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# Capstone of
# Arianna N. Nixon

Submitted in Partial Fulfillment of the Requirements for the Degree of

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NOVA SOUTHEASTERN UNIVERSITY

HALMOS COLLEGE OF ARTS AND SCIENCES

# A Shark Conservationists Toolbox: Current DNA Methods and Techniques Aiding in the Conservation of Sharks

By

Arianna Nixon

Submitted to the Faculty of
Halmos College of Arts and Sciences
in partial fulfillment of the requirements for
the degree of Master of Science with a specialty in:

Marine Biology

Nova Southeastern University

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***Abstract***

Elasmobranchs are important members of their community. Many sharks are important apex predators that help maintain the health of their ecosystem. However, shark populations are globally declining. This is partially due to the fact that sharks are highly targeted for their fins, meat, liver oil, teeth, and skin. However, they are also killed from anthropogenic effects such as habitat destruction and pollution. Most shark species have life history characteristics that also make them more vulnerable to overfishing. Sharks are also difficult to study due to their elusive nature and identification issues. That is why molecular tools are increasingly becoming important for studying sharks. This paper discusses four different types of molecular tools: mitochondrial and nuclear DNA, environmental DNA, sequence-based, and PCR-based tools. All of these techniques are currently being used to help study and conserve sharks. These techniques can obtain important ecological information for a given species. The majority of the research has been conducted on species identification. Specifically, you can use these tools to identify a particular species of importance, or to classify the global fin trade, or even to identify species in highly processed samples. Species identification isn't the only useful information that can be obtained however. Molecular tools can also help us better understand the species composition, stock structure, mating system, or population size of a given area. Molecular tools are still a growing area of research. In the future these techniques will continue to improve, and the information that we can learn will continue to grow. One of the biggest hurdles for this type of research is a lack of communication between geneticists and fishery managers and policy makers. Molecular tools have the potential to help with current and future policy and management. That is why it is important for anyone interested in the conservation of elasmobranchs to have a better understanding of molecular techniques.

Keywords: sharks, elasmobranchs, conservation, DNA, DNA barcoding, eDNA, mtDNA, microsatellites, multiplex PCR, forensics

***Introduction***

Shark populations around the world are rapidly declining, mostly because they are a heavily targeted species. Sharks are killed for many of their parts including fins, meat, liver, teeth, jaws, and skin (Dulvy et al. 2008). The fins are the most prized part of the shark, because they are worth a substantial amount of money in Asian markets (Cardeñosa and Chapman 2018; Fields et al. 2018). Sharks are also killed for other reasons, including bycatch, habitat degradation, and pollution (Dulvy et al. 2014). Sharks are at a greater disadvantage due to many of their life history characteristics (Cortés 2000). Similar to other top predators, they are more k-selected. This means that sharks typically take longer to reach maturity, have longer gestation periods, and have fewer and larger offspring (Parry 1981). This makes them particularly vulnerable to extinction.

There are many challenges we must overcome in order to recover or save these populations from extinction. Sharks are particularly difficult to study. They are a very diverse group occupying most regions of the world at various depths. Additionally, many species are quite mobile, with some undergoing circumglobal migrations. Some species are quite elusive, meaning sampling numbers are typically low. Additionally, it can be hard to identify species from morphological characteristics alone, with even the most trained scientists may confuse some distinctions between species (Tillett et al. 2012; Wong, Shivji, and Hanner 2009; Dulvy et al. 2017). This is especially a problem in markets where sharks are sold for parts and are missing key morphological characteristics. In order to overcome these challenges a variety of approaches will be necessary. Molecular Biology techniques are a more recent branch of science that has changed the way we can study sharks.

There are a number of molecular techniques that can be used to study sharks, including mitochondrial DNA, microsatellite loci, environmental DNA, sequenced-based species identification, and Real-Time PCR-based species identification (Domingues, Hilsdorf, and Gadig 2018). All these are current practices that can be used to help better understand, as well as conserve shark species. These techniques can also be used to identify species of importance, species in the global fin trade, or species in highly processed samples like commercial products. Additionally, they can be used to study species composition, stock structure, mating systems, and population size. This paper aims to discuss how these techniques are being used in these studies, as well as their potential uses in future analyses.

***Why are sharks at risk, and why does it matter?***

Most shark and ray species are vulnerable to overexploitation. This is a result of various life history traits, including low fecundity, late maturity, a long gestation period, slow growth, and a long life span (Compagno 1990). Fecundity refers to the potential to reproduce. Sharks and rays generally have few pups per reproductive cycle, as well as long time periods between these cycles, which results in a low fecundity. Sharks also typically don't reach maturity until later in life, meaning even fewer reproductive cycles, as well as the potential to be caught and killed before having gone through a single reproductive cycle. Additionally, sharks have a long gestation period, which means that the pups take a long time to reach a size capable of surviving outside the mother's womb (Parry 1981; Cortés 2000). The shark's slow growth is part of the reason why they have delayed maturity. It also means that larger, targeted sharks are quite old, and also the most likely reproductive. Another way of explaining this is that sharks are a K-selected species (Parry 1981). K-selected species reproduce slowly and produce few offspring, which means that they have a more stable population size, but they are particularly sensitive to overfishing (O'Bryhim, Parsons, and Lance 2017; Haas, Fedler, and Brooks 2017). Because fishing practices remove sharks rapidly from the water, they are unable to recover their numbers as fast as they are being removed which results in a population crash. All of these life history characteristics makes shark populations very vulnerable to collapse with minimal fishing pressure when compared to bony fish.

Elasmobranchs face a high level of exploitation, which coupled with their life history traits is becoming a huge problem. Sharks are harvested both in targeted fisheries and by-catch for their meat, fins, skin, oil, and cartilage (Hellberg, Isaacs, and Hernandez 2019). However, sharks' fins are the most lucrative, and worth the most money. This is because in Asia, shark fin soup is a symbol of status, and as the middle class has grown, so has the demand for shark fin soup (Sembiring et al. 2015). The global shark market results in the death of ~ 70-100 million sharks a year (Palumbi, Robinson, and Van Houtan 2018).

Unfortunately, their fins are not the only reason sharks are being targeted. Shark meat is used for consumption, especially in third world countries because the meat is relatively inexpensive due to its low quality (Dulvy et al. 2017). Because the meat is less expensive, it is also commonly mislabeled as being a fish filet in more affluent countries. Another valuable part of the shark is its liver. Sharks are known for having very large oily livers. This oil is commonly

used in lubricants, cosmetics, and vitamin A supplements. Cartilage pills are also a very important shark product on the market, as many consumers believe that it will prevent cancer (Gingras et al. 2000). Additionally, their skin is used for leather, and their jaws and teeth are common in the curio trade. Many of the shark's parts are destined for different markets, resulting in parts being imported and re-exported, complicating the ability to track where these parts came from (Dulvy et al. 2017). Therefore, managing the shark trade is not as easy as managing the shark fin trade, as there is a very high demand for shark products globally.

Another big problem that sharks face is bycatch, with an estimated 12 million elasmobranchs being caught as bycatch each year. Longlines and gillnets are particularly dangerous to sharks yet are widely used. For longlines, the mortality rate typically depends on how long the lines are left in the water, while gillnets typically have a high mortality regardless. Unfortunately, even if the fisherman does everything right, post-release mortality is still considered very high for many species (Whitney et al. 2016). Additionally, most elasmobranchs fisheries target multiple species, and they use low species selective gear, which results in the capture of protected species (Oliver et al. 2015). This can create a situation of illicit trade for the fisherman. One extreme case of this is India, as they have no regular shark fishery, yet they export a practically steady number of fins each year (~ 70,000 tons), all caught as bycatch (Verlecar, Desai, and Dhargalkar 2007). Therefore, bycatch is a global problem that needs to be addressed.

As is the case with many other marine animals, there are other threats facing sharks besides fisheries. Anthropogenic effects, such as pollution and habitat degradation or loss are also big threats to sharks. The warming of waters results in a shift in their habitat, northwards. Their prey will also have to shift and adapt as well, which means the sharks will have to follow. Additionally, as the oceans become more acidic this can have negative effects on many aspects of shark ecology and biology (Rosa, Rummer, and Munday 2017). Studies are beginning to show that it can have negative effects on the survival of shark pups, as well as affecting their sense of smell (Pistevos et al. 2017). Elasmobranchs are known to have a sensitive and powerful olfactory system that is used for finding food, mates, predators, homing, and navigation. Increased $CO_2$ levels are being used to demonstrate that it is potentially harder for species to find food using these olfactory cues (Dixson et al. 2015). Additionally, many shark species rely upon estuaries and coastal areas as a nursery. The destruction of these areas can be direct, such as the

destruction of mangroves, or indirect, such as pollution. Unfortunately, there are a lot of potential threats to sharks due to anthropogenic effects (Dulvy et al. 2008).

Sharks are important apex predators in their environment. Therefore, they play a very important role by helping to maintain species diversity as well as the health of their ecosystem. By removing sharks, this will have important top down effects on the rest of the ecosystem. When sharks are removed from a coral reef environment, the next large predator will have a temporary bloom, where their population will expand so rapidly that they will have no prey left to eat. This destructive chain of events typically leads to an increase of algae, which will choke the reef, causing it to die (Roff et al. 2016). This example is not only relevant for coral reef species. Studies have shown that the collapse of shark populations would have disastrous effects on an ocean wide scale (Heupel et al. 2014). Therefore, the removal of these apex predators will greatly affect their entire ecosystem, making them important species to conserve.

***Challenges for reducing overfishing and improving management***

Since we know how important sharks are to their ecosystem, why is it so hard to improve the management of their fisheries? One of the biggest issues is a basic lack of understanding the status of shark populations, which greatly hampers the development and implementation of appropriate conservation measures (Shivji et al. 2002). Another big issue is the identification of landed species. Identification can be close to impossible for many landed species, making management and enforcement of conservation policies impossible. Additionally, marine jurisdiction of landed species is very complicated, which makes monitoring and regulation complicated. Finally, many countries rely upon shark fishing for income, which makes regulation extremely hard because there is no political pressure to do so. While molecular tools cannot fix, or solve all of these problems, molecular tools have the potential to reduce some of these issues and help assist in increasing shark conservation efforts before it is too late.

In order to decide what organisms are in need of increased management and policies, we need to know which species are threatened. The International Union for Conservation of Nature's Red List of Threatened Species (IUCN) provides some of the most comprehensive information to aid in the global conservation of many different animal and plant species. Unfortunately, approximately 50% of shark species are listed as data deficient (DD) (IUCN 2020; Dulvy et al. 2014). Sharks are incredibly hard to study due to their elusive, and highly mobile nature. Understanding aspects of the biology, habitat use, and population demographics is

the first step towards improved conservation and management of sharks and rays (Pazmiño et al. 2018). Therefore, the fact that 50% of all shark species don't even have enough information to determine whether they are threatened or not, is a major concern from a conservation standpoint. While sharks have the potential to be harvested sustainably, this lack of information is an enormous problem that needs to be addressed. While many elasmobranch species are relatively large in size, finding enough individuals to complete a statistically sound study can be very challenging, depending on the species and methodology used. This has become a major challenge to conservation measures, because without information about the status of a species, policy makers cannot take action.

The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) is an international treaty, which aims to not only eliminate trade of species with a high extinction risk, but also to encourage sustainable trade of wildlife. CITIES operates under three main principles: legality, sustainability, and traceability. Species are proposed for listing by at least one nation and is then listed under one of two appendices (Appendix I or II) by vote (CITIES 2020). Species listed under Appendix I are prohibited from international trade, with rare exceptions. For species listed in Appendix II the exporting party must issue a permit that certifies that the trade is not detrimental to the species survival based on a non-detriment finding, and that the traded specimen was legally obtained and traceable through the supply chain. There are currently 12 shark species listed on Appendix II: the whale shark (*Rhincodon typus*), basking shark (*Cetorhinus maximus*), great white (*Carcharodon carcharias*), porbeagle shark (*Lamna nasus*), scalloped hammerhead (*Sphyrna lewini*), great hammerhead (*S. mokarran*), smooth hammerhead (*S. zygaena*), oceanic whitetip shark (*Carcharhinus longimanus*), silky shark (*C. falciformis*), bigeye thresher shark (*Alopias superciliosus*), pelagic thresher shark (*A. pelagicus*), and common thresher shark (*A. vulpinus*) For further information on these species please refer to the appendix. Unfortunately, there are major issues to enforcing CITIES regulations. Recently, evidence has exposed low compliance by CITIES parties, most likely due to the inability to monitor and enforce the regulations (Cardeñosa, Merten, and Hyde 2019). Many sharks are sold in pieces, meaning that the species lack basic morphological characteristics for identification (Palumbi, Robinson, and Van Houtan 2018). Another major issue is the difficulty it is to access the landing points, which is compounded by a lack of port inspectors (Velez-Zuazo et al. 2015). Unfortunately, most of the countries with the greatest shark landings are the ones with the least

amount of monitoring in place. While international treaties such as CITIES is a great first step, it requires greater regulation and monitoring before it can become truly effective.

