturity (Springer

1967). In contrast, recent research revealed that shark occurrence in coastal waters is diverse and that habitat use by sharks is not segregated into different habitats by ontogeny (Bethea et al. 2015). However, there are still cases showing use of estuaries by pregnant females and young sharks. For example, Bishop et al. (2016) found that shallow estuaries are used by pregnant females during warmer months due to thermal physiological advantages. When in marine waters, elasmobranchs maintain an internal salinity that is slightly hyperosmotic to their environment with nitrogenous compounds in their blood (Hopkins and Cech 2003). However, this mechanism requires significant metabolic energy and the balance cannot be maintained in lower salinities (Pang et al. 1977). Abundance of sharks in moderate salinity compared to seawater may be in effort to reduce energetic costs associated with osmoregulation, allowing for increased growth rates (Froeschke et al. 2010a). In dilute seawater, elasmobranchs have decreased blood levels of urea, sodium, and chloride in comparison to when they are in marine waters. However, their body fluids still maintain a higher osmolarity than the surrounding water. This mechanism could indicate a relatively recent occupation into freshwater even though it may be considered an osmotic disadvantage compared to freshwater fishes (Pang et al. 1977).

In 1994, NOAA began the GULFSPAN surveys in the northeastern Gulf of Mexico. The purpose of the GULFSPAN surveys is to help identify essential habitats for juvenile sharks in coastal areas of the Gulf of Mexico (Southeast Fisheries Science Center, 2020). The dynamic, shallow waters of the Northern Gulf of Mexico provide nursery habitat for several shark species and support a diverse shark community (Froeschke et al. 2010b; Hoffmayer et al. 2013). Abundance and distribution of estuarine fishes, including elasmobranchs, are influenced by abiotic factors such as temperature, dissolved oxygen (DO), salinity, and proximity to tidal inlets (Hopkins and Cech 2003; Froeschke et al. 2010a; Bishop et al. 2016; Bangley and Rulifson

2016). Dynamic estuaries of the Northern Gulf of Mexico range from near-oceanic to shallow-brackish and are subject to warm and cold seasonal temperatures as well as wide salinity fluctuations (Bethea et al. 2014; Drymon et al. 2014). In the Gulf of Mexico, most pupping occurs in late spring and early summer, and nurseries can be inhabited by young-of-the-year (YOTY) into fall (Hueter and Tyminski 2007). Ward-Paige et al. (2014) found that six shark species utilize the Northern Gulf of Mexico during early life stages. These species showed distinct habitat preferences which were characterized by physical characteristics such as salinity, temperature, and depth. For example, the Blacktip shark preferred a mid-depth around 5.5 m and temperatures above 30C while the Scalloped hammerhead shark also preferred higher temperatures along with high salinity (>35 PSU) (Ward-Paige et al. 2014). Contrastingly, juvenile Atlantic sharpnose sharks at Crooked Island Sound did not appear to have habitat preferences and were found in all habitat types and would even move through deeper waters, leaving the protected areas (Carlson et al. 2008). However, there are still considerable gaps in knowledge of habitat use and seasonal distribution of elasmobranchs within estuaries, including in the northern Gulf of Mexico (Curtis et al. 2011).

Although essential habitats for elasmobranchs have not been identified, it is known that elasmobranchs travel into estuaries. Bull sharks are one of the few completely euryhaline sharks, often being captured in shallow freshwater creeks and the Mobile and Tensaw Rivers and they are able to remain for extended periods of time (Jensen and Schwartz 1994; Yeiser et al. 2008; Curtis et al. 2011; Drymon et al. 2014). Bethea et al. (2015) found the greatest shark species diversity in areas with the highest salinity fluctuations, such as river mouths. Life-stages such as YOTY, juvenile, and adult, of the indicated shark species were also influenced by geographic area rather than season in the northeastern Gulf of Mexico (Bethea et al. 2015). Bangley and

Rulifson (2017) found that Blacknose sharks were most often found close to inlets in the Black and Core sounds in North Carolina, while Bonnethead sharks have also been observed in freshwater habitats (Jensen and Schwartz 1994). Additionally, Froeschke et al. (2010a) found that salinity, temperature, and river inflow had the greatest influence on habitat use patterns of Bull sharks, Blacktip sharks, and Bonnethead sharks along the Texas Coast. Many sharks avoided hypersaline areas with a salinity greater than 35 PSU, which may reflect the physiological cost of osmoregulation under extreme high salinity (Froeschke et al. 2010a). Furthermore, Scalloped hammerheads are a circumglobal species, occurring in tropical and subtropical waters and are distributed coastally in the northern Gulf of Mexico (Hoffmayer et al. 2013). Specifically, Scalloped hammerheads can tolerate hypoxic waters and large fluctuations in depth and temperature (Jorgensen et al. 2009) but have also been observed in extremely cold waters (5°C).

Dorsoventrally flattened elasmobranchs, such as skates and rays, exhibit similar estuarine distributions as sharks. Specifically, Ajemian and Powers (2016) found that Cownose rays have a higher abundance west of Mobile Bay where salinity is generally lower due to large estuarine outflow compared to regions to the east of the bay, despite poorer water quality along the west. This may be due to increased polychaete worms in this area which are consumed by Cownose rays. Ajemian and Powers (2016) also show ontogenic partitioning of habitat in coastal waters by Cownose rays and an increase in abundance of adult rays in late winter to early spring. Additionally, Yeiser et al. (2008) found Cownose rays and Bonnethead sharks, along with Lemon and Bull sharks, utilize Pine Island Sound, located in lower Charlotte Harbor, Florida, with some staying for several months. Additionally, Lemon sharks present in Pine Island Sound

estuary were all late juveniles, suggesting that this estuary is used as a maturation-specific segregation (Yeiser et al. 2008).

Elasmobranchs play an important role in maintaining a healthy ecosystem (Bakker et al. 2017), and account for a significant portion of the total biomass in a system (Hopkins and Cech 2003). As such, elasmobranchs have direct and indirect predatory impacts throughout the food web (Cortes 1999; Bangley and Rulifson 2016), and larger sharks, such as Bull sharks and Great hammerheads, influence habitat use of smaller species (Heithaus et al. 2007). Sharks are large marine consumers that forage at the top of the food chain (Hopkins and Cech 2003) and influence aquatic communities they inhabit (Cortes 1999). Bottom communities are also influenced by estuarine batoids by aerating sediment, excavating disturbance pits, and stabilizing prey populations (Bishop et al. 2016). Increased studies of elasmobranch habitat use in estuaries is necessary to evaluate their ecological importance in coastal ecosystems and contribute to managing fisheries and coastal development (Bangley and Rulifson 2016).

According to the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (2014), a quarter of the world's elasmobranchs are threatened, with ray species at higher risk than sharks. Only 23% of elasmobranchs are categorized as Least Concern, while the remaining were categorized as near threatened or endangered, making this group of fish one of the highest at-risk groups of animals on Earth. Elasmobranchs are slow growing, have low fecundity, and occur in low densities, making them vulnerable to exploitation (Froeschke et al. 2010a). Larger species of rays and sharks living in shallow waters are most vulnerable, with the greatest threat being overfishing (IUCN 2014). Sharks and rays are often killed as bycatch and are sometimes killed for their fins for shark fin soup (IUCN 2014). Many species of elasmobranch commonly found in the northern Gulf of Mexico are classified as near threatened,

such as the Cownose ray (IUCN 2006), Bull shark, Blacktip shark (IUCN 2009), and the Lemon shark (IUCN 2015), while the Scalloped and Great hammerheads are both classified as critically endangered and are showing trends of decreasing populations (IUCN 2019). Currently, population assessments of many elasmobranch species are lacking with little or no fisheries management plans available to protect them.

Lack of basic knowledge of habitat use for many elasmobranch species is causing a worldwide struggle for scientists and fisheries management. This struggle is in part due to morphologically similar species and inadequate identification of species caught (Holmes et al. 2009). In particular, for large animals, predicting distribution based on habitat characteristics can be difficult and requires data with adequate temporal and spatial coverage (Froeschke et al. 2010a). Elasmobranchs are often large, highly mobile animals, making it difficult to obtain accurate results using traditional survey methods, such as longlines or gill nets. Simpfendorfer et al. (2005) and Heuter and Tyminski (2007) surveyed similar areas, but each found a different species to be most abundant. This difference in species composition could be due to the difference in gear usage for shark capture. For example, Curtis et al. (2011) used gill nets to survey Bull sharks and found a reduced frequency in larger sharks compared to smaller sharks, most likely due to gear bias. Heithaus et al. (2007) also found that gear and bait type affected catch rates for some elasmobranch species, which affected diversity results. Further, gill nets can also be fatal for sharks and may only capture rays if they become entangled by their dorsal spines (Hopkins and Cech 2003). One resolution to gear bias and lack of distribution information lies with using molecular tools such as environmental DNA metabarcoding.

Environmental DNA, Metabarcoding, and Elasmobranchs

Environmental DNA (eDNA) is a trace of cellular or free DNA molecules in the environment from tissue or excreted cells. Organisms continuously leave a trace of DNA as they interact with their environment. Environmental DNA can be extracted from an environmental sample used for detection without direct capture of the target organism. Within the last decade, this process of sampling, extracting, and analyzing eDNA has been a major technological advancement and continues to be successful in monitoring terrestrial and aquatic animals (Taberlet et al. 2012a; Taberlet et al. 2012b; Thomsen et al. 2012; Thomsen and Willerslev 2015; Civade et al. 2016; Gargan et al. 2017).

Environmental DNA dates back to 1987 and was first used to extract microbial DNA from sediments (Taberlet et al. 2012b). The term eDNA was then used by microbiologists in the 2000s, who termed the analysis of eDNA "metagenomics" (Coissac et al. 2012; Taberlet et al. 2012b). An environmental DNA metagenomic approach has since been used to successfully study microbial eukaryotes, and more recently marine benthic and planktonic communities (Creer et al. 2010; Guardiola et al. 2015; Thomsen and Willerslev 2015).

Current methods for obtaining elasmobranch DNA are invasive to the animal and resource intensive (Thomsen et al. 2012; Bakker et al. 2017), while eDNA is cost-effective, non-invasive, and more sensitive than conventional survey methods (Civade et al. 2016; Gargan et al. 2017). Environmental DNA is useful for monitoring and conservation purposes and is being used more widely by ecologists (Thomsen et al. 2012; Civade et al. 2016; Yamamoto et al. 2017). For example, Boussarie et al. (2018) found that eDNA detected 44% more shark species than traditional underwater visual and baited video methods and revealed the presence of previously un-observed species in areas impacted by humans. Moreover, Thomsen et al. (2012) found that

eDNA metabarcoding characterized fish diversity better than nine conventionally used methods for marine fish surveys. Due to degradation of eDNA within hours to weeks depending on the environment, it can provide approximate real-time data on species presence in an environment and is increasingly being used to monitor and detect rare and invasive species in aquatic habitats (Thomsen and Willerslev 2015; Janosik and Johnston 2015; Pfleger et al. 2016; Bakker et al. 2017; Gargan et al. 2017). Thomsen et al. (2012) found that small eDNA fragments consisting of 100-bp degraded within days. However, sea currents and species predators can cause eDNA to be distributed beyond areas where the target species occurs (Thomsen et al. 2012).

Environmental DNA can be used with a species-specific marker to detect a single invasive or endangered species, referred to as DNA barcoding (Civade et al. 2016). This method simplifies the taxonomic aspect of determining species presence in an ecosystem but does not reduce sampling effort (Coissac et al. 2012). DNA barcoding also does not fully satisfy the needs of ecologists because it is designed to detect a single species with DNA that is more or less intact and typically requires isolation of the target organism to be analyzed (Taberlet et al. 2012a). Another approach is eDNA metabarcoding, which refers to the identification of multiple species from a single environmental sample using a generalist molecular marker (Coissac et al. 2012; Taberlet et al. 2012a; Civade et al. 2016). However, due to environmental degradation of eDNA, only small fragments, generally less than 150 bp, of DNA can be amplified. Also, it is important for the employed universal primers to be highly versatile in order to amplify several species in the same polymerase chain reaction (PCR) experiment (Coissac et al. 2012; Taberlet et al. 2012a; Guardiola et al. 2015).

Biodiversity assessment is key to understanding the relationship between biodiversity and ecosystem functioning (Creer et al. 2010). The field of biodiversity assessment has recently been

revolutionized by the use of eDNA metabarcoding and next-generation sequencing (NGS) (Guardiola et al. 2015). DNA metabarcoding provides an opportunity to easily produce large amounts of data on biodiversity (Coissac et al. 2012). Compared to traditional Sanger sequencing, NGS can provide at least five orders of magnitude more sequence reads in a single experiment (Taberlet et al. 2012a). These DNA sequences produced have the potential to represent the biodiversity present in the collected sample (Coissac et al. 2012). Civade et al. (2016) confirmed that eDNA metabarcoding is more efficient than traditional *in situ* sampling when assessing species richness while also providing a more faithful description of local fish biodiversity. Yamamoto et al. (2017) detected 128 fish species after a 6-hour eDNA collection period, compared to the 80 fish species detected by visual census over a 14-year period. Environmental DNA metabarcoding methods will potentially become the standard for surveying fish communities and will enhance marine ecosystem-related research (Yamamoto et al. 2017).

Objectives

The objective of this study was to use eDNA metabarcoding to characterize elasmobranch seasonal usage in the Pensacola Bay System. We expect that the majority of elasmobranch species will be found in Pensacola Bay close to river mouths during late Spring and early Summer, as this is when pupping occurs.

CHAPTER II

METHODS

Study Area

Pensacola Bay, Florida, located in the northern Gulf of Mexico, is a combination of a drowned river and bar-built estuary with the largest amount of freshwater input coming from Escambia River (Hagy et al. 2006). The Pensacola Bay System is the fourth largest estuary in Florida and comprises five subsystems: East Bay, Escambia Bay, Pensacola Bay, Santa Rosa Sound, and Big Lagoon (Lewis et al. 2008).

Sample Locations

Water samples were collected from 22 sites (Figures 1 and 2), with sites starting offshore in the Northern Gulf of Mexico and continuing inshore into Escambia and Blackwater Rivers. Specifically, three sites were located offshore at artificial reefs, three sites were located in Pensacola Bay, three sites were located in East Bay, five sites were located in Blackwater Bay and River, and eight sites were located in Escambia Bay and River. Sites were selected to represent a decreasing salinity gradient moving from the Gulf of Mexico into rivers.

Water Collection

Water samples were collected from each site (n=22) quarterly in Fall 2018 on October 24, 27, November 2, and December 12; in Winter 2019 on March 7, 2, 30, and April 11; Spring 2019 on June 4, 5, and 30; and Summer 2019 on August 29, September 4, 18, and 22. Offshore samples could not be collected in Spring 2019 due to unsafe water conditions. Surface and bottom water samples were collected at each site and dissolved oxygen (DO), salinity, temperature, and pH were tested using a YSI multimeter. Bottom water was collected offshore by diving and inshore using a Van-Dorn sampler. Specifically, at each site, three replicates of 15

mL of water were collected using sterile 15 mL falcon tubes. Samples were preserved in a sterile 50 mL falcon tube containing 1.5 mL of 3M sodium acetate and 33.5 mL of 95% ethanol. A control containing DI water was included for each sampling trip to ensure no contamination occurred during water collection. Samples were stored at room temperature in a dark location until DNA extraction.

eDNA Extraction

Preserved samples were centrifuged for 30 minutes at 3500*g* and at 4°C to precipitate DNA (Ficetola et al. 2008). The supernatant was discarded, and the precipitated pellet was extracted using the DNeasy Blood and Tissue (Qiagen) kit. A generalist elasmobranch primer set was used for amplification of a portion of the 12S mitochondrial rDNA (Taberlet et al. 2018). The primers consisted of an elasmobranch specific forward: "Elas02" 5'-GTTGGTHAATCTCGTGCCAGC-3' and an elasmobranch specific reverse: "Elas02" 5'-CATAGTAGGGTATCTAATCCTAGTTTG-3' (Taberlet et al. 2018), yielding an amplicon of 170-185 basepairs.

PCR Analysis and Library Prep

For PCR amplification, the reaction mixture included the following: a total volume of 20 µl, 10 µl KapaHiFi, 0.8 µl of each forward and reverse 5 mM primer (including Nextera tags), 5.9 µl sterile water, 0.5 µl bovine serum albumin (BSA), and 2 µl DNA template. The PCR profile included an initial denaturing step at 95˚C for 15 minutes, then 35 cycles of 94˚C for 1 minute, 55-60˚C for 1 minute, 72˚C for 1 minute, and a final extension step of 72˚C for 5 minutes (Bakker et al. 2017). A positive skate fin clipping control was included to ensure the PCR reactions were successful. Negative controls were included for each PCR performed to ensure no contamination occurred during the PCR process. The amplicon was then sequenced

using a Nexterra primer tag. Libraries were normalized using the SequalPrep Normalization Plate Kit (Invitrogen, Life Technologies) before pooling samples. Libraries were quantified using qPCR and the KAPA Illumina Library Quantification Kit on a LightCycler Real-Time PCR according to manufacturer guidelines. Libraries were run on an Illumina HiSeq platform at the Hubbard Center for Genomics, Sequencing Core Facility (Durham, NH).

