CHARACTERIZING REEF FISH IN THE GULF OF MEXICO USING ENVIRONMENTAL DNA METABARCODING

by

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ABSTRACT

CHARACTERIZING REEF FISH IN THE GULF OF MEXICO USING ENVIRONMENTAL DNA METABARCODING

Juliana Giraldo-Meneses

A wide range of environmental pressures and anthropogenic stressors, such as overfishing, climate change, and coastal development, have negatively affected fish communities in the Gulf of Mexico, specifically reef fish inhabiting natural or artificial reefs. Fish communities in the Gulf of Mexico vary both spatially and temporally due to movements in the water column, migrations, and environmental pressures making conventional survey sampling challenging to perform. Conventional methods can often be costly, time-consuming and invasive to the target organism. A possible resolution to overcome these challenges to inventory reef fish species lies with environmental DNA (eDNA) metabarcoding. Water samples were collected from artificial and natural reefs in the Gulf of Mexico. The 12s rRNA mitochondrial gene was amplified using elasmobranch and MiFish primers. Only elasmobranch primers were able to provide valuable reads. Amplicon libraries generated by PCR were sequenced using Illumina HiSeq. Environmental DNA metabarcoding revealed 4 reef-associated species out of 12 detected species, the Rough scad (*Trachurus lathami*), Sand diver (*Synodus intermedius*), Pearly razorfish (*Xyrichtys novacula*) and the invasive Lionfish (*Pterois spp.*). Fish species detections across nine sites were grouped by habitat and IUCN status. Surface and benthic fish detections were grouped by temperature and salinity. A generalized linear model and linear regression were used to test for correlation. This project demonstrates the utility of eDNA and metabarcoding as a valuable tool for characterization of reef fish species in the Gulf of Mexico.

CHAPTER I

INTRODUCTION

Reef fish in the Gulf of Mexico

Global biodiversity in the ocean is declining at an alarming rate (Valentini et al., 2016). As a result of human threats, further extensive biodiversity losses are predicted within the next half century, and many species might be lost without our knowledge (Yamamoto et al., 2017). For example, Yamamoto et al. (2017), demonstrated that 20% of fish species still remain to be described. Thus, understanding marine fish diversity is essential and is the first step in making management decisions. Marine ecosystems are in need of conservation and management because these ecosystems are currently facing rapid changes influenced by anthropogenic impacts and global warming (Jiao et al., 2015), leading to alterations in marine communities (Li et al., 2019). Specifically, fish populations are being affected by overfishing, organic pollution, toxic contamination, coastal degradation, climate change, and ocean acidification (Garcia & Rosenberg, 2010).

Effective biodiversity monitoring is crucial for predicting marine ecosystem changes (DiBattista et al., 2019) across multiple spatial and temporal scales (Stat et al., 2017). In order to invest more in conservation policies and better manage natural resources, we need to effectively understand and evaluate biodiversity (Olivier et al., 2018). With biodiversity being threatened by anthropogenic impacts, there is a need to stimulate the use of taxonomic inventories for evaluating global diversity and distribution of marine fish (Mora et al., 2008). Understanding the number of species present, and providing accurate taxonomic identification are key elements to address species interactions, food dynamics and ecosystem functioning (Olds et al., 2016).

Marine fishes are highly diverse vertebrates that help stabilize local community structures and encompass a wide range of ecological roles (Olivier et al., 2018). For example, deep fish vertically transfer nutrients and energy for the offshore community, estuarine fish play a role in sustaining productivity in coastal fisheries, and pelagic fish are the key for large trophic interactions across habitats (Fautin et al., 2010). Further, reef fish herbivores play a role in sediment removal, bioerosion, and algal cover regulation (Edwards et al., 2014), while also providing protein sources for several reef fish predators, which directly regulates fish biomass (Olivier et al., 2018). Understanding the importance of the role of reef fish in marine ecosystems highlights the need for conservation efforts focused on these populations (Faletti et al., 2019).

In the Gulf of Mexico (GoM), reef fish represent a good model for taxonomic analysis and biodiversity indicators and research into spatial patterns of fish biodiversity may suggest candidate sites for protected areas (Olivier et al., 2018). The GoM is a semi-enclosed body of water surrounded by continental landmasses on its three sides. Because of its geographic and oceanographic conditions, the GoM encompasses high organismal biodiversity with a large number of fish species that are constantly changing due to migration patterns, and environmental conditions. Hence, the GoM encompasses a large number of reef-dependent species as well as demersal, pelagic and coastal species that are key elements for ecosystem functioning (Chen, 2017). In the GoM, reef fish populations also support commercial and recreational fisheries (Fujiwara et al., 2019) by providing nutrition, livelihood, and cultural values to coastal communities (Teneva et al., 2018). Thus, GoM reef fisheries contribute globally to food security and are important for human sustainability (Garcia & Rosenberg, 2010). Reef fish play important ecological roles by regulating the top-down controls in the food web. For example, in the eastern GoM, foraging reef fish play a role in transferring energy and nutrients through the food web

(Faletti et al., 2019). Reef fish also serve as major prey for large inshore and offshore fish, seabirds, and marine mammals (Faletti et al., 2019). Specifically, pinfish (*Lagodon rhomboides*) in the GoM transfer nitrogen, from inshore to offshore higher trophic levels (Faletti et al., 2019). Additionally, herbivorous reef fish play a major ecological role in benthic communities because they help regulate the balance between coral and macroalgae growth (Dromard et al., 2015). Reef fish are known to be associated with other non-reef habitats such as seagrass beds, mangrove habitats, open ocean which they use to spawn, nurse and for shelter (Bellwood, 1998).

As reef fish play important ecological roles, they have been the focus of several studies that show fish distributional ranges expanding from warmer-water towards higher latitudes in the Northeastern United States, North Sea, and Mediterranean Sea due to the effects on climate change (Fujiwara et al., 2019). Changes in fish distributions can be associated with environmental variables such as salinity, sea level, dissolved oxygen and temperature in the ocean (Fujiwara et al., 2019). Specifically, in the GoM, the range of many fish species has expanded in recent years across the coast, with drastic changes in species distribution and composition, potentially resulting in introduced species, and alteration of existing fish communities (Fujiwara et al., 2019). Because ecosystems are changing as a result of anthropogenic impacts, and much biodiversity remains undocumented, an effective fish-based assessment tool is necessary to evaluate the current conditions of ecosystems, and improve the ecological quality of degraded or disturbed systems (Li et al., 2019).

Current fish surveys often rely on conventional methods to acquire information about fish populations. These factors can be very destructive and harmful to aquatic ecosystems as they may rely on trawling, electrofishing, gillnetting, or spearfishing (Li et al., 2019). Conventional sampling methods may have low precision and accuracy; specifically, in some cases, low

visibility conditions may prevent fish capture (Fujii et al., 2019), and even species occurring in low densities would be difficult to detect by the use of these methods (Ruppert et al., 2019). Additionally, conventional methods generally are unable to provide information of an entire ecosystem due in part to the small fishes that remain concealed in reefs (Mitsuhiro et al., 2017). Conventional sampling requires taxonomic professional expertise for taxonomic identification during sampling, preventing the collection of multiple species at a rapid time resolution (Ushio et al., 2017). Hence, these methods are often time consuming, and highly costly. Additionally, conventional capture methods may miss detection of cryptic, rare, or even invasive species.

Currently, our knowledge of biodiversity in marine ecosystems is limited due to challenges associated with conventional survey methods (Grey et al., 2018). With ecosystems being altered by anthropogenic impacts and climate change, biodiversity surveys are crucial for understanding these impacts and changes, but may be often difficult to standardize across many taxa and sites (Grey et al., 2018). Thus, continual investigation of marine communities is essential in order to properly manage fisheries and marine resources (Yamamoto et al., 2017). An effective sampling tool is currently needed to improve the accuracy of species detection and monitor fish biodiversity in marine ecosystems (Olds et al., 2016).

Environmental DNA metabarcoding

Environmental DNA (eDNA) metabarcoding with next-generation sequencing is one possible solution for improving species detections for monitoring surveys. This tool can be used as a holistic technique to assess the presence of organisms in the ocean and has been successfully used to determine fish biodiversity assessments over a short period of time (DiBattista et al., 2019). Environmental DNA can originate from feces, scales, gametes, metabolic waste, damaged tissues, or dead individuals containing information about the species identity (Ushio et al., 2018).

Metabarcoding, a multi-specific approach, can be combined with eDNA, providing the ability to evaluate multiple species from a water sample (Ruppert et al., 2019). The utility of eDNA metabarcoding for characterizing biodiversity of marine species serves to establish a baseline to compare past and future samples obtained from marine ecosystems (Sawaya et al., 2019).

Environmental DNA has been utilized since the mid-1980s for detecting microbial communities (Ogram et al., 1987). The first environmental DNA samples occurred during microbiology studies, where DNA was extracted from marine sediments to evaluate microbial communities. In the 1990's, eDNA sampling was used to monitor phytoplankton blooms and evaluate bacterial communities (Diaz-Ferguson et al., 2014). Similar methods were then applied for eukaryotes to assess fecal contamination in aquatic ecosystems (Layton et al., 2006). In 2008, French researchers determined the presence of invasive species (*Rana catesbiana*) from water samples in an aquatic system (Diaz-Ferguson et al., 2014). Foote et al. (2012), conducted the first eDNA study in the marine environment for detecting marine mammals and assessing marine fish biodiversity.

The recently developed approaches of high-throughput sequencing technology in eDNA metabarcoding are novel powerful approaches for fish assessments. Specifically, DNA can be extracted from small volumes of water, amplified using universal PCR primers, and then sequenced with next-generation sequencing to generate sample reads (Ruppert et al., 2019). The outputs are then subjected to the HiSeq paired-end sequencing followed by standard nucleotide blast searches using Genbank (https://www.ncbi.nlm.nih.gov/genbank/) for taxonomic assignments (Miya et al., 2015). For effective metabarcoding of water samples containing fish eDNA, a set of universal primers can be used for amplifying mitochondrial target regions (Sato et al., 2018), the MiFish primers (Miya et al., 2015) and elasmobranch primers (Taberlet et al.,

2018). These primers are targeted to amplify a hypervariable region of the mitochondrial 12S rRNA gene (163–185 bp), surrounded by two highly conservative regions (Miya et al., 2015; Taberlet et al., 2018). Target regions are often amplified from degraded eDNA material using universal primers and high throughput sequencing technologies (Yamamoto et al., 2017). MiFish are universal for the majority of bony fish species in the world (Miya et al., 2020), and thus used for global fish biodiversity assessments (Ruppert et al., 2019) and for detecting local fish fauna (Mitsuhiro et al., 2017; Miya et al., 2020). Elasmobranch primers are universal for cartilaginous fish and may also amplify bony fish (Miya et al., 2015; Taberlet et al., 2018). Nonetheless, these primers can identify fishes to their genus and species level, but may also limit the ability to distinguish closely related congeners (Miya et al., 2015). With these advancements, eDNA metabarcoding in aquatic ecosystems is becoming an increasingly useful approach for characterizing fish communities (Yamamoto et al., 2017) and providing local fish inventories (Mitsuhiro et al., 2017).

Environmental DNA, as a non-invasive genetic monitoring tool (Miya et al., 2015), has the ability to minimize disturbances to ecosystems, and optimize conservation measures in fish habitats (Ruppert et al., 2019). Further, eDNA metabarcoding can be employed with and complementary to conventional methods. When the objective of the study is to characterize and taxonomically identify species, eDNA is the most efficient technique to use. However, when the information needed is related to the sample age, size, sex and other parameters, then traditional sampling methods should be applied.

Moreover, eDNA metabarcoding has the ability to evaluate and quickly monitor large numbers of sites for the presence of invasive, endangered, and rare species (Lacoursière-Roussel et al., 2016). This is particularly important, because the recognition of critically endangered

species at risk of extinction is highly needed due to biodiversity losses occurring at rapid rates (Minamoto et al., 2012). Additionally, eDNA sampling methods can often provide complementary information about cryptic and rare species, and are especially powerful when combined with conventional sampling methods (Miya et al., 2015).

One of the main challenges associated with eDNA methods is the risk of obtaining PCR biases that derive from primer and template mismatches (Miya et al., 2015). Another challenge is the sample contamination during water collection, sample extraction and amplification protocols leading to taxonomic misidentifications. Thus, taking precautions during the field and laboratory assays are essential to minimize errors (Valentini et al., 2016). To minimize contamination, as well as PCR primer and template biases, optimization with sample collection and extraction procedures, and proper bioinformatics tools are necessary (Ruppert et al., 2019).