Another major issue of concern for shark conservation is the complication of international jurisdiction when dealing with highly mobile species such as elasmobranchs. Quite a few sharks are protected by local or national legislation, however, these different legislations can often be complicated. In the United States, some species are only protected within 3 miles of shore by state legislation (Cardeñosa and Chapman 2018). However, nationally speaking, each country has jurisdiction over the use of marine resources extending 200 miles from shore, which is called the Exclusive Economic Zone (EEZ). Therefore, a species can be protected within any countries given EEZ. Any joint organizational treaties across national lines, have the complication that the responsibility of enforcement is placed on the individual countries, which may not have the proper legislation in place (Ovenden et al. 2018). One of the biggest jurisdictional issues is knowledge of where the species was caught. Was it caught locally, nationally, or outside the EEZ? Both of these problems are further complicated by the fact that many shark species are quite mobile. Therefore, they are traveling across jurisdictional lines constantly, making it hard to know where they were caught. It has been shown that there is a positive correlation between the number of EEZs a species spans, and its extinction risk (Dulvy et al. 2017). Additionally, it makes it hard to put enough protection in place for a given species, because even if they are protected in one part of their habitat, they could be heavily exploited in another. Therefore, international cooperation among all countries on a given species migration route is essential for successful conservation (Lascelles et al. 2014) Finally, fishing in international waters is very hard to monitor and regulate, because no one country has jurisdiction, so no one country can control it. Marine jurisdiction is very complicated and must be carefully considered when new legislation is introduced.

Unfortunately, there are many countries that rely upon sharks either as food, income, or both. Therefore, careful consideration must be taken in these cases, in order to maintain the countries and the people's ability to survive. In some cases, alternatives could be introduced, such as shark ecotourism, but some countries do not have that option. In all of these cases, public support is going to be a major factor, because without public support, the conservation initiatives will most likely fail.

***Molecular biology techniques introduction***

In this section, the four major techniques; mitochondrial and nuclear DNA, environmental DNA, sequence-based techniques, and PCR techniques will be introduced. Each technique will be described in terms of basic concept and methodology, advantages, disadvantages, and commonly used primers. However, in order to understand any of these techniques a basic understanding of PCR (polymerase chain reaction) and target primers is necessary.

*PCR and Target Primers*

PCR (polymerase chain reaction) is a technique used to make millions of copies of a specific gene region, which allows this region to be further studied (Mullis and Faloona 1987). In order for PCR to be successful, a primer is an essential component (Van Pelt-Verkuil, Van Belkum, and Hays 2008). A primer is a short sequence of nucleotides that are a starting point for DNA synthesis. Typically, two primers are used so that they can flank the target region by complementary base pairing (Van Pelt-Verkuil, Van Belkum, and Hays 2008). After the primers bind, an enzyme, Taq polymerase extends the primers. Each cycle of PCR doubles the number of copies of the gene. Therefore, the cycle is repeated until millions of identical copies of the target sequence are created. Each cycle of PCR consists of a series of temperature changes. The first step is denaturation, where the temperature is increased (95°C) in order to disrupt the hydrogen bonds between the two strands, resulting in single stranded DNA. The second step is annealing, where the temperature is lowered (45-60°C) in order to promote the binding of the primers. The third step is extension, where the temperature is increased (72°C) for optimal DNA polymerase activity, allowing for the primers to be extended. This process of PCR is depicted in figure 1.

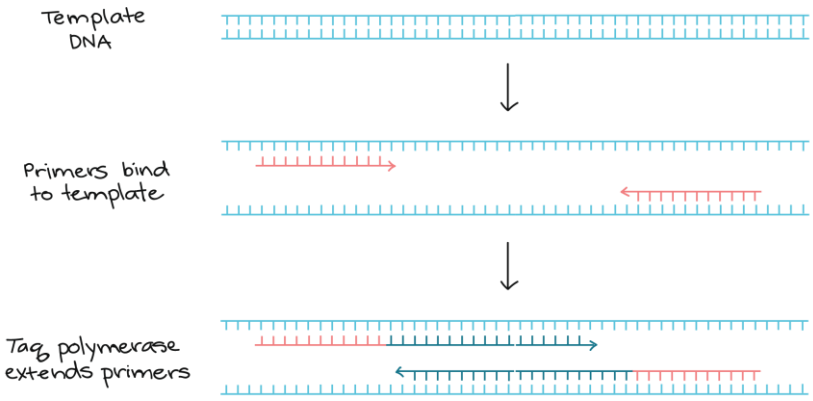


Figure 1: Depicts the simplified process of PCR. The light blue represents the template DNA, the red represents the primers, and the dark blue represents the copied DNA.

When performing a genetic study, one of the most important steps is selecting the correct primer, for the gene or region of interest. Primers should be specific to the target species, while taking into account differences with sequences of related organisms. Unsatisfactory primer specificity can lead to under or over estimation of species, especially with closely related species that may be present (Le Port et al. 2018). Typically, a species-specific primer is designed in order to attach to a specific, targeted locus within the DNA. Typically, the targeted loci are within the mitochondrial genome because of its greater abundance within a cell, and it is more widely used meaning primers for more species are already available. Each mitochondrion contains dozens of copies of its genome, and each cell has multiple mitochondria (Van Pelt-Verkuil, Van Belkum, and Hays 2008). Therefore, each cell contains thousands of copies of mitochondrial DNA compared to the one copy of nuclear DNA found in the nucleus. Additionally, the mitochondria play an important role in cellular respiration for all eukaryotes which is why all animals have conserved mitochondrial DNA. However, within the mtDNA, it does have a high enough mutation rate to make it useful for studying evolutionary relationships. Mitochondria are descended from specialized energy producing bacteria and early on became incorporated into the cytoplasm of all living cells today (Raven et al. 2020). That is why their genome is so similar between species. Selecting the correct gene region will depend on the intra- and interspecies variability. The locus must be highly conserved, but also have enough variation to be able to distinguish between species, making one universal locus very hard to find.

A commonly targeted locus in the mitochondrial genome is the cytochrome oxidase subunit I gene (COI). The COI gene (~650 bp) has been shown to have sufficient interspecies variation, while maintaining low intraspecific variation for most species (Moftah et al. 2011). Additionally, the COI gene has a high enough mutation rate to allow for species level identification in most cases. However, its biggest advantage is the fact that it is supported by a large database of sequence information due to the Barcode of Life Imitative (Hellberg, Isaacs, and Hernandez 2019). Therefore, the BOLD (Barcode of Life Data System) database is a very valuable tool for many different types of studies (Ratnasingham and Hebert 2007). However, there are some limitations within the COI gene. It has been observed that high rates of sequence variability impair the design of truly universal primers and hamper bioinformatics analysis (Le Port et al. 2018).

Another commonly targeted locus is the nuclear ribosomal DNA Internal Transcribed Spacer 2 (ITS2). This is one of the only nuclear DNA loci that is commonly used. The ITS2 region is highly conserved but contains enough interspecific variation to allow species level identification (Cardeñosa et al. 2018). However, there is insufficient sequence data available for most shark species in addition to the limited quantities of nuclear DNA (Caballero et al. 2012). Unfortunately, this makes the ITS2 locus unsatisfactory for many studies, unless the primers were previously designed. Primer design is labor intensive work and requires extensive testing to ensure validity. The result is that ITS2 studies are not as common.

There are many other loci and methods used in very specific cases. Other common mitochondrial loci include: cytochrome *b*, nicotine adenine dinucleotide dehydrogenase subunit 4 (NADH4), 16S, and 12S (Le Port et al. 2018). These markers are used in more specific types of analysis, such as eDNA analysis, that require the greater quantity of DNA available that only a mitochondrial locus can offer. There are significantly more copies of mitochondrial than nuclear DNA in cells, so in eDNA studies targeting a specific species it might be more advantageous to either select a less commonly used loci. There are other loci used for genetic studies, such as the 18S locus. However, the 18S locus is highly conserved, making it almost impossible to distinguish between the species or genus level (Le Port et al. 2018). The 16S rRNA gene is generally used in identification of prokaryotes, whereas the 18S rRNA gene is most times used for detecting microbial eukaryotes. Therefore, when designing an experiment, selecting an appropriate locus for the study is important, and multiple options should be considered.

*Mitochondrial and Nuclear DNA*

Genomic Mitochondrial DNA (mtDNA) is maternally inherited, haploid, and is located within the mitochondria organelles whereas nuclear DNA is diploid and located within the nucleus. The microsatellite portions of nuclear DNA are typically targeted for study. Microsatellites are a tract of repetitive DNA, where certain motifs are repeated. While valuable information can be learned from studying either type, some important information can be discerned by studying and comparing the two within a given population. By comparing the maternally inherited DNA to the nuclear DNA one can study more complex relationships including population structure, population size, and genetic relationships (Ovenden et al. 2018).

The greatest advantage of studies utilizing both mtDNA and microsatellites are the diversity of applications. mtDNA studies can, and are used for species ID. However, when combined with microsatellite information mtDNA can also be used to better understand the mating systems of a given species, and its global movements. Therefore, unlike the other techniques this one has the greatest current potential for ecological studies. The combination of both types of markers has been shown to yield a high degree of intraspecific resolution, making it useful to determine lineages and distinct populations (Spaet et al. 2015). Therefore, mtDNA and nuclear DNA studies are currently being used to better understand how species are moving through and using their environment, allowing for better understanding of how to protect them.

While mtDNA and nuclear DNA techniques can obtain some very useful information, they have some disadvantages. These methods can be time consuming, and sometimes complicated to understand. In order to obtain the most accurate results, many samples from different sampling areas are necessary (Pazmiño et al. 2018) and the selection of loci to study is very important. Additionally, a greater understanding of genetics is necessary in order to analyze the data and interpret the results. Therefore, there are greater challenges in addressing the knowledge gap between research scientists and the people who work in the social, political, and economic field in order to implement research findings into conservative actions (Ovenden et al. 2018).

*Environmental DNA*

All organisms continuously leave traces of themselves behind in the environment in the form of shed skin cells, bodily fluids, metabolic waste, gametes, or blood (Le Port et al. 2018).

Environmental DNA (eDNA) analysis is based on the retrieval of this naturally released genetic material from the environment (Lodge et al. 2012). eDNA is collected from bulk environmental samples such as soil, water, or air, making it non-invasive (Barnes and Turner 2016). There is substantially more mitochondrial than nuclear DNA per cell, making mtDNA the typical target for eDNA studies (Le Port et al. 2018).

Environmental DNA has been shown to be a reliable detection method, with many advantages over traditional methods. Traditional methods of DNA collection include blood and tissue samples, which can have negative effects on the target organism (Adams et al. 2019). Because eDNA techniques are non-invasive, it is not necessary for the target species to be disturbed or caught making this method advantageous (Le Port et al. 2018). The sensitivity of eDNA techniques make them ideal for detecting the presence of endangered, invasive, or rare species (Adams et al. 2019). Therefore, eDNA analysis can offer considerable time and cost benefits.

