5-2023

Development of a novel environmental DNA (eDNA) tool for monitoring Vulnerable Freckled Guitarfish, Pseudobatos lentiginosus, in the Western Central Atlantic

Sarah Toepfer

Development of a novel environmental DNA (eDNA) tool for monitoring Vulnerable Freckled Guitarfish, *Pseudobatos lentiginosus*, in the Western Central Atlantic

by

Sarah Toepfer

A Thesis
Submitted to the Honors College of
The University of Southern Mississippi
in Partial Fulfillment
of Honors Requirements

May 2023

Approved by:

Nicole M. Phillips, Ph.D., Thesis Advisor,
School of Biological, Environmental and Earth Sciences

Jake F. Schaefer, Ph.D., Director,
School of Biological, Environmental and Earth Sciences

Sabine Heinhorst, Ph.D., Dean
Honors College

ABSTRACT

Rhino-rays are the most threatened group of elasmobranchs, having experienced widespread declines due to mortalities in fisheries and habitat degradation. Within the Western Central Atlantic, there are two extant species of Rhino-rays, the Critically Endangered Smalltooth Sawfish, *Pristis pectinata*, and the Vulnerable Freckled Guitarfish, *Pseudobatos lentiginosus*. Although there is research committed to *P. pectinata* in this region, less is known about the distribution status of *P. lentiginosus*. Over the past 50 years, *P. lentiginosus* have undergone a presumed range contraction in U.S. waters; once found from North Carolina to Texas, and historically common in the north central Gulf of Mexico, they are now only abundant in Florida. Their occurrence in the north-central Gulf of Mexico remains uncertain, and they have not been sighted in the Mississippi Sound in 15 years. Therefore, a highly sensitive, species-specific Droplet Digital™ PCR environmental DNA (eDNA) assay was designed to detect the presence of this species, targeting a 174 base pair portion of the mitochondrial 16S rRNA gene. The assay detects DNA from only *P. lentiginosus*, and not from other co-occurring closely related species. This tool can be used in future eDNA surveys across the northern Gulf of Mexico and Western Central Atlantic to inform the current distribution of this threatened species and implement conservation action.

Keywords: Atlantic Guitarfish, Freckled Guitarfish eDNA, *Pseudobatos lentiginosus*, ddPCR,

DEDICATION

This thesis is dedicated to Mom, Dad, Vicky, and my dear Mark for listening to my seemingly endless rambling about guitarfish and eDNA. I love you all so much.

ACKNOWLEDGMENTS

This project was funded and supported by the University of Southern Mississippi as well as Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103476.

Thank you to my amazing thesis advisor, Dr. Nicole Phillips for your tremendous support and patience throughout my undergraduate career. I was so lucky to get the absolute best possible thesis advisor there is. Thank you to Annmarie Fearing and Emma Humphreys for showing me qPCR and ddPCR, while also answering my calls on a whim about different reagents and thermocycler settings. Thanks to the guitarfish master, Bryan Huerta Beltrán for answering my endless questions about guitarfish and providing supplementary information, such as maps. Thank you to Dr. Joana Días and Ryan Lehman for your wonderful feedback and support on my first drafts. Thanks to Claire Gauci for introducing me to eDNA in the first place.

Finally, thanks again to my wonderful support group, Mark, Vicky, close friends, and my dear parents, who have always inspired and supported my love of the sea my whole life. I don't know where I would be without you guys.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

LIST OF ABBREVIATIONS

BLAST	Basic Local Alignment Search Tool
bp	Base Pair
ddPCR™	Droplet Digital™ Polymerase Chain Reaction
DNA	Deoxyribonucleic Acid
eDNA	Environmental DNA
gDNA	genomic DNA
IUCN	International Union for Conservation of Nature
MT	Manual Threshold
mtDNA	mitochondrial DNA
NCBI	National Center for Biotechnology Information
NTC	No Template Control
PCR	Polymerase Chain Reaction
qPCR	Quantitative Polymerase Chain Reaction
RED	Rare Event Detection
RFU	Relative Fluorescent Unit
RNA	Ribonucleic Acid
rRNA	Ribosomal RNA
USD	United States Dollars
UV	Ultraviolet

CHAPTER I: INTRODUCTION

Elasmobranchs at risk

Elasmobranchs, a subclass of Chondrichthyans which includes sharks, rays, and skates, are ecologically, economically, and culturally important to many communities. Elasmobranchs provide ecosystem services as upper-trophic level predators, buffering against invasive species, decreasing transmission of diseases, and maintaining biodiversity (Barría et al., 2015). Elasmobranchs may directly prey on invasive species, inhibiting their potential for exponential growth (see Triay-Portella et al., 2022) and can buffer against the transmission of disease by predating on infected individuals (see Packer et al., 2003). Their upper-trophic level control decreases overabundant lower-level species and invasive species, maintaining biodiversity (Barría et al., 2015).

Elasmobranchs are economically important through commercial and artisanal fisheries as well as ecotourism (Barrowclift et al., 2017; Haas et al., 2017). In 2013, Cisneros-Montemayor *et al*. estimated the global economic impact of elasmobranchs was $314 million Unites States Dollars (USD) per year. In The Bahamas alone, $112.6 million USD is gained from diving ecotourism and $1.2 million USD from fisheries (Haas et al., 2017). In Zanzibar, elasmobranchs are directly fished for fins, skin, cartilage, meat, liver, jaws, and teeth (Barrowclift et al., 2017). In a study done by Barrowclift *et al*., all fishers interviewed stated that fishing was their primary occupation, and the majority stated that 76–100% of their household income came from fishing (2017). In the same study, one-third of the fishers that caught elasmobranchs stated that 41–60% of their income came from elasmobranchs (Barrowclift et al., 2017).

In terms of cultural significance, sharks are present in most cultures with a history of ocean use, such as Hawaiian and Aboriginal cultures. In Hawaiian culture, ‘aumakua, a personal or family god or ancestor, may present itself as manō, a shark (Puniwai, 2020). Many Aboriginal Torres Strait Islander clans have shark or stingray family totems. Hammerhead sharks are also included in many Torres Strait Islander stories as hunters keeping the “law” of the ecosystem (Gerhardt, 2018).

The primary threats faced by elasmobranchs are mortalities in fisheries, habitat loss, and degradation (Dulvy et al., 2021; Haas et al., 2017). Overfishing threatens all one-third of elasmobranch species threatened with extinction, being the lone risk to 67.3% of these species (Dulvy et al., 2021). Habitat loss threatens 31.2% of these species (Dulvy et al., 2021). They are targeted through direct fishing for their meat and high-value fins and are also unintentionally caught as bycatch; 99.6% of Chondrichthyes assessed by Dulvy *et al*. were threatened by overfishing (Dulvy et al., 2021; Kyne et al., 2020; Shepherd and Myers, 2005; Zea-de la Cruz et al., 2021). Elasmobranchs are susceptible to overexploitation due to their life history characteristics, such as low fecundity and late maturity (Barrowclift et al., 2017; Shepherd and Myers, 2005; Zea-de la Cruz et al., 2021). Almost all overfished Chondrichthyes were harvested for use in human consumption for food, liver oil, the fin industry, or for aquaria (Dulvy et al., 2021). Habitat loss and degradation due to various reasons such as coastal development, fisheries (Magris and Giarrizzo, 2020), aquaculture, energy mining, or transportation negatively affects 31.2% of threatened elasmobranchs (Dulvy et al., 2021). Climate change also threatens them through habitat destruction from coral reef loss, causing decline of temperate elasmobranchs in equatorial latitudes due to rising temperatures

creating unsuitable habitat (Dulvy et al., 2021). The loss of productive and quality habitats, such as mangroves and seagrass, has accelerated in the past 100 years and is attributed to pollution from human interruptions (Dulvy et al., 2021; Gallagher et al., 2012).