Understanding, environmental conditions, duration and persistence of eDNA in the environment is essential for determining species presence at that particular time and location (Collins et al., 2018). As in some cases with conventional sampling, species that are present may not be detected due to their low density at that particular location (false negatives). DNA can be transported from one location to other locations providing potentially false positives, when species are truly absent in that particular location (Collins et al., 2018). Contrastingly, even small fragments of DNA, which are typically used for eDNA studies, have been shown to degrade beyond detectability in seawater samples within a few days (Thomsen et al., 2012). Additionally, differences in chemical composition, pH, temperature, and amount of DNA released by individuals play a role with eDNA detection in aquatic systems (Collins et al., 2018). Environmental DNA degradation in seawater is known to be 1.6x faster in the inshore than offshore environments due to potential freshwater inputs and lower pH**,** though no evidence for

seasonal differences in degradation rates has been found yet. Estimated rates for most marine eDNA decay vary between 10 and 50 hours and the lowest decay rates are associated with colder temperatures (Collins et al., 2018). Hence, eDNA can be recovered up to 10 kilometers from the original source location and may persist in the environment for several days (Deiner & Altermatt, 2014). However, some eDNA samples may last for at least two weeks (Lacoursière-Roussel et al., 2016). The recognition of biological and environmental factors that may impact eDNA surveys are crucial when interpreting results and for comparing species richness (Rees et al., 2015).

Objectives/Hypothesis

The objective of this study was to use eDNA metabarcoding to characterize reef fish communities in the GoM, specifically cryptic, rare and elusive species for efficient, precise, and standardized monitoring of marine fish biodiversity in the GoM. Although eDNA metabarcoding has been used successfully in other vertebrate applications, to my knowledge the eDNA evidence presented in this study is the first use of this technique for reef fish identification in the GoM.

I hypothesized that reef fish and non-reef fish species present across sampling sites in the GoM would be detected using eDNA metabarcoding. Surface and benthic fish detections would remain consistent between sites. I expected more taxonomic variation of reef fish species between habitat types than within habitat types. For instance, a previous study determined that 92.2% of variance occurred due to between site differences and the remaining percentage belonged to the within habitat variances (Port et al., 2016). Additionally, this molecular tool would likely detect the majority of cryptic and/or invasive species present throughout collection sites. Environmental DNA metabarcoding would determine if the reef fish species present are

consistent across the GoM, and if this presence varies in response to habitat, temperature and salinity.

CHAPTER II

METHODS

Collection

Environmental DNA samples were collected on a NOAA Reef Fish Video (SRFV) survey cruise in May and July 2019. Surface and benthic water samples were collected from predetermined, and randomized sites in the GoM. Sampling locations included the following reefs: DeSoto Canyon Area, West Florida Shelf, Madison-Swanson, The Edges, Florida Middle Ground, Christmas Tree Ridge, Pulley Ridge, Riley's Hump and Dry Tortugas (Figure 1**)**. Prior to sample collection, abiotic data were collected for temperature (°C), depth, and salinity. Collection protocol incorporated application of a 3M sodium acetate buffer to the water samples (Ficetola et al., 2008; Thomsen et al., 2012). Water was collected at each site by filling three 50-mL Falcon tubes containing 1.5 mL of pre-prepared sodium acetate buffer solution with 15 mL of surface water and 33.5 mL of 95% ethanol. Each 15 mL water sample was collected with a sterile 15 ml conical tube and poured into a 50 ml conical tube containing 1.5 ml of 3 M sodium acetate and 33.5ml of ethanol. Upon collection samples were placed in plastic bags and stored in a cooler at room temperature until extraction. A field control tube was placed in each cooler to ensure no contamination at the sampling site. Water samples were transported to and analyzed in the Janosik Lab at the University of West Florida.

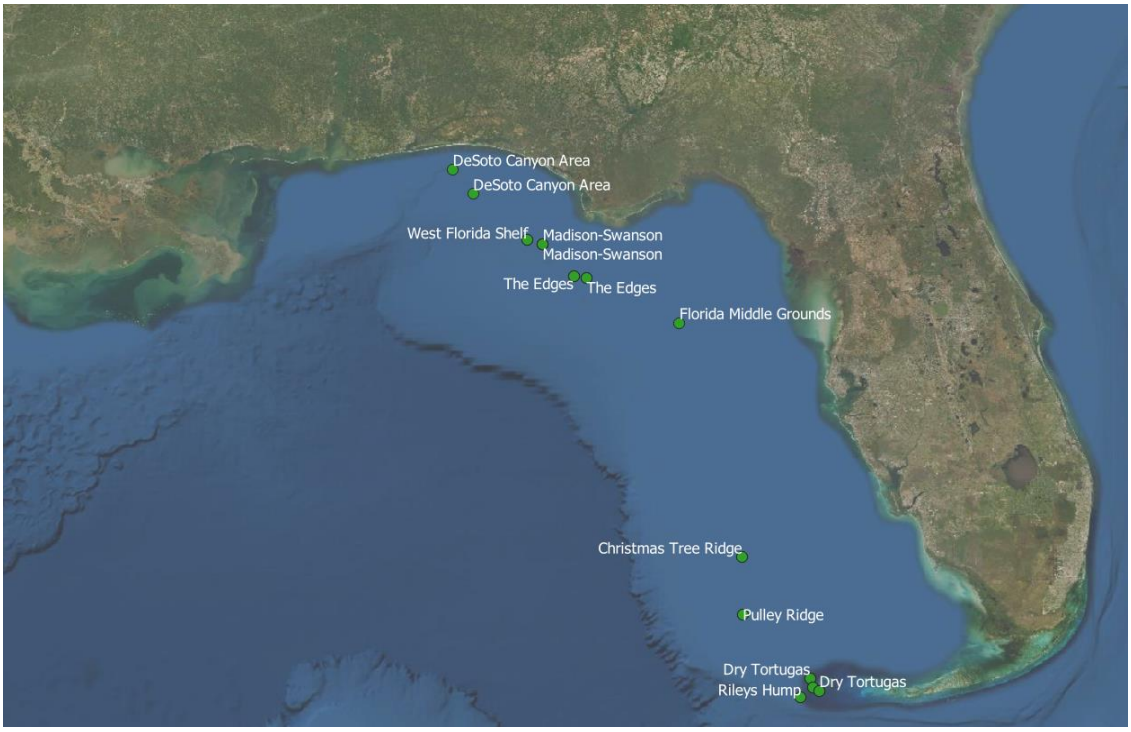

Figure 1. Satellite map of the sampling locations collected aboard the NOAA research vessel in the Gulf of Mexico. Surface and benthic samples were collected in May and July 2019.

DNA extraction

Precipitated water samples were placed in a -20℃ for cellular debris pellet formation, and then centrifuged for 30 minutes at 3500xg. Supernatant for each sample was discarded, cellular debris pellets were removed, and replicates were pooled (Hebert, 2021; Sigsgaard et al., 2015). DNA extraction was performed using the DNeasy Blood & Tissue kit (Qiagen ®, Hilden, Germany). An extraction control was used for each set of extractions to ensure no contamination occurred during the extraction step (Hebert, 2021).

PCR Analysis and Library Preparation

Samples were subjected to polymerase chain reaction (PCR) for amplification with the use of two universal primers sets, specifically fish (MiFish) primers to target the mitochondrial 12S rRNA gene (163-185bp) (Miya et al., 2015). PCR reactions were carried out as in Miya et al.

(2015), with 12 µl reactions with a small modification in the annealing temperatures and annealing time. A total of nineteen eDNA samples contained the following reaction: 6.0 µl 2x KAPA HiFi HotStart ReadyMix, 0.7 µl 5µM forward primer (5′-ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT NNN NNN GTC GGT AAA ACT CGT GCC AGC -3), 0.7 µl 5µM reverse primer (5′-GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC TNN NNN NCA TAG TGG GGT ATC TAA TCC CAG TTT G-3'), 2.0 µl of eDNA template, and 2.6 µl sterile distilled H_2O. Five samples were prepared using 0.3 µl BSA and 2.3 µl sterile distilled instead of 2.6 µl. For successful amplification and to adjust for DNA inhibition in the sample a PCR inhibition kit buffer was employed for these five samples. The nineteen samples were amplified using the following thermal cycling profile: 95℃ for 3 min; 35 cycles 98℃ for 20 s, 57.5℃ for 30s, and 72℃ for 15s. A final extension step was employed at 72℃ for 5 minutes. The thermal cycler profile for the remaining five samples was modified to 58 ℃ annealing, and 40 cycles.

Additionally, elasmobranch primers were used to amplify a section of the 12S mitochondrial rRNA (170-185bp) (Taberlet et al., 2018). The primers consisted of an elasmobranch specific forward (Elas02_F_NX): (5’- TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG GTT GGT HAA TCT CGT GCC AGC-3’) and an elasmobranch specific reverse (Elas02_R_NX): (5’- GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GCA TAG TAG GGT ATC TAA TCC TAG TTT G-3’) (Hebert, 2021; Taberlet et al., 2018). The reaction mixture included 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. Samples were amplified using the following thermal cycler profile: 95˚C for 15 minutes, 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; Hebert, 2021). Thirty-two elasmo samples were sent for sequencing. Negative controls were performed for each PCR performed to ensure no contamination occurred during the process. All PCR products were visualized on a 2% agarose via gel electrophoresis. Samples were optimized and amplification steps in the thermal cycler were modified until each band was clearly visible on the gel. Upon visualization, each DNA sample was quantified on a qubit reader. Libraries were normalized using the SequalPrep Normalization Plate Kit (Invitrogen, Life Technologies), quantified using qPCR and the KAPA Illumina Library Quantification Kit (Hebert, 2021), and run on an Illumina HiSeq platform at Hubbard Center for Genomics, Sequencing Core Facility (Durham, NH).

Bioinformatics Analysis

A total of 6,464,619 reads were provided from Illumina HiSeq which were then filtered, denoised, and duplicated. Paired-end reads were merged and chimeras were filtered out using QIIME 2 (Caporaso et al., 2010) and QIIME 2 plugin DADA2 (Callahan et al., 2016; Hebert, 2021), resulting in 2,355,700 final reads. Reads were truncated at 190 and taxonomic assignments were performed using QIIME2 and nucleotide BLAST (Johnson et al., 2008). Sequences were assigned to the top BLAST hit species if the top-hit was ≥ 90%. Taxonomic assignments were modified due to closely related species being indistinguishable using the 12S rRNA gene. Sequences that did not hit the database were discarded (Hebert, 2021).

eDNA fish community Analysis

Fish species detections were listed, presented and compared across collection sites. Fish species detections were grouped by habitat type. Surface and bottom species richness was grouped by temperature, salinity, location (latitude and longitude) and tested for correlation. Non-target species (e.g., sponges, corals, etc.) were analyzed further. Both target and non-target

species were grouped based on their International Union for Conservation of Nature (IUCN) status and richness.

Statistical Analysis

Benthic and surface species richness detections among sites were tested for correlation with temperature, salinity, latitude and longitude using a generalized linear model (glm) with a Poisson distribution and a linear regression analysis. A single-factor ANOVA was used to test mean (richness) similarity among sites and to test for significant differences between reef fish associates and pelagic fish detections from surface and benthic samples.

CHAPTER III

RESULTS

Environmental DNA metabarcoding revealed a total of 12 fish taxa belonging to 11 families across the nine sampling sites and all fish taxa were identified to the species level (Table 1). Of these fish detections, the majority were marine, some were coastal or brackish, and some were associated with pelagic, demersal (benthic) or reef environments (Table 1). Four reef associated species were detected from different sites: Rough scad, *Trachurus lathami* (DeSoto Canyon Area), Sand diver, *Synodus intermedius* (DeSoto Canyon Area), Pearly razorfish, *Xyrichtys novacula* (DeSoto Canyon Area), and invasive lionfish, *Pterois miles* (DeSoto Canyon Area, West Florida Shelf, Christmas Tree Ridge, and Dry Tortugas). Other interesting detections were for the Duckbill eel (*Nessorhamphus ingolfianus*) in Dry Tortugas and Florida Middle Ground, the Snake eel (*Apterichtus moseri*) in the West Florida Shelf, and the freshwater Ironcolor shiner (*Notropis chalybaeus*) detected in Dry Tortugas. The Bay anchovy (*Anchoa mitchilli*), the Gulf killifish (*Fundulus grandis*), and the Rough silverside (*Membras martinica*) were the most detected fish species and were detected in seven of nine sites (Table 1, Figure 2). Bay anchovy was detected in the DeSoto Canyon Area, West Florida Shelf, Madison-swanson, Edges, Pulley Ridge, Riley's Hump and Dry Tortugas. Gulf killifish was detected in DeSoto Canyon Area, West Florida Shelf, Madison-swanson, Florida Middle Ground, Pulley Ridge, Riley's Hump, and Dry tortugas. Rough silverside was detected in the DeSoto Canyon Area, West Florida Shelf, Madison-Swanson, Florida Middle Ground, Edges, Christmas Tree Ridge, and Dry Tortugas.