While the non-invasive nature of this technique offers many advantages, there are a few important caveats. It cannot be determined if the organism is living or dead. Additionally, the size, movement, condition, development, or sex of the individuals cannot be distinguished. Hybrids are also impossible to distinguish. Due to the highly sensitive nature of this technique, contamination is a potentially big issue. While shedding rates appear to be positively correlated to biomass, the complex factors that influence shedding rates are hard to study (Barnes et al. 2014). DNA characteristics, abiotic, and biotic factors all influence the degradation of eDNA (Barnes and Turner 2016). Additionally, there are habitat and ecosystem effects to consider. The transport of the eDNA can be hard to understand in lotic systems. Higher temperature, salinity, and UV radiation can result in higher degradation rates (Senapati et al. 2018). Therefore, with further refinements eDNA techniques could potentially be able to provide more information on taxonomic diversity and population abundance than current techniques (Lodge et al. 2012).

*Sequence-Based Techniques*

Sequence-based techniques are typically called DNA barcoding, and it is the most commonly used genetic identification method. DNA barcoding can be implemented in most scenarios at any point of the supply chain. DNA barcoding depends on the DNA sequence variation within species being lower than variation between species, which enables comparison

between the sequence derived from a product and reference sequences to infer the identity of the unknown species (Cardeñosa and Chapman 2018). In this technique, the DNA is isolated, which is followed by PCR, running on an agarose gel to check for amplification, a clean up step, DNA sequencing, sequence editing, and analysis. There are two main databases used to compare sequences to reference sequences resulting in a percentage of identity; GenBank Basic Local Alignment Search Tool (BLAST) or the Barcode of Life Data System (BOLD), which are comprised of the cytochrome *c* oxidase subunit 1 (COI) reference genes.

DNA barcoding has many advantages over similar techniques. One major advantage is that there are a wide range of species available for detection (Hellberg, Isaacs, and Hernandez 2019). Additionally, the BOLD and BLAST databases are always growing, improving their ability to accurately detect a wide variety of species from a global database (B. H. Holmes, Steinke, and Ward 2009). DNA barcoding can be used for a wide range of studies, especially when there is a wide range of possible species.

There are a few disadvantages to the barcoding technique. One big issue is the reliance on a database. If the database is incomplete, or if there are incorrectly identified individuals included in the database, then there could be potentially big impacts on the results. Additionally, there is controversy due to the exclusive reliance on the mitochondrial genome barcode. Sections of the mitochondrial DNA have been found to reside as pseudogenes, and mitochondria show heteroplasmy frequently resulting in an unknown level of identification errors ( Shivji 2010). A pseudogene is a section of DNA that resembles a functional gene, but is actually nonfunctional as a result of many mutation errors over time. Finally, there are three species pairs which have either recently diverged or have experience recent gene flow, and therefore cannot be unambiguously identified using a single locus: dusky and Galapagos shark (Carcharhinus obscurus and c. galapagensis), blacktip and Australian blacktip shark (C. limbatus and C. tilstoni), and sandbar and bignose shark (C. plumbeus and C. altimus) (Cardeñosa and Chapman 2018). For further information of these species please refer to appendix.

*PCR-Based*

Polymerase chain reaction-based (PCR) tests are designed to identify a target species or a group of species. PCR-based tests are accomplished by designing species-specific PCR primers that bind to a matching target sequence (Cardeñosa and Chapman 2018). The COI or internal transcribed spacer 2 (ITS2) loci are typically used for this method. If the target species DNA is

not present, the primer will not anneal and PCR amplification will not occur. A universal primer is typically included as an internal positive control. Multiple species-specific primers can be run together, with each primer having its own position generating size differences between fragments to enable species identification (Shivji et al. 2002).

PCR has several advantages over other techniques. It is streamlined and comparatively inexpensive. It only requires three steps: DNA extraction, multiplex PCR reaction, and running on an agarose gel or some other method of visualizing the fragments (Cardeñosa and Chapman 2018). This technique is also very easy to perform with simple to read results. Additionally, there is minimum equipment necessary. All that is required is a thermal cycler, gel electrophoresis, and visualization apparatus (Shivji 2010). Furthermore, all of this equipment is compact, and easily portable making it advantageous for many situations.

PCR techniques also have some disadvantages compared to other techniques. One of the biggest disadvantages is the species-specific primer that must be designed. There are a limited number of primers available, and primer development requires intensive research and development before they can be used (Shivji 2010). The primer must be designed and tested against a robust number of individuals from as much of the species range as possible to confirm that it does not amplify non-target species (Chapman et al. 2003). Additionally, the correct multiplex of species must be picked for the target area before beginning in order to minimize cost and time. Therefore, these techniques are most useful when there is a narrow suite of species of interest, such as looking for highly endangered CITIES species (Cardeñosa and Chapman 2018).

***What information can be obtained from molecular biology techniques?***

This section discusses five major areas that can be studied using the four techniques described above. Most studies have used these techniques for species identification. Some species are important for one reason or another and are selected for study. For the fin and meat trade identification it is very important for management of the species, so these techniques have been increasingly studied and used. Additionally, highly processed samples, such as cosmetics or pet food may contain shark DNA but require special methodologies for study. Species identification isn't the only thing that molecular tools are useful for when studying sharks.

Molecular tools can also be used for studying species composition, stock structure, mating structure, and population size.

*Species ID*

The ability to properly identify the species of a shark specimen is as important, as it is difficult. The identification of species represents the first basic step for biodiversity monitoring and conservation (Moftah et al. 2011). Many species lack distinct enough morphological information, or differentiation for easy identification (B. H. Holmes, Steinke, and Ward 2009). Many skilled scientific observers still struggle at identification to the species level, regardless of experience (Tillett et al. 2012). Additionally, many landed sharks are missing parts, mainly due to the fin trade, that make identification impossible (Velez-Zuazo et al. 2015). Therefore, molecular methods are important for the identification of landed sharks in order to better understand and assess how specific species are being targeted.

Species of Importance

While all of the techniques mentioned could potentially be used for the identification of a specific species of interest, there are some methods that are better suited for this purpose. As previously mentioned, PCR techniques are relatively inexpensive and easy to perform an analysis (Cardeñosa and Chapman 2018). Additionally, a limited number of species can be run on one agarose gel, making this technique ideal for some types of studies. However, eDNA techniques can also be beneficial when looking for rare or highly endangered species, as the techniques are non-invasive and highly sensitive (Lodge et al. 2012).

While the basking shark (*Cetorhinus maximus*) has a circumglobal distribution, it is very sensitive to exploitation due to its low recovery potential. For further information on this species refer to the appendix. The high demand for their fins combined with the international efforts to conserve these species make this species a prime candidate for molecular techniques to aid in protective regulation (Magnussen et al. 2007). A bi-organelle (ITS2 and cyt *b*) primer was designed and tested on dried shark fins from Hong Kong and Japan (Magnussen et al. 2007). They found that, as suspected, the Chinese trader category ‘Nuo Wei Tian Jiu’ is most likely the basking shark, as 16 out of 19 tested fins were positively identified as basking sharks (Magnussen et al. 2007). It is hypothesized that the DNA of the other fins were too degraded. This study provides a useful tool for assessing basking shark fins from the Hong Kong auction

records by demonstrating the link between the trade name and basking shark DNA. Additionally, this study demonstrated how powerful of a tool this can be for the rapid identification of an endangered, and highly traded individual that needs increased management.

The daggernose shark (*Isogomhodon oxyrhynchus*) is under intense fishing pressure and is listed as critically endangered (CR) by the IUCN. For further information on this species refer to the appendix. An ITS2 primer was designed in order to identify daggernose shark products at market (Nachtigall et al. 2017). 51 samples were tested at market, with four being listed by the sellers as being from daggernose shark. However, this screening showed that they were actually a sympatric species, not the daggernose shark (Nachtigall et al. 2017). Sympatric species are closely related species that inhabit the same geographic area. Not only did they create a primer useful for the identification of a critically endangered (CR) shark for inexpensive and rapid identification, but they also showed how incorrect and unreliable the seller or fishermen's information can be.

The genus *Rhizoprionodon* comprises seven small shark species, commonly called the sharpnose sharks. Not only do all seven species have a similar range and habitat, but they are also hard to distinguish using morphological characteristics (Pinhal et al. 2012). Unfortunately, they are currently listed as data deficient (DD) or of least concern (LC) by the IUCN due to their life history characteristics. However, globally they are landed in very large quantities by both commercial and artisanal fisheries (Pinhal et al. 2012). Therefore, these species still need to be monitored and assessed at the species-level. A multiplex PCR format was used in order to determine which species a particular specimen belonged to, in a single reaction tube using species-specific primers based off of the ITS2 locus (Pinhal et al. 2012). Due to the high productivity of the sharpnose shark, they could be sustainable fished in moderate fishing (Pinhal et al. 2012). Therefore, this study provides a fast and inexpensive test that can be used to monitor the catch of the sharknose-shark in order to determine and regulate sustainable fishing levels.

The white shark (*Carcharodon carcharias*) is one species that is typically targeted for these types of studies because it is one of the most widely protected elasmobranchs in the world (Bowlby and Gibson 2020). For more information on this species please refer to the appendix. While the white shark  is heavily protected internationally, there still remains a high demand for their body parts (Chapman et al. 2003). Unfortunately monitoring of the trade of white sharks is extremely difficult (Bowlby and Gibson 2020). PCR techniques have been shown to be useful

for the identification of white shark body parts (Chapman et al. 2003; Shivji et al. 2005). From a legal standpoint, the identification must be as accurate as possible. That is why a bi-organelle (ITS2 and cyt *b*) primer was designed for the white shark (Chapman et al. 2003). The term bi-organelle is used to demonstrate that the primer comes from two different organelles (mitochondria and nucleus). Therefore, for a false-positive to occur, the non-target shark would have to be similar enough in DNA at both the ITS2 and cyt *b* loci, which is highly unlikely given the fact that the primers are located in different organelles (Chapman et al. 2003).

In 2003, 900kg of dried fins were confiscated on the U.S. east coast, which were intended for export to Asian markets. These fins were identified using a bi-organelle (ITS2 and cyt *b*) PCR technique in order to determine if any white shark fins were purposefully mislabeled (Shivji et al. 2005). Additionally, the fin morphometrics were collected in order to estimate the size of shark at time of capture. All of the 21 fins that were suspected to be white shark were confirmed as such (Shivji et al. 2005). Additionally, many of the fins were small in size, indicating they were very young. Therefore, they were most likely caught in a nursery area along the Atlantic coast of the U.S. (Shivji et al. 2005). Using the bi-organelle approach, they were able to find that these heavily protected sharks are being exploited in a region with some of the most extensive regulations in the world (Chapman et al. 2003). Therefore, these techniques can have important ramification not only from a legal standpoint, but also as a conservation tool.

eDNA techniques can also be beneficial for the detection of heavily protected and endangered species, such as the white shark (*C. carcharias*). Southern California beaches are important nursery habitats for juvenile white sharks (Lafferty et al. 2018). Traditional methods such as tagging and direct observation are typically used but can have problems with the detection of the white shark. eDNA has the potential to help expand the options for the surveillance and monitoring of white sharks in this area. In order to test this, eDNA samples were taken at two locations where juveniles were known to be, and two where they are presumably absent (Lafferty et al. 2018). They found that their primer design allowed for the detection of white shark DNA, while excluding other elasmobranchs in the area (Lafferty et al. 2018). This study is a great first step towards the use of eDNA techniques to be used to enhance the surveillance and monitoring of the white shark, not only in California, but globally (Lafferty et al. 2018).

Currently most eDNA studies have the disadvantage of having to return the field samples to a laboratory after collection, creating a delay in information which can create challenges for management strategies (Truelove, Andruszkiewicz, and Block 2019). The most recent advance in technology is the portable Oxford Nanopore MinION Sequencer. The MinION sequencer allows for sequencing to be real-time, resulting in immediate results (Tyler et al. 2018). The MinION was used in order to successfully detect white sharks (*C. carcharias*) in the open ocean in ~ 48 hours (Truelove, Andruszkiewicz, and Block 2019). This has important implications for future research, and the ability to inform conservation efforts while at sea. These types of studies allow the researcher to rapidly determine if the organism of interest was in the immediate area, which allows for better decisions to be made in the field in regard to where to sample further, or where to allocate resources. The biggest disadvantage to this technique is that it is still new, meaning that the protocols and accuracy are constantly changing and improving (Tyler et al. 2018). Therefore, with future study and development, the MinION sequencer has the potential to change the way we conduct studies in the field.