Bioinformatic Analysis

A total of 343,775,480 reads were provided from Illumina HiSeq which were then denoised, duplicated, and chimeras were filtered out using QIIME 2 (Caporaso et al. 2010) and QIIME 2 plugin DADA2 (Callahan et al. 2016), resulting in 787 final reads. Low quality regions of the sequences were removed by trimming reads at zero and truncating at 170. Reads were then assigned to OTUs using QIIME2 and nucleotide BLAST (Johnson et al. 2008). Sequences were assigned to the top BLAST hit species if the top hit was ≥90% and the similarity between queries was ≥30%. The taxonomic assignment was modified if the program made clear incorrect assignments for taxa or due to closely related species being indistinguishable using the 12S rDNA gene. Any sequences that did not hit in the database were discarded.

Elasmobranch Community Analysis

Detected species were grouped based on season (fall, winter, spring, summer) when collected and salinity of the water sample as well as by overall species detection. To test for significance in shark preference of surface or bottom water, a chi-squared analysis was done. Species were grouped based on their International Union for Conservation of Nature (IUCN) status, and species of special interest (endangered, vulnerable, or threatened) were further investigated. A bar plot representing relative abundance of reads for every elasmobranch Molecular Operational Taxonomic Unit (MOTU) detected (Bakker et al. 2017), and pie charts

were made representing species detected in each sampling location (Bethea et al. 2015). Lastly, bar graphs were made representing species and salinity, and species and temperature (season).

CHAPTER III

RESULTS

Environmental DNA metabarcoding revealed 266 total detections from 57 fish species in 31 families (Table 1). Of these fish detections, 61% were brackish, 31% were freshwater, and 8% were marine (Figure 3). The most species were detected in spring, while the least were detected in summer (Figure 4). Tables 2-5 list the abiotic conditions at each sampling location in each season. Environmental DNA metabarcoding detected only three Elasmobranch species, which include the Clearnose skate (*Rostroraja eglanteria*), Atlantic stingray (*Hypanus sabinus*), and American cownose ray (*Rhinoptera bonasus*) (Figure 5). A map of elasmobranch usage in the Pensacola Bay System can be found in figure 6. Overall, DNA from elasmobranchs was primarily detected in spring, with only one detection in both winter and summer and no detections in fall (Figure 7). Specifically, *Rhinoptera bonasus* DNA was only detected once in Bayou Texar in summer. This bottom site location had a temperature of 29.1C and a salinity of 18.31 at the time of sampling (Table 5). *Hypanus sabinus* was detected only in spring in Pensacola Bay, and upper Escambia and East Bays. *Hypanus sabinus* was detected at sites with a wide range in salinity from 1.98-15.52 (Table 4). While the most abundant Elasmobranch was *Rostroraja eglanteria* with a total of seven DNA detections in Bayou Texar, Pensacola Bay, Escambia Bay, and upper East Bay in spring and upper Escambia Bay in winter. Elasmobranchs were mainly detected in locations with a temperature around 30°C with the exception of *R. eglanteria,* which was detected in winter with a temperature of 13.4°C (Table 3). Salinity did not appear to have an effect on elasmobranch detection, with detections in locations with salinities ranging from 1.98-18.31. Elasmobranchs were detected more frequently at bottom sites than surface (Figure 8) (X^2=5.22, df=2, p=0.074).

Figures 9-12 show all aquatic vertebrate detections in each season. Aquatic vertebrates do not appear to have a preference between surface and bottom water (Figure 13) and there also does not appear to be any relationship between species presence and abiotic conditions (Figures 14-20). The Red drum (*Sciaenops ocellatus*) was the only species detected at either offshore location. This species was detected at the USS Catherine in summer. The endangered American eel (*Anguilla rostrata*) was detected once in the Escambia River in summer. The vulnerable Blueback herring (*Alosa aestivalis*) was the most ubiquitous with a total of 38 detections throughout the Pensacola Bay System in fall, spring, and summer. The invasive Red lionfish (*Pterois volitans*) was detected in Bayou Texar, Escambia Bay, Escambia River, and in the far east and upper portions of East Bay. Further, the invasive Flathead catfish (*Pylodictis olivaris*) was detected once in summer in the Escambia River.

In addition to the 57 fish species, two aquatic non-fish vertebrates were detected. The Bottlenose dolphin (*Tursiops truncatus*) was detected in both spring and summer in two different locations within Escambia Bay. The American alligator (*Alligator mississippiensis*) was detected once in Escambia River near Thompson's Bayou in summer. A list of non-fish vertebrates can be found in table 6. Overall, DNA from domesticated animals such as the dog, cat, and chicken were detected at several locations in each season.

CHAPTER IV

DISCUSSION

Elasmobranchs in the Pensacola Bay System

Elasmobranch DNA was detected in the Pensacola Bay System using environmental DNA metabarcoding. Through the duration of this study, DNA of three elasmobranch species was detected, with the majority of elasmobranch DNA detections in the spring season. Specifically, the Atlantic stingray (*Hypanus sabinus*), a common nearshore and estuarine species in the Gulf of Mexico, was detected at three bottom sites in spring (SGB, SKB, and SMB). *Hypanus sabinus* has been documented entering freshwater habitats, with a resident freshwater population on the Atlantic Coast of Florida in the St. Johns River (Johnson and Snelson 1996). Like many other elasmobranch species, *H. sabinus* ovulates during spring and pups in mid- to late summer (Soulen et al. 2019). *Hypanus sabinus* was only detected in late spring, which were likely pregnant females. *Hypanus sabinus* is an ecosystem engineer, and primarily feeds on small invertebrates including polychaete worms and crustaceans (Cook 1994). Stingrays create pits by shifting bottom sediments, causing displacement of the invertebrate community structure. These pits can also be used by other species, such as harpacticoid copepods possibly searching for healthier mates or better food (Reidenauer and Thistle 1981; Soulen et al. 2019). All *Hypanus sabinus* environmental DNA was detected from bottom sampling locations. It is likely these fish were foraging for benthic invertebrates or creating ray pits.

Although *H. sabinus* are not a large part of commercial or recreational fisheries, they are occasionally harvested for bait in shark and crab fisheries and sometimes fished for human consumption (Adams et al. 2003). *Hypanus sabinus* are also predated upon by larger piscivorous species including juvenile Bull sharks (*Carcharhinus leucas*) (Soulen et al. 2019). *Hypanus*

sabinus is currently classified as least concern by the IUCN Red List of Threatened Species (2016) but has not been assessed since 2006 and the current population trends are unknown.

In the summer water collections for this study, eDNA from the American cownose ray (*Rhinoptera bonasus*) was detected at one site (RBB). *Rhinoptera bonasus* occurs in coastal waters and is common in bays in estuaries, with some individuals extending in the lower reaches of coastal rivers (Collins et al. 2007; Collins et al. 2008; Ogburn et al. 2018). Ogburn et al. (2018) found that *R. bonasus* is non-migratory in summer because they are pupping, thus detected DNA from this species was likely from either pupping female(s) or juvenile(s). Due to a highly modified jaw, *R. bonasus* primarily feeds on hard molluscan prey such as oysters and other bivalves (Collins et al. 2007; Collins et al. 2008). Environmental DNA for *Rhinoptera bonasus* was only detected at a bottom site location, meaning this species was likely foraging for benthic invertebrates. They are also known as a nuisance species as their foraging in several estuaries has caused destruction to commercial shellfish (Collins et al. 2008; Goodman et al. 2011). *Rhinoptera bonasus* is currently classified as near threatened by the IUCN Red List of Threatened Species (2006), but species status has not been assessed since 2006 and their current population trend is unknown.

The Clearnose skate (*Rostroraja eglanteria*) was the most detected elasmobranch species with six detections in spring (SAB, SAS, SBS, SCS, SFB, and SLS) and one detection in winter (WMS). *Rostroraja eglanteria* is commonly found along the Atlantic Coast of Florida and in the Gulf of Mexico, ranging from mid-Florida around the Gulf Coast to Eastern Texas (Luer and Gilber 1985; Schwartz 1996). In Sarasota, Florida, *R. eglanteria* is commonly found nearshore during winter months for mating and egg deposition and then individuals migrate offshore to cooler, deeper waters as the coastal water warms (Luer and Gilber 1985). Timing of the

reproductive cycle of *R. eglanteria* is most likely based on temperature rather than season (Luer et al. 2007). Fitz and Daiber (1963) found that in Delaware Bay, *R. eglanteria* lay eggs in early spring, with pups hatching in late spring. In the Gulf of Mexico, however, egg deposition occurs in the winter (Luer and Gilber 1985). Winter eDNA detection of *R. eglanteria* was presumably an adult female traveling into the Pensacola Bay System to deposit eggs, while the spring eDNA detections were likely newly hatched juveniles migrating out of the warm coastal waters. It is unlikely that spring detections were pregnant females preparing to deposit eggs as the water temperature was too warm at the time of sampling, and the eggs would not have survived (Luer et al. 2007). *Rostroraja eglanteria* primarily feeds on small fish and invertebrates such as crustaceans and mollusks (Schwartz 1996). Broadstriped anchovy (*Anchoa hepsetus*) and Spot croaker (*Leiostomus xanthurus*) found in the stomachs of *R. eglanteria* off the Atlantic coast in North Carolina (Schwartz 1996). The presence of environmental DNA of these two species suggests that the Pensacola Bay System contains potential food sources for *R. eglanteria.* Due to their small size, *R. eglanteria* is not commonly fished for food. Population status of *R. eglanteria* was last assessed in 2008 and is currently classified as "least concern" by the IUCN Red List of Threatened Species (2009).

Seagrass beds are an important habitat for many marine organisms, including elasmobranchs. Specifically, *H. sabinus* often inhabits seagrass beds along the Atlantic Coast (Soulen et al. 2019). The Pensacola Bay System is home to a variety of seagrass species including *Halodule wrightii* (Shoal grass) and *Thalassia testudinum* (Turtle grass) (Lewis et al. 2008). However, seagrass beds are rapidly declining. In the past 50 years, the Gulf of Mexico seagrass coverage has been reduced 20-100% (Dawes et al. 2004) and this decline is mostly attributed to anthropogenic activities such as declining water quality, overfishing, global climate

change, and dredging. However, anthropogenic pollution from point and nonpoint sources is the largest cause of seagrass decline (Ralph et al. 2006; Schwenning et al. 2006). Although seagrass beds were not sampled during this study, future studies in seagrass beds may show that these declining habitats are a possible cause for few elasmobranch detections.

In the northern Gulf of Mexico, dredging of the Gulf Intracoastal Waterway began in 1900 which runs east to west through the Pensacola Pass. The Army Corps of Engineers (ACOE) has since overseen occasional dredging of the Pensacola Pass to maintain its length, width, and depth dimensions (Lang 2015). This dredging causes increased turbidity along with destruction of the benthic community, which can lead to decreased biodiversity. Additionally, dredging within Pensacola Bay along the northern end of the Pensacola Bay Bridge in 1951 caused a complete disappearance of seagrass beds in that area (Schwenning et al. 2006). The disruption of the benthic community caused by dredging may have contributed to the low detection of elasmobranch eDNA in this study.

High mobility and migration patterns of larger elasmobranch species may be another possibility for the lack of eDNA detections in this study. The Pensacola Bay System consists of five subsections, while sampling only occurred in three. Many elasmobranchs undergo large movements within an estuary (Froeschke et al. 2010a); therefore, it is likely that not enough area of the Pensacola Bay System was sampled. Due to the quick degradation of eDNA in the system (Gargan et al. 2017), more frequent sampling or greater geographic coverage might have yielded increased elasmobranch eDNA detections. However, this is not the case for all elasmobranchs. Some batoids, such as *Rhinoptera bonasus* have shown a lack of seasonal migration in the Gulf of Mexico (Collins et al. 2008; Ajemian and Powers 2016), increasing their time spent in the system. Environmental DNA detections of other, highly mobile, elasmobranch species may have

been missed due to not enough sampling and their frequent migrations within and out of the system.

Teleosts in the Pensacola Bay System

Interestingly Gulf sturgeon (*Acipenser oxyrinchus*) DNA was detected in the Blackwater River in spring. This species of sturgeon often travels into coastal rivers in spring and then migrates out into bays and estuaries in fall for feeding. Rivers within the Pensacola Bay System are home to one of four populations of Gulf Sturgeon in the northeastern Gulf of Mexico. However, there is very little known about the seasonal movements of this specific population (Duncan et al. 2011). Continuing eDNA studies specifically targeting *Acipenser oxyrinchus* in the Pensacola Bay System would help illuminate this species' migration patterns in the system.

Environmental DNA metabarcoding only detected three elasmobranch species, at-risk and invasive species of interest were also detected. The endangered American eel (*Anguilla rostrata*) was detected in the summer season at a bottom location where Bayou Texar and Pensacola Bay intersect. American eels populations have been decreasing for the past several decades with abundances at a historic low (ASMFC 2017; Warshafsky et al. 2018). *A. rostrata* is a facultative catadromous species, remaining in estuaries or rivers until they reach maturity (Bonvechio et al. 2018). In addition to the endangered *A. rostrata*, two invasive species were also detected. *Pylodictis olivaris* (Flathead catfish) was detected once in this study using eDNA in summer in the Escambia River. This species was first seen in Escambia River in 1980 and has not been detected in the Escambia River since 2014 (USGS NAS 2020), demonstrating that this species is still in the area. DNA from the Red Lionfish (*Pterois volitans*), a widespread invasive species from tropical coral reefs to subtropical estuaries was also detected in this study (Harris et al. 2020). Lionfish were first reported in the northern Gulf of Mexico in summer 2010 (Dahl and

Patterson 2014). In coral reef environments, *P. volitans* has been shown to have damaging effects on native reef fish populations. However, little is known about how the presence of *P. volitans* will affect fish communities in the northern Gulf of Mexico (Dahl and Patterson 2014). In addition to *P. volitans* DNA detection, *Gobiosoma sp.* were also detected, a known prey item of Red lionfish in this area (Dahl and Patterson 2014). Continuation of eDNA metabarcoding studies in this area could highlight potential prey for these invasive fish. Additionally, a 2017 study using eDNA to detect Lionfish in the northern Gulf of Mexico also found Lionfish presence in Escambia Bay in all season except for spring (Brower, 2019). This study further supports the effectiveness of using eDNA to detect invasive species. Environmental DNA from another invasive species, *Alosa aestivalis* (Blueback herring), was also detected more frequently than any other species in this study. *Alosa aestivalis* was first recorded off the coast of Florida in the northern Gulf of Mexico in 1962. Although this species has been accidentally introduced in several areas as bait by fisherman, it is suggested by USGS NAS (2015) that this area may be a part of this species invasive range. DNA detections of *Alosa aestivalis* in this study indicate that this species is present in the Pensacola Bay System.

Conclusions

Overall, these results suggest that the Pensacola Bay System may not be an essential habitat for many elasmobranch species, potentially due to habitat loss, DNA degradation, or problems with eDNA methodology. This study did demonstrate that three elasmobranch species (*Rostroraja eglanteria, Hypanus sabinus,* and *Rhinoptera bonasus*), are utilizing the Pensacola Bay System potentially for reproductive/ breeding and feeding grounds. This system is also being used frequently by other invasive and endangered species. Environmental DNA metabarcoding detected an invasive species in Escambia River that has not been seen in the area

for six years, further emphasizing the importance of eDNA studies. Furthermore, DNA detection of elasmobranchs, as well as other vulnerable and endangered species, highlights the importance of proper habitat protection and restoration. Protecting the Pensacola Bay System from anthropogenic activities such as dredging may help improve water quality, creating a more suitable environment for these species to live. Additionally, seagrass restoration may increase elasmobranch usage and subsequently increase eDNA detections. Continuing eDNA metabarcoding studies in the Pensacola Bay System will not only provide a better understanding of the various species that utilize this area but will also aid in determining which areas should be better regulated and protected.

Figure 1: Satellite map of 19 eDNA water sampling locations within the Pensacola Bay System. Water samples were collected from each location quarterly from Fall 2018-Summer 2019. Surface and Bottom samples were taken at each location.