Guitarfish

The rhino-rays (Rhinopristiformes) are the most imperiled of the elasmobranchs (Last et al., 2016; Moore, 2017; Naylor et al., 2012). These "shark-like rays" include the guitarfishes, wedgefishes, sawfishes, giant guitarfishes, and banjo rays (Marramà et al., 2021; Naylor et al., 2012). Many rhino-rays, such as giant guitarfish, and wedgefish are directly targeted for their "high-value" fins (Moore, 2017; Kyne, 2020). In addition, because most rhino-rays are benthic, trawls easily destroy their habitats and contribute to bycatch (Moore, 2017). After decline, their recovery speed is slow because of their k-selected life-history; they are slow to mature and reproduce in small numbers (Moore, 2017).

The guitarfishes include six genera and 38 species (Marramà et al., 2021) in tropical and temperate oceans to depths of 400 meters (m) (Last et al., 2016). Genus *Pseudobatos* includes nine species distributed in coastal waters of the Western Atlantic and the Eastern Pacific of the Americas (Charvet et al., 2019). Eight of the nine species are listed under a threatened category (i.e., Critically Endangered, Endangered, or Vulnerable) on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Charvet et al., 2019) (Table 1). Of these species, only 2 occur in the Central Western Atlantic (*Pseudobatos lentiginosus* and *Pseudobatos percellens*)

(Charvet et al., 2019), and only one of these are co-occurring in the Northern Gulf of Mexico, *P. lentiginosus*.

Table 1: International Union for Conservation of Nature Red List of Threatened Species statuses of guitarfish.

Species Name	Common Name	Exclusion/Target	IUCN Status
Pseuboatos buthi	Spadenose Guitarfish	Not used in study	Vulnerable
Pseudobatos glaucostigmus	Speckled Guitarfish	Not used in study	Vulnerable
Pseudobatos horkelli	Brazilian Guitarfish	Not used in study	Critically Endangered
Pseudobatos lentiginosus	Atlantic Guitarfish	Target species	Vulnerable
Pseudobatos leurcorhynchus	Whitesnout Guitarfish	Not used in study	Vulnerable
Pseudobatos percellens	Chola Guitarfish	Not used in study	Endangered
Pseudobatos planiceps	Pacific Guitarfish	Not used in study	Vulnerable
Pseudobatos prahli	Gorgana Guitarfish	Not used in study	Vulnerable
Pseudobatos productus	Shovelnose Guitarfish	Exclusion species	Near Threatened

Atlantic Guitarfish

Distribution and Habitat Use

The Atlantic Guitarfish, *Pseudobatos lentiginosus*, is an inshore marine species found in the Western Atlantic from the United States (North Carolina) to Nicaragua (Hensley et al., 1998; Last et al., 2016; Charvet et al., 2019) (Figure 1). However, they have become rare in the Northern Gulf of Mexico outside Florida in the past 50 years (Charvet et al., 2019) and have not been reported near Louisiana nor the Mississippi Sound in the past 15 years (Jill Hendon pers. comm., 2022; Bryan Huerta Beltrán pers. comm., 2022). An isolated population of guitarfish exists in Brazil but there is uncertainty whether this is a population of *P. lentiginosus* due to the absence of their characteristic white dorsal spots (Last et al., 2016; *Shorefishes—The Fishes—Species*, 2015). Their preferred adult habitats are soft, benthic habitats of 30–100 meters in depth (Charvet et al., 2019; Last et al., 2016; Moore, 2017).

Figure 1: Global Distribution of the Atlantic guitarfish, Pseudobatos lentiginosus. Map Created by Bryan Huerta Beltrán

Reproduction and Diet

P. lentiginosus reproduce via aplacental yolk viviparity, with early spring to late summer parturition (Hamlett et al., 1998; Hensley et al., 1998) and a mean litter size of seven individuals (Hensley et al., 1998). Although little is known about their life history, other *Pseudobatos* spp. exhibit biennial reproduction (Rocha and Gadig, 2013) and aggregate for parturition in benthic, sandy, shallow coastal waters where young remain for their first year before moving into deeper waters as adults (Anderson et al., 2021). Throughout their life, *P. lentiginosus* feed on crustaceans through suction feeding (Shibuya et al., 2005; Wilga and Motta, 1998) and have a hyostylic jaw that assists in their suction feeding and prey manipulation (Wilga and Motta, 1998).

Decline, Threats, and Knowledge Gaps

P. lentiginosus are listed as Vulnerable (Table 1) on IUCN Red List of Threatened Species due to a population reduction of 30–49% in the past three generations and fragmented occurrences (Charvet et al., 2019). These declines are rooted in various causes such as bycatch and habitat loss throughout their known distribution. Like other elasmobranchs, shrimp trawling in the Gulf of Mexico contributes to both bycatch and habitat loss because the trawl damages the loose benthic sediment habitat (Charvet et al., 2017; Magris and Giarrizzo, 2020). Additional habitat loss results from Atlantic and Gulf of Mexico oil spills (Charvet et al., 2019; Magris and Giarrizzo, 2020; Moore et al., 2017; Zea-de la Cruz et al., 2021). For example, an oil spill off the coast of Brazil interfered with the benthic habitat quality of *P. lentiginosus* (Magris and Giarrizzo, 2020).

The decline of *P. lentiginosus* in their known range and the lack of recent documented occurrences in the northern Gulf of Mexico call for highly sensitive survey techniques to assess their status in these waters. This is necessary for more accurate

information regarding current distributions and population declines or location extinctions, which can be achieved via environmental DNA (eDNA) surveys.

Environmental DNA

Environmental DNA is trace DNA left behind by organisms in the water column that can be captured via water collection and filtration methods (Ficetola et al., 2008). Potential sources of eDNA include skin cells within mucus, waste, skin, or blood from the target species (see Schweiss et al., 2019). Environmental DNA surveys that use water samples typically filter onto membranes to capture particulate material. Compared to traditional methods, such as tagging, eDNA surveys are non-invasive, because they do not require handling the target species (Kirshtein et al., 2007). Further, handling an individual may cause excess stress and may lead to injury or mortality, which is problematic if surveying an at-risk species (Schweiss et al., 2019). These assays also eliminate the time and expenses necessary to locate rare species (Ficetola et al., 2008). Because eDNA techniques are more sensitive than traditional methodologies and do not require handling, they are often used to detect rare and imperiled species (Ficetola et al., 2008; Schweiss et al., 2019). The information gained from eDNA surveys can allow inferences of ecological qualities such as: habitat use, abundance, and species distributions (Schweiss et al., 2019).