The number of species detected varied across sites (Fig. 2). The DeSoto Canyon Area (n=8) along with the West Florida Shelf (n=8) had the highest number of fish detections. Dry Tortugas

had the second highest number of detections (n=7) followed by Florida Middle Grounds (n=4), Madison-Swanson (n=4), Pulley Ridge (n=3), Riley's Hump (n=3), Edges (n=2), and Christmas Tree Ridge (n=2).

The West Florida Shelf (n=6), DeSoto Canyon Area (n=6), and Dry Tortugas (n=6) had the highest number of surface fish species detections, followed by Florida Middle Ground (n=2), Pulley Ridge (n=2), and Madison-Swanson (n=1) (Table 2). Similarly, West Florida Shelf (n=5), DeSoto Canyon Area (n=5), and Dry Tortugas (n=5) and the highest number of benthic fish species detections followed by Florida Middle Ground (n=3), Madison-Swanson (n=3), Pulley Ridge (n=1) (Table 3). Surface and benthic richness of fish species were directly correlated to each other (p=0.03), and their means were statistically the same and non-significant (ANOVA, F-stat= 0.04, p=0.83). Specifically, the sites with the highest number of surface detections (n=6) such as DeSoto Canyon Area and West Florida Shelf, also had the highest number of benthic (n=6) detections among the seven other sites. This correlation was not only due to overlapping species (the same species at both benthic and surface), but also due to the presence of only benthic or surface fishes at each site (Fig. 4).

In addition to fish species richness comparisons, significant differences between reef fish associates and pelagic fish detections from surface and benthic samples was examined. No significant differences (ANOVA, F-stats=1.2, p=0.3) were found when comparing benthic and surface reef fish detections across sites, and no significant differences (ANOVA, F-stats= 0, p=1) were found between benthic and surface pelagic fish detections. Therefore, neither benthic samples had significantly more reef fish species than pelagic, nor surface samples had significantly more pelagic detections than reef fish.

Fish species were also analyzed for relationships with temperature and salinity across sites (Fig. 3, A, B). No significant difference was found ($p=0.8$ and $p=0.4$) when benthic species richness was compared to temperature and salinity, suggesting that the number of detected species (richness) in benthic sites are not directly correlated to these abiotic factors. Additionally, no significant difference was found ($p=0.2$ and $p=0.1$) between surface species richness and temperature and salinity, suggesting that the number of detected species (richness) in surface sites is not directly correlated with these two factors. Species were also grouped by latitude and longitude at each site (Table 5) and tested for correlation. The number of detected species (richness) in both benthic and surface sites was not directly correlated with latitude or longitude ($p=0.5, 0.3$). Therefore, benthic and surface richness were not dependent on temperature, salinity, latitude and longitude.

In addition to the detected fish, 28 non-target species were detected across the sampling sites (Table 6). Interestingly, there were no elasmobranch detections using the elasmobranch primers, but other reef builders such as corals and sponges were revealed. The Leathery Barrel sponge (*Geodia neptuni*), the Giant Barrel sponge (*Xestospongia muta*), the Erect Rope sponge (*Amphimedon compressa*), the Azure Vase sponge (*Callyspongia plicifera*), the encrusting sponge (*Halisarca harmelin*) and the soft coral (*Leptogorgia capverdensis*) were detected at the benthic site in Dry Tortugas. In the DeSoto Canyon Area, the hydrozoan (*Liriope tetraphylla*) was the only surface non-fish species detected. Additionally, the coastal bird, the Least tern (*Sternula antillarum*), was detected at the surface site West Florida Shelf site (Table 7, Figure 5). Other non-target species such as algae, fungi, and bacteria are listed in Table 6. Overall, non-target species were detected more frequently in benthic sites than in surface sites. Similarly, to fish species detections, surface and benthic richness of non-fish species are directly correlated to

each other (p=0.001), and not correlated to temperature (p=0.2), salinity (p=0.2), latitude (p=0.4), longitude(p=0.4). All detected species (target and non-target fish species) are illustrated in Figure 6.

Table 1. Fish species detected by eDNA metabarcoding grouped by habitat.

Common name	Species	Habitat	IUCN	N detections
Ophichthidae (Family)	*Apterichtus moseri*	marine; demersal over sandy bottoms	NE	1
Duckbill oceanic eel	*Nessorhamphus ingolfianus*	marine; bathy/mesopelagic	NE	2
Atlantic menhaden	*Brevoortia tyrannus*	coastal; pelagic	LC	2
Bay anchovy	*Anchoa mitchilli*	marine; pelagic-neritic	LC	7
Broad-striped anchovy	*Anchoa hepsetus*	marine; pelagic-neritic	LC	6
Rough scad	*Trachurus lathami*	marine; reef-associated	LC	1
Sand diver	*Synodus intermedius*	marine; reef-associated	LC	1
Spot	*Leiostomus xanthurus*	coastal; demersal over sandy bottoms	LC	1
Rough silverside	*Membras martinica*	marine; pelagic-neritic	LC	7
Lionfish	*Pterois miles*	marine; reef-associated	LC	4
Pearly Razorfish	*Xyrichtys novacula*	marine; reef-associated	LC	1
Gulf Killifish	*Fundulus grandis*	brackish; benthopelagic	LC	7

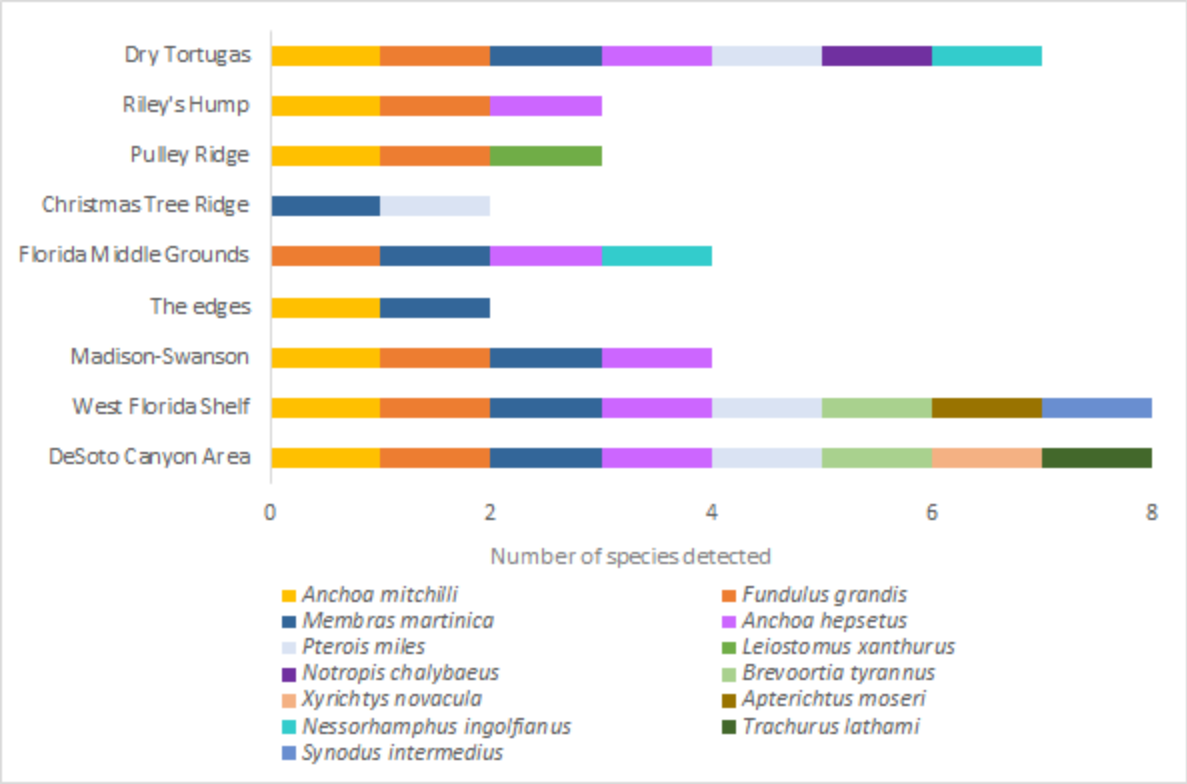

Figure 2. Number of fish species detected at each site.

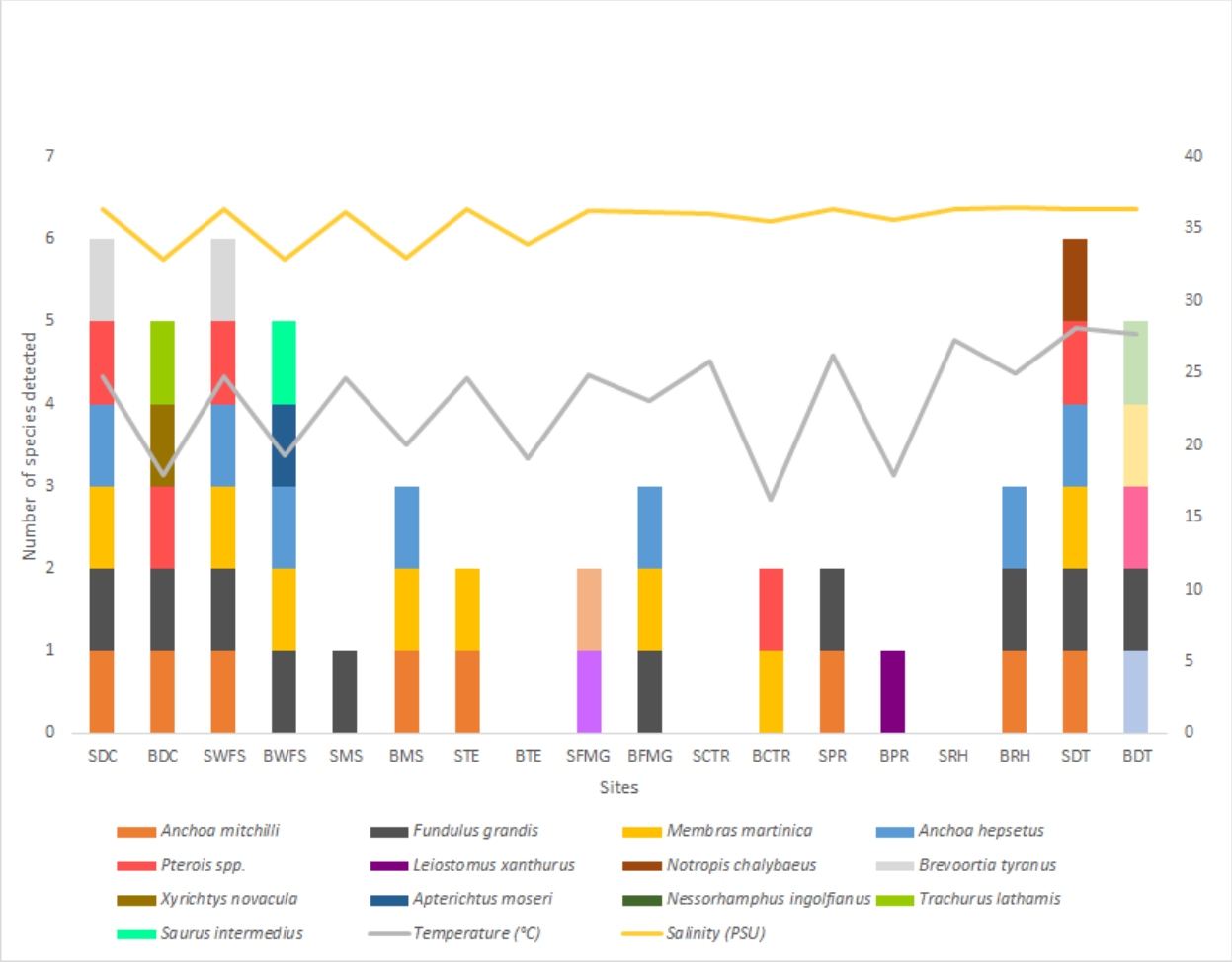

Figure 3. Bottom and surface detected species across sampling sites. This graph also depicts salinity and temperature of the water samples upon collection in each site. Surface sites are indicated by an "S" and benthic sites are indicated by a "B." DeSoto Canyon Area is indicated by the abbreviation "DC." The West Florida Shelf and Florida Middle ground sites are indicated by the abbreviation "WFS" and "FMG" respectively. The Madison-Swanson and Christmas Tree Ridge are indicated by the abbreviation "MS" and "CTR" respectively. The Edges is indicated by the abbreviation "TE." Pulley Ridge, Riley's Hump and Dry Tortugas are indicated by the abbreviation "PR", "RH", and "DT" respectively.