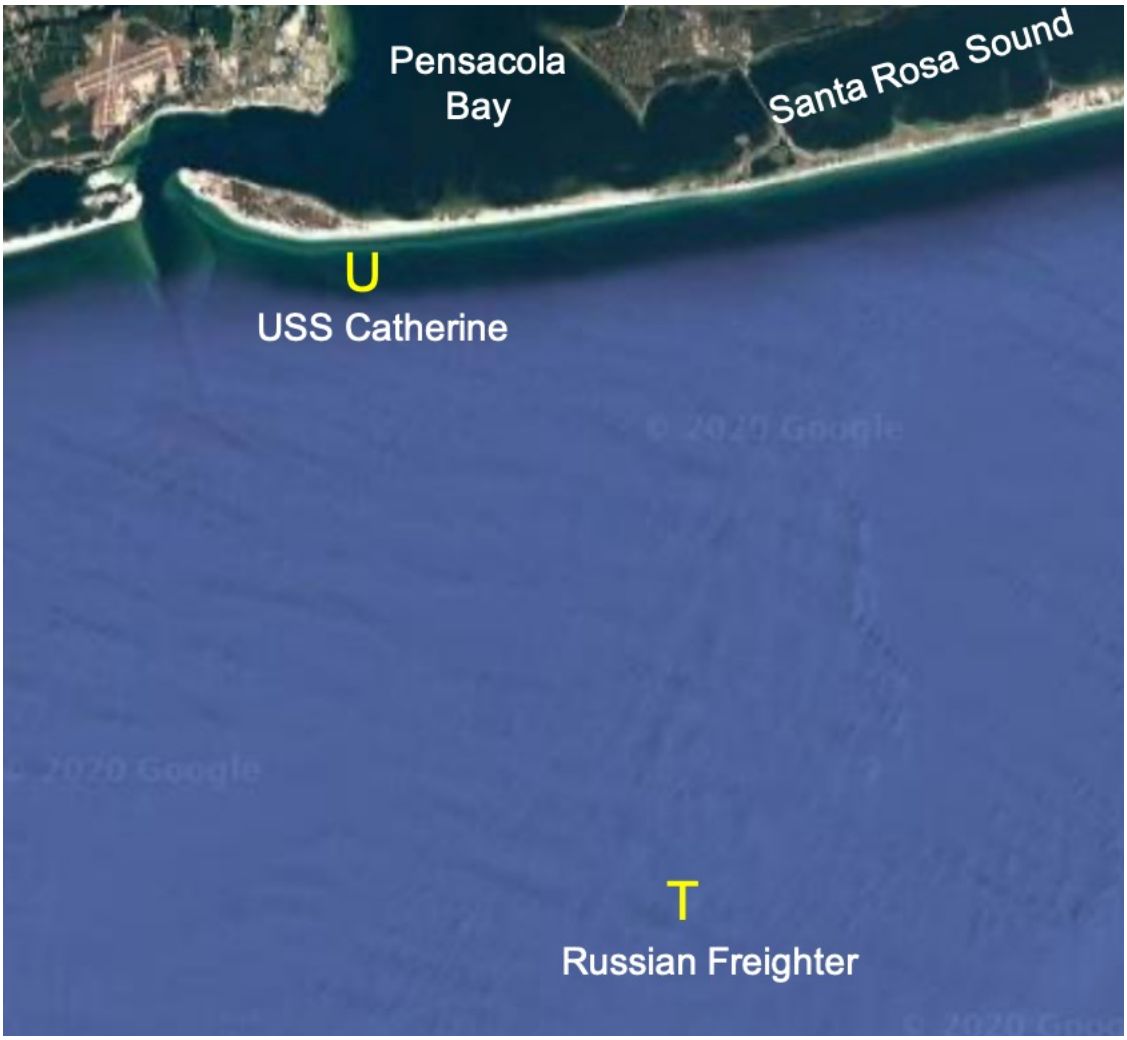

Figure 2: Map of two offshore eDNA water sampling locations in the Gulf of Mexico. Water samples were collected quarterly from Fall 2018-Summer 2019 with the exception of Spring 2019 due to dangerous water conditions. Surface samples were collected at each location and bottom samples were collected by diving. Two bottom samples were collected at the Russian Freighter (one at the bow and one at the stern) due to the large size of the sunken ship.

Table 1: List of all fish species detected by eDNA metabarcoding. International Union for Conservation of Nature (IUCN) categories are endangered (EN), near threatened (NT), vulnerable (VU), and least concern (LC). Species that are not classified by the IUCN are listed as not applicable (N/A).

Order	Family	Species	Common Name	IUCN
Acipenseriformes	Acipenseridae	*Acipenser oxyrinchus*	Gulf sturgeon	NT
Amiiformes	Amiidae	*Amia calva*	Bowfin	LC
Anguilliformes	Anguillidae	*Anguilla rostrata*	American eel	EN
Atheriniformes	Atherinopsidae	*Membras martinica*	Rough silverside	LC
Atheriniformes	Atherinopsidae	*Menidia beryllina*	Inland silverside	LC
Clupeiformes	Clupeidae	*Alosa aestivalis*	Blueback herring	VU
Clupeiformes	Clupeidae	*Dorosoma cepedianum*	Gizzard shad	LC
Clupeiformes	Clupeidae	*Dorosoma petenense*	Threadfin shad	LC
Clupeiformes	Clupeidae	*Harengula jaguana*	Scaled herring	LC
Clupeiformes	Engraulidae	*Anchoa hepsetus*	Broad-striped anchovy	LC
Clupeiformes	Engraulidae	*Anchoa mitchilli*	Common anchovy	LC
Cypriniformes	Catostomidae	*Erimyzon oblongus*	Creek chubsucker	LC
Cypriniformes	Catostomidae	*Minytrema melanops*	Spotted sucker	LC
Cypriniformes	Catostomidae	*Moxostoma poecilurum*	Blacktail redhorse	LC
Cypriniformes	Cyprinidae	*Cyprinella venusta*	Blacktail shiner	LC
Cypriniformes	Cyprinidae	*Hybopsis winchelli*	Clear chub	LC
Cypriniformes	Cyprinidae	*Luxilus cornutus*	Common shiner	LC

Cypriniformes	Cyprinidae	*Notemigonus crysoleucas*	Golden shiner	LC
Cypriniformes	Cyprinidae	*Notropis baileyi*	Rough shiner	LC
Cypriniformes	Cyprinidae	*Opsopoeodus emiliae*	Pugnose minnow	LC
Cyprinodontiformes	Fundulidae	*Fundulus grandis*	Gulf killifish	LC
Cyprinodontiformes	Poeciliidae	*Gambusia holbrooki*	Eastern mosquitofish	LC
Elopiformes	Elopidae	*Elops saurus*	Ladyfish	LC
Esociformes	Esocidae	*Esox niger*	Chain pickerel	LC
Lepisosteiformes	Lepisosteidae	*Atractosteus spatula*	Alligator gar	LC
Lepisosteiformes	Lepisosteidae	*Lepisosteus osseus*	Longnose gar	LC
Mugiliformes	Mugilidae	*Mugil cephalus*	Flathead grey mullet	LC
Mugiliformes	Mugilidae	*Mugil curema*	White mullet	LC
Myliobatiformes	Dasyatidae	*Hypanus sabinus*	Atlantic stingray	LC
Myliobatiformes	Rhinopteridae	*Rhinoptera bonasus*	American Cownose ray	NT
Perciformes	Carangidae	*Caranx latus*	Horse-eye jack	LC
Perciformes	Centrarchidae	*Ambloplites rupestris*	Rock bass	LC
Perciformes	Centrarchidae	*Lepomis gulosus*	Warmouth	LC
Perciformes	Centrarchidae	*Lepomis macrochirus*	Bluegill	LC
Perciformes	Centrarchidae	*Lepomis megalotis*	Longear sunfish	LC
Perciformes	Centrarchidae	*Lepomis miniatus*	Redspotted sunfish	LC
Perciformes	Centrarchidae	*Lepomis punctatus*	Spotted sunfish	LC
Perciformes	Centrarchidae	*Micropterus salmoides*	Largemouth bass	LC

Perciformes	Centrarchidae	*Pomoxis nigromaculatus*	Black crappie	LC
Perciformes	Elassomatidae	*Elassoma zonatum*	Banded pygmy sunfish	LC
Perciformes	Gobiidae	*Gobiosoma sp.*	Gobies	N/A
Perciformes	Percidae	*Etheostoma swaini*	Gulf darter	LC
Perciformes	Percidae	*Percina copelandi*	Channel darter	LC
Perciformes	Sciaenidae	*Bairdiella chrysoura*	Silver perch	LC
Perciformes	Sciaenidae	*Cynoscion nebulosus*	Spotted seatrout	LC
Perciformes	Sciaenidae	*Leiostomus xanthurus*	Spot croaker	LC
Perciformes	Sciaenidae	*Micropogonias undulatus*	Atlantic croaker	LC
Perciformes	Sciaenidae	*Sciaenops ocellatus*	Red drum	LC
Perciformes	Scombridae	*Acanthocybium solandri*	Wahoo	LC
Perciformes	Sparidae	*Lagodon rhomboides*	Pinfish	LC
Percopsiformes	Aphredoderidae	*Aphredoderus sayanus*	Pirate perch	LC
Rajiformes	Rajidae	*Rostroraja eglanteria*	Clearnose skate	LC
Scorpaeniformes	Cottidae	*Cottus carolinae*	Banded sculpin	LC
Scorpaeniformes	Scorpaenidae	*Pterois volitans*	Red lionfish	LC
Siluriformes	Ariidae	*Bagre marinus*	Gafftopsail catfish	LC
Siluriformes	Ictaluridae	*Pylodictis olivaris*	Flathead catfish	LC
Syngnathiformes	Syngnathidae	*Syngnathus fuscus*	Northern pipefish	LC

Table 2: Abiotic water conditions at each sampling location in Fall 2018. Surface locations are indicated with an "S" at the end of each site name and bottom locations are indicated with a "B" at the end of each site name. Abiotic data were collected using a YSI multimeter which was calibrated before each sampling trip.

Site	Depth (m)	Temperature (℃)	Salinty (PSU)	DO (%)	pH
F-AS		21.1	12.39	95.0	7.97
F-AB	3.05	22.1	16.66	64.9	8.01
F-BS		21.1	13.88	88.7	8.13
F-BB	1.49	22.0	18.19	67.0	8.23
F-CS		21.6	19.84	80.9	8.33
F-CB	1.40	21.4	19.87	71.0	8.29
F-DS		20.9	20.03	84.1	8.32
F-DB	2.29	20.9	19.92	67.1	8.27
F-KS		21.1	17.29	84.3	8.37
F-KB	1.46	22.4	20.61	75.0	8.44
F-LS		23.4	23.25	67.4	8.02
F-LB	1.83	23.8	23.68	33.2	7.97
F-MS		23.2	22.74	60.6	7.99
F-MB	0.98	23.1	22.70	44.8	8.03
F-NS		23.1	15.91	47.2	7.70
F-NB	1.19	23.1	16.40	27.0	7.61
F-OS		20.8	4.38	67.5	7.75
F-OB	0.91	21.5	8.87	61.6	7.57
F-PS		21.6	5.84	61.0	7.59
F-PB	0.73	22.1	10.65	51.9	7.41
F-RS		20.5	0.05	80.2	8.73
F-RB	5.55	20.2	0.06	64.2	8.16
F-SS		20.6	0.15	73.6	7.14
F-SB	3.57	23.1	6.99	19.2	6.70
F-QS		21.0	6.08	92.2	7.59
F-QB	1.77	21.3	10.09	67.5	7.63
F-ES		22.6	19.54	82.2	8.01
F-EB	0.61	22.7	19.34	73.5	8.09
F-JS		20.3	0.67	71.2	6.71
F-JB	3.96	21.3	5.73	41.0	6.92
F-IS		20.4	1.24	68.5	6.81
F-IB	3.66	21.4	6.08	37.8	6.97
F-HS		21.0	3.46	63.3	7.12
F-HB	1.83	21.6	6.21	54.1	7.24
F-GS		21.6	5.97	66.2	7.41
F-GB	1.83	21.9	7.91	62.9	7.23
F-FS		20.8	2.23	51.0	6.90
F-FB	1.22	20.8	3.82	49.6	7.03
F-TS		16.7	32.50	89.7	8.40
F-TB Stern	20.42				
F-TB Bow	21.34				
F-US		16.7	31.87	87.0	8.52
F-UB	6.10				

Table 3: Abiotic water conditions at each sampling location in Winter 2019. Surface locations are indicated with an "S" at the end of each site name and bottom locations are indicated with a "B" at the end of each site name. Abiotic data were collected using a YSI multimeter which was calibrated before each sampling trip.

Site	Depth (m)	Temperature (℃)	Salinity (PSU)	DO (%)	pH
W-AS		12.6	10.19	99.7	7.64
W-AB	1.98	15.6	17.33	91.5	7.88
W-BS		12.8	10.35	102.1	7.71
W-BB	1.55	14.1	13.69	95.6	7.82
W-CS		13.1	16.07	98.4	7.68
W-CB	0.98	13.8	18.04	91.8	7.89
W-DS		12.7	13.41	100.9	7.89
W-DB	0.91	12.8	13.51	93.3	7.92
W-KS		13.3	10.72	100.6	7.89
W-KB	0.88	14.2	13.96	101.8	7.92
W-LS		14.4	17.04	101.8	7.96
W-LB	1.22	16.0	19.47	90.3	8.01
W-MS		13.4	7.83	97.7	7.92
W-MB	0.61	14.7	16.31	97.7	7.95
W-NS		14.3	12.45	102.9	7.85
W-NB	0.61	14.5	13.76	95.8	7.99
W-OS		15.3	0.73	93.0	8.42
W-OB	0.61	15.8	9.84	108.3	7.63
W-PS		14.1	3.01	93.3	7.96
W-PB	0.46	14.2	3.26	90.6	7.84
W-RS		12.7	0.03	95.9	8.67
W-RB	4.88	12.7	0.03	90.4	8.21
W-SS		15.1	0.03	89.2	7.51
W-SB	2.44	14.0	0.04	81.6	7.35
W-QS		14.1	3.41	104.3	6.68
W-QB	1.52	16.4	23.93	97.6	7.34
W-ES		23.0	9.25	93.7	7.65
W-EB	0.61	23.4	9.22	71.6	7.60
W-JS		16.2	1.21	106.6	7.38
W-JB	3.05	17.8	14.69	67.3	6.61
W-IS		16.3	3.36	87.4	7.10
W-IB	1.52	16.4	3.87	82.1	7.08
W-HS		15.9	2.78	97.7	7.50
W-HB	1.52	15.9	3.06	83.3	7.32
W-GS		16.7	4.40	93.9	7.10
W-GB	1.83	17.1	7.29	83.2	7.08
W-FS		15.5	0.11	100.9	8.69
W-FB	0.61	15.4	0.11	90.8	8.42
W-TS		20.7	33.56	101.6	7.87
W-TB Sterm	21.03				
W-TB Bow	23.47				
W-US		21.0	32.85	97.0	8.01
W-UB	5.18				

Table 4: Abiotic water conditions at each sampling location in Spring 2019. Surface locations are indicated with an "S" at the end of each site name and bottom locations are indicated with a "B" at the end of each site name. Abiotic data were collected using a YSI multimeter which was calibrated before each sampling trip. Offshore locations could not be collected this quarter due to dangerous water conditions.

Site	Depth (m)	Temperature (℃)	Salinity (PSU)	DO (%)	pH
S-AS		29.9	14.45	78.3	7.72
S-AB	2.38	29.9	16.34	61.3	7.76
S-BS		30.0	13.42	73.6	7.89
S-BB	0.98	29.9	13.64	70.3	7.92
S-CS		30.1	12.87	82.6	8.03
S-CB	1.37	29.9	13.43	75.1	8.05
S-DS		29.9	12.93	74.6	7.93
S-DB	1.74	29.6	14.25	65.5	7.76
S-KS		30.5	12.18	77.1	7.98
S-KB	1.4	30.6	15.52	82.1	8.05
S-LS		31.0	8.65	75.4	7.94
S-LB	1.8	31.0	9.80	67.2	7.88
S-MS		31.6	9.67	66.8	7.76
S-MB	0.98	31.1	8.38	77.7	7.80
S-NS		30.1	4.66	57.2	7.75
S-NB	1.16	31.1	5.88	67.3	7.39
S-OS		31.1	1.98	102.7	8.43
S-OB	0.67	30.1	2.18	89.5	8.21
S-PS		30.8	3.44	104.6	8.37
S-PB	0.61	30.6	5.83	86.3	8.07
S-RS		29.7	0.06	71.2	8.54
S-RB	4.54	29.2	0.06	69.0	7.87
S-SS		29.5	0.19	67.3	7.46
S-SB	3.41	27.6	2.00	60.8	6.88
S-QS		30.9	4.92	88.0	7.95
S-QB	1.68	30.4	8.07	65.9	7.65
S-ES		30.5	9.14	63.7	7.52
S-EB	0.61	30.7	15.86	41.0	7.31
S-JS		28.1	0.06	74.8	6.57
S-JB	3.66	27.8	0.07	71.4	6.60
S-IS		28.1	0.17	73.7	7.10
S-IB	4.57	27.7	0.24	71.2	6.84
S-HS		29.4	1.02	70.8	7.28
S-HB	2.13	29.2	1.12	77.5	7.18
S-GS		29.2	1.16	75.3	7.57
S-GB	1.83	29.5	1.98	67.6	7.23
S-FS		29.8	3.06	37.7	7.53
S-FB	0.91	29.5	2.87	30.8	7.33

Table 5: Abiotic water conditions at each sampling location in Summer 2019. Surface locations are indicated with an "S" at the end of each site name and bottom locations are indicated with a "B" at the end of each site name. Abiotic data were collected using a YSI multimeter which was calibrated before each sampling trip.