Here, a species-specific droplet digital PCR (ddPCR)™ eDNA assay was designed to detect DNA from the Vulnerable Atlantic Guitarfish from water samples collected in the northern Gulf of Mexico. This ddPCR™ assay is used instead of quantitative PCR (qPCR) or metabarcoding to provide higher sensitivity and better quantification of target eDNA (see Yang et al., 2014). This assay could be used in future

studies to assess the status of this species in United States (U.S.) waters, potentially identifying areas of location extinction events.

CHAPTER II: METHODS

Laboratory Controls

To prevent contamination, laboratory controls were used throughout assay development (see Goldberg et al., 2016; Schweiss et al., 2020). All plastics (i.e., ice blocks, tube racks, waste bins) were cleaned with 10% bleach and run under ultraviolet (UV) light for 15 minutes before use. All PCR reactions were set up in a clean PCR hood with designated pipettes, which were run under UV light for 15 minutes before each PCR cycle. To prevent contamination of the PCR reaction reagents, the DNA template was stored separately, and DNA was added using a separate pipette than the reaction set up. Negative controls, e.g., reactions without DNA template, were included in each PCR to test for contamination, which were run in replicates of three.

Droplet Digital™ PCR Assay

Primers were designed to amplify a 174 base-pair (bp) fragment of the mitochondrial DNA (mtDNA) *16S* ribosomal RNA (rRNA) gene in *P. lentiginosus*, but not in other closely related, co-occurring elasmobranchs species. To design these primers, *16S* sequences for *P. lentiginosus* (GenBank® accession No. AY830717) and 5 closely related co-occurring species (Table 2) were downloaded from GenBank® and aligned using CodonCode v 6.0.2. Forward (Plen16SF: 5'-CTAGTATAGGTGATAGAACGG-3') and reverse (Plen16SR: 5'-CTAATATGCTGCTCCGAA-3') primers, and an internal PrimeTime® double-quenched ZEN/IOWA Black™ probe labeled with FAM-6 (5'-6-FAM/CAGAGATTA/ZEN/GTCCTCGTA-3') were created with at least one bp difference between the target species (*P. lentiginosus*) and exclusion species (Table 2) in areas with over 50 cytosine and guanine percentage.

To confirm that the primers and probe amplified the desired fragment, quantitative PCR (qPCR) was conducted using genomic DNA (gDNA) extracted from a *P. lentiginosus* fin clip obtained in the Eastern Gulf of Mexico from the Florida Fish and Wildlife Conservation Commission and stored in ethanol. Reaction mixtures included: 1.1 microliter (µL) of target DNA (25 ng/µL), 900 nanomolar (nM) per primer, 250 nM of probe, 10.84 µL of PCR-grade water, and 1X Bio-Rad® iTaq™ universal probe supermix for a total reaction volume of 22 µL. The cycling conditions included enzyme activation at 95°C for 5 minutes followed by 44 cycles of: 94°C for 30 seconds and 60°C for two minutes. Finally, an enzyme deactivation step was performed at 98°C for 10 minutes, all with a ramp rate of 1°C/s. The PCR amplicon was cleaned using QIAGEN® QIAquick PCR purification kit following the manufacturer's protocols except all centrifugations were conducted at 12,000 revolutions per minute (rpm) and sent to Eurofins® Genomics for sequencing in a forward and reverse direction. A consensus sequence was assembled using CodonCode v 6.0.2. This sequence was analyzed using the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST).

The assay was optimized for the Bio-Rad® QX200™ AutoDG™ Droplet Digital™ PCR platform (droplet generator instrument no. 773BR3222; droplet reader instrument no. 771BR1496) by adjusting annealing temperature (60 °C–62 °C) and two supermixes (i.e., Bio-Rad® ddPCR™ supermix for probes (no dUTP's) and Bio-Rad® multiplex ddPCR™ supermix) to produce clear separation of positive and negative droplet relative fluorescent units (RFUs) with minimal "droplet rain" (i.e., the droplets lying between positive and negative bands). Optimized ddPCR™ reaction mixtures consisted of 1.1 µL

of target DNA (0.20 ng/µL), 900 nM of each primer, 250 nM of probe, 10.84 µL of PCR-grade water, and 4X Bio-Rad® multiplex ddPCR™ supermix to produce a reaction volume of 22 µL. DdPCR droplets were generated by the Bio-Rad® QX200™ AutoDG™ Droplet Digital™ PCR by extracting 20 µL of the reaction mixture and adding the oils. Optimized cycling conditions consisted of enzyme activation at 95℃ for 10 minutes, followed by 40 cycles of: 94℃ for 30 seconds and 60℃ for 2 minutes. A final enzyme deactivation was performed at 98℃ for 10 minutes, with a ramp rate of 1℃/s. To ensure the assay was species specific, it was cross-tested using the optimized reaction conditions and 0.20 ng/µL gDNA from one individual of each of the most closely related species, including two species of sawfish (*Pristis* spp.) and another closely related guitarfish (*Pseudobatos productus*) (Table 2) in replicates of three.

Table 2: Exclusion and target species GenBank® accession numbers and available collection locations for both cross-testing and assay design.

Species	GenBank® Accession Number	Collection Location (Cross-testing)	Collection Location (Assay Design)
Pseudobatos lentiginosus (target)	AY830717	Florida, Gulf of Mexico	South Carolina, Western Atlantic
Aetobatus narinari	KX151649.1	Not used	unknown
Mobula birostris	KM364991.1	Not used	La Paz, Mexico, Gulf of California
Pristis pectinata	MF682494.1	Africa	Veracruz, Mexico, Gulf of Mexico
Pristis pristis	NC_039438	Australia	Australia, Timor Sea
Pseudobatos productus	HM140461	California, U.S.	Panama, Central Eastern Pacific

Data Analysis

All ddPCR ™ data were analyzed using two criteria for a positive detection: 1) the droplet amplitude must be greater than the manual threshold (MT) of 2000 RFUs, and 2) droplets above the MT fall within a range of positive droplet population of 5000–7000 RFUs, based on analysis of a known positive sample. The analysis was performed using Bio-Rad® Quantasoft™ software with the Rare Event Detection (RED) option. The MT was determined by running a No Template Control (NTC) plate without DNA template on the ddPCR™ system with the optimized cycling conditions and reaction. Controls were considered negative if zero of the two criteria were met: the amplitude was not greater than the MT and droplets did not fall in the positive droplet range.

CHAPTER III: RESULTS

The negative controls did not produce an amplicon on qPCR (Figure 2b) and did not meet any of the criteria for a positive detection on ddPCR™. Specifically, there were no "positive" droplets above the defined MT of 2000 RFUs (Figure 3), nor were there any "positive" droplets within the positive droplet range. This means the assay did not amplify any negative controls on qPCR nor ddPCR™.

The genetic assay (e.g., primers and probe) designed was verified to amplify the target gene, *16S*, in *P. lentiginosus* using the NCBI BLAST function, in which the sequenced amplicon was 98.82% like *P. lentiginosus* from the Central Western Atlantic (GenBank accession No. AY830717.1).