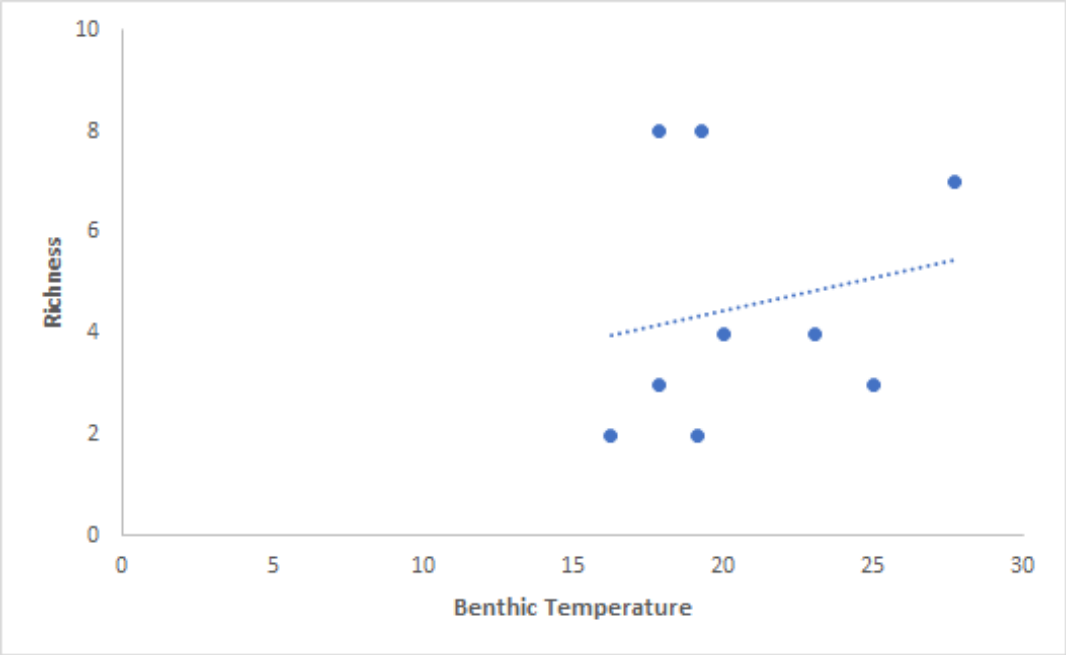

Figure 3. (A) Total number of fish species detections grouped by benthic temperature.

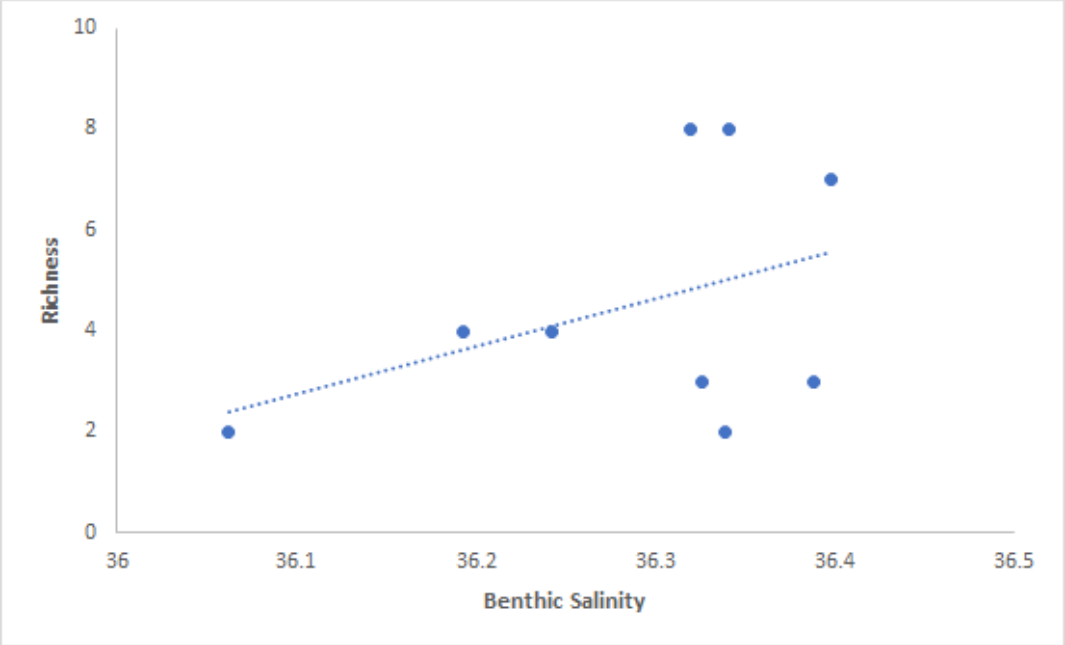

Figure 3. (B) Total number of fish species detections grouped by benthic salinity.

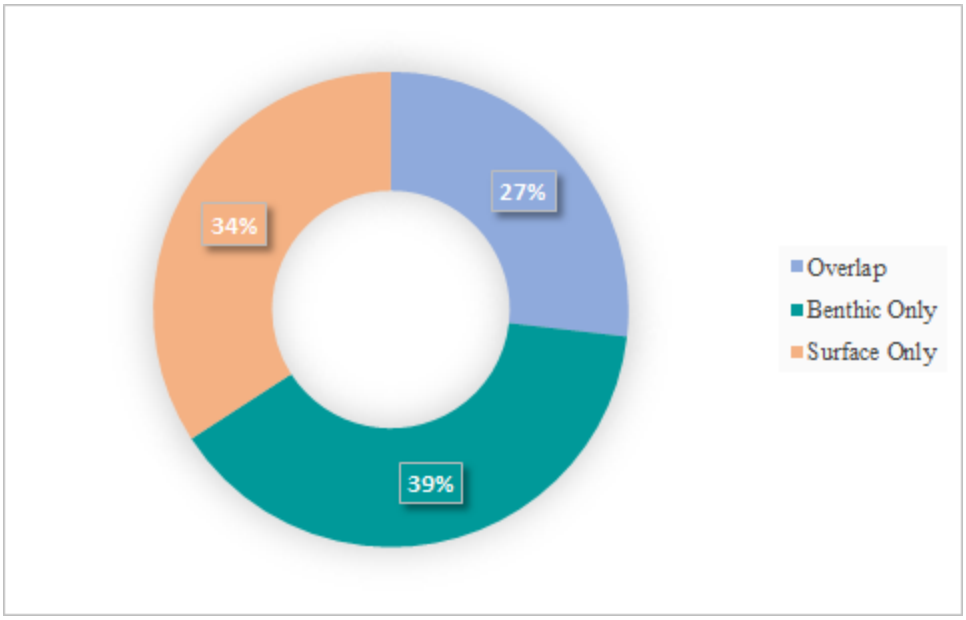

Figure 4. Percent of total number of fish detections across all sites according to benthic and surface species detections.

Table 2. List of surface fish species grouped by temperature and salinity. Species in "BOLD" were also detected in benthic sites.

Site	Species	Temperature (°C)	Salinity (PSU)
West Florida Shelf	*Anchoa mitchilli*	24.784	32.876
West Florida Shelf	*Pterois miles*	24.784	32.876
West Florida Shelf	*Brevoortia tyrannus*	24.784	32.876
West Florida Shelf	***Fundulus grandis***	24.784	32.876
West Florida Shelf	***Membras martinica***	24.784	32.876
West Florida Shelf	***Anchoa hepsetus***	24.784	32.876
DeSoto Canyon	*Membras martinica*	24.784	32.876
DeSoto Canyon	*Anchoa hepsetus*	24.784	32.876
DeSoto Canyon	*Brevoortia tyrannus*	24.784	32.876
DeSoto Canyon	***Fundulus grandis***	24.784	32.876
DeSoto Canyon	***Pterois miles***	24.784	32.876
DeSoto Canyon	***Anchoa mitchilli***	24.784	32.876

Dry Tortugas	*Pterois miles*	28.096	36.397
Dry Tortugas	***Fundulus grandis***	28.096	36.397
Dry Tortugas	***Membras martinica***	28.096	36.397
Dry Tortugas	***Anchoa mitchilli***	28.096	36.397
Dry Tortugas	***Anchoa hepsetus***	28.096	36.397
Florida Middle Ground	*Nessorhamphus ingolfianus*	24.843	36.13
Florida Middle Ground	***Fundulus grandis***	24.843	36.13
Madison-Swanson	*Fundulus grandis*	24.624	32.947
Pulleys Ridge	*Anchoa mitchilli*	26.201	35.613
Pulleys Ridge	*Fundulus grandis*	26.201	35.613
The Edges	*Anchoa mitchilli*	24.669	33.884
The Edges	*Membras martinica*	24.669	33.884

Table 3. List of benthic fish species grouped by temperature and salinity. Species in "BOLD" were also detected in surface sites.

Site	**Species**	**Temperature (°C)**	**Salinity (PSU)**
DeSoto Canyon Area	*Apterichtus moseri*	19.287	36.319
DeSoto Canyon Area	*Synodus intermedius*	19.287	36.319
DeSoto Canyon Area	***Fundulus grandis***	19.287	36.319
DeSoto Canyon Area	***Membras martinica***	19.287	36.319
DeSoto Canyon Area	***Anchoa hepsetus***	19.287	36.319
West Florida Shelf	***Fundulus grandis***	17.875	36.341
West Florida Shelf	***Pterois miles***	17.875	36.341
West Florida Shelf	***Anchoa mitchilli***	17.875	36.341
West Florida Shelf	*Trachurus lathami*	17.875	36.341
West Florida Shelf	*Xyrichtys novacula*	17.875	36.341
Dry Tortugas	***Fundulus grandis***	27.706	36.398
Dry Tortugas	***Membras martinica***	27.706	36.398

Dry Tortugas	***Anchoa mitchilli***	27.706	36.398
Dry Tortugas	***Anchoa hepsetus***	27.706	36.398
Dry Tortugas	*Nessorhamphus ingolfianus*	27.706	36.398
Florida Middle Ground	***Fundulus grandis***	23.038	36.242
Florida Middle Ground	*Membras martinica*	23.038	36.242
Florida Middle Ground	*Anchoa hepsetus*	23.038	36.242
Madison-swanson	*Anchoa hepsetus*	19.997	36.193
Madison-swanson	*Membras martinica*	19.997	36.193
Madison-swanson	*Anchoa mitchilli*	19.997	36.193
Pulley Ridge	*Leiostomus xanthurus*	17.896	36.326
Riley's Hump	*Fundulus grandis*	24.995	36.388
Riley's Hump	*Anchoa mitchilli*	24.995	36.388
Riley's Hump	*Anchoa hepsetus*	24.995	36.388
Christmas Tree Ridge	*Membras martinica*	16.272	36.062
Christmas Tree Ridge	*Pterois miles*	16.272	36.062

Table 4. List of latitude and longitude at each collection site.

Reef name	**Latitude**	**Longitude**
DeSoto Canyon Area	30.1253	-86.7577
DeSoto Canyon Area	29.8652	-86.5438
West Florida Shelf	29.3753	-85.9713
West Florida Shelf	29.3753	-85.9713
Madison-Swanson	29.3307	-85.8113
Madison-Swanson	29.3307	-85.8113
Madison-Swanson	29.3262	-85.8133
Madison-Swanson	29.3262	-85.8133
The Edges	28.9858	-85.4865
The Edges	28.9858	-85.4865
The Edges	28.9693	-85.3517

The Edges	28.9693	-85.3517
Florida Middle Ground	28.4925	-84.3732
Christmas Tree Ridge	25.9977	-83.7155
Christmas Tree Ridge	25.9977	-83.7155
Pulley Ridge	25.3877	-83.7067
Pulley Ridge	25.3877	-83.7067
Riley's Hump	24.5127	-83.0975
Riley's Hump	24.5127	-83.0975
Dry Tortugas	24.7077	-82.9963
Dry Tortugas	24.7077	-82.9963
Dry Tortugas	24.6153	-82.968
Dry Tortugas	24.6153	-82.968
Dry Tortugas	24.5765	-82.9013
Dry Tortugas	24.5765	-82.9013

Table 5. List of non-target fish species detected by eDNA metabarcoding.