Site	Depth (m)	Temperature (°C)	Salinty (PSU)	DO (%)	pH
R-AS		29.1	13.89	84.7	7.97
R-AB	1.58	29.1	18.18	70.8	8.05
R-BS		29.2	18.22	78.3	8.26
R-BB	0.88	29.1	18.31	64.4	8.28
R-CS		29.1	16.98	80.8	8.32
R-CB	1.52	29.5	21.75	63.0	8.25
R-DS		28.8	15.88	72.0	8.25
R-DB	1.22	28.7	15.96	60.0	8.19
R-KS		28.2	17.36	65.4	8.06
R-KB	1.22	29.1	20.66	42.0	7.99
R-LS		29.9	22.67	41.6	7.91
R-LB	1.68	30.0	23.87	21.1	7.88
R-MS		28.5	11.92	69.9	8.20
R-MB	0.91	29.2	21.04	42.2	7.94
R-NS		28.1	8.56	54.3	7.98
R-NB	1.10	29.1	11.82	36.0	7.77
R-OS		28.0	3.85	67.8	8.10
R-OB	0.52	28.1	4.33	62.9	7.96
R-PS		28.5	4.18	62.1	7.91
R-PB	0.43	28.9	7.52	61.5	7.73
R-RS		27.9	0.06	56.7	8.13
R-RB	4.57	27.2	0.06	55.9	7.88
R-SS		28.0	0.41	53.7	7.61
R-SB	3.29	28.8	9.50	41.0	6.91
R-QS		28.6	9.79	88.8	8.27
R-QB	1.52	29.0	16.77	51.7	7.95
R-ES		29.3	18.34	63.2	7.99
R-EB	0.61	29.1	18.36	61.9	8.04
R-JS		27.2	1.83	60.0	8.25
R-JB	4.27	29.9	17.20	27.5	7.23
R-IS		27.4	3.35	56.3	7.88
R-IB	3.66	29.7	21.54	17.2	7.25
R-HS		27.8	5.22	57.7	7.72
R-HB	1.83	30.1	19.16	22.8	7.34
R-GS		29.8	12.28	69.2	7.93
R-GB	1.83	30.1	23.66	31.7	7.67
R-FS		28.9	11.99	45.2	7.55
R-FB	0.91	29.6	19.32	43.2	7.53
R-TS		29.8	28.67	91.1	8.24
R-TB Stern	21.03	25.0			
R-TB Bow	23.47				
R-US		30.2	28.06	88.6	8.32
R-UB	5.18				

Table 6: Non-fish vertebrates detected by eDNA metabarcoding. International Union for Conservation of Nature (IUCN) categories are least concern (LC).

Common Name	Scientific Name	Site	Season	IUCN
Domestic chicken	*Gallus gallus*	FES	Fall	LC
Domestic chicken	*Gallus gallus*	FGB	Fall	LC
Domestic chicken	*Gallus gallus*	FGS	Fall	LC
Domestic chicken	*Gallus gallus*	FIB	Fall	LC
Domestic chicken	*Gallus gallus*	FJS	Fall	LC
Domestic dog	*Canis lupus familiaris*	FES	Fall	LC
Domestic dog	*Canis lupus familiaris*	FFB	Fall	LC
Domestic dog	*Canis lupus familiaris*	FIB	Fall	LC
Domestic dog	*Canis lupus familiaris*	FIS	Fall	LC
House mouse	*Mus musculus*	FAB	Fall	LC
House mouse	*Mus musculus*	FAS	Fall	LC
House mouse	*Mus musculus*	FBS	Fall	LC
House mouse	*Mus musculus*	FES	Fall	LC
House mouse	*Mus musculus*	FFB	Fall	LC
House mouse	*Mus musculus*	FGB	Fall	LC
House mouse	*Mus musculus*	FGS	Fall	LC
House mouse	*Mus musculus*	FHB	Fall	LC
House mouse	*Mus musculus*	FIB	Fall	LC
House Mouse	*Mus musculus*	FIS	Fall	LC
House Mouse	*Mus musculus*	FNB	Fall	LC
House Mouse	*Mus musculus*	FNS	Fall	LC

House Mouse	*Mus musculus*	FPB	Fall	LC
House Mouse	*Mus musculus*	FQS	Fall	LC
House Mouse	*Mus musculus*	FRB	Fall	LC
Domestic cow	*Bos taurus*	WEB	Winter	LC
Domestic cow	*Bos taurus*	WOS	Winter	LC
Domestic dog	*Canis lupus familiaris*	WEB	Winter	LC
Domestic dog	*Canis lupus familiaris*	WLB	Winter	LC
Domestic dog	*Canis lupus familiaris*	WMB	Winter	LC
House mouse	*Mus musculus*	WBS	Winter	LC
House mouse	*Mus musculus*	WDS	Winter	LC
House mouse	*Mus musculus*	WFB	Winter	LC
House mouse	*Mus musculus*	WFS	Winter	LC
House mouse	*Mus musculus*	WGB	Winter	LC
House mouse	*Mus musculus*	WGS	Winter	LC
House mouse	*Mus musculus*	WHS	Winter	LC
House mouse	*Mus musculus*	WHB	Winter	LC
House mouse	*Mus musculus*	WLB	Winter	LC
House mouse	*Mus musculus*	WMS	Winter	LC
House mouse	*Mus musculus*	WOB	Winter	LC
House mouse	*Mus musculus*	WOS	Winter	LC
House mouse	*Mus musculus*	WPS	Winter	LC
House mouse	*Mus musculus*	WQS	Winter	LC
House mouse	*Mus musculus*	WRB	Winter	LC

House mouse	*Mus musculus*	WSB	Winter	LC
House mouse	*Mus musculus*	WSS	Winter	LC
Wild turkey	*Meleagris gallopavo*	WEB	Winter	LC
Bottlenose dolphin	*Tursiops truncatus*	SLB	Spring	LC
Domestic cat	*Felis catus*	SAB	Spring	LC
Domestic cat	*Felis catus*	SAS	Spring	LC
Domestic cat	*Felis catus*	SDB	Spring	LC
Domestic chicken	*Gallus gallus*	SFB	Spring	LC
Domestic chicken	*Gallus gallus*	SGS	Spring	LC
Domestic chicken	*Gallus gallus*	SKB	Spring	LC
Domestic cow	*Bos taurus*	SAB	Spring	LC
Domestic cow	*Bos taurus*	SAS	Spring	LC
Domestic cow	*Bos taurus*	SGB	Spring	LC
Domestic dog	*Canis lupus familiaris*	SBB	Spring	LC
Domestic dog	*Canis lupus familiaris*	SDB	Spring	LC
Domestic dog	*Canis lupus familiaris*	SDS	Spring	LC
Domestic dog	*Canis lupus familiaris*	SFB	Spring	LC
Domestic dog	*Canis lupus familiaris*	SGB	Spring	LC
Domestic dog	*Canis lupus familiaris*	SHB	Spring	LC
Domestic dog	*Canis lupus familiaris*	SJS	Spring	LC
Domestic dog	*Canis lupus familiaris*	SLB	Spring	LC
Domestic dog	*Canis lupus familiaris*	SMS	Spring	LC
Domestic dog	*Canis lupus familiaris*	SNB	Spring	LC

Domestic dog	*Canis lupus familiaris*	SNS	Spring	LC
Domestic dog	*Canis lupus familiaris*	SOS	Spring	LC
Domestic dog	*Canis lupus familiaris*	SQB	Spring	LC
Domestic dog	*Canis lupus familiaris*	SRB	Spring	LC
Domestic dog	*Canis lupus familiaris*	SSB	Spring	LC
Domestic pig	*Sus scrofa domesticus*	SAB	Spring	LC
Domestic pig	*Sus scrofa domesticus*	SAS	Spring	LC
House mouse	*Mus musculus*	SAB	Spring	LC
House mouse	*Mus musculus*	SAS	Spring	LC
House mouse	*Mus musculus*	SBB	Spring	LC
House mouse	*Mus musculus*	SBS	Spring	LC
House mouse	*Mus musculus*	SCB	Spring	LC
House mouse	*Mus musculus*	SCS	Spring	LC
House mouse	*Mus musculus*	SDS	Spring	LC
House mouse	*Mus musculus*	SES	Spring	LC
House mouse	*Mus musculus*	SFB	Spring	LC
House mouse	*Mus musculus*	SHB	Spring	LC
House mouse	*Mus musculus*	SIB	Spring	LC
House mouse	*Mus musculus*	SJB	Spring	LC
House mouse	*Mus musculus*	SKS	Spring	LC
House mouse	*Mus musculus*	SLB	Spring	LC
House mouse	*Mus musculus*	SLS	Spring	LC
House mouse	*Mus musculus*	SMB	Spring	LC

House mouse	*Mus musculus*	SNS	Spring	LC
House mouse	*Mus musculus*	SPB	Spring	LC
House mouse	*Mus musculus*	SPS	Spring	LC
House mouse	*Mus musculus*	SQB	Spring	LC
House mouse	*Mus musculus*	SRB	Spring	LC
House mouse	*Mus musculus*	SRS	Spring	LC
House mouse	*Mus musculus*	SSB	Spring	LC
House mouse	*Mus musculus*	SSS	Spring	LC
Wild boar	*Sus scrofa*	SHB	Spring	LC
Wild boar	*Sus scrofa*	SMB	Spring	LC
American alligator	*Alligator mississippiensis*	RSB	Summer	LC
Bottlenose dolphin	*Tursiops truncatus*	RMS	Summer	LC
Domestic cat	*Felis catus*	RJB	Summer	LC
Domestic cat	*Felis catus*	RJS	Summer	LC
Domestic cat	*Felis catus*	RRS	Summer	LC
Domestic chicken	*Gallus gallus*	RDB	Summer	LC
Domestic chicken	*Gallus gallus*	REB	Summer	LC
Domestic chicken	*Gallus gallus*	RIS	Summer	LC
Domestic chicken	*Gallus gallus*	RQB	Summer	LC
Domestic chicken	*Gallus gallus*	RRB	Summer	LC
Domestic cow	*Bos taurus*	REB	Summer	LC
Domestic cow	*Bos taurus*	RES	Summer	LC
Domestic dog	*Canis lupus familiaris*	RAB	Summer	LC

Domestic dog	*Canis lupus familiaris*	RBS	Summer	LC
Domestic dog	*Canis lupus familiaris*	RDB	Summer	LC
Domestic dog	*Canis lupus familiaris*	REB	Summer	LC
Domestic dog	*Canis lupus familiaris*	RES	Summer	LC
Domestic dog	*Canis lupus familiaris*	RFS	Summer	LC
Domestic dog	*Canis lupus familiaris*	RHB	Summer	LC
Domestic dog	*Canis lupus familiaris*	RRS	Summer	LC
Domestic pig	*Sus scrofa domesticus*	RGB	Summer	LC
Grey squirrel	*Sciurus carolinensis*	RNB	Summer	LC
House mouse	*Mus musculus*	RAB	Summer	LC
House mouse	*Mus musculus*	RAS	Summer	LC
House mouse	*Mus musculus*	RBS	Summer	LC
House mouse	*Mus musculus*	RCB	Summer	LC
House mouse	*Mus musculus*	RCS	Summer	LC
House mouse	*Mus musculus*	RDB	Summer	LC
House mouse	*Mus musculus*	REB	Summer	LC
House mouse	*Mus musculus*	RES	Summer	LC
House mouse	*Mus musculus*	RFB	Summer	LC
House mouse	*Mus musculus*	RFS	Summer	LC
House mouse	*Mus musculus*	RGB	Summer	LC
House mouse	*Mus musculus*	RGS	Summer	LC
House mouse	*Mus musculus*	RHB	Summer	LC
House mouse	*Mus musculus*	RHS	Summer	LC

House mouse	*Mus musculus*	RIB	Summer	LC
House mouse	*Mus musculus*	RIS	Summer	LC
House mouse	*Mus musculus*	RJB	Summer	LC
House mouse	*Mus musculus*	RKB	Summer	LC
House mouse	*Mus musculus*	RKS	Summer	LC
House mouse	*Mus musculus*	RLS	Summer	LC
House mouse	*Mus musculus*	RMB	Summer	LC
House mouse	*Mus musculus*	RMS	Summer	LC
House mouse	*Mus musculus*	RNS	Summer	LC
House mouse	*Mus musculus*	RPB	Summer	LC
House mouse	*Mus musculus*	RQB	Summer	LC
House mouse	*Mus musculus*	RRB	Summer	LC
House mouse	*Mus musculus*	RRS	Summer	LC
House mouse	*Mus musculus*	RSB	Summer	LC
House mouse	*Mus musculus*	RSS	Summer	LC
House mouse	*Mus musculus*	RUB	Summer	LC
Racoon	*Procyon lotor*	RKB	Summer	LC
Wild boar	*Sus scrofa*	RDB	Summer	LC

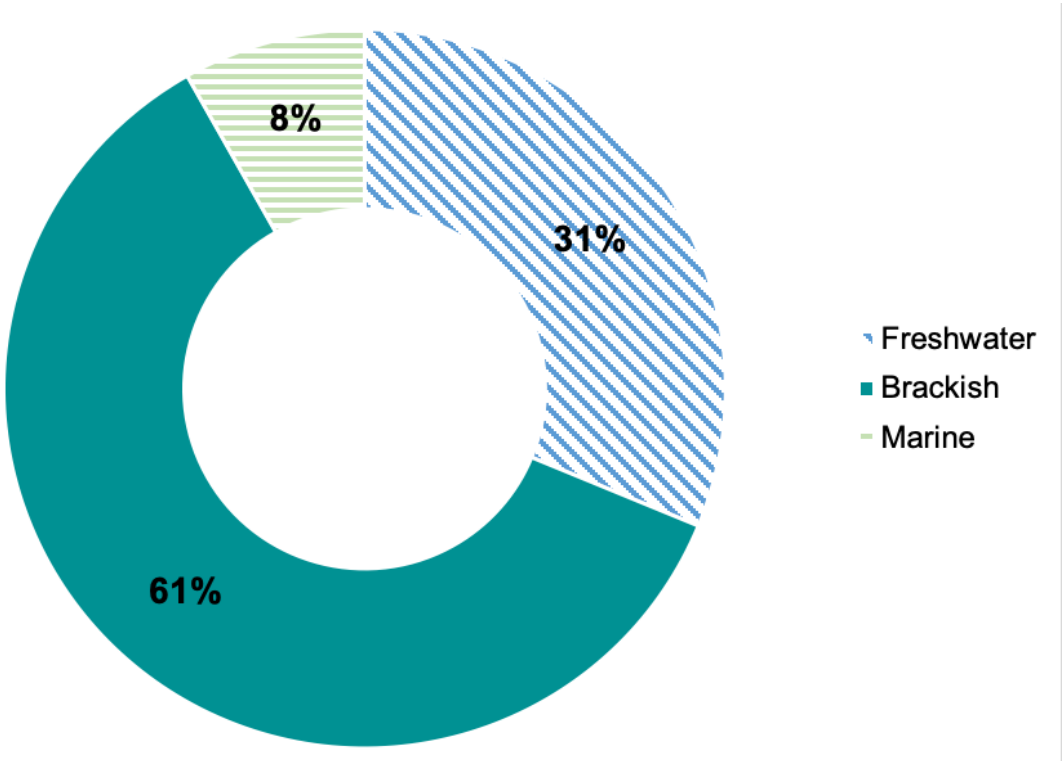

Figure 3: eDNA fish composition grouped by salinity classification in the Pensacola Bay System.

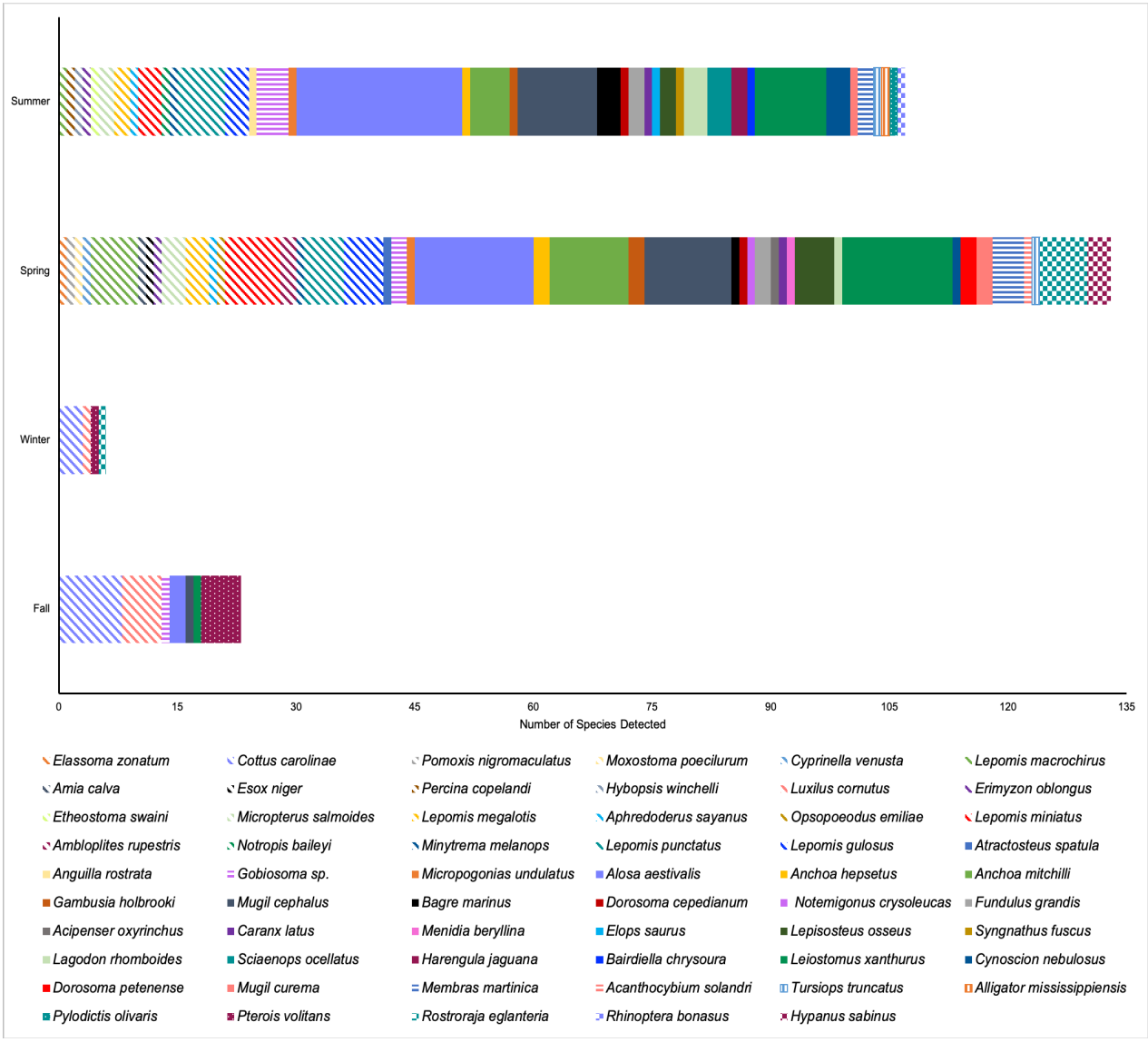

Figure 4: Season comparison of all species detected by eDNA metabarcoding excluding terrestrial vertebrates. Freshwater species are indicated by diagonal lines, brackish species are indicated by solid colors, marine species are indicated by horizontal lines, aquatic non-fish vertebrates are indicated by vertical lines with a border, elasmobranchs are indicated by a checkered pattern, and invasive species are indicated by solid colors with polka dots.