MinION technologies have also been used for more advanced, Next Generation Sequencing. One study used MinION technology to study a CITIES appendix II species, *Carcharhinus falciformis*, in India, a region with little current genetic research on the species (Johri et al. 2019). For more information about this species refer to the appendix. They used a technique called 'genomic skimming'. Genomic skimming uses low-pass shallow sequencing to generate fragments. These high copy number fragments are sections of RNA, plastomes, mtDNA, and microsatellites. This allows for the collection and accumulation of genetic information about a species in real time. In this study they took samples of an unknown fin at market that was believed to belong to a Carcharhinus shark, and they identified them as belonging to *Carcharhinus falciformis* (Johri et al. 2019). In addition to a successful identification, they were able to gain valuable insight about this species in the area in order to better protect it in the future. The advantages of this technique is that it only requires the gDNA extraction (~ 3.5 hrs), library preparation/loading time (~15-30 min), and finally sequencing with MinION and a laptop (Johri et al. 2019). Therefore, this methodology, while more complicated, allows for more information to be gained in a much quicker time span. This is a potentially important tool in any marketplace that is in need of more information and better conservation practices.

Seamounts have been shown to sometimes be globally important ecosystems, where productivity, biomass, and biodiversity are high (Gargan et al. 2017). However, the detection of these hotspots is difficult, even at a regional scale. The Chilean devil ray (*Mobula tarapacana*) is not only known to form aggregations at some seamounts but is also listed as vulnerable by the IUCN (Gargan et al. 2017). For more information on this species refer to the appendix. This makes them a great target for eDNA studies not only to identify them in the marine environment, but also to identify these important hotspots of biodiversity for conservation measures. eDNA samples were taken in conjunction with visual identification in order to test the ability of these techniques to identify the Chilean devil ray out of a highly biodiverse environment (Gargan et al. 2017). qPCR techniques are advantageous in these situations over traditional PCR methods because of the use of a fluorescent dye (SYBR™ Green) or a fluorescently labeled probe, which allows for the amplification of the target sequence to be monitored in real time by comparison with against a standard curve (Le Port et al. 2018). qPCR is advantageous because it increases specificity and sensitivity making it easier to identify low abundance species. The disadvantage to this technique is that it is limited to only a few species at a time (Le Port et al. 2018). Additionally, this method is prone to non-specific fluorescence in low-concentration targets, so multiple primers are used in order to maintain the increased specificity. (Le Port et al. 2018).

Global Fin Trade

Elasmobranchs are globally declining largely in part to the demand for shark and ray products in many Asian communities (Wainwright et al. 2018). Specifically, their fins are typically worth the most as they are used in shark fin soup (Sembiring et al. 2015). However, the meat and liver oils also have a high demand, fueling a nearly 1 billion USD industry (Wainwright et al. 2018). Additionally, most elasmobranchs have life history traits that make them highly susceptible to overfishing, such as: low fecundity, late maturation, and a long gestation period (Sembiring et al. 2015). Elasmobranchs are already hard to visually identify due to similar morphology, but identification issues are compounded with the fact that whole sharks are rarely landed at commercial ports (Sembiring et al. 2015). Therefore, there is an increased need for tools that are able to monitor the global shark trade that are reliable, fast, and easy (Caballero et al. 2012). PCR and sequence-based techniques are the most common for these types of studies due to their ease of use and relatively inexpensive nature. However,

mitochondrial and nuclear DNA techniques can be used as well. eDNA techniques are not necessarily appropriate for these types of studies, because at the point of study, the elasmobranchs are already dead.

As previously mentioned, PCR techniques have the advantage of being relatively inexpensive. However, the downside is that there is a limited number of species that can be run on a traditional agarose gel. Therefore, the design of the study has to be well thought out for these global fin trade studies.

In order to aid in the identification of shark species in the Colombian Pacific two multiplex PCR tests were designed using the COI gene (Caballero et al. 2012). The first multiplex gel identified the sample as belonging to one of four major genera (*Sphyrna*, *Alopias*, *Isurus*, and *Carcharhinus*) After that, the sample was run on a multiplex gel that distinguished between a few major species in each category (11 different species in total). This methodology was used to successfully identify 407 samples to either the genus or species level, resulting in an 89% success rate (Caballero et al. 2012). The samples that did not amplify could either be due to degraded DNA, or it belonged to species outside the scope of the study. They found that 96% of the samples belong to the genus *Alopias*, with 367 of those samples belonging to *A. pelagicus* (pelagic thresher shark) (Caballero et al. 2012). For more information on this species refer to the appendix. Therefore, they found that *Alopias pelagicus* is by far the most exploited species in the Colombian Pacific. This study is important because it identifies a species which will need further research, and protection in this area, giving biologists and policy makers an important species to watch in this region.

Species listed under CITIES Appendix II must be documented and traceable throughout the supply as being legally obtained, and its trade must not be detrimental to the survival of the species. Currently 12 species of sharks are listed under Appendix II: the whale shark (*Rhincodon typus*), basking shark (*Cetorhinus maximus*), great white (*Carcharodon carcharias*), porbeagle shark (*Lamna nasus*), scalloped hammerhead (*Sphyrna lewini*), great hammerhead (*S. mokarran*), smooth hammerhead (*S. zygaena*), oceanic whitetip shark (*Carcharhinus longimanus*), silky shark (*C. falciformis*), bigeye thresher shark (*Alopias superciliosus*), pelagic thresher shark (*A. pelagicus*), and common thresher shark (*A. vulpinus*). Enforcing CITIES regulations is very challenging, but very important. That is why a multiplex PCR test was designed for identifying nine out of twelve (excluding whale shark, basking shark, and oceanic

whitetip shark) Appendix II listed species using the ITS2 locus (Cardeñosa et al. 2018). This test can be accomplished in 4 hours for 94 samples, and only costs about $0.94 USD per sample (Cardeñosa et al. 2018) .The purpose and advantage of this test is to rapid, reliable, fast, and cost effective test in order to justify holding shipments based on the illegal presence of CITIES listed species (Cardeñosa et al. 2018).

Hong Kong represents at least 50% of the global trade on shark fins. Unfortunately, these markets rely upon Chinese market categories for the fins which are primarily organized by the quality rather than distinguishing features. One study attempted to study the species compilation of 11 market categories, which represent about 46% of the auctioned fins in order to better understand which trade names relate to which species (Clarke et al. 2006). Clarke designed a species-specific ITS2 primer for 11 different species, corresponding to which species they believed each market category was mainly composed of (Clarke et al. 2006). Overall, they found that approximately 14 species make up 34 – 45% of the fin trade in Hong Kong. The blue shark (*Prionace glauca*) was found to compose the most significant portion of the trade, accounting for ~ 17.3%. The three species of hammerheads (*Sphyrna lewini, Sphyrna mokarran, Sphyrna zygaena)* collectively account for ~ 5.9%. The other 10 species (*Isurus oxyrinkchus, Alopias superciliosus, A. pelagicus, A. vulpinus, Galeocerdo cuvier, Carcharhinus falciformis, C. plumbeus, C. leucas, C. longimanus, C. obscurus)* account for less than 4% of the trade individually. For more information on these species refer to the appendix. Another major finding of this study is that many of the trade names are in fact composed of a variety of species.

While DNA barcoding techniques are not as fast and easy as PCR techniques, they have the advantage of being able to identify any species within the BOLD or BLAST database. Therefore, they are advantageous in situations where there isn't one particular species or group of species of interest, but rather a comprehensive idea of an entire fishery. This technique has been used to study the South African fisheries by sampling catches from a variety of catch sources (trawl and commercial, rod and handline, and longline) (Kuguru et al. 2018). They obtained a 99% sequence match in BOLD for all 75 specimens, identifying as 23 different species of elasmobranchs (Kuguru et al. 2018). However, only 72% of these samples matched their original morphological identification, demonstrating how common and easy it is to misidentify a species. Misidentification rates were highest for the houndsharks (genus *Mustelus*), with 50% of *M. palumbes* and 38.5% of *M. mustelus* being misidentified (Kuguru et al. 2018).

For more information on these species refer to the appendix. The houndsharks are not only important commercial species in this area, but they are also listed as threatened by the IUCN. Therefore, misidentification is a big problem in this region that needs to be addressed in order for these commercially important species to be properly monitored.

Brazil is one of the largest exporters of shark meat, making it an important player in the global shark trade. A major issue to the monitoring of trade in Brazil is the use of the common name "cação" in order to describe multiple species. Additionally, many consumers do not even know what "cação" is shark meat, but rather believe it to be a cheap "thornless fish" (Almerón-Souza et al. 2018). The term "thornless fish" is translated from a portugese word, which tells the consumer that the fish is boneless. DNA barcoding was used in order to identify "cação" to the species level in local markets in South Brazil (Almerón-Souza et al. 2018). They found that 18 Elasmobranchii and two Actinopterigii species among 63 different samples of "cação" in Southern Brazilian fish markets. The most abundant species were *Prionace glauca* (23.8%) and *Sphyrna lewini* (22%) (Almerón-Souza et al. 2018). For more information on these species refer to the appendix. Despite being listed as endangered (EN), *P. glauca* is still heavily fished, representing 56% of the total catch of pelagic sharks. Southern Brazil is a hot spot for shark diversity, with a high level of endemism. The results of this study, coupled with the fact that many of the species identified in this study are still listed as data deficient (DD), demonstrate the need for increased research and monitoring of the trade in this area (Almerón-Souza et al. 2018). The mislabeling of many species as "cação" imposes many barriers for the protection and conservation of these species, as many consumers do not even know they are eating protected species.

Singapore is considered the second-largest importer and re-exporter of shark fins in terms of value, as well as being a large consumer of shark products themselves. DNA barcoding techniques were used in order to identify elasmobranch products that are available to consumers in Singapore (Wainwright et al. 2018). 28 elasmobranch species were identified out of 207 samples, with two species being listed as Endangered (EN), ten as Vulnerable (VU) (VU), and eight listed under CITIES Appendix II (Wainwright et al. 2018). Only 34 samples were not able to be positively identified as they returned as contrasting species or multiple species in GenBank and BOLD. One troubling finding was that 38% of all shark products were identified as belonging to a species of guitar fish (*Rhynchobatus australiae)* (Wainwright et al. 2018). For

more information on this species refer to the appendix. Guitar fish are highly prized for their fins, and are considered endangered (EN), in not already locally extinct in some areas. Therefore, this study shows the importance of increasing monitoring of protected species.

Indonesia contains approximately 30% of the world's elasmobranch species. Therefore, the growing fin trade has resulted in a growth in the shark-fishing industry in Indonesia. DNA barcoding techniques were used in order to understand the targeted species in these fisheries (Sembiring et al. 2015). Unfortunately, five species (silky, scalloped hammerhead, blue, big eye thresher, and thresher shark) represented more than 50% of all of the 582 fins sampled (Sembiring et al. 2015). For more information of these species refer to the appendix. This suggests that this is the result of large-scale targeted fisheries. Additionally, 92% of the samples were listed as endangered (EN), vulnerable (VU), or near threatened (NT) (Sembiring et al. 2015). This demonstrates how unsustainable the Indonesian fisheries are. 83% of the species are considered pelagic species, while only 17% are reef sharks (Sembiring et al. 2015). Typically, the greatest fishing pressure is on reef sharks, because they are usually easier to get to and have a greater abundance of species. Therefore, the increased number of pelagic species indicates that the reef-shark populations have most likely collapsed due to overfishing and are potentially ecologically extinct (Sembiring et al. 2015). The results of this study are alarming, and not only demonstrate how important conservation policy and enforcement is in Indonesia, but it also demonstrates how much information can be gained from DNA barcoding studies.