Common name	**Scientific name**	**Site**	**IUCN**
House Mouse	*Mus musculus*	DeSoto Canyon Area	LC
House Mouse	*Mus musculus*	West Florida Shelf	LC
House Mouse	*Mus musculus*	Madison-Swanson	LC
House Mouse	*Mus musculus*	The edges	LC
House Mouse	*Mus musculus*	Florida Middle Grounds	LC
House Mouse	*Mus musculus*	Christmas Tree Ridge	LC
House Mouse	*Mus musculus*	Pulley Ridge	LC
House Mouse	*Mus musculus*	Rileys Hump	LC
House Mouse	*Mus musculus*	Dry Tortugas	LC
Ostreococcus algae	*Ostreococcus tauri*	West Florida Shelf	LC
Ostreococcus algae	*Ostreococcus tauri*	Madison-Swanson	LC
Ostreococcus algae	*Ostreococcus tauri*	The edges	LC

Ostreococcus algae	*Ostreococcus tauri*	Florida Middle Grounds	LC
Ostreococcus algae	*Ostreococcus tauri*	Christmas Tree Ridge	LC
Ostreococcus algae	*Ostreococcus tauri*	Pulley Ridge	LC
Ostreococcus algae	*Ostreococcus tauri*	Riley's Hump	LC
Ostreococcus algae	*Ostreococcus tauri*	Dry Tortugas	LC
Bathycoccus	*Bathycoccus prasinos*	West Florida Shelf	LC
Bathycoccus	*Bathycoccus prasinos*	Florida Middle Grounds	LC
Bathycoccus	*Bathycoccus prasinos*	Christmas Tree Ridge	LC
Bathycoccus	*Bathycoccus prasinos*	Pulley Ridge	LC
Bathycoccus	*Bathycoccus prasinos*	Riley's Hump	LC
Bathycoccus	*Bathycoccus prasinos*	Dry Tortugas	LC
Skeletonema	*Skeletonema marinoi*	West Florida Shelf	LC
Skeletonema	*Skeletonema marinoi*	The edges	LC
Skeletonema	*Skeletonema marinoi*	Christmas Tree Ridge	LC
Skeletonema	*Skeletonema marinoi*	Pulley Ridge	LC
Skeletonema	*Skeletonema marinoi*	Dry Tortugas	LC
marine diatom	*Pseudo-nitzschia multiseries*	Madison-Swanson	LC
marine diatom	*Pseudo-nitzschia multiseries*	Christmas Tree Ridge	LC
marine diatom	*Pseudo-nitzschia multiseries*	Riley's Hump	LC
marine diatom	*Pseudo-nitzschia multiseries*	Dry Tortugas	LC
Picozoa	*Picobiliphyte sp. Ms584-11*	West Florida Shelf	LC
Picozoa	*Picobiliphyte sp. Ms584-11*	Madison-Swanson	LC
Picozoa	*Picobiliphyte sp. Ms584-11*	The edges	LC
Picozoa	*Picobiliphyte sp. Ms584-11*	Christmas Tree Ridge	LC
marine diatom	*Thalassiosira pseudonana*	Pulley Ridge	LC
marine diatom	*Thalassiosira pseudonana*	Dry Tortugas	LC
Algae	*Bigelowiella natans*	Madison-Swanson	LC
Algae	*Bigelowiella natans*	Florida Middle Grounds	LC
Algae	*Bigelowiella natans*	Dry Tortugas	LC
domestic dog	*Canis lupus familiaris*	West Florida Shelf	LC

domestic dog	*Canis lupus familiaris*	The edges	LC
domestic dog	*Canis lupus familiaris*	Riley's Hump	LC
domestic dog	*Canis lupus familiaris*	Dry Tortugas	LC
Algae	*Triparma laevis*	DeSoto Canyon Area	LC
Algae	*Triparma laevis*	West Florida Shelf	LC
Algae	*Triparma laevis*	Florida Middle Grounds	LC
Algae	*Triparma laevis*	Dry Tortugas	LC
Fungi	*Parengyodontium album*	Madison-Swanson	LC
Fungi	*Parengyodontium album*	Christmas Tree Ridge	LC
Fungi	*Parengyodontium album*	Dry Tortugas	LC
Fungi	*Penicillium sp.Sh4C*	Christmas Tree Ridge	LC
Diatom	*Phaeodactylum tricornutum*	Christmas Tree Ridge	LC
Fungi	*Hortaea werneckii EXF-2000*	West Florida Shelf	LC
Fungi	*Hortaea werneckii EXF-2000*	The edges	LC
Fungi	*Hortaea werneckii EXF-2000*	Christmas Tree Ridge	LC
Fungi	*Hortaea werneckii EXF-2000*	Dry Tortugas	LC
encrusting sponge	*Halisarca harmelin*	Dry Tortugas	NE
Fungi	*Aspergillus fischeri*	Madison-Swanson	LC
Fungi	*Aspergillus fischeri*	Christmas Tree Ridge	LC
giant barrel sponge	*Xestospongia muta*	Dry Tortugas	NE
giant barrel sponge	*Xestospongia muta*	Christmas Tree Ridge	NE
Fungi	*Aspergillus fischeri*	Christmas Tree Ridge	LC
soft coral	*Leptogorgia capverdensis*	West Florida Shelf	NE
soft coral	*Leptogorgia capverdensis*	Dry Tortugas	NE
erect rope sponge	*Amphimedon compressa*	Riley's Hump	NE
Red jungle	*Gallus gallus*	Christmas Tree Ridge	LC
Azure vase sponge	*Callyspongia plicifera*	Dry Tortugas	NE
Algae	*Micromonas commode*	Dry Tortugas	LC

Leathery Barrel Sponge	*Geodia neptuni*	Dry Tortugas	NE
Fungi	*Fusarium avenaceum*	*Dry Tortugas*	LC
Jewel jellyfish	*Liriope tetraphylla*	DeSoto Canyon Area	NE
Least tern	*Sternula antillarum*	West Florida Shelf	LC

Table 6. List of detected non-target species: coral, hydrozoa, Least tern and sponges. Species are listed along with their corresponding sites and location in the water column (surface/benthic).

Site	**Species name**	**Common name**	**Surface/Benthic**
Dry Tortugas	*Xestospongia muta*	Giant barrel sponge	Surface
Dry Tortugas	*Leptogorgia capverdensis*	soft coral	Benthic
Dry Tortugas	*Amphimedon compressa*	Erect Rope sponge	Benthic
Dry Tortugas	*Callyspongia plicifera*	Azure Vase sponge	Benthic
Dry Tortugas	*Geodia neptuni*	Leathery Barrel Sponge	Benthic
Dry Tortugas	*Halisarca Hamelin*	Encrusting sponge	Benthic
DeSoto Canyon Area	*Liriope tetraphylla*	Hydrozoa	Surface
West Florida Shelf	*Sternula antillarum*	Least Tern	Surface

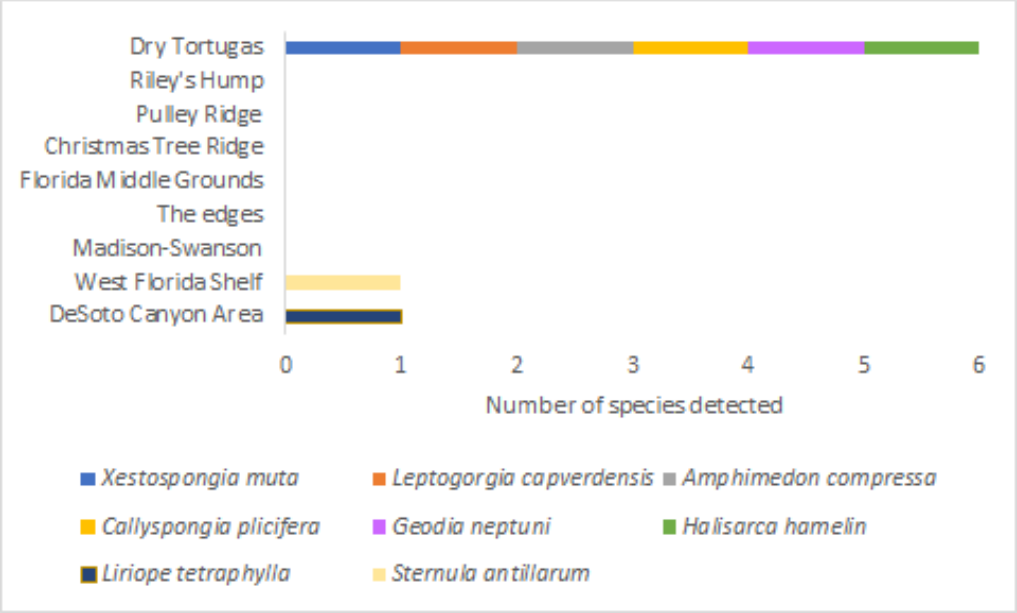

Figure 5. Number of detected non-target species: soft coral, hydrozoan, Least tern, and sponges among sampling sites.

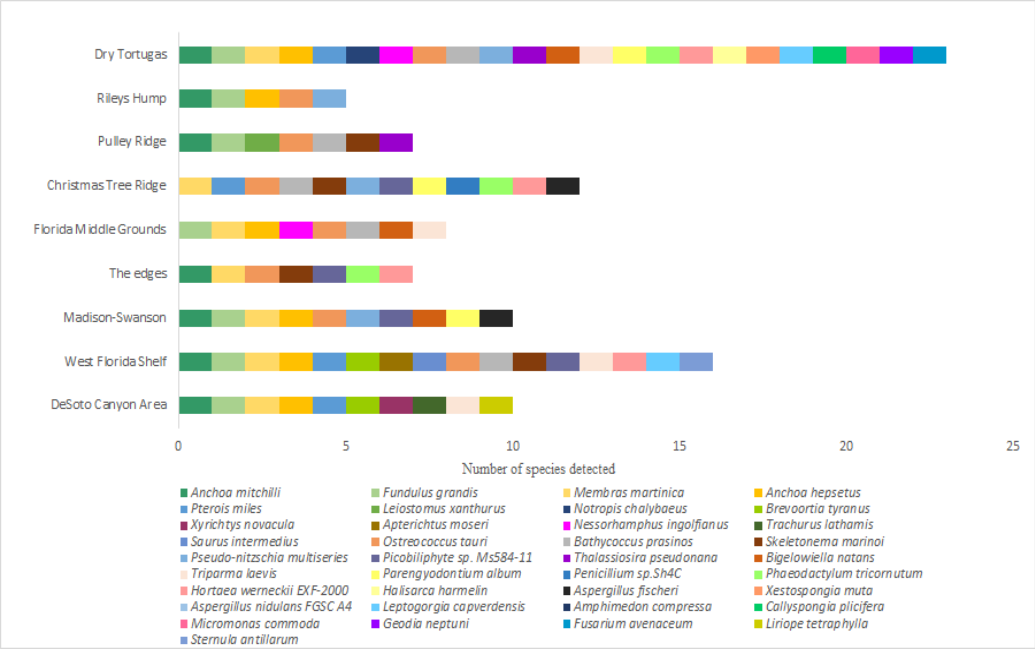

Figure 6. Number of all detected species at each site.

CHAPTER IV

DISCUSSION

Overall eDNA metabarcoding revealed reef fish and other species that are connected to reef ecosystems in the Gulf of Mexico that were present at the time of sampling. Additionally, this tool was able to reveal several reef-building invertebrate species such as sponges and soft coral. The most commonly detected species among the nine sites were Bay anchovy, Gulf killifish, and Rough silverside followed by Striped anchovy. The Bay anchovy (*Anchoa mitchilli*) and the Striped anchovy (*Anchoa hepsetus)* are two of the most abundant pelagic/estuarine species in the Gulf of Mexico (Jackson et al., 2013), so it is not surprising that they were detected in seven out of nine sampling sites. Anchovies play a critical role in transferring of energy from primary producers to higher trophic level fish or directly supporting commercial and recreational fisheries (Chen, 2017). Higher trophic level fish that are known to prey on anchovies include the Bluefish (*Pomatomus saltatrix*), Striped bass (*Morone saxatilis*), Weakfish (*Cynoscion regalis*), the apex predator Atlantic swordfish (*Xiphias gladius*), Red drum (*Sciaenops ocellatus*), the voracious carnivore King mackerel (*Scomberomorus cavalla*), and the shallow coastal water the Smalltooth sawfish (*Pristis pectinata*) (Chen, 2017; Harfmart & Brandt, 1995; Kells & Carpenter, 2011).

The Gulf Killifish (*Fundulus grandis*) was detected in seven out of nine sites. Both killifish and anchovies are forage fish that filter phytoplankton and feed on zooplankton, supporting the forage base in the Gulf of Mexico ecosystem. Gulf killifish may also feed on invertebrates and small fishes while being preyed upon by the Red drum (*Sciaenops ocellatus*), the passive bottom-dweller Southern flounder (*Paralichthys lethostigma*), and the Spotted seatrout (*Cynoscion nebulosus*) (Chen, 2017; Kells & Carpenter, 2011). Flounders and seatrouts are then preyed upon by sharks, cod and sculpins in the Gulf of Mexico. Additionally, Gulf Killifish are

commonly used as a bait source by fishers in the Gulf of Mexico (Phelps et al., 2010), which could also contribute to abundant eDNA detections. The adaptation to a wide range of salinities (euryhaline) of both anchovies and killifish from hypersaline pools to near freshwater streams (Griffith, 1974) is another potential explanation to being one of the most detected species in this study. The Rough silverside (*Membras martinica*) was detected in seven sites. Similarly to anchovies and killifish, silversides inhabit pelagic coastal habitats and are preyed upon larger fishes such as Bluefish (*Pomatomus saltatrix*), Striped bass (*Morone saxatilis*), Weakfish (*Cynoscion regalis*), Red drum (*Sciaenops ocellatus*), and the voracious carnivore Atlantic mackerel (*Scomber scombrus*) (Chen, 2017).