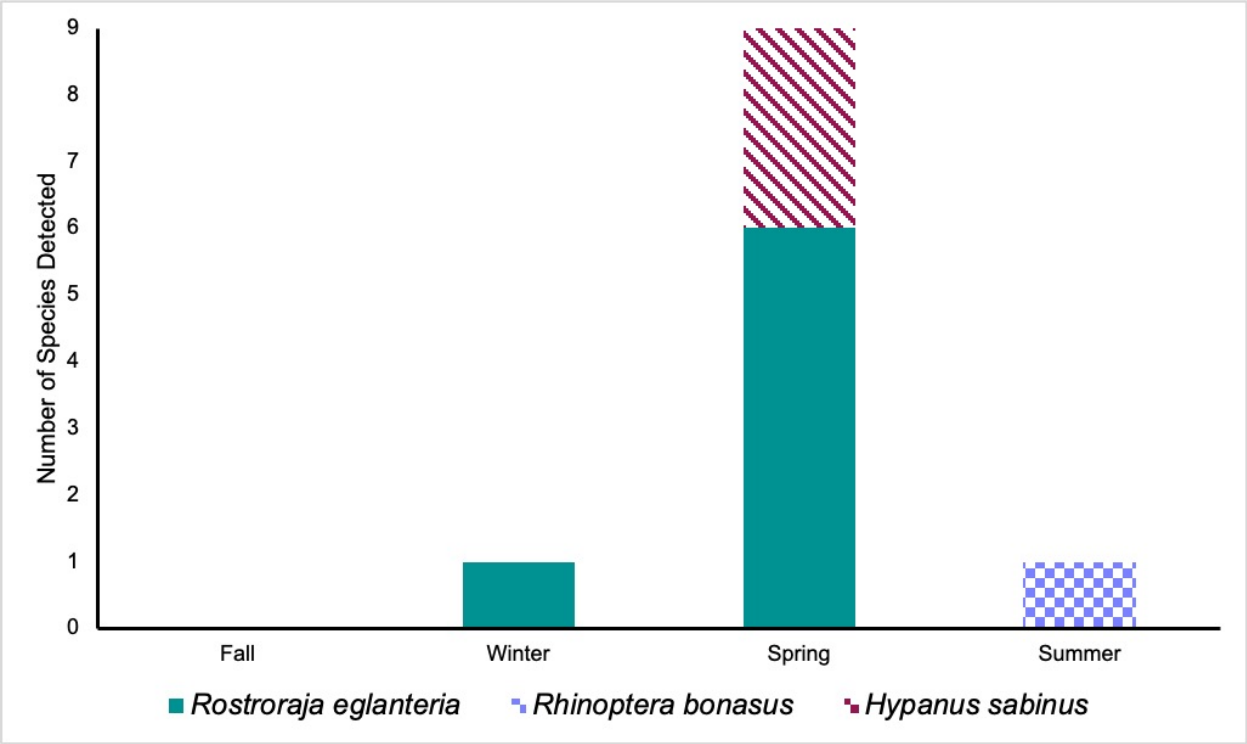

Figure 5: Overall elasmobranch eDNA detection during each sampling season in the Pensacola Bay System. *Rostroraja eglanteria* detections are represented by a solid teal color, *Rhinoptera bonasus* detections are represented by a checkered purple color, and *Hypanus sabinus* detections are represented by diagonal maroon lines.

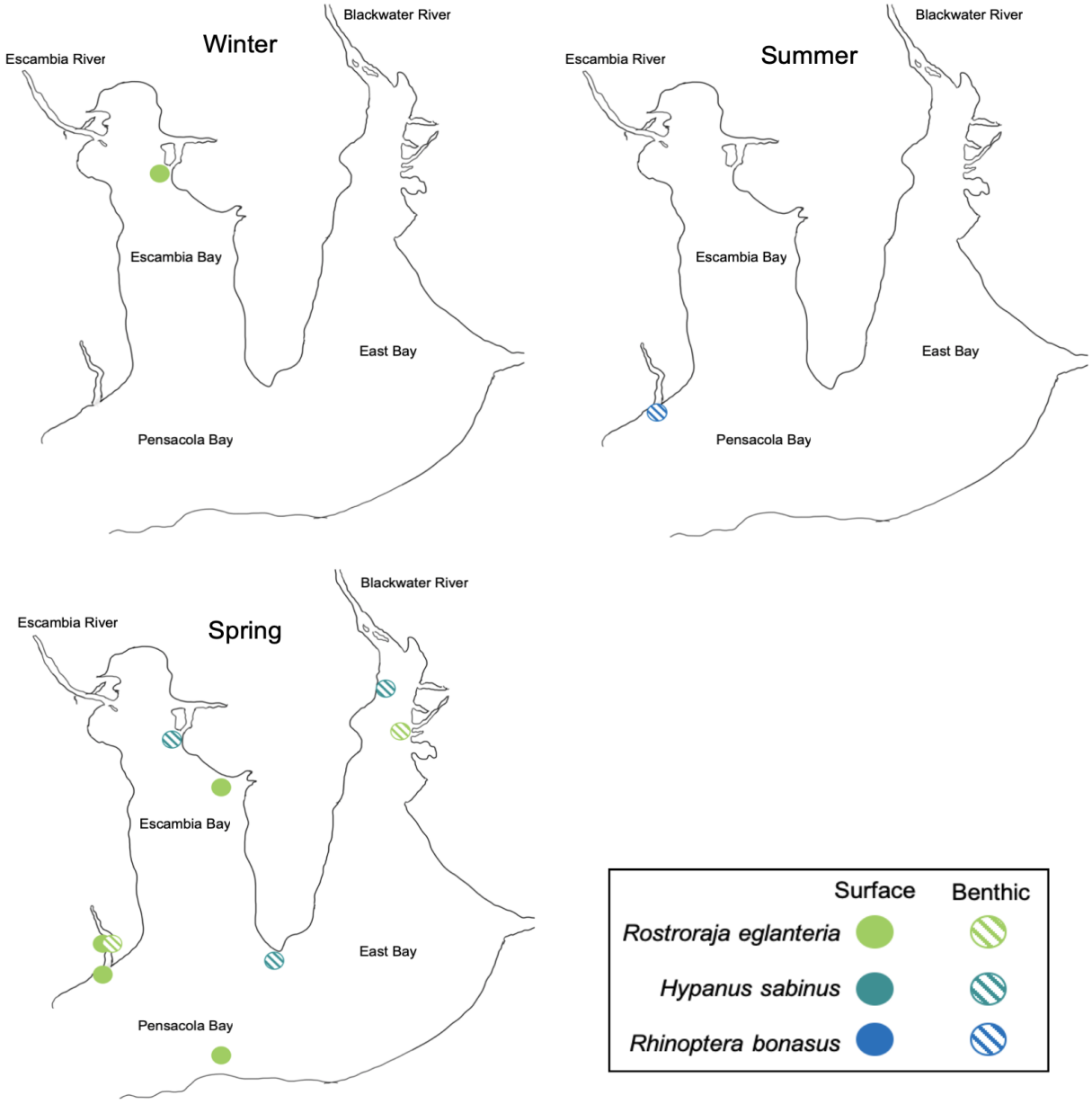

Figure 6: Map of elasmobranch usage from eDNA detections in the Pensacola Bay System. Surface detections are indicated by solid colors and bottom detections are indicated by diagonal lines. No elasmobranchs were detected during the fall sampling trip.

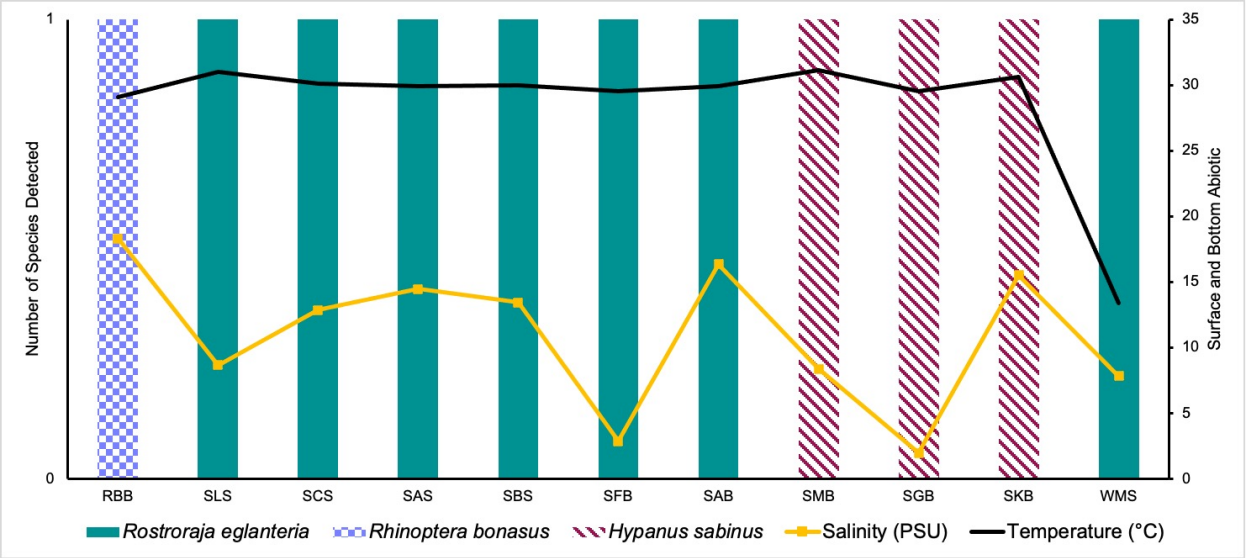

Figure 7: Elasmobranch eDNA detections plotted against salinity and temperature at time of sampling. At the beginning of each site name, summer sites are indicated with an "R", spring sites are indicated with an "S", and winter sites are indicated with a "W". At the end of each site name, surface sites are indicated with an "S" and bottom sites are indicated with a "B".

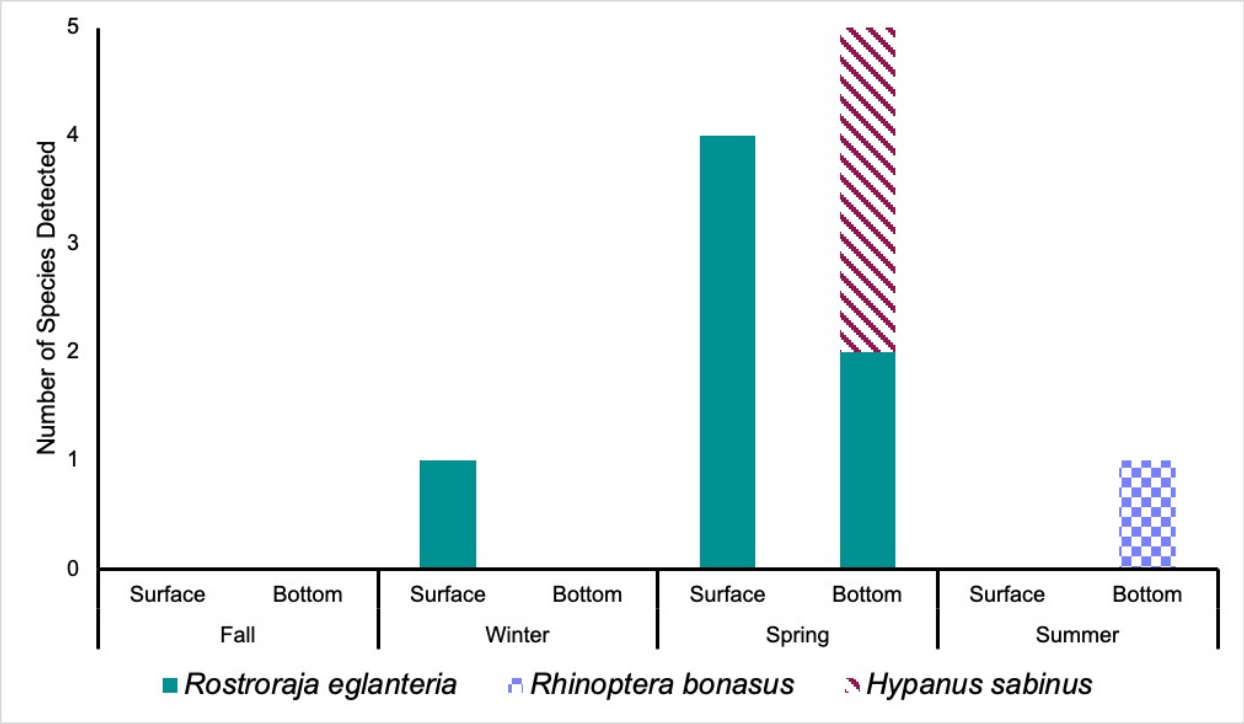

Figure 8: Surface and bottom elasmobranch eDNA detection comparison during each sampling season.

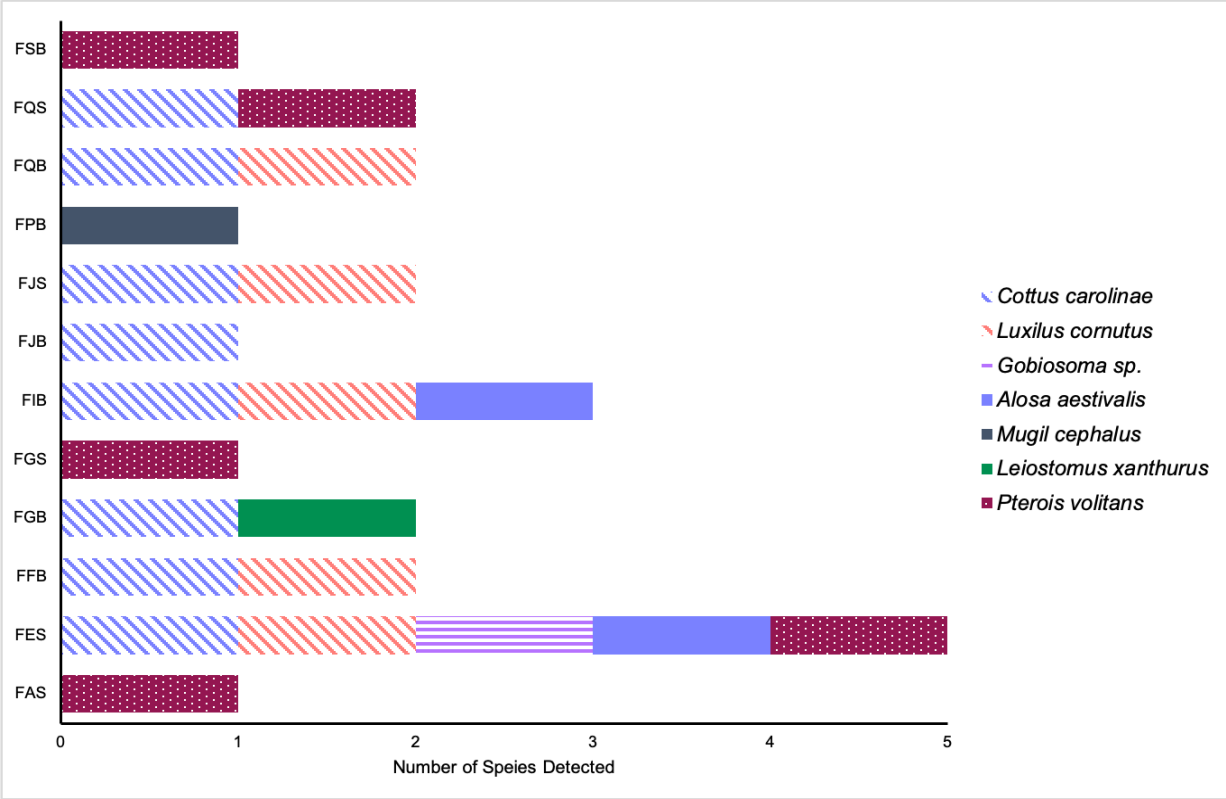

Figure 9: All species detected during fall sampling season. Samples were collected on October 24, October 27, November 2, and December 12 of 2018. Surface sites are indicated by an "S" and bottom sites are indicated by a "B" at the end of each sample name. Freshwater species are indicated by diagonal lines, brackish species are indicated by solid colors, marine species are indicated by horizontal lines, and invasive species are indicated by solid colors with polka dots.