Classical mitochondrial and nuclear DNA techniques are not typically used in studies outside a laboratory situation having to do with the global trade of sharks, because these methods are more complicated, expensive, and not as quickly performed, however, they certainly are definitive in most cases. One study used the NADH4 region in the mitochondrial DNA genome in order to assess the accuracy of fishery-observer identification in northern Australia (Tillett et al. 2012). They tested the ability of the observer to distinguish between five common species of carcharhinids: Australian black-tip (*Carcharhinus tilstoni*), bull shark (*C. leucas*), Pigeye shark (*C. amboinesis*), spot-tail shark (*C. sorrah*), spinner shark (*C. brevipinna*). For more information on these species please refer to the appendix. Observers were found to incorrectly identify 30% of black-tips, 14.8% of bull sharks, 12.8% of spinner sharks, 13.5% of pigeye sharks, and 7.3% of spot-tail sharks, giving an overall 19.8% error (Tillett et al. 2012). Figure 2 depicts the probability of identification error for each of the five species, as well as the overall species error.

The species had the greatest influence over misidentification; however, misidentification did increase with size, and males were found to be less likely to be misidentified than females. Surprisingly, they found little influence of observer experience on identification error, demonstrating just how similar all of the species look (Tillett et al. 2012). Overall, this study demonstrated how big of an issue misidentification is, and how important it is that we work towards resolving this issue using microbiology techniques.

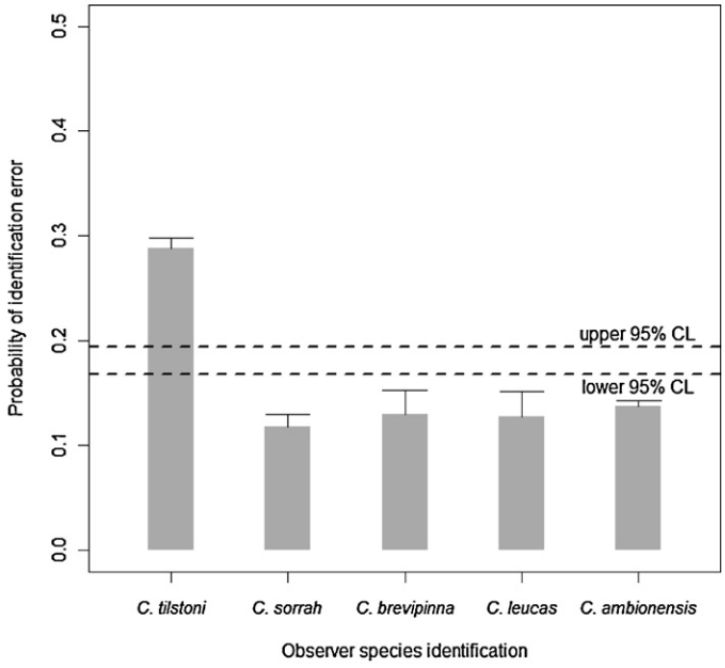


Figure 2: This figure was obtained from Tillett et al. 2012 demonstrating the probability of identification error for five different species. The species-specific variation is represented by individual error bars. The upper and lower 95% confidence limits are shown for the average probability of species misidentification

As conservationists, the global fin trade is an important area to not only understand but be able to regulate. Understanding which species are being targeted, and what their conservation status is, will help scientists communicate with regulators which species are of increased concern to protect. Many studies focus on a small area of study. That is why the data of ten different studies executed across the globe was combined, synthesized, and studied. The studies were conducted in ten different areas across the globe, including: Indonesia (Sembiring et al. 2015), Singapore (Wainwright et al. 2018), South Africa (Kuguru et al. 2018), Taiwan (Chuang et al. 2016), Brazil (Almerón-Souza et al. 2018), Australia (B. H. Holmes, Steinke, and Ward 2009),

Hong Kong (Fields et al. 2018), Guyana (Kolmann et al. 2017), Costa Rica (O'Bryhim, Parsons, and Lance 2017), and Peru (Velez-Zuazo et al. 2015). Table 1 demonstrates which species were identified, how individuals of each were identified, their current IUCN status, and how many different locations they were found at.

Table 1: List of all of the species found in the ten studies, including: the number of samples of species (N), their current IUCN listing ((Critically Endangered (CR), Endangered (EN), Vulnerable (VU), Near Threatened (NT), Least Concern (LC), and Data Deficient (DD)), and how many locations (L) they were found at. The highlighted species are the species of interest in the following graphs. For more information of the highlighted species refer to the appendix.

| Species | N | IUCN | L | Species | N | IUCN | L |
|---|---|---|---|---|---|---|---|
| *Prionace glauca* | 1969 | NT | 6 | *Carcharhinus plumbeus* | 6 | VU | 3 |
| *Carcharhinus falciformis* | 1227 | VU | 8 | *Chiloscyllium punctatum* | 6 | NT | 2 |
| *Sphyrna lewini* | 361 | CR | 8 | *Anoxypristis cuspidata* | 6 | EN | 1 |
| *Carcharhinus limbatus* | 274 | NT | 7 | *Carcharhinus isodon* | 6 | LC | 1 |
| *Sphyrna zygaena* | 222 | VU | 7 | *Deania quadrispinosa* | 6 | NT | 1 |
| *Isurus oxyrinchus* | 183 | EN | 4 | *Carcharhinus porosus* | 5 | DD | 2 |
| *Carcharhinus sorrah* | 117 | NT | 4 | *Mustelus palumbes* | 5 | DD | 1 |
| *Alopias pelagicus* | 115 | EN | 5 | *Negaprion brevirostris* | 5 | NT | 1 |
| *Alopias superciliosus* | 101 | VU | 4 | *Rhizoprionodon oligolinx* | 5 | LC | 1 |
| *Carcharhinus leucas* | 95 | NT | 5 | *Carcharhinus galapagensis* | 4 | LC | 2 |
| *Rhizoprionodon acutus* | 94 | LC | 5 | *Mustelus lunulatus* | 4 | LC | 2 |
| *Dasyatis longa* | 85 | DD | 1 | *Deania profundorum* | 4 | LC | 1 |
| *Carcharhinus brevipinna* | 78 | NT | 6 | *Hemitriakis indroyonoi* | 4 | DD | 1 |
| *Carcharhinus amboinensis* | 69 | DD | 5 | *Holohalaelurus regani* | 4 | LC | 1 |
| *Carcharhinus longimanus* | 63 | EN | 3 | *Lamiopsis temminckii* | 4 | EN | 1 |
| *Sphyrna mokarran* | 61 | CR | 3 | *Carcharias taurus* | 3 | VU | 2 |
| *Carcharhinus dussumieri* | 57 | EN | 2 | *Mobula tarapacana* | 3 | EN | 2 |
| *Dalatias licha* | 54 | VU | 2 | *Rhynchobatus djiddensis* | 3 | CR | 2 |
| *Mobula mobular* | 54 | EN | 2 | *Stegostoma fasciatum* | 3 | EN | 2 |
| *Carcharhinus obscurus* | 51 | EN | 6 | *Dasyatis chrysonota* | 3 | LC | 1 |
| *Carcharhinus macloti* | 49 | NT | 3 | *Galeus sauteri* | 3 | DD | 1 |
| *Rhynchobatus australiae* | 48 | CR | 4 | *Mustelus canis* | 3 | NT | 1 |
| *Galeocerdo cuvier* | 45 | NT | 6 | *Nasolamia velox* | 3 | DD | 1 |
| *Galeorhinus galeus* | 43 | VU | 4 | *Scyliorhinus capensis* | 3 | NT | 1 |
| *Carcharhinus coatesi* | 38 | LC | 2 | *Alopias vulpinus* | 2 | VU | 1 |
| *Negaprion acutidens* | 30 | VU | 2 | *Gymnura altavela* | 2 | VU | 1 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Rhizoprionodon taylori* | 29 | LC | 3 | *Haploblepharus edwardsii* | 2 | NT | 1 |
| *Rhizoprionodon lalandii* | 29 | DD | 2 | *Maculabatis gerrardi* | 2 | VU | 1 |
| *Carcharhinus tilstoni* | 27 | LC | 1 | *Poroderma africanum* | 2 | NT | 1 |
| *Carcharhinus amblyrhynchos* | 25 | NT | 4 | *Poroderma pantherinum* | 2 | DD | 1 |
| *Carcharhinus brachyurus* | 23 | NT | 4 | *Pseudobatos horkelii* | 2 | CR | 1 |
| *Rhizoprionodon porosus* | 23 | LC | 3 | *Rhinobatos typus* | 2 | CR | 1 |
| *Mustelus mustelus* | 23 | VU | 2 | *Rostroraja alba* | 2 | EN | 1 |
| *Hemigaleus australiensis* | 21 | LC | 3 | *Squalus blainville* | 2 | DD | 1 |
| *Lamna ditropis* | 17 | LC | 1 | *Squalus mitsukurii* | 2 | DD | 1 |
| *Squalus hemipinnis* | 17 | NT | 1 | *Squalus montalbani* | 2 | VU | 1 |
| *Carcharhinus sealei* | 16 | NT | 3 | *Zapteryx brevirostris* | 2 | VU | 1 |
| *Hemipristis elongata* | 16 | VU | 3 | *Apristurus macrorhynchus* | 1 | DD | 1 |
| *Isurus paucus* | 16 | EN | 3 | *Atelomycterus marmoratus* | 1 | NT | 1 |
| *Carcharhinus acronotus* | 16 | NT | 2 | *Carcharhinus tjutjot* | 1 | VU | 1 |
| *Etmopterus pusillus* | 16 | LC | 1 | *Chiloscyllium hasseltii* | 1 | NT | 1 |
| *Lamna nasus* | 15 | VU | 3 | *Etmopterus brachyurus* | 1 | DD | 1 |
| *Hemigaleus microstoma* | 15 | VU | 2 | *Heptranchias perlo* | 1 | NT | 1 |
| *Mustelus punctulatus* | 14 | DD | 1 | *Hexanchus griseus* | 1 | NT | 1 |
| *Carcharhinus melanopterus* | 13 | NT | 3 | *Himantura hortlei* | 1 | VU | 1 |
| *Carcharhinus altimus* | 12 | DD | 2 | *Leucoraja wallacei* | 1 | LC | 1 |
| *Scoliodon laticaudus* | 12 | NT | 2 | *Mustelus californicus* | 1 | LC | 1 |
| *Mustelus mosis* | 12 | NT | 1 | *Mustelus henlei* | 1 | LC | 1 |
| *Triaenodon obesus* | 11 | NT | 3 | *Myliobatis aquila* | 1 | DD | 1 |
| *Rhynchobatus laevis* | 11 | CR | 2 | *Myliobatis goodei* | 1 | DD | 1 |
| *Mobula thurstoni* | 10 | EN | 2 | *Narcine brasiliensis* | 1 | DD | 1 |
| *Rhina ancylostoma* | 10 | CR | 2 | *Odontaspis ferox* | 1 | VU | 1 |
| *Centrophorus niaukang* | 10 | NT | 1 | *Pastinachus sephen* | 1 | NT | 1 |
| *Loxodon macrohinus* | 9 | LC | 3 | *Pseudocarcharias kamoharai* | 1 | LC | 1 |
| *Carcharhinus albimarginatus* | 9 | VU | 1 | *Raja straeleni* | 1 | DD | 1 |
| *Manta alfredi* | 9 | VU | 1 | *Rhinobatos punctifer* | 1 | NT | 1 |
| *Rhizoprionodon longurio* | 8 | DD | 2 | *Rhizoprionodon terraenovae* | 1 | LC | 1 |
| *Nebrius ferrugineus* | 8 | VU | 1 | *Sphyrna media* | 1 | DD | 1 |
| *Sphyrna tudes* | 8 | VU | 1 | *Squalus cubensis* | 1 | DD | 1 |
| *Sphyrna tiburo* | 7 | LC | 3 | *Squalus megalops* | 1 | DD | 1 |
| *Eusphyra blochii* | 7 | EN | 2 | *Squatina californica* | 1 | NT | 1 |
| *Centrophorus granulosus* | 7 | DD | 1 | *Squatina guggenheim* | 1 | EN | 1 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Hemitriakis falcata* | 7 | LC | 1 | *Squatina occulta* | 1 | CR | 1 |
| *Mustelus lenticulatus* | 7 | LC | 1 | *Triakis megalopterus* | 1 | NT | 1 |

First, the data was visually examined by determining which species had the highest number of samples. Approximately 31% of the total number of samples belonged to one species, *Prionace glauca*, which was found at six out of ten locations. This species is listed as near threatened (NT). When combining the fact that this species is highly migratory, and it accounts for almost a third of all of the samples, a reconsidering of this species IUCN status and increased legal protections is probably warranted. The second highest species, *Carcharhinus falciformis*, accounts for approximately 20% of the total number of samples and is found at eight of the ten locations. This species is listed as vulnerable (VU) by the IUCN, demonstrating how important it is that these species are further protected globally. The top eight species, and the number of locations they are found at are demonstrated in figure 3. This figure shows how heavily the two species were caught compared to the others.