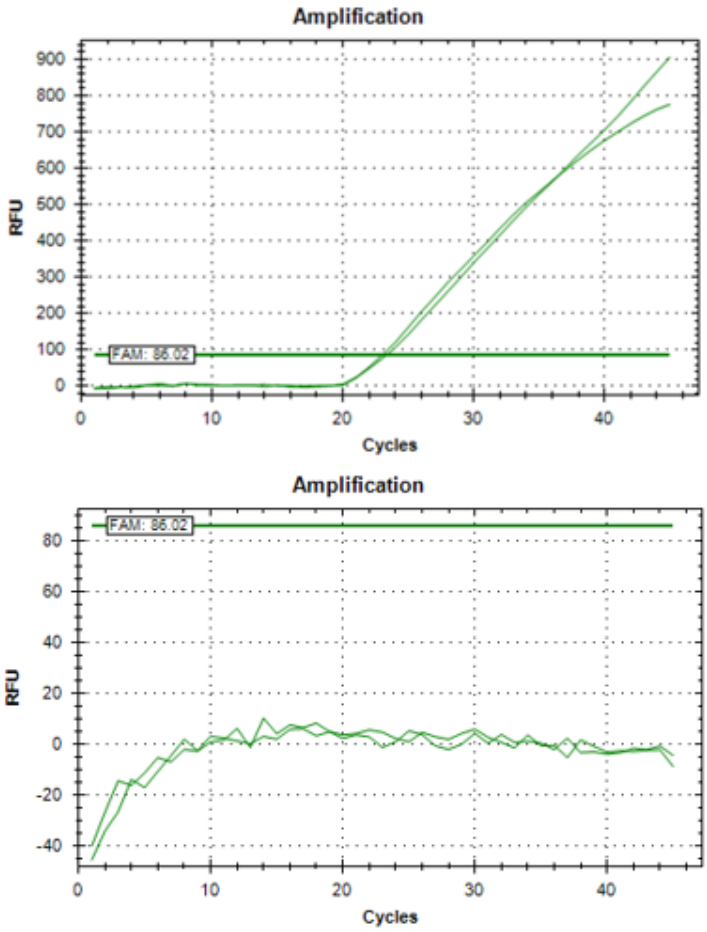

Figure 2: Quantitative PCR analysis for negative and positive controls using genomic Pseudobatos lentiginosus DNA targeting the mitochondrial DNA gene 16s. The figure shows an automatic threshold set at ~86 relative fluorescent units (RFUs), for both a and b with RFU measured on the y-axis and the number of cycles on the x-axis. Figure 2a shows 2 positive controls with a maximum RFU amplification of 900 RFU. Figure 2b shows the two negative replicates showing no amplification of the target 16s gene.

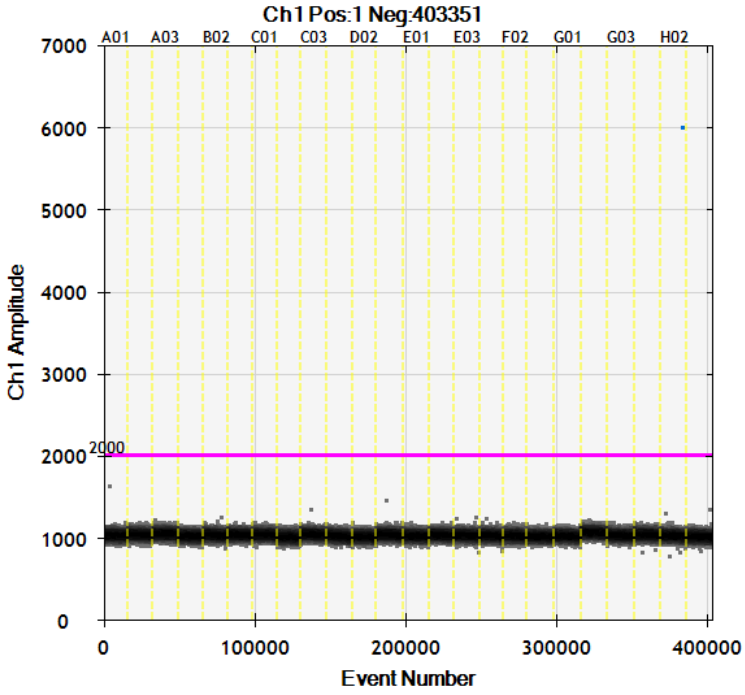

Figure 3: Digital Droplet PCR™ analysis completed on negative control plate of genomic Pseudobatos lentiginosus DNA targeting the mitochondrial 16S gene. The figure shows the amplitude in relative fluorescent units (RFUs) on the y-axis and the event number on the x-axis. The manual threshold (MT) is presented as the pink line in 2000 RFUs, and gray dots represent negative control data. This analysis is done using Bio-Rad® QX200™ Droplet Reader, QuantaSoft™ software and the Rare Event Detection (RED) analysis setting.

The optimized qPCR assay amplified DNA in all replicates of P. lentiginosus, reaching a maximum of 900 RFUs (Figure 2a). Additionally, the optimized ddPCR™ assay also amplified DNA in all replicates of *P. lentiginosus* DNA (Figure 4). Both criteria were met on positive controls; the droplets fell above the MT of 2000 RFUs and droplets also fell within the positive droplet range of 5000–7000. The average concentration of DNA was 49.2 ng/μL. Additionally, it did not amplify any of the replicates of the exclusion species (Figure 5). This is supported by the fact that none of the exclusion species had amplification over the MT nor did they fall within the positive range of 5000–7000. Although *P. productus* showed one PCR artifact over the MT, this droplet did not fall within the positive droplet range and does not show a typical ddPCR™ "band".

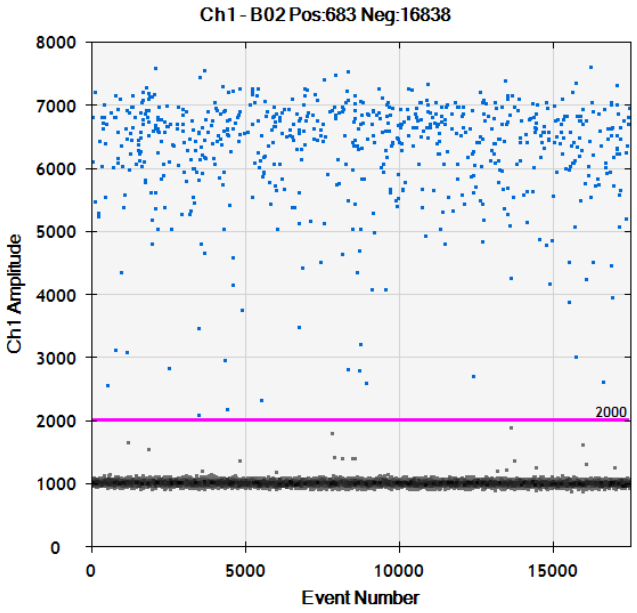

Figure 4: The optimized assay for genomic Pseudobatos lentiginosus DNA targeting the mitochondrial 16S gene shown on the Droplet Digital PCR™ platform. The pink line represents the manual threshold of 2000. The y-axis measures amplitude in relative fluorescent units (RFUs), while the x-axis shows the event number. Blue dots above the minimum threshold (MT) of 2000 RFUs are positive, while the gray dots below the MT are negative. The concentrated band of positive droplets between 5000–7000 indicate the range of positives, analyzed using Bio-Rad® QX200™ Droplet Reader, QuantaSoft™ software and the Rare Event Detection (RED) analysis setting.