The Spot (*Leiostomus xanthurus*) was detected at the benthic site in Pulley Ridge (May) before they migrate closer to estuaries in the summer and fall. The Spot is an ecologically important species influencing the structure and function of estuarine systems representing a significant link in the transfer of energy from the estuary to coastal waters (Winemiller, 2015). They feed primarily on bottom-dwelling worms and crustaceans, and just like anchovies, are then are preyed on by Southern flounder (*Paralichthys lethostigma*) (Burke & Rice, 2002), Striped bass, Bluefish, Weakfish and Red drum becoming increasingly important to their diets (Harfmart & Brandt, 1995; Kells & Carpenter, 2011). The Weakfish (*Cynoscion regalis*) is then preyed upon by the voracious predator King mackerel (*Scomberomorus cavalla*), while the Bluefish (*Pomatomus saltatrix*) is preyed upon by Atlantic Bluefin tuna (*Thunnus thynnus*).

The Atlantic menhaden (*Brevoortia tyrannus*) was detected at the surface sites of DeSoto Canyon Area and West Florida Shelf. The Atlantic menhaden is a species that is typically found in offshore waters as adults (Chen, 2017). Atlantic menhaden (*Brevoortia tyrannus*) play an essential role for both consumers and prey in the nearshore and coastal pelagic ecosystem of the

Gulf of Mexico. Similarly to anchovies, Atlantic menhaden feed primarily on phytoplankton, and as they get larger, transition to feeding on zooplankton, which are then preyed by the piscivorous inshore fish, such as Ladyfish (*Elops saurus*) and offshore fishes such as the Bluefish (*Pomatomus saltatrix*), Weakfish (*Cynoscion regalis*), Blue runner (*Caranx crysos*), Spotted seatrout (*Cynoscion nebulosus*), Red drum (*Sciaenops ocellatus*), and sharks (Harfmart & Brandt, 1995; Leaf et al., 2018). The Red drum (*Sciaenops ocellatus*) are then preyed upon by larger predators such as barracudas and sharks (reef associated) regulating the energy flow between organisms from estuarine, coastal habitats to reefs (Chen, 2017). Menhaden have a more efficient energy transfer source to higher trophic levels than jellyfish accounting for a great part of piscivorous fish diets and are considered to be the most important fish in the USA in regards to their economic and ecological value (Sagarese et al., 2016). Overall, resident and non-resident reef fish consume pelagic school fish such as anchovies, menhaden and killifish moving between mangroves and seagrass beds ecosystems.

It is essential to understand the role of reef fish in the trophic level because they often consume a large number of commercially or ecologically important fish such as the anchovies, killifish and menhaden (Cruz-Escalona et al., 2005). The Rough scad (*Trachurus lathami*), Sand diver (*Synodus intermedius*), Pearly razorfish (*Xyrichtys novacula*), and Lionfish (*Pterois miles*) were the four reef-associated species in this study, all play important ecological roles in the food web as both prey and predators. Rough scad are schooling fish often preyed on by the Western comb grouper (*Mycteroperca acutirostris*) (Bonaldo et al., 2004), a reef-associated species inhabiting the rocky bottoms and shallow reefs in the Northwest Gulf of Mexico (Kells & Carpenter, 2011). In this study, the Rough scad (*Trachurus lathami*) was detected in the northwestern site, DeSoto Canyon Area, precisely where the Western comb groupers are known

to be present. Due to their association with reefs, Rough scad feed mainly on small invertebrates (Bonaldo et al., 2004), but may also eat eggs and larvae of small estuarine fish such as the anchovies and killifish detected at all sites of the study. Rough scad may also become an alternative fish as a result of declining fisheries of other small pelagic fish such as sardines, *Sardinella brasiliensis* (Braicovic et al., 2012) and/or the Atlantic thread herring, *Opisthonema oglinum* (Ruas & Vaz-dos-Santos, 2017).

Also detected in this study using eDNA, the demersal fish, the Sand diver (*Synodus intermedius*) is a piscivorous ambush predator found over coralline sandy bottoms in the Gulf of Mexico, feeding mainly on small prey such as Striped anchovy as juveniles, while adults consume larger fish such as Gulf killifish. However, prey items of the Sand diver can also include invertebrates, mainly shrimp (Cruz-Escalona et al., 2005; Kells & Carpenter, 2011). Interestingly, both the Sand diver (*Synodus intermedius*) and prey items, Striped anchovy (*Anchoa hepsetus*), were detected at the same site (DeSoto Canyon Area), and the other prey, *Trachurus lathami* was detected in the closest site to DeSoto Canyon Area (West Florida Shelf). Previous studies have positioned Synodontidae species as apex predators due to their high trophic position as hunters feeding on predatory fishes, but juveniles may still occupy a mid-trophic position as they inhabit inshore estuaries and lagoons consuming mainly penaeid shrimp (Cruz-Escalona et al., 2005). A common predator of juvenile *Synodus* is the Red drum *(Sciaenops ocellatus*) (Chen, 2017).

The Pearly razorfish (*Xyrichtys novacula*), also detected in this study, inhabits sandy and coral bottoms from shore to about 260ft in the Gulf of Mexico, and feeds on benthic invertebrates. This reef fish takes advantage of the physical structures in coral reefs for feeding and shelter but also dives quickly into sand burrows to hide from predators (Katsanevakis, 2005). Coral debris is

used for nest building. The Pearly razorfish is a benthic feeder dependent on the community of well-sorted fine sand for foraging of molluscs and echinoderms (Kells & Carpenter, 2011). The West Florida Shelf, where the Pearly razorfish was detected, has a benthic habitat of well-sorted fine sand. In the Gulf of Mexico, Pearly razorfish mainly prey upon the invasive lionfish (*Pterois spp.*), which was also found at the benthic and surface sites of the West Florida Shelf (Castriota et al., 2005; Dahl & Patterson, 2014).

The detection of invasive lionfish (*Pterois spp.*) DNA at multiple sites in this study is of immediate conservation and management concern due to the implications of an invasive species. As effective predators, the invasion and spread of lionfish is one of the most immediate conservation concerns in the world (Sutherland et al., 2010). The presence of *Pterois* spp. in the northern Gulf of Mexico is not well known (Dahl & Patterson, 2014), and the fact that Lionfish were detected at both benthic and surface sites in DeSoto Canyon Area, West Florida Shelf (Northwest Florida) and at the benthic sites in Christmas Tree Ridge, and Dry Tortugas is of immediate concern. Lionfish were detected at the same sites where their prey Pearly razorfish, Sand diver and Rough scad were also detected (DeSoto Canyon Area and West Florida Shelf) calling for immediate attention to manage these sites.

This study shows the ability of eDNA metabarcoding to reveal species habitat (benthic and/or pelagic). For instance, the benthic, reef associated fish species, Rough scad, Sand diver, and Pearly razorfish were only detected in benthic samples. Additionally, the demersal Snake eel which inhabits sandy bottoms, was only detected in benthic samples. The foraging Atlantic menhaden, known to inhabit pelagic areas, was detected from surface sites only. The most detected fish species in this study, Bay anchovy, Gulf killifish and Rough silverside, are known to inhabit pelagic areas, but were detected in both surface and benthic samples. A possible

explanation could be that they are the prey for many reef associated species residing in benthic habitats, and highly migratory fish such as tunas and mackerels.

DNA of the freshwater fish, the Rough shiner (*Notropis chalybaeus*) was detected in Dry Tortugas. This species is likely not actively present in Dry Tortugas because these reefs are far from the coast and no freshwater fishes should be present. A potential explanation for detection of Rough shiner DNA could be that DNA from *Notropis chalybaeus* was transported from coastal runoff or another source.

Two additional non-native species were detected in this study. The Japanese snake eel, *Apterichtus moseri*, was detected at Desoto Canyon Area, and the Duckbill snake eel (*Nessorhamphus ingolfianus*) was detected at Florida Middle Ground and Dry Tortugas. Both species have not previously been recorded in the Gulf of Mexico, or Western Atlantic, so they could be potentially underrepresented exotic species. Another potential explanation could be the lack of snake eel sequences (12s rRNA) from the GOM reported in BLASTn. Thus, there is no way to tell if these snake eels are exotic or closely related species.

Though not a fish, the marine bird, the endangered Least tern (*Sternula antillarum*) was detected in the surface site of the West Florida Shelf. The Least tern is a migratory bird that breeds throughout the coastal beaches of the US, Central and South America. Because of steep population decline, the Least Tern is classified as "endangered" in the USA, and under special protection in Mexico (Burger, 1989; Schweitzer et al., 2000; Zuarth et al., 2016). These declines are primarily due to the rapid loss of breeding and feeding grounds, egg predation, weather extremes, and human activities (Zuarth et al., 2016). The detection of the Least Tern at the West Florida Shelf site, is of great importance to better acknowledge the distribution of this species, and to reevaluate the habitat management strategies for protection of this species.

Although not the focus of this study, sponges and soft coral species playing an important role in reef structure and function were detected. The Leathery barrel sponge (*Geodia neptuni*), Giant barrel sponge (*Xestospongia muta*), Erect rope sponge (*Amphimedon compressa*), Azure vase sponge (*Callyspongia plicifera*), and the encrusting sponge (*Halisarca harmelin*) were all detected in this study and play critical roles in substrate deposition and nutrient cycling in reef ecosystems in the Gulf of Mexico and Caribbean. Specifically, these invertebrates contribute to reef fish biodiversity by providing habitat (McMurray et al., 2015). The Giant barrel sponge (*Xestospongia muta*), detected in Dry Tortugas, has been identified as the second most abundant sponge in the Caribbean contributing to the flux of carbon and nutrients in coral reefs (McMurray et al., 2015). The Erect rope sponge (*Amphimedon compressa*), also detected in Dry Tortugas, is grazed upon, non-fatally by many reef fish species, and by the critically endangered Hawksbill turtle (*Eretmochelys imbricata*) (Leon & Bjorndal, 2002; Zuñiga-Marroquin & Espinosa de los Monteros, 2017). Interestingly, eDNA from sponges and soft coral species was only detected in benthic samples implying that eDNA metabarcoding can reveal species habitat. Due to the lack of taxonomic expertise, sponges are often not considered in monitoring and conservation programs (Calcinai et al., 2017), thus implementing eDNA metabarcoding tools could help to overcome this limitation. While populations of hard corals in the Florida Keys and elsewhere in the Caribbean are declining in abundance due to environmental stressors, sponges, such as *Xestospongia muta*, and soft corals, such as *Leptogorgia cavernensis*, both detected in Dry Tortugas, may be experiencing population growth (McMurray et al., 2015; Silvestri et al., 2019). Therefore, eDNA metabarcoding highlights the need to further consider the ecology of sponges, and soft corals on Caribbean and Gulf of Mexico coral reefs.

Additionally, the jellyfish, *Liriope tetraphylla*, detected in this study, is one of the most abundant jellyfish in the Southern Gulf of Mexico, and is ecologically important due to its role in trophic chains feeding mainly on diatoms, dinoflagellates, fish eggs and larvae, and all zooplanktonic groups (Flores-Coto et al., 2016). Interestingly, *Liriope tetraphylla* was detected only in the surface sites of the DeSoto Canyon Area, where the number of fish detections were the highest among all sites. The detection of the non-target species is highly valuable as future studies could potentially inventory reef building species in order to quickly inform management and conservation strategies for these sites.

Overall, surface and benthic fish species richness were directly correlated to each other. Additionally, no significant differences were found when comparing surface and benthic pelagic fish detections. This could be explained by species migrating up and down the water column to feed or to go from warmer to cooler temperatures. This could also explain why there was no correlation between surface/benthic fish species richness and temperature as many fish are highly mobile and likely to migrate in the water column when temperatures rise or cool down. No correlation was found between surface/benthic fish species richness with salinity, which may be due to the fact that many fish are tolerant to salinity changes (euryhaline), such as anchovies, killifish (detected in all sites), and the Atlantic menhaden using brackish waters at some point in life. However, this correlation is not only due to the overlapping of the same species but from different species detections in surface and benthic sites. This highlights the importance of collecting both surface and benthic samples in order to recover a higher diversity of species and acquire a more complete inventory. The analysis of non-target species goes beyond the scope of the study. However, it is still important to acknowledge the presence of several algae and/or

fungi species as they can both be vital to coastal nutrient cycling processes and food webs in the Gulf of Mexico.