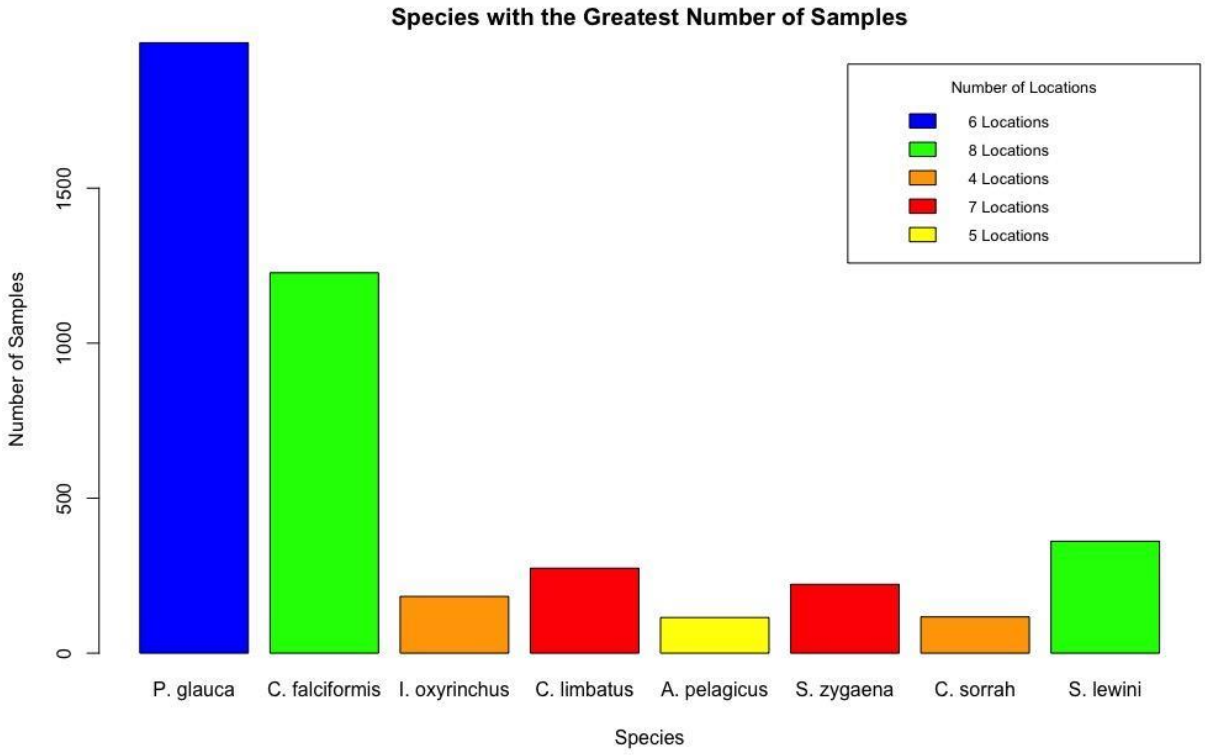

Figure 3: This graph demonstrates eight species which had the greatest number of samples, regardless of how many locations they were found at.

Second, these data were visually examined by looking at which species were found at the greatest number of locations. The most locations a species was found at was eight out of the ten, which was the case for *Carcharhinus falciformis* and *Sphyrna lewini*. As previously mentioned, *Carcharhinus falciformis* is listed as vulnerable (VU) by the IUCN. However, *Sphyrna lewini* is listed as critically endangered (CR). While *Sphyrna lewini* only accounts for about 6% of the total number of samples, it was the third highest species found. This coupled with its critically endangered status demonstrates a lack of protective measures globally, even for a critically endangered species. The species found at six, seven, and eight locations are all found in figure 4. This figure demonstrates all of the species found at more than half of the studied locations, indicating their presence in the global fin market.

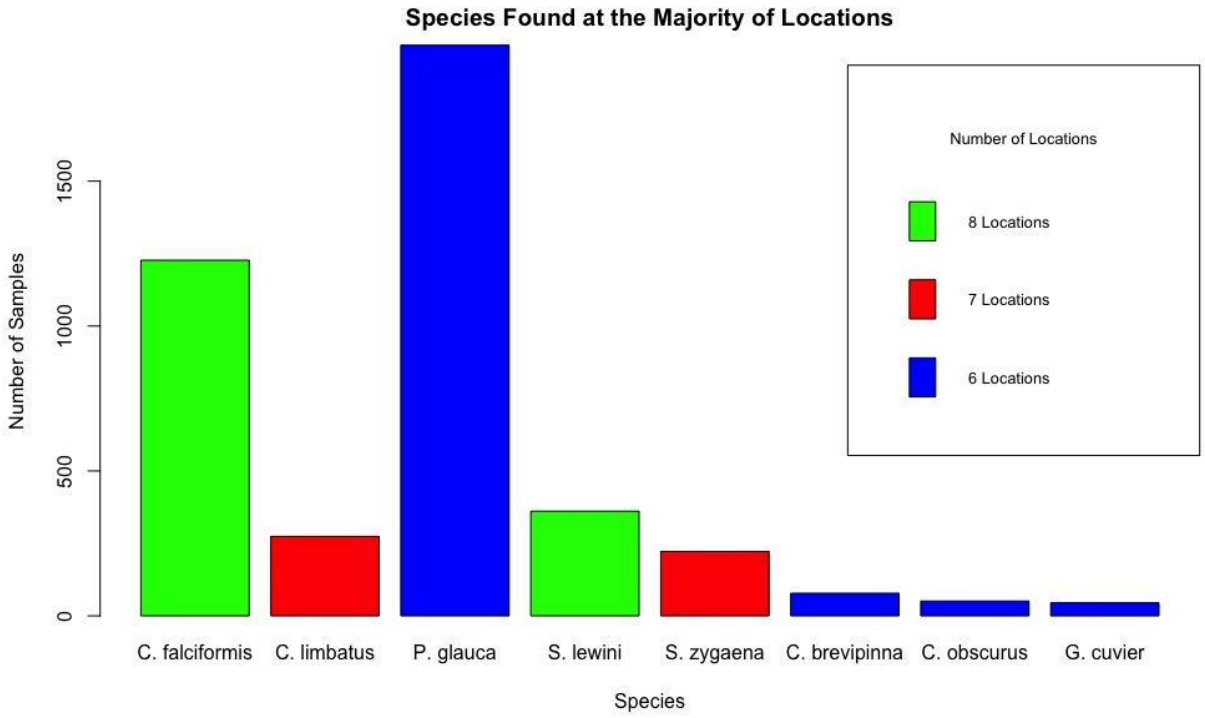


Figure 4: This graph demonstrates the top eight species found at the greatest number of locations. 10 different studies at 10 different locations were compared. Two species were found at eight locations, two at seven, and four at six. Additionally, this graph demonstrates the number of samples of each species Five species overlap with those found in figure 3 (*Prionace glauca, Carcharhinus falciformis, Sphyrna lewini, Carcharhinus limbatus, and Sphyrna zygaena*).

Third, the data was visually examined by looking specifically at the species found that are listed as critically endangered (CR). critically endangered (CR) species are considered at the greatest risk of extinction and require the most immediate protections. critically endangered (CR) species were found at eight, four, three, two, and one location. The number of samples found of these species ranged from 1 to 361. All nine of the critically endangered (CR) species can be found in figure 5. This figure demonstrates how some critically endangered (CR) species are either being captured in high numbers still or are not. The question is, whether the species found in lower numbers are due to increased protections, or decreased stock levels. Further studies into the current status and conservation of all nine of these species should be conducted.

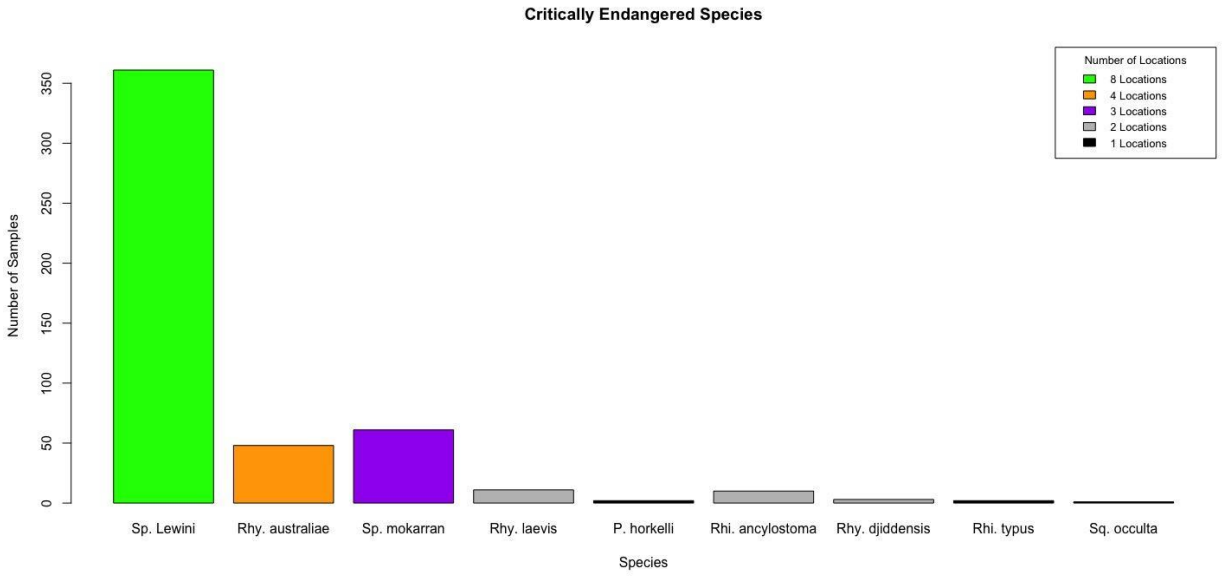


Figure 5: This graph demonstrates the number of samples and the number of locations that the nine species listed as critically endangered (CR) by the IUCN were found in. Only one species that overlaps with Figure 3 and 4, S. lewini.

Finally, the data was examined to determine if endangered species are caught more frequently. This was performed in R using a one-way ANOVA. It was found that the IUCN status of the species did not significantly affect the number of species caught ($p = 0.1355$). While not statistically significant, the data was still visualized in order to see how many different species and samples were found for each IUCN category (Figure 6). Near threatened (NT) by far had the greatest number of both samples, as well as different species. While there are the fewest number of species found that are listed as critically endangered (CR), there were still fewer

samples of least concern (LC) and data deficient (DD) species. This demonstrates how the species of least concern, are staying of least concern, because they are not targeted or caught as often as other species. Overall, this study demonstrates how global the fin market really is. Many of the most at risk and targeted species are mobile enough to be caught in many different areas, only further complicating their management. That is why these types of studies are important, because they give us greater insight into which species might need further protecting.