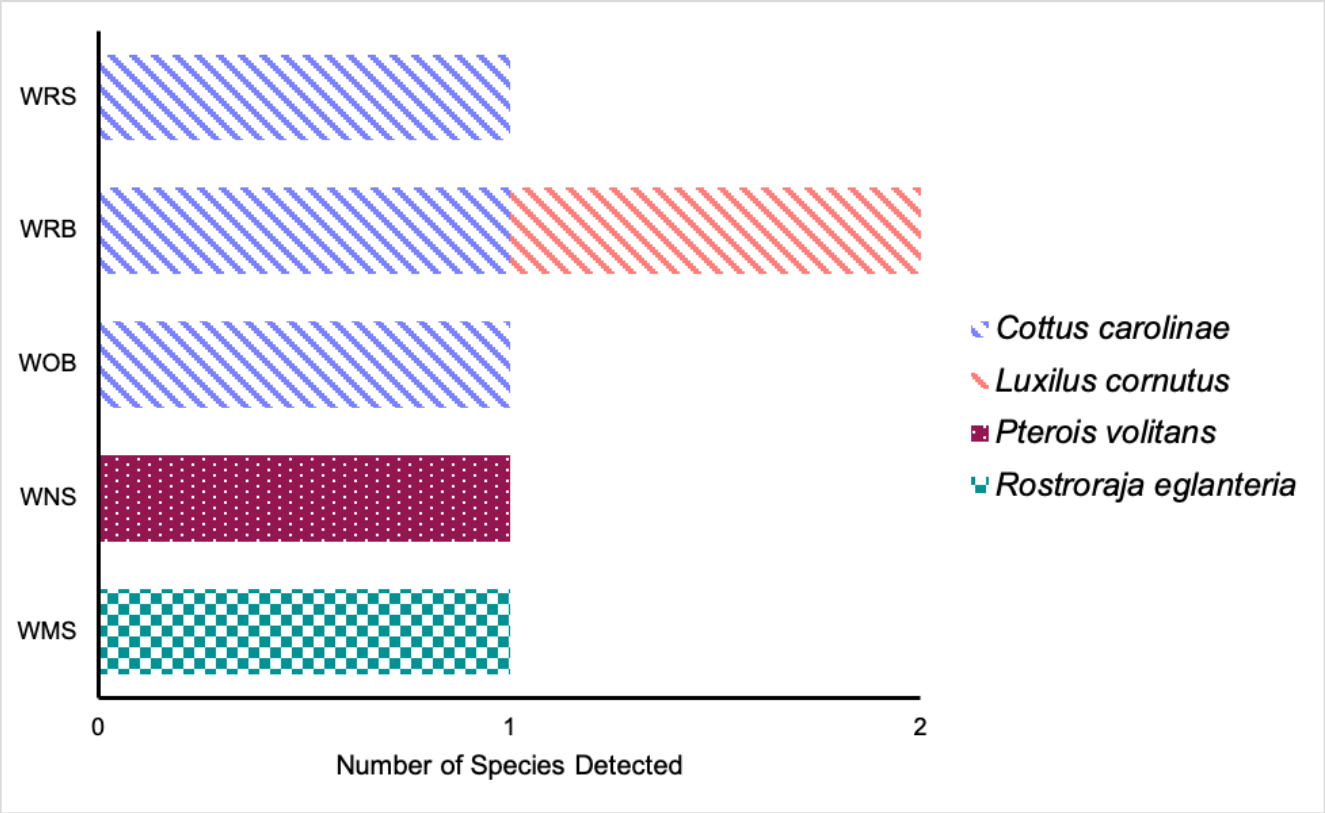

Figure 10: All species detected during winter sampling season. Samples were collected on March 7, March 21, March 30, and April 11 of 2019. Surface sites are indicated by an "S" and bottom sites are indicated by a "B" at the end of each sample name. Freshwater species are indicated by diagonal lines, elasmobranchs are indicated by a checkered pattern, and invasive species are indicated by solid colors with polka dots.

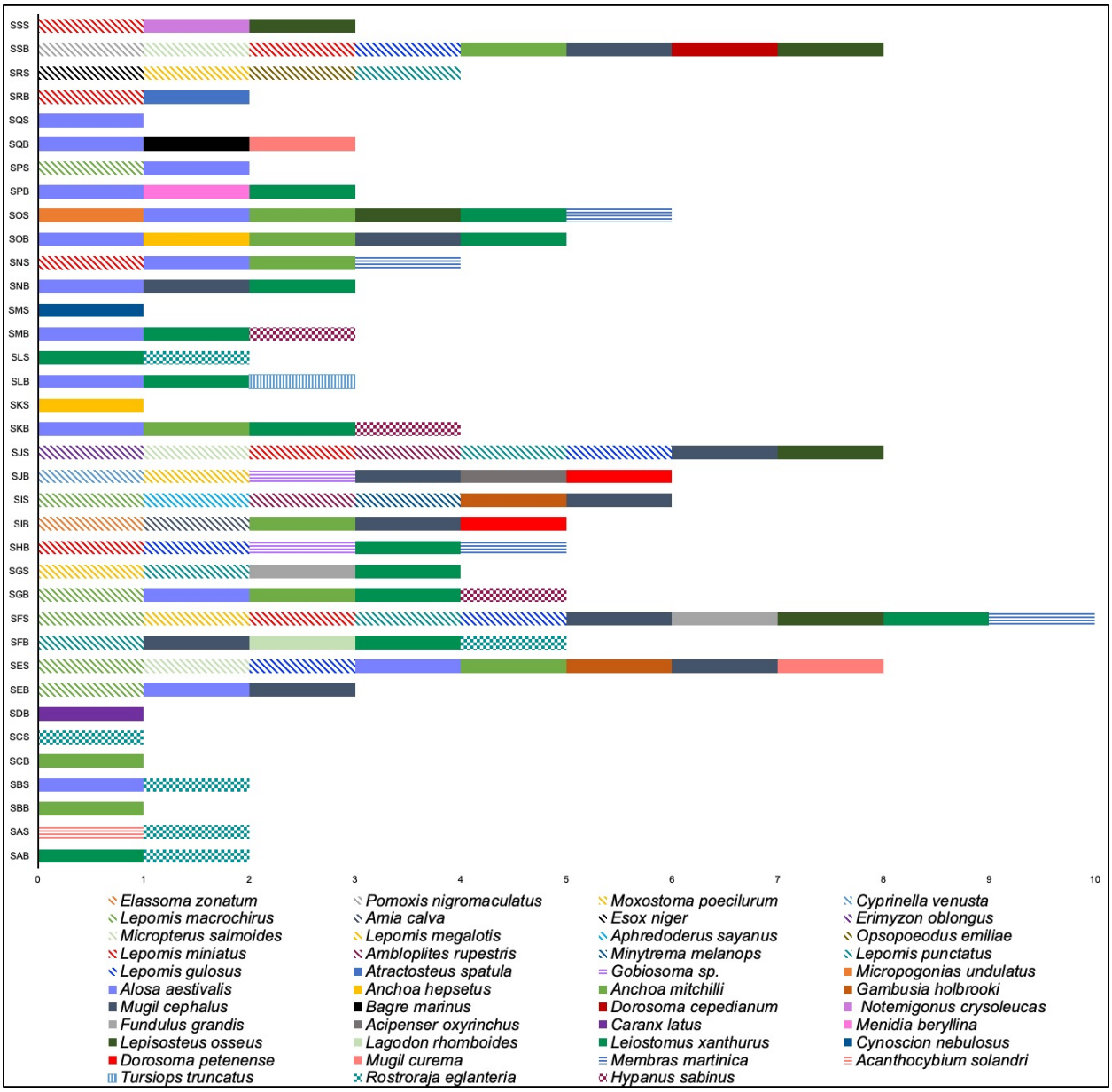

Figure 11: All species detected during spring sampling season. Samples were collected on June 4, June 5, and June 30 of 2019. Due to dangerous water conditions, offshore samples could not be collected during this season. Surface sites are indicated by an "S" and bottom sites are indicated by a "B" at the end of each sample name. Freshwater species are indicated by diagonal lines, brackish species are indicated by solid colors, marine species are indicated by horizontal lines, aquatic non-fish vertebrates are indicated by horizontal lines with a border, and elasmobranchs are indicated by a checkered pattern.

Figure 12: All species detected during summer sampling season. Samples were collected on August 29, September 4, September 18, and September 22 of 2019. Surface sites are indicated by an "S" and bottom sites are indicated by a "B" at the end of each sample name. Freshwater species are indicated by diagonal lines, brackish species are indicated by solid colors, marine species are indicated by horizontal lines, aquatic non-fish vertebrates are indicated by horizontal lines with a border, elasmobranchs are indicated by a checkered pattern, and invasive species are represented by a solid color with white polka dots.

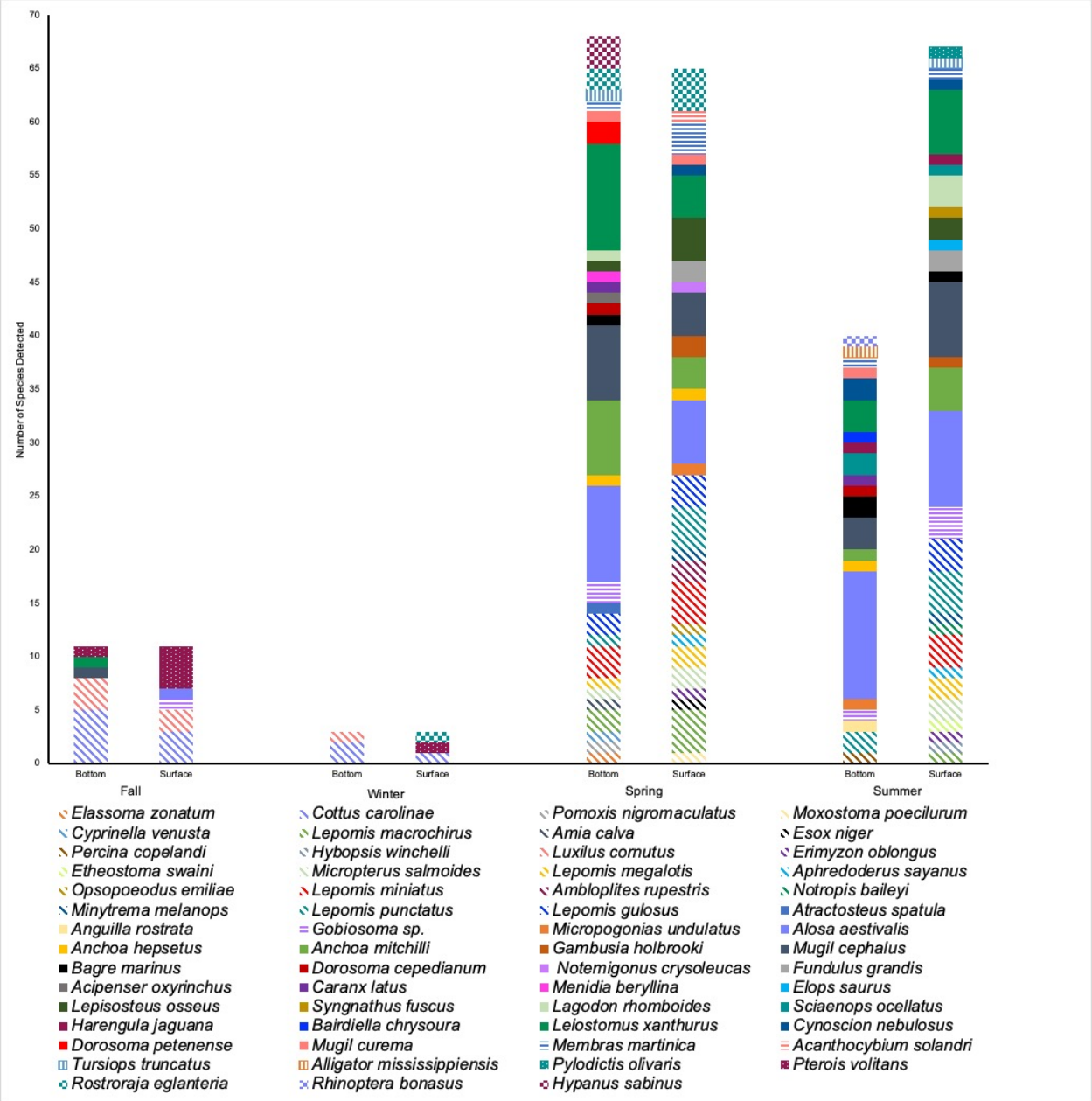

Figure 13: Surface and bottom species detection comparison in each sampling season. Freshwater species are indicated by diagonal lines, brackish species are indicated by solid colors, marine species are indicated by horizontal lines, aquatic non-fish vertebrates are indicated by vertical lines with a border, elasmobranchs are indicated by a checkered pattern, and invasive species are indicated by solid colors with polka dots.

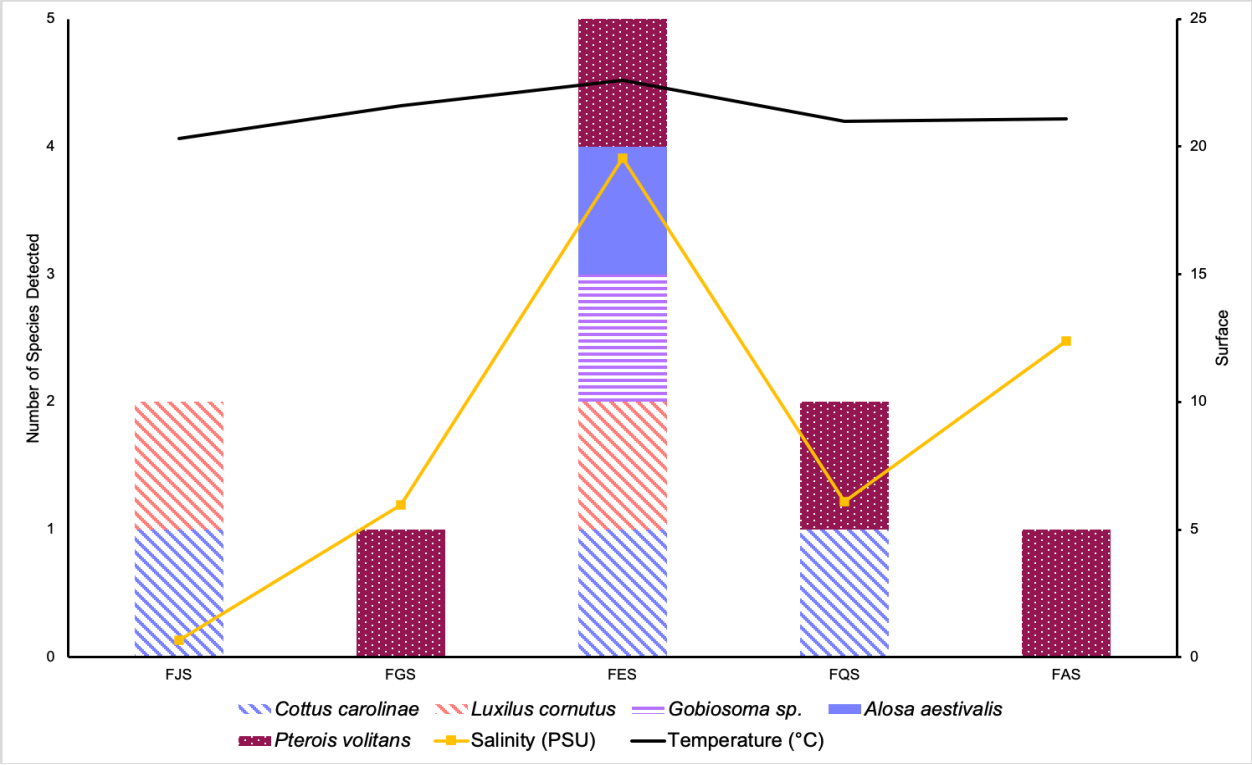

Figure 14: Fall surface species detections plotted against surface salinity and temperature at time of collection. Freshwater species are indicated by diagonal lines, brackish species are indicated by solid colors, marine species are indicated by horizontal lines, and invasive species are indicated by solid colors with polka dots.