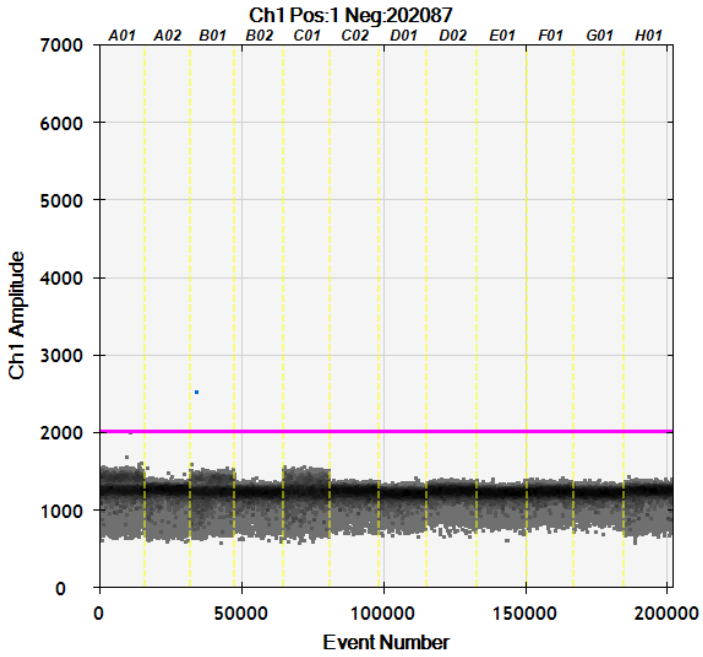

Figure 5: The cross-tested exclusion species shown on Droplet Digital PCR™ platform against genomic Pseudobatos lentiginosus DNA. The y-axis shows the amplitude in relative fluorescent units (RFUs), while the x-axis shows the event number. The manual threshold (MT) is represented by the pink line. The assay was tested against Pseudobatos productus (A01, B01, and C0), Pristis pectinata (A02, G01, and H01), Pristis pristis (D01, E01, and F01). Negative controls are in wells B02, C02, and D02.

CHAPTER IV: DISCUSSION

The development of a novel species-specific assay for *P. lentiginosus* is the first critical step needed to initiate eDNA surveys for this Vulnerable species in the Northern Gulf of Mexico. This assay was cross-tested against closely related elasmobranchs found in U.S. waters and did not amplify the target gene in any of these exclusion species. This eDNA tool is the first to be developed for any species of guitarfish and will support field surveys to assess the status and occurrence of *P. lentiginosus* in the U.S.

The developed eDNA assay may also have utility throughout the range of *P. lentiginosus* in the western Atlantic, which is thought to extend throughout Mexico, Central America, and possibly Brazil (Cruz et al., 2016; Last et al., 2016). Populations of *P. lentiginosus* outside of the U.S. may consist of genetically distinct populations (Cruz et al., 2023), but the assay targets the 16S gene, a highly conserved gene within species of elasmobranchs (Vences et al., 2005). Given this, it is anticipated that the developed assay will amplify DNA from *P. lentiginosus* throughout its range. Regardless, this should be confirmed via testing with individuals from these locations prior to commencing eDNA surveys outside of U.S. waters. This is particularly true for the purported *P. lentiginosus* in Brazil, which have morphological differences, such as the lack of characteristic white spots, and their taxonomy is unresolved, so these may constitute a distinct species (Cruz et al., 2023).

Before the developed eDNA assay can be used outside of U.S. waters, additional cross-testing is needed. Specifically, it should be cross-tested against the Chola Guitarfish, *Pseudobatos percellens*, which is a closely related species whose range

extends from the Yucatán peninsula to northern Brazil (Cruz et al., 2023). The current study was not able to comprehensively test assay function across the range of *P. lentiginosus*, nor to cross-test against *P. percellens* due to the lack of tissue samples from these species and locations.

Use of eDNA to assess *Pseudobatos lentiginosus* occurrence

The developed eDNA assay provides a powerful tool to conduct field surveys for the Vulnerable *P. lentiginosus*. EDNA surveys are beneficial in detecting presence without sighting and handling. This is both useful and cost-effective for rare, at-risk species, which may be unnecessarily stressed and harmed due to traditional tagging techniques (Kirshtein et al., 2007; Schweiss et al., 2019). Assays done for other at-risk elasmobranchs, including *P. pectinata* (Lehman et al., 2020, Lehman et al., 2022) and the Critically Endangered Scalloped Hammerhead, *Sphyrna lewini* (Budd et al., 2021) provided essential population information for management use.

The data given by *P. lentiginosus* eDNA surveys, such as species presence and distribution (Ficetola et al., 2008; Vences et al., 2005), would provide necessary information to enact conservation action for this Vulnerable, declining species (Charvet et al. 2019). Surveys have been used to confirm the presence of *P. pectinata* in historically occupied regions within the Northern Gulf of Mexico, where they were thought locally extinct (Lehman et al., 2022). Because of this confirmation, Mississippi State is now in the process of reinstating *P. pectinata* on endangered species lists and providing protections. Guitarfish, however, are not listed nor protected, so future *P. lentiginosus* eDNA surveys will provide the certainty needed for conservation of this declining, Vulnerable species.

P. lentiginosus were historically common in the Northern Gulf of Mexico (Charvet et al., 2019), but sightings have become rare in the past 15 years within this region outside of Florida (Bryan Huerta-Beltrán pers. comm., 2022; Jill Hendon pers. comm., 2022). Furthermore, there have been no known sightings in the Mississippi sound within the past 15 years, and public sightings are limited to Texas, Alabama, Georgia, and Florida (Jill Hendon pers. comm., 2022; Bryan Huerta-Beltrán pers. comm., 2022; Shepherd and Myers, 2005). The developed assay can provide the necessary quantitative data for historical distributions from which to work towards recovery. This recovery process of *P. lentiginosus* is predicted to be slow based on their k-selected life history of slow maturity and production, showing more need for this assay (Barrowclift et al., 2017; Shepherd and Myers, 2005; Zea-de la Cruz et al., 2021).

The Usefulness of the ddPCR™ Platform

The gaining popularity of integrating ddPCR™ technology in eDNA surveys is due to its sensitivity and higher quantification accuracy compared to traditional and qPCR (Chen et al., 2021; Doi et al., 2015; Falzone et al., 2020). Other developed ddPCR™ assays such as for *P. pectinata* (Lehman et al., 2020) and the Bull Shark, *Carcharhinus leucas* (Schweiss et al., 2020) have found this system capable of detecting as low as 0.25 picograms (pg) of target DNA per reaction. Compared to qPCR, ddPCR™ has been shown as 65% more sensitive (Song et al., 2018; Yang et al., 2014), leading to reduction of false negatives in which target DNA is present, but not detected (Doi et al., 2015) and leading to quicker protection. This is necessary in assays contemplating at-risk species such as manatees, *Trichechus* spp. (Hunter et al., 2018), Critically Endangered Scarce Yellow Scally, *Isogenus nubecula* (Mauvisseau et al., 2019), and Critically

Endangered Smalltooth Sawfish, *P. pectinata* (Lehman et al., 2020; Lehman et al., 2022), in which the surveys lead to conservation actions.