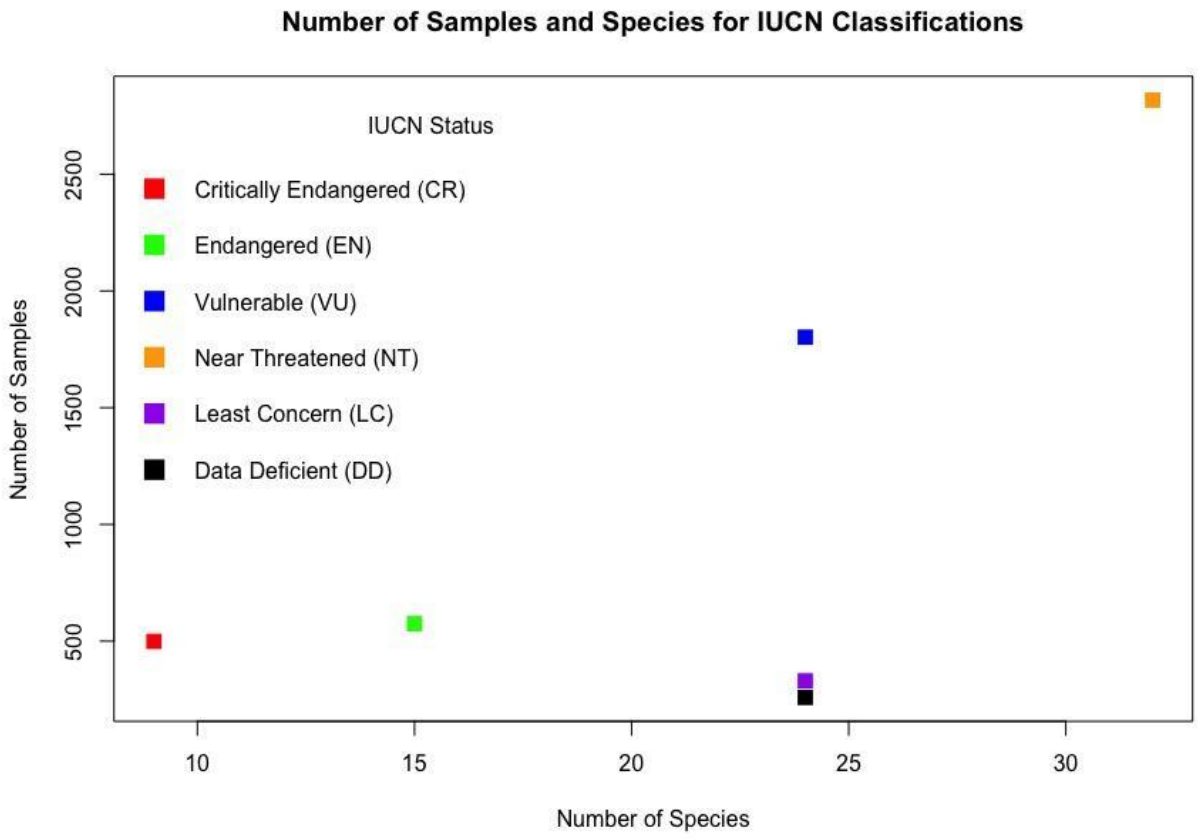


Figure 6: This graph demonstrates the number of samples, as well as the number of species for each IUCN status.

Highly Processed Samples

The mislabeling of seafood products is an emerging problem, which has negative effects on global trade policies and conservation measures. Most cases of mislabeling comes from items that are intentionally mislabeled, for financial gain because they can sell it at a higher price than it is worth, making it so the consumer cannot make informed eco-friendly purchases (Shokralla et al. 2015; Cardeñosa 2019). Unfortunately, many of the processing and preserving methods

used degrade the DNA which limits the ability of molecular biology techniques (Shokralla et al. 2015; Hellberg, Isaacs, and Hernandez 2019). One answer to this problem is the use of a mini-barcoding approach, which focuses on a shorter portion of the targeted gene region. The information from a small mini-barcode (>100 bp) fragment within the COI gene can still provide enough information for the identification to the species level with more than 90% species resolution (Shokralla et al. 2015). This method still requires the development and testing of a primer. The ability to study highly processed sample studies is still fairly new. Therefore, mini-barcoding techniques are currently the most accurate and are beginning to be used more frequently.

DNA mini-barcoding has the potential to be an important tool for food authentication and safety concerns. Mini-barcoding primers were designed, optimized, and used to identify species of commercially processed fish products in the United States (Shokralla et al. 2015). Additionally, they tested all of the samples with the mini-barcoding primers, as well as the standard COI primer in order to compare the two methods. Out of 44 tested products, standard COI barcoding only resulted in species identification of 9 products (20.5%), while mini-barcoding identified 41 products (93.2%) (Shokralla et al. 2015). Another study compared the effectiveness of three different barcoding methods (mini-barcoding, fish full barcoding, and mammalian full barcoding) for the identification of shark species in commercial products (Hellberg, Isaacs, and Hernandez 2019). The commercial products had varying levels of processing: shark jerky, shark fin soup, shark cartilage pills, fresh or grilled shark fillets. They found that out of 35 commercial products, mini-barcoding had the highest identification rate (54.3%), followed by mammalian full-barcoding (45.7%), and fish full-barcoding (8.6%) (Hellberg, Isaacs, and Hernandez 2019). Additionally, they found that the three primer sets are complementary. For example, the fish full-barcoding primer set was the only method that was able to identify the winter skate in shark cartilage pills, while the other primers also showed advantages for the identification of certain species. The shark cartilage pills were most successfully identified using mini-barcoding techniques demonstrating the benefit of these techniques on highly processed samples (Hellberg, Isaacs, and Hernandez 2019). Both of these studies demonstrate not only the advantages of mini-barcoding techniques, but also the importance of using multiple techniques and methodologies for the most accurate identification of these highly processed samples.

When thinking of traded shark products, the fins, meat, and liver oil are the most commonly thought of products. However, shark products are commonly found in cosmetics and pet food. Squalene is commonly used in the cosmetic industry in moisturizing products, but it can also be obtained from plants (Cardeñosa 2019). Unfortunately, most brands do not specify the source of their squalene, hindering the consumers ability to make an informed decision. Additionally, pet food contains meat from unspecific categories, like "ocean fish" or "white fish" (Cardeñosa 2019). One study attempts to determine if products such as cosmetics or pet food products contain traces of elasmobranch DNA using a mini-barcoding PCR protocol focusing on the COI gene region (Cardeñosa 2019). Out of 87 pet food products tested, 63% amplified, but only 33% were of sufficient quantity to identify the sample. Out of the 29 identifications, 21 were shark species, including: the shortfin mako shark (*Isurus oxyrinkchus*), blacktip sharks complex (*C. libatus, C. tilstoni, C. amblyrhynchoides, C. leiodon*) (Cardeñosa 2019). 24 cosmetics were tested, out of which only 12.5% (3) amplified successfully with sufficient quantity for identification, belonging to the blue shark (*Prionace glauca*), the scalloped hammerhead shark (*Sphyrna lewini*), and one of the blacktip sharks in the previously mentioned complex (Cardeñosa 2019). For more information on the species mentioned refer to the appendix. One important caveat is that this protocol has not been tested for amplification biases if the product contains mixed species. More studies are required in order to determine if the lack of beauty product amplification is a result of degraded DNA, or the squalene originating from a plant-based source. This study represents the first evidence of threatened species in pet food. Therefore, more studies are required in order to quantify, and trace this in the international shark trade.

*Species Composition*

The species composition is a similar concept to studying the identity of a species, however these studies typically have greater ecological implications. These studies are typically looking at either the species composition of an area, or the trophic interactions in a given area. Unlike previously discussed studies, there is a potential to better understand how anthropogenic effects are affecting a given population. These studies utilize both eDNA as well as barcoding techniques.

Due to anthropogenic effects, many areas are experiencing “dark diversity”. Dark diversity is defined as a suite of species that should be present within a certain region, based on their habitat requirements and dispersal ability, yet they are absent (Boussarie et al. 2018). High dark diversity not only can have negative implications for ecosystem function but can also represent areas that have the potential for recovery. Due to the already difficult nature of sampling for marine biodiversity, there is the potential that dark diversity is significantly overestimated. Therefore, eDNA techniques offer an important solution. Boussarie et al. compared the results of more traditional techniques to eDNA metabarcoding results to study the dark diversity of sharks in the New Caledonian archipelago. Traditional underwater visual census (UVC) and baited remote underwater video station (BRUVS) are both commonly used techniques to visualize shark diversity. However, they have many disadvantages including being potentially resource extensive, selective, and dependent on taxonomic expertise (Le Port et al. 2018). For example, UBVs and BRUVS have a limited visual area as well as a limited sampling period (Boussarie et al. 2018). Of the 26 historically present species, only 9 species were detected in 2,758 UVCs and 385 BRUVS, with an initial dark diversity estimated at 65%. In contrast, with only 22 eDNA samples 13 species were detected, resulting in a 50% dark diversity (Boussarie et al. 2018). Figure 7 shows four different bar graphs that demonstrate the difference between the sample size, number of species detected, frequency of shark detection, and shark diversity per sample. Therefore, there is an urgent need for increased use of eDNA techniques to compliment traditional methods for the detections of elusive and rare megafauna in order to better protect them.

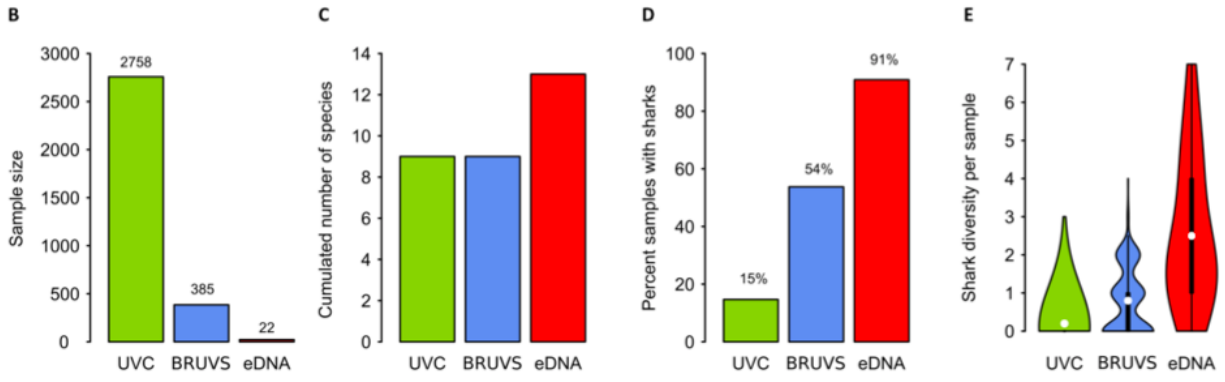


Figure 7: This figure was obtained from Boussarie et al. 2018 demonstrating the sampling analysis across the New Caledonia archipelago. B) sample size C) cumulated number of shark

species detected D) frequency of samples with sharks detected E) violin plot showing detected shark species richness

While whale sharks (*Rhincodon typus*) are very large, and tend to be considered more charismatic organisms, there is still a lot we do not understand about their biology. Whale sharks in Qatar are reported to aggregate at Al Shaheen to feed on mackerel tuna (*Euthynnus affinis*) spawn (Sigsgaard et al. 2016). In order to examine the ability of eDNA to be used as a proxy to study trophic interactions, the eDNA was quantified from both species using qPCR (Sigsgaard et al. 2016). It was found that the concentration of whale shark eDNA strongly correlated with the mackerel tuna eDNA ($P< 0.001$, $R^2 = 0.84$). Therefore, showing that this relationship most likely reflects their predator-prey relationship. The findings from this study are just the beginning of being able to use eDNA techniques in order to examine more complicated trophic interaction when looking at a community level composition.

Anthropogenic impacts as well as conservation efforts can have a significant impact on the diversity of the local shark population. In the Caribbean, there has been a long history of exploitation and anthropogenic pressure. However, the Bahamas has enacted specific shark conservation policies to help combat this. For example, gillnet and long-line fishing have been prohibited since 1991 and their national waters have been a shark sanctuary since 2011 (Bakker et al. 2017). However, in the wider Indo-Pacific region, overfishing and poaching are not well controlled. Additionally, elasmobranchs are still found in high numbers around more remote and isolated locations such as uninhabited atolls and Chagos Archipelago (Bakker et al. 2017). Bakker et al used a metabarcoding eDNA study to examine shark biodiversity in New Caledonian and the Caribbean. They found that the patterns of species richness and abundance of sequence reads followed the level of anthropogenic impact in each location (Bakker et al. 2017). The remote locations (Chesterfield atolls) and the protected Bahamas have the highest species richness and read abundance. Therefore, further demonstrating that eDNA has the potential to be an important tool for rapid environmental monitoring, and thus influence conservation decisions (Le Port et al. 2018).