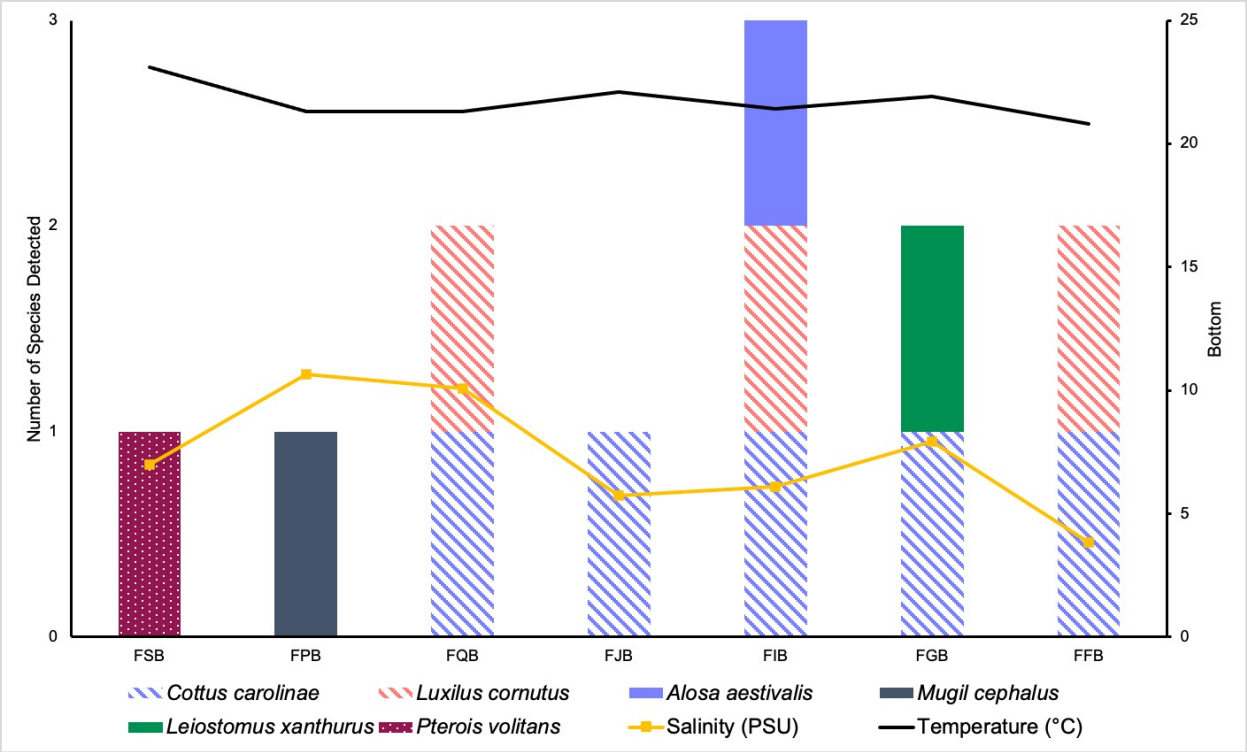

Figure 15: Fall bottom species detections plotted against bottom salinity and temperature at time of collection. Freshwater species are indicated by diagonal lines, brackish species are indicated by solid colors, and invasive species are indicated by solid colors with polka dots.

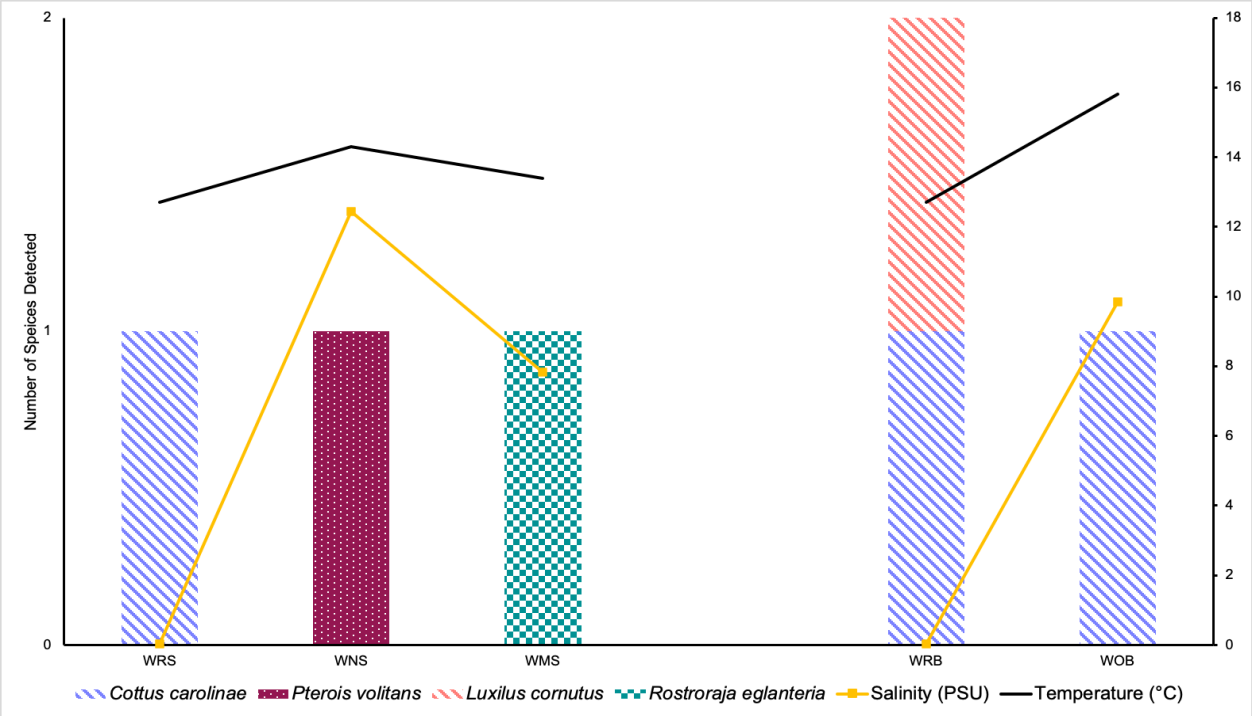

Figure 16: Winter species detections plotted against salinity and temperature at time of collection. Surface sites are indicated by an "S" and bottom sites are indicated by a "B" at the end of the site name. Freshwater species are indicated by diagonal lines, elasmobranchs are indicated by a checkered pattern, and invasive species are indicated by solid colors with polka dots.

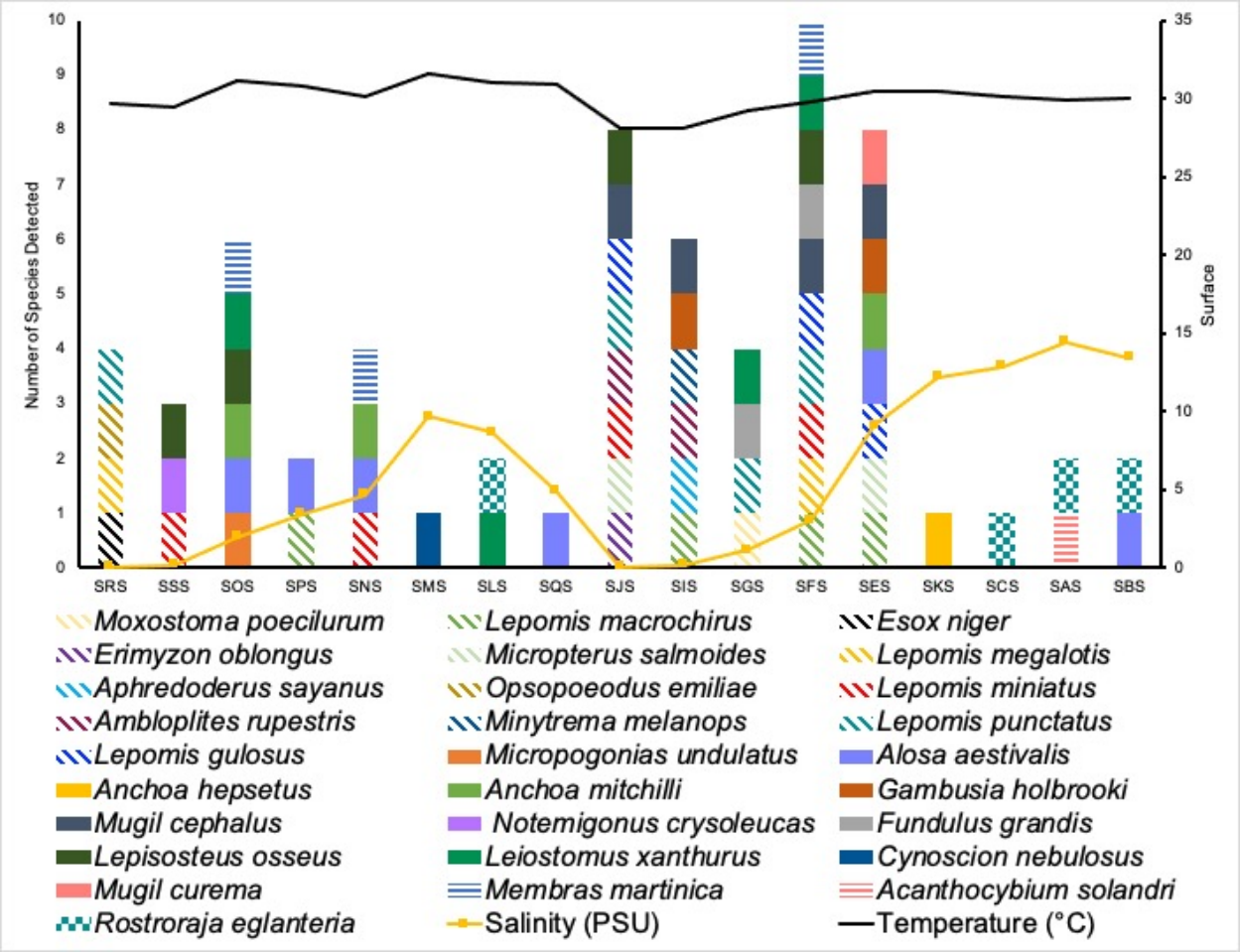

Figure 17: Spring surface species detections plotted against surface salinity and temperature at time of collection. Freshwater species are indicated by diagonal lines, brackish species are indicated by a solid color, marine species are indicated by horizontal lines, and elasmobranchs are indicated by a checkered pattern.

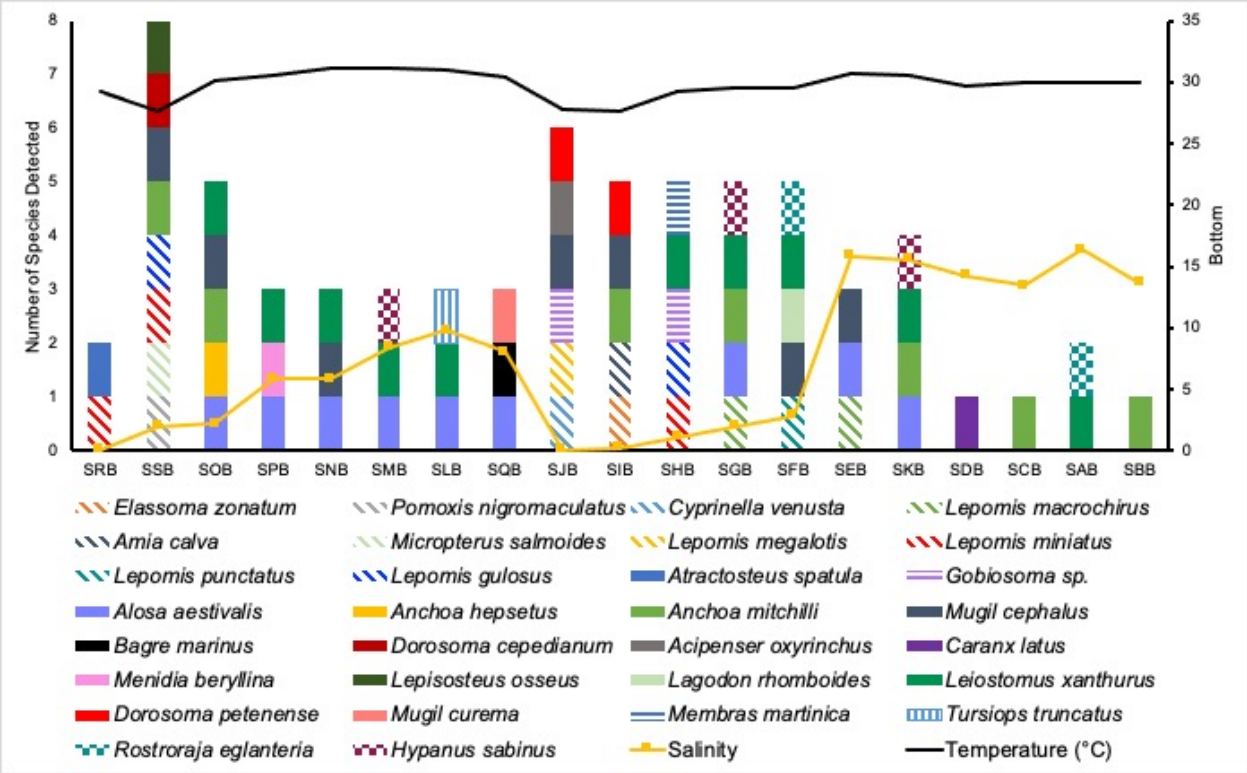

Figure 18: Spring bottom species detections plotted against bottom salinity and temperature at time of collection. Freshwater species are indicated by diagonal lines, brackish species are indicated by a solid color, marine species are indicated by horizontal lines, aquatic non-fish vertebrates are indicated by vertical lines with a border, and elasmobranchs are indicated by a checkered pattern.

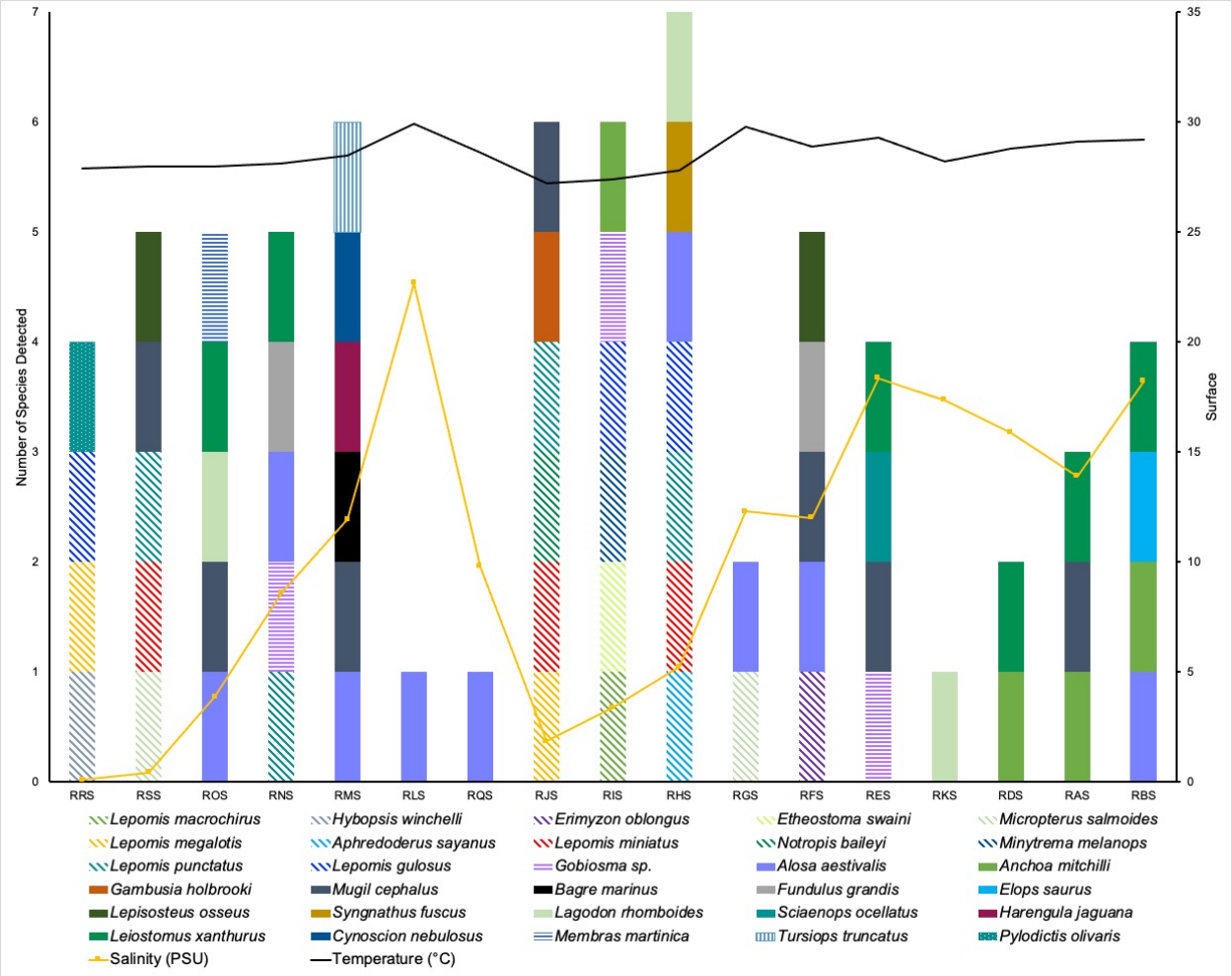

Figure 19: Summer surface species detections plotted against surface salinity and temperature at time of collection. Freshwater species are indicated by diagonal lines, brackish species are indicated by a solid color, marine species are indicated by horizontal lines, aquatic non-fish vertebrates are indicated by vertical lines with a border, and invasive species are indicated by a solid color with white polka dots.