Unlike qPCR, ddPCR™ can detect target eDNA in the presence of inhibitors and highly concentrated, closely related, non-target DNA (Harper et al., 2019; Hunter et al., 2018). Inhibitors, such as tannin and humic compounds, inactivate DNA polymerase and obstruct PCR amplification, causing increased false negatives (Harper et al., 2019; Hunter et al., 2018). Because eDNA surveys commonly target rare species, it is important to reduce the inhibition as much as possible. RED analysis allows ddPCR™ to detect small amounts of target eDNA among highly concentrated non-target DNA (Nyaruaba et al., 2019; Yang et al., 2014). Assays using this analysis can detect rare mutations as low as 0.0005% and rare sequences as low as 0.00031% (Yang et al., 2014). These accomplishments are possible because of ddPCR™'s mechanism of droplet partitioning. DdPCR™ isolates the DNA in up to 20,000 chambers, so only one of two sequences are contained in each chamber, greatly decreasing the competition between target and non-target DNA (Nyaruaba et al., 2019) Using this approach in combination with strict eDNA field and lab controls (see Goldberg et al., 2016) ensures accurate interpretation of eDNA results.

Future Research

This assay is the first step in filling the gaps needed for *P. lentiginosus* management and recovery. The future directions of this assay include determining a limit of detection (LoD) to quantify performance and validation to confirm the performance of the assay (Goldberg et al., 2016). The limit of detection determines the lowest concentration at which DNA can be detected with this assay at 95% probability by testing

the assay against 10-fold dilutions of DNA (Xia et al., 2021). Quantification of the DNA (i.e., the ability to determine accurate concentrations of DNA within a sample) decreases false negatives and gives a basis on which to build the rest of the eDNA protocols (Klymus et al., 2020). Validation of the assay should be performed using eDNA surveys in waters of known populations, such as aquaria or Floridian waters of high population in the Western Atlantic on the east coast of Florida (Charvet et al., 2019; Last et al., 2016). After an LoD and validation of this assay are done, it can be used for Vulnerable *P. lentiginosus* population assessment and management strategies in the Northern Gulf of Mexico. More information on the occurrence of *P. lentiginosus* along the Texas, Louisiana, Mississippi, and Alabama coasts is needed to enact state-wide conservation action and protection of this species.

REFERENCES

Alexandre de-Franco, B., Fernandes Mendonça, F., Oliveira, C., & Foresti, F. (2012). Illegal trade of the Guitarfish Rhinobatos horkelii on the Coasts of Central and Southern Brazil: Genetic Identification to Aid Conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *22*(2), 272–276. https://doi.org/10.1002/aqc.2229

Alverson, D. L., Freeberg, M. H., Murawski, S. A., & Pope, J. G. (1994). *A Global Assessment of Fisheries Bycatch and Discards*. Food & Agriculture Organization.

Anderson, A. B., Fiuza, T. M. J., Araujo, G. S., Canterle, A. M., Canto, L. M. C., Freitas, R. H. A., Gadig, O. B. F., & Floeter, S. R. (2021). A Safe Haven for Potential Reproductive Aggregations of the Critically Endangered Brazilian Guitarfish (Pseudobatos horkelii). *Journal of Fish Biology*, *99*(6), 2030–2034. https://doi.org/10.1111/jfb.14880

Barrowclift, E., Temple, A. J., Stead, S., Jiddawi, N. S., & Berggren, P. (2017). Social, Economic and Trade Characteristics of the Elasmobranch Fishery on Unguja Island, Zanzibar, East Africa. *Marine Policy*, *83*, 128–136. https://doi.org/10.1016/j.marpol.2017.06.002

Barría, C., Coll, M., & Navarro, J. (2015). Unravelling the Ecological role and Trophic Relationships of Uncommon and Threatened Elasmobranchs in the Western Mediterranean Sea. *Marine Ecology Progress Series*, *539*, 225–240. https://doi.org/10.3354/meps11494

Budd, A. M., Cooper, M. K., Port, A. L., Schils, T., Mills, M. S., Deinhart, M. E., Huerlimann, R., & Strugnell, J. M. (2021). First Detection of Critically Endangered Scalloped Hammerhead Sharks (Sphyrna lewini) in Guam, Micronesia, in Five Decades using Environmental DNA. *Ecological Indicators*, *127*, 107649. https://doi.org/10.1016/j.ecolind.2021.107649

Charvet, P., Pollom, R., Blaco-Parra, M. P., Cardenosa, D., Derrick, D., Espinoza, E., Herman, K., Morales-Saldaña, J.M., Naranjo-Elizondo, B., Pacoureau, N., Pérez Jiménez, J. C., Schneider, E. V. C., Simpson, N. J., & Dulvy, N. K. (2019). IUCN Red List of Threatened Species: Pseudobatos lentiginosus. *IUCN Red List of Threatened Species*. https://www.iucnredlist.org/en

Chen, B., Jiang, Y., Cao, X., Liu, C., Zhang, N., & Shi, D. (2021). Droplet Digital PCR as an Emerging Tool in Detecting Pathogens Nucleic Acids in Infectious Diseases. *Clinica Chimica Acta*, *517*, 156–161. https://doi.org/10.1016/j.cca.2021.02.008

Cisneros-Montemayor, A. M., Barnes-Mauthe, M., Al-Abdulrazzak, D., Navarro-Holm, E., & Sumaila, U. R. (2013). Global Economic Value of Shark Ecotourism: Implications for Conservation. *Oryx*, *47*(3), 381–388. https://doi.org/10.1017/S0030605312001718

Cruz, V. P., Rotundo, M. M., Charvet, P., Boza, B. R., Souza, B. C., Cerqueira, N. N. C. D., Oliveira, C., Lessa, R., & Foresti, F. (2023). Investigating an Unknown Biodiversity: Evidence of Distinct Lineages of the Endemic Chola Guitarfish Pseudobatos percellens Walbaum, 1792 in the Western Atlantic Ocean. *Diversity*, *15*(3), Article 3. https://doi.org/10.3390/d15030344

Díaz-Jaimes, P., Bonfil, R., Palacios-Barreto, P., Bolaño-Martinez, N., & Bayona-Vásquez, N. J. (2018). Mitochondrial Genome of the Critically Endangered Smalltooth Sawfish Pristis pectinata from Veracruz, Mexico. *Conservation Genetics Resources*, *10*(4), 663–666. https://doi.org/10.1007/s12686-017-0896-9

Doi, H., Takahara, T., Minamoto, T., Matsuhashi, S., Uchii, K., & Yamanaka, H. (2015). Droplet Digital Polymerase Chain Reaction (PCR) Outperforms Real-Time PCR in the Detection of Environmental DNA from an Invasive Fish Species. *Environmental Science & Technology*, *49*(9), 5601–5608. https://doi.org/10.1021/acs.est.5b00253