This study successfully detected fish DNA in the Gulf of Mexico using eDNA metabarcoding. Amplicon libraries from both Elasmo and MiFish primers were generated. However, only the reads from the Elasmobranch primers were used. MiFish primers failed to generate valuable reads using Illumina High-throughput sequencing technologies. The MiFish paired-end reads by Illumina had significantly low quality scores and short reads preventing the merging of paired-end reads. The MiFish sequences failed to sequence could possibly be explained by having very low quality with highly degraded DNA or could be the result of sequencing errors. Sample preservation techniques after collection should be considered to reduce sample degradation in further eDNA metabarcoding studies. In order to acquire a better resolution output from Illumina platforms, the samples could be run multiple times (Yamamoto et al., 2017) and three PCR replicates or more to resulting in possibly double the detections. Our samples were run multiple times in Illumina Hiseq platforms and three PCR replicates per sample were used to test for amplified products. Additionally, sampling from more locations in the Gulf of Mexico could also be more informative to the species composition. In the future, we expect to see significantly higher detections of reef fish when the purpose of the study is to inventory reef fish in natural and artificial reef sites.

Conclusion

Environmental DNA metabarcoding was not able to successfully provide an inventory of reef fish species, but revealed increasingly valuable reef and non-reef fish species detections as these pelagic schooling fish transfer primary productivity from estuaries, mangroves, and seagrass beds to reef ecosystems, contributing not only to reef fish diets but also to highly migratory fish.

The low number of reef fish detections demonstrated the need to improve preservation steps after collection to reduce eDNA degradation in the water samples and suggested that reference databases are lacking many reference sequences for closely related fish species. Continuing eDNA metabarcoding studies in the Gulf of Mexico, specifically in Dry Tortugas, will provide a better understanding of both sponges and coral species that utilize this area, and that will also help in inventorying species without the need of taxonomic expertise. Additionally, the study highlighted the need to continue sampling at different depths in the water column, and the urgent need to improve reference databases to have complete inventories of reef fish in the GoM. Overall, this work demonstrates the utility of eDNA and metabarcoding to reveal reef fish species in the Gulf of Mexico, but calls for improving 12S rRNA fish databases in the GoM as well as the preservation techniques between the time of collection and extraction. Future studies should continue to optimize this effective tool to quickly monitor reef fish and make informed decisions for habitat protection.

REFERENCES

Bakker, J., Wangensteen, O. S., Chapman, D. D., Boussarie, G., Buddo, D., Guttridge, T. L., Hertler, H., Mouillot, D., Vigliola, L. & Mariani, S. (2017). Environmental DNA reveals tropical shark diversity in contrasting levels of anthropogenic impact. *Scientific Reports*, *7*(1), 16886. https://doi.org/10.1038/s41598-017-17150-2

Bellwood, D. R. (1998). What are reef fishes? - Comment on the report by D. R. Robertson: Do coral-reef fish faunas have a distinctive taxonomic structure? (Coral Reefs 17: 179–186). *Coral Reefs*, *17*(2), 187–189. https://doi.org/10.1007/s003380050114

Bonaldo, R.M., J.P. Krajewski and I. Sazima, 2004. Does the association of young fishes with jellyfishes protect from predation? Report of a failure due to damage to the jellyfish. Neotrop. Ichthyol. 2(2):103-105.

Braicovich, P. E., Luque, J. L., & Timi, J. T. (2012). Geographical patterns of parasite infracommunities in the rough scad, Trachurus lathami Nichols, in the Southwestern Atlantic ocean. *The Journal of Parasitology*, *98*(4), 768–777.

Burger, J. (1989). Least Tern Populations in Coastal New Jersey: Monitoring and Management of a Regionally-Endangered Species. *Journal of Coastal Research, 5*(4), 801-811. Retrieved June 25, 2021, from http://www.jstor.org/stable/4297614

Burke, B. J., & Rice, J. A. (2002). A Linked Foraging and Bioenergetics Model for Southern Flounder. *Transactions of the American Fisheries Society*. 131. 120-131. 10.1577/1548-8659(2002)131.

Calcinai, B., Bastari, A., Bavestrello, G., Bertolino, M., Horcajadas, S. B., Pansini, M., Makapedua, D. M. & Cerrano, C. (2017). Demosponge diversity from North Sulawesi, with

the description of six new species. *ZooKeys*, *680*(680), 105–150. https://doi.org/10.3897/zookeys.680.12135

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A. & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Peña, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., Reeder, J., … Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature methods*, *7*(5), 335–336. https://doi.org/10.1038/nmeth.f.303

Castriota, L., Scarabello, M. P., Finoia, M. G., Sinopoli, M. & Andaloro, F. (2005). Food and feeding habits of pearly razorfish, Xyrichtys novacula (Linnaeus, 1758), in the southern Tyrrhenian Sea: variation by sex and size. *Environmental Biology of Fishes*, *72*(2), 123–133. https://doi.org/10.1007/s10641-004-6576-0

Chen, Y. (2017). Fish Resources of the Gulf of Mexico. In C. H. Ward (Ed.), *Habitats and Biota of the Gulf of Mexico: Before the Deepwater Horizon Oil Spill: Volume 2: Fish Resources, Fisheries, Sea Turtles, Avian Resources, Marine Mammals, Diseases and Mortalities* (pp. 869–1038). Springer New York. https://doi.org/10.1007/978-1-4939-3456-0_1

Collins, R. A., Wangensteen, O. S., O'Gorman, E. J., Mariani, S., Sims, D. W. & Genner, M. J. (2018). Persistence of environmental DNA in marine systems. *Communications Biology*, *1*(1), 185. https://doi.org/10.1038/s42003-018-0192-6

Cruz-Escalona, V. H., Peterson, M. S., Campos-Dávila, L. & Zetina-Rejón, M. (2005). Feeding habits and trophic morphology of inshore lizardfish (Synodus foetens) on the central

continental shelf off Veracruz, Gulf of Mexico. *Journal of Applied Ichthyology*, *21*(6), 525–530. https://doi.org/10.1111/j.1439-0426.2005.00651.x

Dahl, K. A. & Patterson, W. F. (2014). Habitat-Specific Density and Diet of Rapidly Expanding Invasive Red Lionfish, Pterois volitans, Populations in the Northern Gulf of Mexico. *PLoS ONE*, *9*(8), e105852. https://doi.org/10.1371/journal.pone.0105852

Deiner, K. & Altermatt, F. (2014). Transport Distance of Invertebrate Environmental DNA in a Natural River. *PLoS ONE*, *9*(2), e88786. https://doi.org/10.1371/journal.pone.0088786

Díaz-Ferguson, Edgardo E., & Moyer, Gregory R. (2014). History, applications, methodological issues and perspectives for the use environmental DNA (eDNA) in marine and freshwater environments. *Revista de Biología Tropical*, *62*(4), 1273-1284. Retrieved August 02, 2021, from http://www.scielo.sa.cr/scielo.php?script=sci_arttext&pid=S0034-77442014000400002&lng=en&tlng=en

DiBattista, J. D., Reimer, J. D., Stat, M., Masucci, G. D., Biondi, P., Brauwer, M. D. & Bunce, M. (2019). Digging for DNA at depth: rapid universal metabarcoding surveys (RUMS) as a tool to detect coral reef biodiversity across a depth gradient. *PeerJ*, *7*, e6379. https://doi.org/10.7717/peerj.6379

Dromard, C. R., Bouchon-Navaro, Y., Harmelin-Vivien, M. & Bouchon, C. (2015). Diversity of trophic niches among herbivorous fishes on a Caribbean reef (Guadeloupe, Lesser Antilles), evidenced by stable isotope and gut content analyses. *Journal of Sea Research*, *95*, 124–131. https://doi.org/10.1016/j.seares.2014.07.014

Edwards, C. B., Friedlander, A. M., Green, A. G., Hardt, M. J., Sala, E., Sweatman, H. P., Williams, I. D., Zgliczynski, B., Sandin, S. A. & Smith, J. E. (2014). Global assessment of the status of coral reef herbivorous fishes: evidence for fishing effects. *Proceedings of the*

Royal Society B: Biological Sciences, *281*(1774), 20131835. https://doi.org/10.1098/rspb.2013.1835

Faletti, M. E., Chacin, D. H., Peake, J. A., MacDonald, T. C. & Stallings, C. D. (2019). Population dynamics of Pinfish in the eastern Gulf of Mexico (1998-2016). *PLOS ONE, 14*(8), e0221131. https://doi.org/10.1371/journal.pone.0221131

Fautin, D., Dalton, P., Incze, L. S., Leong, J.-A. C., Pautzke, C., Rosenberg, A., Sandifer, P., Sedberry, G., Tunnell, J. W., Abbott, I., Brainard, R. E., Brodeur, M., Eldredge, L. G., Feldman, M., Moretzsohn, F., Vroom, P. S., Wainstein, M. & Wolff, N. (2010). An Overview of Marine Biodiversity in United States Waters. *PLoS ONE*, *5*(8), e11914. https://doi.org/10.1371/journal.pone.0011914

Ficetola, Gentile Francesco & Miaud, Claude & Pompanon, François & Taberlet, Pierre. (2008). Species detection using environmental DNA from water samples. *Biology letters*. 4. 423-5.

Flores-Coto, C., Puente-Tapia, A., Añorve, L. & Fernández-Alamo, M. (2016) Segregated Distribution of *Liriope tetraphylla*, *Aglaura hemistoma* and *Nausithoe punctata* (Cnidaria) in the Southern Gulf of Mexico. *Open Journal of Ecology*, 6, 568-578. doi: 10.4236/oje.2016.69055

Foote, A. D., Thomsen, P. F., Sveegaard, S., Wahlberg, M., Kielgast, J., Kyhn, L. A., Salling, A. B., Galatius, A., Orlando, L. & Gilbert, M. T. P. (2012). Investigating the Potential Use of Environmental DNA (eDNA) for Genetic Monitoring of Marine Mammals. *PLoS ONE, 7*(8), e41781. https://doi.org/10.1371/journal.pone.0041781

Fujii, K., Doi, H., Matsuoka, S., Nagano, M., Sato, H. & Yamanaka, H. (2019). Environmental DNA metabarcoding for fish community analysis in backwater lakes: A comparison of

capture methods. *PLOS ONE*, *14*(1), e0210357. https://doi.org/10.1371/journal.pone.0210357

Fujiwara, M., Martinez-Andrade, F., Wells, R. J. D., Fisher, M., Pawluk, M. & Livernois, M. C. (2019). Climate-related factors cause changes in the diversity of fish and invertebrates in subtropical coast of the Gulf of Mexico. *Communications Biology*, *2*(1), 403. https://doi.org/10.1038/s42003-019-0650-9

Garcia, S. M. & Rosenberg, A. A. (2010). Food security and marine capture fisheries: characteristics, trends, drivers and future perspectives. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1554), 2869–2880. https://doi.org/10.1098/rstb.2010.0171

Grey, E. K., Bernatchez, L., Cassey, P., Deiner, K., Deveney, M., Howland, K. L., Lacoursière-Roussel, A., Leong, S. C. Y., Li, Y., Olds, B., Pfrender, M. E., Prowse, T. A. A., Renshaw, M. A. & Lodge, D. M. (2018). Effects of sampling effort on biodiversity patterns estimated from environmental DNA metabarcoding surveys. *Scientific Reports*, *8*(1), 8843. https://doi.org/10.1038/s41598-018-27048-2

Griffith, R. W. (1974). Environment and Salinity Tolerance in the Genus Fundulus. *Copeia*, *1974*(2), 319–331. https://doi.org/10.2307/1442526

Harfmart, K.J., & Brandt, S. (1995). Predatory demand and impact of striped bass, bluefish, and weakfish in the Chesapeake Bay: applications of bioenergetics models. *Canadian Journal of Fisheries and Aquatic Sciences, 52*, 1667-1687

Hebert, M.M., (2021). Characterizing Elasmobranchs in Pensacola Bay System using Environmental DNA Metabarcoding [published master's thesis]. University of West Florida.

Jackson, J. B. & Jr., J. H. C. (2013). The Effects of Front-Associated Wind Events and Resultant Sediment Resuspension on Dietary Habits and Caloric Intake of Bay Anchovy and Age-0 Atlantic Croaker in Mobile Bay, Alabama. *Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science*, *5*(1), 103–113. https://doi.org/10.1080/19425120.2013.790862

Jiao, N.-Z., Chen, D.-K., Luo, Y.-M., Huang, X.-P., Zhang, R., Zhang, H.-B., Jiang, Z.-J. & Zhang, F. (2015). Climate change and anthropogenic impacts on marine ecosystems and countermeasures in China. *Advances in Climate Change Research*, *6*(2), 118–125.