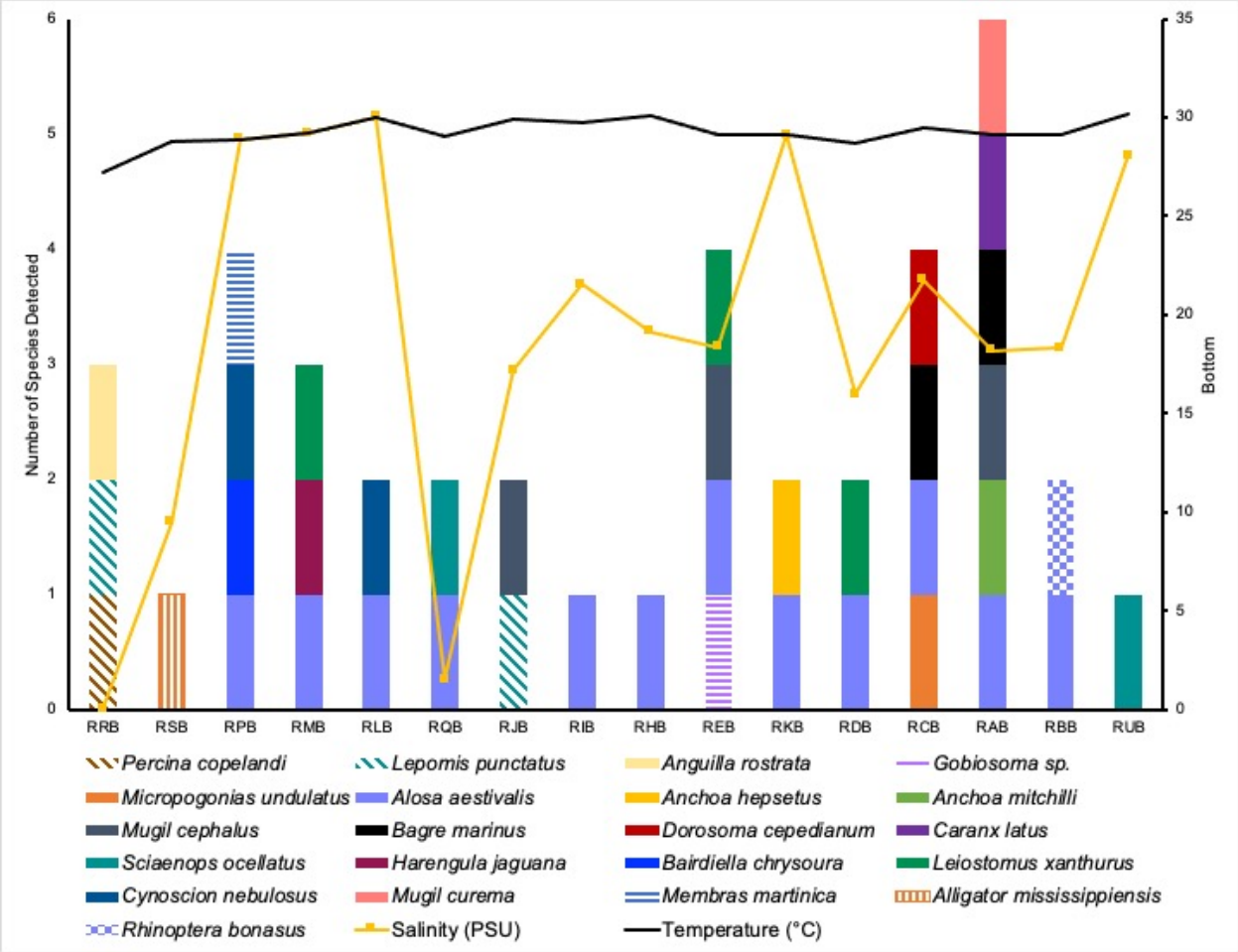

Figure 20: Summer bottom species detections plotted against bottom salinity and temperature at time of collection. Freshwater species are indicated by diagonal lines, brackish species are indicated by a solid color, marine species are indicated by horizontal lines, aquatic non-fish vertebrates are indicated by vertical lines with a border, and elasmobranchs are indicated by a checkered pattern.

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APPENDIX

LIST OF ALL AQUATIC VERTEBRATES DETECTED USING eDNA METABARCODING

Species	Common Name	Sites Detected	Temperature (°C)	Salinitiny (PSU)	IUCN
Acipenser oxyrinchus	Gulf sturgeon	S-JB	27.8	0.07	NT
Amia calva	Bowfin	S-IB	27.7	0.24	LC
Anguilla rostrata	American eel	R-RB	27.2	0.06	EN
Membras martinica	Rough silverside	S-FS	29.8	3.06	LC
		S-HB	29.2	1.12	
		S-NS	30.1	4.66	
		S-OS	31.1	1.98	
		R-OS	28.0	3.85	
		R-PB	28.9	7.52	
Menidia beryllina	Inland silverside	S-PB	30.6	5.83	LC
Alosa aestivalis	Blueback herring	F-ES	22.6	19.54	VU
		F-IB	21.4	6.08	
		S-BS	30.0	13.42	
		S-EB	30.7	15.86	
		S-ES	30.5	9.14	
		S-GB	29.5	1.98	
		S-KB	30.6	15.52	
		S-LB	31.0	9.80	
		S-MB	31.1	8.38	
		S-NB	31.1	5.88	
		S-NS	30.1	4.66	
		S-OB	30.1	2.18	
		S-OS	31.1	1.98	
		S-PB	30.6	5.83	
		S-PS	30.8	3.44	
		S-QB	30.4	8.07	
		S-QS	30.9	4.92	
		R-AB	29.1	18.18	

		R-BB	29.1	18.31	
		R-BS	29.2	18.22	
		R-CB	29.5	21.75	
		R-DB	28.7	15.96	
		R-EB	29.1	18.36	
		R-FS	28.9	11.99	
		R-GS	29.8	12.28	
		R-HB	30.1	19.16	
		R-HS	27.8	5.22	
		R-IB	29.7	21.54	
		R-KB	29.1	20.66	
		R-LB	30.0	23.87	
		R-LS	29.9	22.67	
		R-MB	29.2	21.04	
		R-MS	28.5	11.92	
		R-NS	28.1	8.56	
		R-OS	28.0	3.85	
		R-PB	28.9	7.52	
		R-QB	29.0	16.77	
		R-QS	28.6	9.79	
Dorosoma cepedianum	Gizzard shad	S-SB	27.6	2.00	LC
		R-CB	29.5	21.75	
Dorosoma petenense	Threadfin shad	S-IB	27.7	0.24	LC
		S-JB	27.8	0.07	
Harengula jaguana	Scaled herring	R-MB	29.2	21.04	LC
		R-MS	28.5	11.92	
Anchoa hepsetus	Broad-striped anchovy	S-KS	30.5	12.18	LC
		S-OB	30.1	2.18	
		R-KB	29.1	20.66	
Anchoa mitchilli	Common anchovy	S-BB	29.9	13.64	LC
		S-CB	29.9	13.43	
		S-ES	30.5	9.14	
		S-GB	29.5	1.98	

		S-IB	27.7	0.24	
		S-KB	30.6	15.52	
		S-NS	30.1	4.66	
		S-OB	30.1	2.18	
		S-OS	31.1	1.98	
		S-SB	27.6	2.00	
		R-AB	29.1	18.18	
		R-AS	29.1	13.89	
		R-BS	29.2	18.22	
		R-DS	28.8	15.88	
		R-IS	27.4	3.35	
Erimyzon oblongus	Creek chubsucker	S-JS	28.1	0.06	LC
		R-FS	28.9	11.99	
Minytrema melanops	Spotted sucker	S-IS	28.1	0.17	LC
		R-IS	27.4	3.35	
Moxostoma poecilurum	Blacktail redhorse	S-GS	29.2	1.16	LC
Cyprinella venusta	Blacktail shiner	S-JB	27.8	0.07	LC
Hybopsis winchelli	Clear chub	R-RS	27.9	0.06	LC
Luxilus cornutus	Common shiner	F-ES	22.6	19.54	LC
		F-FB	20.8	3.82	
		F-IB	21.4	6.08	
		F-JS	20.3	0.67	
		F-QB	21.3	10.09	
		W-RB	12.7	0.03	
Notemigonus crysoleucas	Golden shiner	S-SS	29.5	0.19	LC
Notropis baileyi	Rough shiner	R-JS	27.2	1.83	LC
Opsopoeodus emiliae	Pugnose minnow	S-RS	29.7	0.06	LC
Fundulus grandis	Gulf killifish	S-FS	29.8	3.06	LC

		S-GS	29.2	1.16	
		R-FS	28.9	11.99	
		R-NS	28.1	8.56	
Gambusia holbrooki	Eastern mosquitofish	S-ES	30.5	9.14	LC
		S-IS	28.1	0.17	
		R-JS	27.2	1.83	
Elops saurus	Ladyfish	R-BS	29.2	18.22	LC
Esox niger	Chain pickerel	S-RS	29.7	0.06	LC
Atractosteus spatula	Alligator gar	S-RB	29.2	0.06	LC
Lepisosteus osseus	Longnose gar	S-FS	29.8	3.06	LC
		S-JS	28.1	0.06	
		S-OS	31.1	1.98	
		S-SB	27.6	2.00	
		S-SS	29.5	0.19	
		R-FS	28.9	11.99	
		R-SS	28.0	0.41	
Mugil cephalus	Flathead grey mullet	F-PB	22.1	10.65	LC
		S-EB	30.7	15.86	
		S-ES	30.5	9.14	
		S-FB	29.5	2.87	
		S-FS	29.8	3.06	
		S-IB	27.7	0.24	
		S-IS	28.1	0.17	
		S-JB	27.8	0.07	
		S-JS	28.1	0.06	
		S-NB	31.1	5.88	
		S-OB	30.1	2.18	
		S-SB	27.6	2.00	
		R-AB	29.1	18.18	
		R-AS	29.1	13.89	
		R-EB	29.1	18.36	
		R-ES	29.3	18.34	
		R-FS	28.9	11.99	
		R-JB	29.9	17.20	

		R-JS	27.2	1.83	
		R-MS	28.5	11.92	
		R-OS	28.0	3.85	
		R-SS	28.0	0.41	
Mugil curema	White mullet	S-ES	30.5	9.14	LC
		S-QB	30.4	8.07	
		R-AB	29.1	18.18	
Hypanus sabinus	Atlantic stingray	S-GB	29.5	1.98	LC
		S-KB	30.6	15.52	
		S-MB	31.1	8.38	
Rhinoptera bonasus	American Cownose ray	R-BB	29.1	18.31	NT
Caranx latus	Horse-eye jack	S-DB	29.6	14.25	LC
		R-AB	29.1	18.18	
Ambloplites rupestris	Rock bass	S-IS	28.1	0.17	LC
		S-JS	28.1	0.06	
Lepomis gulosus	Warmouth	S-ES	30.5	9.14	LC
		S-FS	29.8	3.06	
		S-HB	29.2	1.12	
		S-JS	28.1	0.06	
		S-SB	27.6	2.00	
		R-HS	27.8	5.22	
		R-IS	27.4	3.35	
		R-RS	27.9	0.06	
Lepomis macrochirus	Bluegill	S-EB	30.7	15.86	LC
		S-ES	30.5	9.14	
		S-FS	29.8	3.06	
		S-GB	29.5	1.98	
		S-IS	28.1	0.17	
		S-PS	30.8	3.44	
		R-IS	27.4	3.35	
Lepomis megalotis	Longear sunfish	S-FS	29.8	3.06	LC

		S-JB	27.8	0.07	
		S-RS	29.7	0.06	
		R-JS	27.2	1.83	
		R-RS	27.9	0.06	
Lepomis miniatus	Redspotted sunfish	S-FS	29.8	3.06	LC
		S-HB	29.2	1.12	
		S-JS	28.1	0.06	
		S-NS	30.1	4.66	
		S-RB	29.2	0.06	
		S-SB	27.6	2.00	
		S-SS	29.5	0.19	
		R-HS	27.8	5.22	
		R-JS	27.2	1.83	
		R-SS	28.0	0.41	
Lepomis punctatus	Spotted sunfish	S-FB	29.5	2.87	LC
		S-FS	29.8	3.06	
		S-GS	29.2	1.16	
		S-JS	28.1	0.06	
		S-RS	29.7	0.06	
		R-HS	27.8	5.22	
		R-JB	29.9	17.20	
		R-JS	27.2	1.83	
		R-NS	28.1	8.56	
		R-RB	27.2	0.06	
		R-SS	28.0	0.41	
Micropterus salmoides	Largemouth bass	S-ES	30.5	9.14	LC
		S-JS	28.1	0.06	
		S-SB	27.6	2.00	
		R-GS	29.8	12.28	
		R-SS	28.0	0.41	
Pomoxis nigromaculatus	Black crappie	S-SB	27.6	2.00	LC
Elassoma zonatum	Banded pygmy sunfish	S-IB	27.7	0.24	LC
Gobiosoma sp.	Gobies	F-ES	22.6	19.54	N/A

		S-HB	29.2	1.12	
		S-JB	27.8	0.07	
		R-EB	29.1	18.36	
		R-ES	29.3	18.34	
		R-IS	27.4	3.35	
		R-NS	28.1	8.56	
Etheostoma swaini	Gulf darter	R-IS	27.4	3.35	LC
Percina copelandi	Channel darter	R-RB	27.2	0.06	LC
Bairdiella chrysoura	Silver perch	R-PB	28.9	7.52	LC
Cynoscion nebulosus	Spotted seatrout	S-MS	31.6	9.67	LC
		R-LB	30.0	23.87	
		R-MS	28.5	11.92	
		R-PB	28.9	7.52	
Leiostomus xanthurus	Spot croaker	F-GB	21.9	7.91	LC
		S-AB	29.9	16.34	
		S-FB	29.5	2.87	
		S-FS	29.8	3.06	
		S-GB	29.5	1.98	
		S-GS	29.2	1.16	
		S-HB	29.2	1.12	
		S-KB	30.6	15.52	
		S-LB	31.0	9.80	
		S-LS	31.0	8.65	
		S-MB	31.1	8.38	
		S-NB	31.1	5.88	
		S-OB	30.1	2.18	
		S-OS	31.1	1.98	
		S-PB	30.6	5.83	
		R-AS	29.1	13.89	
		R-BS	29.2	18.22	
		R-DB	28.7	15.96	
		R-DS	28.8	15.88	
		R-EB	29.1	18.36	

		R-ES	29.3	18.34	
		R-MB	29.2	21.04	
		R-NS	28.1	8.56	
		R-OS	28.0	3.85	
Micropogonias undulatus	Atlantic croaker	S-OS	31.1	1.98	LC
		R-CB	29.5	21.75	
Sciaenops ocellatus	Red drum	R-ES	29.3	18.34	LC
		R-QB	29.0	16.77	
		R-UB			
Acanthocybium solandri	Wahoo	S-AS	29.9	14.45	LC
Lagodon rhomboides	Pinfish	S-FB	29.5	2.87	LC
		R-HS	27.8	5.22	
		R-KS	28.2	17.36	
		R-OS	28.0	3.85	
Aphredoderus sayanus	Pirate perch	S-IS	28.1	0.17	LC
		R-HS	27.8	5.22	
Rostroraja eglanteria	Clearnose skate	W-MS	13.4	7.83	LC
		S-AB	29.9	16.34	
		S-AS	29.9	14.45	
		S-BS	30.0	13.42	
		S-CS	30.1	12.87	
		S-FB	29.5	2.87	
		S-LS	31.0	8.65	
Cottus carolinae	Banded sculpin	F-ES	22.6	19.54	LC
		F-FB	20.8	3.82	
		F-GB	21.9	7.91	
		F-IB	21.4	6.08	
		F-JB	21.3	5.73	
		F-JS	20.3	0.67	
		F-QB	21.3	10.09	
		F-QS	21.0	6.08	

		W-OB	15.8	9.84	
		W-RB	12.7	0.03	
		W-RS	12.7	0.03	
Pterois volitans	Red lionfish	F-AS	21.1	12.39	LC
		F-ES	22.6	19.54	
		F-GS	21.6	5.97	
		F-QS	21.0	6.08	
		F-SB	23.1	6.99	
		W-NS	14.3	12.45	
Bagre marinus	Gafftopsail catfish	S-QB	30.4	8.07	LC
		R-AB	29.1	18.18	
		R-CB	29.5	21.75	
		R-MS	28.5	11.92	
Pylodictis olivaris	Flathead catfish	R-RS	27.9	0.06	LC
Syngnathus fuscus	Northern pipefish	R-HS	27.8	5.22	LC
Tursiops truncatus	Bottlenose dolphin	S-LB	31.0	9.80	LC
		R-MS	28.5	11.92	
Alligator mississippiensis	American alligator	R-SB	28.8	9.50	LC