Dulvy, N. K., Pacoureau, N., Rigby, C. L., Pollom, R. A., Jabado, R. W., Ebert, D. A., Finucci, B., Pollock, C. M., Cheok, J., Derrick, D. H., Herman, K. B., Sherman, C. S., VanderWright, W. J., Lawson, J. M., Walls, R. H. L., Carlson, J. K., Charvet, P., Bineesh, K. K., Fernando, D., … Simpfendorfer, C. A. (2021). Overfishing Drives Over one-third of All Sharks and Rays Toward a Global Extinction Crisis. *Current Biology*, *31*(21), 4773-4787.e8. https://doi.org/10.1016/j.cub.2021.08.062

Falzone, L., Musso, N., Gattuso, G., Bongiorno, D., Palermo, C. I., Scalia, G., Libra, M., & Stefani, S. (2020). Sensitivity Assessment of droplet digital PCR for SARS-CoV-2 Detection. *International Journal of Molecular Medicine*, *46*(3), 957–964. https://doi.org/10.3892/ijmm.2020.4673

Ficetola, G. F., Miaud, C., Pompanon, F., & Taberlet, P. (2008). Species Detection Using Environmental DNA from Water Samples. *Biology Letters*, *4*(4), 423–425. https://doi.org/10.1098/rsbl.2008.0118

Gallagher, A. J., Kyne, P. M., & Hammerschlag, N. (2012). Ecological Risk Assessment and its Application to Elasmobranch Conservation and Management. *Journal of Fish Biology*, *80*(5), 1727–1748. https://doi.org/10.1111/j.1095-8649.2012.03235.x

Gerhardt, K (2018). *Indigenous knowledge and cultural values of hammerhead sharks in Northern Australia*. Report to the National Environmental Science Program, Marine Biodiversity Hub. James Cook University.

Goldberg, C., Turner, C., Deiner, K., Klymus, K., Thomsen, P. F., Murphy, M., Spear, S., McKee, A., Oyler-McCance, S., Cornman, R., Laramie, M., Mahon, A., Lance, R., Pilliod, D., Strickler, K., Waits, L., Fremier, A., Takahara, T., Herder, J., & Taberlet, P. (2016). Critical Considerations for the Application of Environmental DNA Methods to Detect Aquatic Species. *Methods in Ecology and Evolution*, *7*, 1299–1307.

Greig, T. W., Moore, K. M., Woodley, C. M., & Quattro, J. M. (2005). Mitochondrial Gene Sequences Useful for Species Identification of Western North Atlantic Ocean Sharks. *Fishery Bulletin*, *103*(3), 516–523.

Haas, A. R., Fedler, T., & Brooks, E. J. (2017). The Contemporary Economic Value of Elasmobranchs in The Bahamas: Reaping the Rewards of 25 years of Stewardship and Conservation. *Biological Conservation*, *207*, 55–63. https://doi.org/10.1016/j.biocon.2017.01.007

Hamlett, W.C., Hysell, M.K., Galvin, J., & Spieler, R. (1998). Reproductive Accommodations for Gestation in the Atlantic Guitarfish, Rhinobatos

lentiginosus, Rhinobatidae. *Journal of the Elisha Mitchell Scientific Society*, *114*(4), 199–208.

Harper, L. R., Buxton, A. S., Rees, H. C., Bruce, K., Brys, R., Halfmaerten, D., Read, D. S., Watson, H. V., Sayer, C. D., Jones, E. P., Priestley, V., Mächler, E., Múrria, C., Garcés-Pastor, S., Medupin, C., Burgess, K., Benson, G., Boonham, N., Griffiths, R. A., & Hänfling, B. (2019). Prospects and Challenges of Environmental DNA (eDNA) Monitoring in Freshwater Ponds. *Hydrobiologia*, *826*(1), 25–41. https://doi.org/10.1007/s10750-018-3750-5

Hensley, R. A., McCoid, M. J., & Luer, C. A. (1998). Litter Variation in the Atlantic Guitarfish (Rhinobatidae: Rhinobatos lentiginosus) with Comments on Distribution in the Gulf of Mexico. *The Southwestern Naturalist*, *43 No. 4*(1998), 501–504.

Hunter, M., Meigs-Friend, G., Ferrante, J., Takoukam Kamla, A., Dorazio, R., Keith-Diagne, L., Luna, F., Lanyon, J., & Reid, J. (2018). Surveys of Environmental DNA (eDNA): A new Approach to Estimate Occurrence in Vulnerable manatee populations. *Endangered Species Research*, *35*, 101–111. https://doi.org/10.3354/esr00880

Kirshtein, J. D., Anderson, C. W., Wood, J. S., Longcore, J. E., & Voytek, M. A. (2007). Quantitative PCR Detection of Batrachochytrium dendrobatidis DNA from Sediments and Water. *Diseases of Aquatic Organisms*, *77*, 11–15. https://doi.org/10.3354/dao01831

Klymus, K. E., Merkes, C. M., Allison, M. J., Goldberg, C. S., Helbing, C. C., Hunter, M. E., Jackson, C. A., Lance, R. F., Mangan, A. M., Monroe, E. M., Piaggio, A. J.,

Stokdyk, J. P., Wilson, C. C., & Richter, C. A. (2020). Reporting the Limits of Detection and Quantification for Environmental DNA Assays. *Environmental DNA*, *2*(3), 271–282. https://doi.org/10.1002/edn3.29

Kyne, P., Jabado, R., Rigby, C., Dharmadi, Gore, M., Pollock, C., Herman, K., Cheok, J., Ebert, D., Simpfendorfer, C., & Dulvy, N. (2020). The Thin Edge of the Wedge: Extremely High Extinction risk in Wedgefishes and Giant Guitarfishes. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *30*(7), 1337–1361.

Kyne, P. M., Wang, J.-J., Xiang, D., Chen, X., & Feutry, P. (2018). The Phylogenomic Position of the Critically Endangered Largetooth Sawfish Pristis pristis (Rhinopristiformes, Pristidae), Inferred from the Complete Mitochondrial Genome. *Mitochondrial DNA Part B*, *3*(2), 970–971. https://doi.org/10.1080/23802359.2018.1501315

Last, P. R., White, W. T., de Carvalho, B. S., Stehmann, M. F. W., & Naylor, G. J. P. (Ed.). (2016). *Rays of the World*. CSIRO.

Lee, B. R., Bullock, D., Hoover, A. L., & Olsen, N. A. (Eds.). (2019). *US National Bycatch Report First Edition Update 3*. US Department of Commerce, NOAA.