Johnson, M., Zaretskaya, I., Raytselis, Y., Merezhuk, Y., McGinnis, S., & Madden, T. L. (2008). NCBI BLAST: a better web interface. *Nucleic Acids Research*, *36*(suppl_2), W5–W9. https://doi.org/10.1093/nar/gkn201

Katsanevakis, S. (2005). Habitat use by the pearly razorfish, Xyrichtys novacula (Pisces: Labridae). *Scientia Marina*, *69*, 223-229.

Kells, V., & Carpenter, K. (2011). *A field Guide to Coastal Fishes from Maine to Texas*. The John Hopkins University Press.

Lacoursière-Roussel, A., Côté, G., Leclerc, V. & Bernatchez, L. (2016). Quantifying relative fish abundance with eDNA: a promising tool for fisheries management. *Journal of Applied Ecology*, *53*(4), 1148–1157. https://doi.org/10.1111/1365-2664.12598

Layton, A., McKay, L., Williams, D., Garrett, V., Gentry, R., & Sayler, G. (2006). Development of Bacteroides 16S rRNA gene TaqMan-based real-time PCR assays for estimation of total, human, and bovine fecal pollution in water. *Applied and environmental microbiology*, *72*(6), 4214–4224. https://doi.org/10.1128/AEM.01036-05

Leaf, R. T., Trushenski, J., Brown-Peterson, N. J. & Andres, M. J. (2018). Temporal dynamics of lipid and fatty acid characteristics of Gulf Menhaden, Brevoortia patronus, in the northern Gulf of Mexico. *Regional Studies in Marine Science*, *24*, 1–9. https://doi.org/10.1016/j.rsma.2018.06.011

León, Yolanda & Bjorndal, KA. (2002). Selective feeding in the hawksbill turtle, an important predator in coral reef ecosystems. *Marine Ecology-progress Series*, *245*, 249-258. 10.3354/meps245249.

Li, J., Hatton-Ellis, T. W., Handley, L. L., Kimbell, H. S., Benucci, M., Peirson, G. & Hänfling, B. (2019). Ground-truthing of a fish-based environmental DNA metabarcoding method for assessing the quality of lakes. *Journal of Applied Ecology*, *56*(5), 1232–1244. https://doi.org/10.1111/1365-2664.13352

McMurray, S. E., Finelli, C. M. & Pawlik, J. R. (2015). Population dynamics of giant barrel sponges on Florida coral reefs. *Journal of Experimental Marine Biology and Ecology*, *473*, 73–80. https://doi.org/10.1016/j.jembe.2015.08.007

Minamoto, T., Yamanaka, H., Takahara, T., Honjo, M. N. & Kawabata, Z. (2012). Surveillance of fish species composition using environmental DNA. *Limnology*, *13*(2), 193–197. https://doi.org/10.1007/s10201-011-0362-4

Mitsuhiro, A., Satoquo, S., Tetsuya, S., & Masaki, M. (2017). Environmental DNA metabarcoding with MiFish primer reveals marine fish fauna of Tsushima Island, Nagasaki for establishing a marine protected area. 日本水産学会大会講演要旨集, 2017 (CD-ROM), 4–17.

Miya, M., Gotoh, R. O. & Sado, T. (2020). MiFish metabarcoding: a high-throughput approach for simultaneous detection of multiple fish species from environmental DNA and other samples. *Fisheries Science*, *86*(6), 939–970. https://doi.org/10.1007/s12562-020-01461-x

Miya, M., Sato, Y., Fukunaga, T., Sado, T., Poulsen, J. Y., Sato, K., Minamoto, T., Yamamoto, S., Yamanaka, H., Araki, H., Kondoh, M. & Iwasaki, W. (2015). MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: detection of more than 230 subtropical marine species. *Royal Society Open Science*, *2*(7), 150088. https://doi.org/10.1098/rsos.150088

Mora, C., Tittensor, D. P. & Myers, R. A. (2008). The completeness of taxonomic inventories for describing the global diversity and distribution of marine fishes. *Proceedings of the Royal Society B: Biological Sciences*, *275*(1631), 149–155. https://doi.org/10.1098/rspb.2007.1315

Ogram, A., Sayler, G. S. & Barkay, T. (1987). The extraction and purification of microbial DNA from sediments. *Journal of Microbiological Methods*, *7*(2–3), 57–66. https://doi.org/10.1016/0167-7012(87)90025-x

Olds, B. P., Jerde, C. L., Renshaw, M. A., Li, Y., Evans, N. T., Turner, C. R., Deiner, K., Mahon, A. R., Brueseke, M. A., Shirey, P. D., Pfrender, M. E., Lodge, D. M. & Lamberti, G. A. (2016). Estimating species richness using environmental DNA. *Ecology and Evolution*, *6*(12), 4214–4226. https://doi.org/10.1002/ece3.2186

Olivier, D., Loiseau, N., Petatán-Ramírez, D., Millán, O. T., Suárez-Castillo, A. N., Torre, J., Munguia-Vega, A. & Reyes-Bonilla, H. (2018). Functional-biogeography of the reef fishes of the islands of the Gulf of California: Integrating functional divergence into marine conservation. *Global Ecology and Conservation*, *16*(Ecol. Indicat. 52 2015), e00506. https://doi.org/10.1016/j.gecco.2018.e00506

Phelps, R. P., Daniels, W. H., Sansing, N. R. & Brown, T. W. (2010). Production of Gulf Killifish in the Black Belt Region of Alabama Using Saline Groundwater. *North American Journal of Aquaculture, 72*(3), 219–224. https://doi.org/10.1577/a09-025.1

Port, J. A., O'Donnell, J. L., Romero-Maraccini, O. C., Leary, P. R., Litvin, S. Y., Nickols, K. J., Yamahara, K. M., & Kelly, R. P. (2016). Assessing vertebrate biodiversity in a kelp forest ecosystem using environmental DNA. *Molecular ecology*, *25*(2), 527–541. https://doi.org/10.1111/mec.13481

Rees, H. C., Gough, K. C., Middleditch, D. J., Patmore, J. R. M. & Maddison, B. C. (2015). Applications and limitations of measuring environmental DNA as indicators of the presence of aquatic animals. *Journal of Applied Ecology*, *52*(4), 827–831. https://doi.org/10.1111/1365-2664.12467

Ruas, L. C. & Vaz-dos-Santos, A. M. (2017). Age structure and growth of the rough scad, Trachurus lathami (Teleostei: Carangidae), in the Southeastern Brazilian Bight. *Zoologia*, *34*, 1–11. https://doi.org/10.3897/zoologia.34.e20475

Ruppert, K. M., Kline, R. J. & Rahman, M. S. (2019). Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. *Global Ecology and Conservation*, *17*, e00547. https://doi.org/10.1016/j.gecco.2019.e00547

Sagarese, S. R., Nuttall, M. A., Geers, T. M., Lauretta, M. V., III, J. F. W. & Serafy, J. E. (2016). Quantifying the Trophic Importance of Gulf Menhaden within the Northern Gulf of Mexico Ecosystem. *Marine and Coastal Fisheries*, *8*(1), 23–45. https://doi.org/10.1080/19425120.2015.1091412

Sato, Y., Miya, M., Fukunaga, T., Sado, T. & Iwasaki, W. (2018). MitoFish and MiFish Pipeline: A Mitochondrial Genome Database of Fish with an Analysis Pipeline for Environmental DNA Metabarcoding. *Molecular Biology and Evolution*, *35*(6), 1553–1555. https://doi.org/10.1093/molbev/msy074

Sawaya, N. A., Djurhuus, A., Closek, C. J., Hepner, M., Olesin, E., Visser, L., Kelble, C., Hubbard, K. & Breitbart, M. (2019). Assessing eukaryotic biodiversity in the Florida Keys National Marine Sanctuary through environmental DNA metabarcoding. *Ecology and Evolution*, *9*(3), 1029–1040. https://doi.org/10.1002/ece3.4742

Schweitzer, S. H. (2000). Stage-specific Survival Rates of the Endangered Least Tern (Sterna antillarum) in Northwestern Oklahoma. *Proceedings of the Oklahoma Academy of Science*, 53–60.

Sigsgaard, E. E., Carl, H., Møller, P. R. & Thomsen, P. F. (2015). Monitoring the near-extinct European weather loach in Denmark based on environmental DNA from water samples. *Biological Conservation*, *183*, 46–52. https://doi.org/10.1016/j.biocon.2014.11.023

Silvestri, S., Figueroa, D. F., Hicks, D. & Figueroa, N. J. (2019). Mitogenomic phylogenetic analyses of Leptogorgia virgulata and Leptogorgia hebes (Anthozoa: Octocorallia) from the Gulf of Mexico provides insight on Gorgoniidae divergence between Pacific and Atlantic lineages. *Ecology and Evolution*, *9*(24), 14114–14129. https://doi.org/10.1002/ece3.5847

Stat, M., Huggett, M. J., Bernasconi, R., DiBattista, J. D., Berry, T. E., Newman, S. J., Harvey, E. S. & Bunce, M. (2017). Ecosystem biomonitoring with eDNA: metabarcoding across the tree of life in a tropical marine environment. *Scientific Reports*, *7*(1), 12240. https://doi.org/10.1038/s41598-017-12501-5

Sutherland, W. J., Clout, M., Côté, I. M., Daszak, P., Depledge, M. H., Fellman, L., Fleishman, E., Garthwaite, R., Gibbons, D. W., Lurio, J. D., Impey, A. J., Lickorish, F., Lindenmayer, D., Madgwick, J., Margerison, C., Maynard, T., Peck, L. S., Pretty, J., Prior, S., … Watkinson, A. R. (2010). A horizon scan of global conservation issues for 2010. *Trends in Ecology & Evolution*, *25*(1), 1–7. https://doi.org/10.1016/j.tree.2009.10.003

Taberlet, P., Bonin, A., Zinger. L., Coissac, E. (2018) Environmental DNA: For biodiversity research and monitoring. Oxford: Oxford University Press. doi.org/10.1093/oso/9780198767220.001.0001

Teneva L, Schemmel E, Kittinger J (2018). State of the plate: Assessing present and future contribution of fisheries and aquaculture to hawaii's food security. *Marine Policy, 94,* 28-38. Doi:10.1016/j.marpol.2018.04.025

Thomsen, P. F., Kielgast, J., Iversen, L. L., Møller, P. R., Rasmussen, M. & Willerslev, E. (2012). Detection of a Diverse Marine Fish Fauna Using Environmental DNA from Seawater Samples. *PLoS ONE*, *7*(8), e41732. https://doi.org/10.1371/journal.pone.0041732

Ushio, M., Fukuda, H., Inoue, T., Makoto, K., Kishida, O., Sato, K., Murata, K., Nikaido, M., Sado, T., Sato, Y., Takeshita, M., Iwasaki, W., Yamanaka, H., Kondoh, M. & Miya, M. (2017). Environmental DNA enables detection of terrestrial mammals from forest pond water. *Molecular Ecology Resources*, *17*(6). https://doi.org/10.1111/1755-0998.12690

Ushio, M., Murakami, H., Masuda, R., Sado, T., Miya, M., Sakurai, S., Yamanaka, H., Minamoto, T. & Kondoh, M. (2018). Quantitative monitoring of multispecies fish environmental DNA using high-throughput sequencing. *Metabarcoding and Metagenomics*, *2*, e23297. https://doi.org/10.3897/mbmg.2.23297

Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P. F., Bellemain, E., Besnard, A., Coissac, E., Boyer, F., Gaboriaud, C., Jean, P., Poulet, N., Roset, N., Copp, G. H., Geniez, P., Pont, D., Argillier, C., Baudoin, J.-M., … Dejean, T. (2016). Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Molecular Ecology*, *25*(4), 929–942. https://doi.org/10.1111/mec.13428

Winemiller, Kirk. (2015). Habitat use and diets of juvenile spot (Leiostomus xanthurus) and Atlantic croaker (Micropogonias undulatus) in a small estuary at Mad Island Marsh, Texas. *Texas Journal of Science*. 64. 3-31

Yamamoto, S., Masuda, R., Sato, Y., Sado, T., Araki, H., Kondoh, M., Minamoto, T. & Miya, M. (2017). Environmental DNA metabarcoding reveals local fish communities in a species-rich coastal sea. *Scientific Reports*, *7*(1), 40368. https://doi.org/10.1038/srep40368

Zuarth, C. G., Vallarino, A. & Botello, A. V. (2016). Breeding biology of the Atlantic Least Tern (Sternula antillarum antillarum) in a colony of the south of the Gulf of Mexico: new perspectives for its threat status. *Revista Brasileira de Ornitologia*, *24*(4), 323–328. https://doi.org/10.1007/bf03544361

Zuñiga-Marroquin, T., & Espinosa de los Monteros, A. (2017). Genetic characterization of the Critically Endangered hawksbill turtle (Eretmochelys imbricata) from the Mexican Pacific region. *Latin American Journal of Aquatic Research*, *45*(3), 555–562. https://doi.org/10.3856/vol45-issue3-fulltext-5