Lehman, R. N., Poulakis, G. R., Scharer, R. M., Hendon, J. M., Court, A. G., Wooley, A. K., Williams, A. M., Ajemian, M. J., Hadden, J. P., Beal, J. L., McCallister, M. M., & Phillips, N. M. (2022). Environmental DNA evidence of the Critically Endangered Smalltooth Sawfish, Pristis pectinata, in Historically Occupied US waters. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *32*(1), 42–54. https://doi.org/10.1002/aqc.3721

Lehman, R. N., Poulakis, G. R., Scharer, R. M., Schweiss, K. E., Hendon, J. M., & Phillips, N. M. (2020). An Environmental DNA tool for Monitoring the Status of the Critically Endangered Smalltooth Sawfish, Pristis pectinata, in the western Atlantic. *Conservation Genetics Resources*, *12*(4), 621–629. https://doi.org/10.1007/s12686-020-01149-5

Magris, R. A., & Giarrizzo, T. (2020). Mysterious Oil Spill in the Atlantic Ocean Threatens Marine Biodiversity and Local People in Brazil. *Marine Pollution Bulletin*, *153*, 110961. https://doi.org/10.1016/j.marpolbul.2020.110961

Marramà, G., Carnevale, G., Naylor, G. J. P., Varese, M., Giusberti, L., & Kriwet, J. (2021). Anatomy, Taxonomy, and Phylogeny of the Eocene Guitarfishes from the Bolca Lagerstätten, Italy, Provide new Insights into the Relationships of the Rhinopristiformes (Elasmobranchii: Batomorphii). *Zoological Journal of the Linnean Society*, *192*(4), 1090–1110. https://doi.org/10.1093/zoolinnean/zlaa125

Mauvisseau, Q., Davy-Bowker, J., Bulling, M., Brys, R., Neyrinck, S., Troth, C., & Sweet, M. (2019). Combining ddPCR and Environmental DNA to Improve Detection Capabilities of a Critically Endangered Freshwater Invertebrate. *Scientific Reports*, *9*(1), Article 1. https://doi.org/10.1038/s41598-019-50571-9

Moore, A. (2017). Are Guitarfishes the Next Sawfishes? Extinction Risk and an Urgent Call for Conservation Action. *Endangered Species Research*, *34*, 75–88. https://doi.org/10.3354/esr00830

Naylor, G., Caira, J., Jensen, K., Rosana, K., Straube, N., & Lakner, C. (2012). *Elasmobranch Phylogeny* (pp. 31–56). https://doi.org/10.1201/b11867-4

Nyaruaba, R., Mwaliko, C., Kering, K. K., & Wei, H. (2019). Droplet Digital PCR Applications in the Tuberculosis world. *Tuberculosis*, *117*, 85–92. https://doi.org/10.1016/j.tube.2019.07.001

Packer, C., Holt, R. D., Hudson, P. J., Lafferty, K. D., & Dobson, A. P. (2003). Keeping the Herds Healthy and Alert: Implications of Predator Control for Infectious disease. *Ecology Letters*, *6*(9), 797–802.

Poortvliet, M., Olsen, J. L., Croll, D. A., Bernardi, G., Newton, K., Kollias, S., O'Sullivan, J., Fernando, D., Stevens, G., Galván Magaña, F., Seret, B., Wintner, S., & Hoarau, G. (2015). A Dated Molecular Phylogeny of Manta and Devil Rays (Mobulidae) based on Mitogenome and Nuclear Sequences. *Molecular Phylogenetics and Evolution*, *83*, 72–85. https://doi.org/10.1016/j.ympev.2014.10.012

Puniwai, N. (2020). Pua ka wiliwili, nanahu ka manō: Understanding Sharks in Hawaiian Culture. *Human Biology*, *92*(1), 11–17. https://doi.org/10.13110/humanbiology.92.1.03

Rocha, F., & Gadig, O. B. F. (2013). Reproductive Biology of the Guitarfish Rhinobatos percellens (Chondrichthyes, Rhinobatidae) from the São Paulo Coast, Brazil, Western South Atlantic Ocean. *Journal of Fish Biology*, *82*(1), 306–317. https://doi.org/10.1111/j.1095-8649.2012.03493.x

Schweiss, K. E., Lehman, R. N., Drymon, J. M., & Phillips, N. M. (2020). Development of Highly Sensitive Environmental DNA Methods for the Detection of Bull Sharks, Carcharhinus leucas (Müller and Henle, 1839), using Droplet Digital™ PCR. *Environmental DNA*, *2*(1), 3–12. https://doi.org/10.1002/edn3.39

Shepherd, T. D., & Myers, R. A. (2005). Direct and Indirect Fishery Effects on Small Coastal Elasmobranchs in the Northern Gulf of Mexico. *Ecology Letters*, *8*(10), 1095–1104. https://doi.org/10.1111/j.1461-0248.2005.00807.x

Shibuya, A., de Souza Rosa, R., & Costa Soares, M. (2005). Note on the Diet of the Guitarfish Rhinobatos percellens (Walbaum, 1792) (Elasmobranchii: Rhinobatidae) from the coast of Paraíba, Brazil. *Acta Biologica Leopoldensia*, *27*(1), 63–74.

Shorefishes—The Fishes—Species. (2015). Smithsonian Tropical Research Institute. https://biogeodb.stri.si.edu/caribbean/en/thefishes/species/2726

Song, N., Tan, Y., Zhang, L., Luo, W., Guan, Q., Yan, M., Zuo, R., Liu, W., Luo, F., & Zhang, X.-L. (2018). Detection of Circulating *Mycobacterium tuberculosis* - Specific DNA by Droplet Digital PCR for Vaccine Evaluation in Challenged Monkeys and TB Diagnosis. *Emerging Microbes & Infections*, *7*(1), 1–9. https://doi.org/10.1038/s41426-018-0076-3

Thomsen, P. F., & Willerslev, E. (2015). Environmental DNA – An Emerging Tool in Conservation for Monitoring Past and Present Biodiversity. *Biological Conservation*, *183*, 4–18. https://doi.org/10.1016/j.biocon.2014.11.019

Triay-Portella, R., Martín, J. A., Luque, L., & Pajuelo, J. G. (2022). Relevance of Feeding Ecology in the Management of Invasive Species: Prey Variability in a Novel Invasive Crab. *Estuarine, Coastal and Shelf Science*, *274*, 107949. https://doi.org/10.1016/j.ecss.2022.107949

Vences, M., Thomas, M., van der Meijden, A., Chiari, Y., & Vieites, D. R. (2005). Comparative Performance of the 16S rRNA gene in DNA barcoding of amphibians. *Frontiers in Zoology*, *2*(1), 5. https://doi.org/10.1186/1742-9994-2-5

Wilga, C. D., & Motta, P. J. (1998). *Feeding Mechanism of the Atlantic Guitarfish Rhinobatos Lentiginosus: Modulation of Kinematic and Motor Activity*. 19.

Xia, Z., Zhan, A., Johansson, M. L., DeRoy, E., Haffner, G. D., & MacIsaac, H. J. (2021). Screening Marker Sensitivity: Optimizing eDNA-based Rare Species Detection. *Diversity and Distributions*, *27*(10), 1981–1988. https://doi.org/10.1111/ddi.13262

Yang, R., Paparini, A., Monis, P., & Ryan, U. (2014). Comparison of Next-Generation Droplet Digital PCR (ddPCR) with Quantitative PCR (qPCR) for Enumeration of Cryptosporidium Oocysts in Faecal Samples. *International Journal for Parasitology*, *44*(14), 1105–1113. https://doi.org/10.1016/j.ijpara.2014.08.004

Zea-de la Cruz, H., Tovar-Ávila, J., Meiners-Mandujano, C., Jiménez-Badillo, L., & Oviedo-Pérez, J. L. (2021). Determining Potential Management Strategies for the Elasmobranchs Bycatch of the Mexican Shrimp Trawl Fishery of the Gulf of Mexico Through a Vulnerability Analysis. *Regional Studies in Marine Science*, *42*, 101626. https://doi.org/10.1016/j.rsma.2021.101626