Unfortunately, the shark composition of the Egyptian Mediterranean waters has only been studied once in 1974 (Mazhar 1974). That is why Moftah et al. decided to use DNA barcoding techniques to not only build an Egyptian waters specific COI database, but also study the species composition of the area. Out of 51 samples, they were able to positively assign all

organisms eight different species belonging to three different orders: Squaliformes, Squantiniformes, and Carcharhiniformes (Moftah et al. 2011). Additionally, while it was not the aim of the study, they were able to observe some phylogenetic relationships by observing neighbor-joining trees as well as by assigning bootstrap values. This demonstrates another potential avenue of future research.

In a previous section the ability of DNA barcoding techniques to identify species from the fin trade was demonstrated. However, one study took this concept one step further. Taiwan's fleet has the fourth largest catch in the world, as well as the highest species diversity of sharks in the world (Liu et al. 2013). Liu et al. collected tissues from harbor loadings, fish markets, supermarkets, street vendors, and restaurants. They were able to not only identify what County the samples were collected in, but also determine the percentages of catches that are listed by CITIES and the IUCN. They found that four different species (*Alopias pelagicus, Carcharhinus falciformis, Isurus oxyrinchus,* and *Prionace glauca*) represent 80% of all the shark meat (Liu et al. 2013). Refer to the appendix for more information on these species. They also found that 5% of the samples were listed under CITIES Appendix II. Following the IUCN classification, 2.5% were endangered (EN), 50% were vulnerable (VU), 24.5% were near threatened (NT), with 23% being classified as least concern (LC) (Liu et al. 2013). Using the results of this study, the four most heavily targeted species were able to be identified, resulting in a very clear idea of where they are potentially being targeted, and how important it is to monitor these species for future conservation measures. This study really shows the power of DNA barcoding as being able to be a powerful tool for shark conservation.

*Stock Structure*

Most elasmobranchs are highly mobile and hard to find, making it very important to understand where shark populations are located not only locally, but also globally in terms of conservation. While mark and recapture methods and tagging studies have had some success at determining survival rates and long-range movements, there are still limitations for determining population size and population exchange (Ahonen, Harcourt, and Stow 2009). Molecular tools have been successful at determining the population structure in a given area (Ovenden et al. 2018). For the purpose of this study, the population structure is defined as the spatial extent of populations. By determining where one distinct population begins and ends, we can have a better

idea at where conservation efforts need to be placed or increased. Mitochondrial and nuclear microsatellite genes have shown to be a very useful technique for these types of studies, and therefore are very commonly used. eDNA techniques have the potential and are starting to be used for this type of study as well.

It has been demonstrated that species that are capable of long-distance migrations will display greater genetic connectivity than less vagile species that generally exhibit distinct populations (Hull et al. 2019). Patterns of genetic population structure in sharks range from localized genetic subdivision, to population structuring in relatively small geographic scales, to population differentiation that is only detectable across oceanic basins, and to nearly global panmixia (Spaet et al. 2015). Gene flow is more likely to occur in populations in close proximity, resulting in distant populations becoming genetically distinct. Therefore, the population structure observed is usually due to isolation by distance (Hull et al. 2019). However, biogeographical barriers can also limit gene flow between populations, including; oceanic currents, temperature variation, and the occurrence of suitable, continuous habitat (Ovenden et al. 2018).

The grey nurse shark (*Carcharias taurus*) has a widespread, but disjunct distribution in water-temperate inshore waters (Ahonen, Harcourt, and Stow 2009). For more information on this species refer to the appendix. Wide and deep ocean basins are believed to inhibit their dispersal, with little to no gene flow expected between populations. In order to study the global stock structure of the grey nurse shark, samples were taken from each of the three ocean basins (Pacific: eastern Australia and Japan; India: western Australia and South Africa; Atlantic: Northwest Atlantic and Brazil) in order to sequence their mitochondrial DNA six microsatellite sites (Ahonen, Harcourt, and Stow 2009). Each of the six populations defined by geographic regions were substantially divergent at both mtDNA and microsatellite sites. The strongest differentiation was found between the Northern and the Southern Hemisphere populations supporting the idea that the warm equatorial waters may be a major barrier to the grey nurse shark's dispersal (Ahonen, Harcourt, and Stow 2009). Overall, there appears to be low exchange among populations with high genetic divergence, demonstrating that each population should be regarded as evolutionarily significant and thus needs to be managed regionally (Ahonen, Harcourt, and Stow 2009).

The common smoothhound (*Mustelus mustelus*) is a commercially valuable species which has a wide distribution ranging from the north Atlantic and Mediterranean Sea, to the

south-west Indian Ocean along the coast of southern Africa (Hull et al. 2019). Despite their wide distribution range, the common smoothhound has been shown to have a high degree of residency and site fidelity within a small area. For more information on this species please refer to the appendix. Samples of common smoothhound were collected across their wide distribution (Mediterranean Sea, west Africa, and southern Africa) in order to determine the global population structure for this species (Hull et al. 2019). This species was found to have low to moderate nuclear diversity but high levels of mitochondrial diversity, demonstrating that the genetic variation at the different loci could be due to different evolutionary processes (Hull et al. 2019). For this species, there was no statistically significant correlation found between nuclear differentiation and total geographic distance, suggesting that the open ocean is a major barrier of gene flow for this species. Additionally, this study documents little to no present-day connectivity among extant populations (Hull et al. 2019). Finally, it was found that the Mediterranean population exhibited the lowest mitochondrial diversity. There are many things that can result in low mitochondrial diversity. First, it could be indicative of female gene flow between populations. However, this would require sharks to migrate across the open ocean, so this is not likely. Unfortunately, it is more likely that it is a result of large declines in population size within the region. Reductions in population size have been shown to accelerate the loss of unique female lineages, resulting in a low mitochondrial diversity (Hull et al. 2019). This study demonstrates how powerful these techniques are for determining previously unknown population structure as well as areas of conservation concern.

The Galapagos shark (*Carcharhinus galapagensis*) is a circumtropically distributed species with a preference for isolated oceanic islands and seamounts in tropical water (Pazmiño et al. 2018). However, information about population structure and connectivity of this species is still limited. Both nuclear genome-wide SNPs and mtDNA sequences were used to assess the stock structure of the Galapagos shark across the Pacific Ocean (Pazmiño et al. 2018). A Single Nucleotide Polymorphism (SNP) is an area of the genome where the type of nucleotide (Adenine, Guanine, Cytosine, or Thymine) is variable between individuals (Raven et al. 2020). Figure 8 depicts what a SNP might look like. SNPs can be unique, or occur in many individuals, making them important markers for genetic studies. There are typically 4 -5 million SNPs in the human genome (Raven et al. 2020). More research is necessary for an accurate estimation of the number of SNPs in sharks' genomes. Samples were collected from nine locations across the

Pacific. Evidence suggests at least two distinct populations, the east tropical Pacific and central-west Pacific. With further evidence, there is an indication for possibly three or four distinct populations (Pazmiño et al. 2018). Unfortunately, the geographic scale of analysis influences the results, with subtle differences being more significant on a regional than global scale (Pazmiño et al. 2018). Hawaii was found to have the highest diversity, with many mutations, indicating multiple colonization events. Therefore, Hawaii is believed to be an important location that links the east (Mexico) and west (New Zealand) Pacific populations (Pazmiño et al. 2018). While the Galapagos shark is capable of crossing the open ocean, they heavily rely on shelf habitats, with long-term fidelity to several closely adjacent islands. This potentially explains the population structure seen for this species. Therefore, oceanic islands, such as Hawaii are important connecting steps for these species' dispersal (Pazmiño et al. 2018).

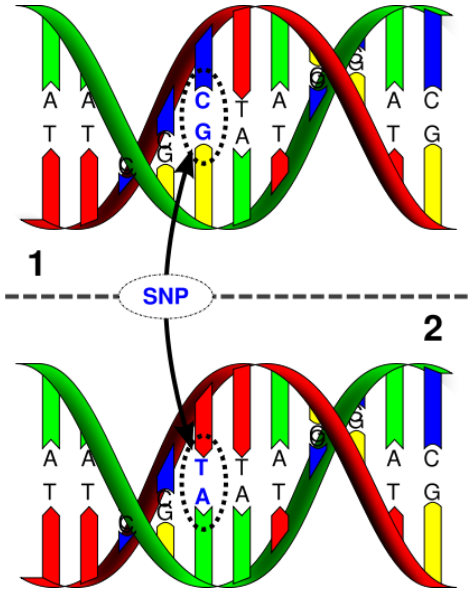


Figure 8: Diagram of a Single Nucleotide Polymorphism (SNP) (Sukhumsirichart 2018)

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The Arabian region has been shown to have a substantial proportion of taxa that are genetically distinct from their close relatives in neighboring regions with a number of taxa displaying biological heterogeneity in terms of distribution, behavior, morphology, and population genetics (Spaet et al. 2015). The Arabian Peninsula consists of the Arabian Sea, the Gulf of Oman, the Red Sea, and the Arabian/Persian Gulf. A combination of mitochondrial and microsatellite markers were used to study the stock structure of four species within the Arabian

region, including: the blacktip shark (*Carcharhinus limbatus*), the spot-tail shark (*Carcharhinus sorrah*), the milk shark (*Rhizoprionodon acutus*), and the scalloped hammerhead shark (*Sphyrna lewini*) (Spaet et al. 2015). The four species are known to have different biological, ecological, and life-history characteristics. For more information on these species please refer to the appendix. Additionally, all of the species, except for the milk shark are believed to have a high dispersal capacity. There has not been any movement study for the milk shark, however it is the smallest species, implying that it has the lowest vagility. There were two major findings in this study. First, there was no obvious barrier to gene flow found around the Arabian Peninsula for the species of interest. This indicates that any existing barriers (upwelling, ocean currents, ect.) are not likely to influence the connectivity (Spaet et al. 2015). Therefore, the populations are sufficiently connected within the Arabian Peninsula to be considered one stock. Second, the ecological, morphological, and life-history differences between species did not significantly affect the population structure, indicating that populations of all other species are likely to function in a similar manner (Spaet et al. 2015). Unfortunately, the currently unsustainable harvesting level and connectedness of stocks means that unregulated exploitation in even one country is likely to result in uniform depletion of the stock.

eDNA techniques have the potential to be used to examine stock structure as well. To date there have only been two studies that attempt to use eDNA techniques to examine the genetic variation. The first study examined the mtDNA control region of an aggregation of whale sharks (*Rhincodon typus*) in Qatar using eDNA from seawater samples (Sigsgaard et al. 2016). They found that the population in the Indo-Pacific is genetically distinct from the Atlantic Ocean population (Sigsgaard et al. 2016). The second paper also examined whale shark populations in the Atlantic Ocean. However, they used a very different technique, that is an offshoot of the eDNA approach. Invertebrate-derived DNA (iDNA) involves the extraction of genetic material of animals from flesh-eating or hematophagous invertebrates that parasitize them (Meekan et al. 2017). Additionally, this is the first iDNA study in the marine environment. These techniques are non-invasive, so they offer ethical as well as practical advantages. Additionally, these techniques potentially allow for the recovery of the complete mitogenome and ribosomal nuclear genes, which is not possible for most eDNA samples (Meekan et al. 2017). The copepod *Pandarus rhincodonicus* was used for this study, it is believed to be a commensal that feeds off microorganisms on the skin of the shark. Copepods were scraped off fins and lips of the whale

sharks by a snorkeler, and the DNA extraction was performed on the entire copepod (Meekan et al. 2017). Similar to the first study, they found two distinct populations, one in the Indo-Pacific and the other in the Atlantic (Meekan et al. 2017). This study is very important, because it shows the potential of iDNA techniques for the study of large marine vertebrates.

*Mating Systems*

Elasmobranchs have demonstrated a wide range of mating behaviors. However, studying the mating systems of elasmobranch species has been very difficult for some of the more elusive solitary species, with most of our understanding relying on aquariums or shallow water observational studies. However, mitochondrial and nuclear DNA methodology has allowed for the study of mating systems. This methodology has been used to study multiple paternity, philopatry, and parthenogenesis. When testing for multiple paternity, look at how many parental alleles are present in littermates. If the female mated with one male there will only be four parental alleles at each nuclear genetic locus, and if there are five or more it indicated that the female mated with multiple males (Ovenden et al. 2018). Philopatry is defined as the tendency of an organism to stay in or habitually return to a particular area. There are two main types: regional and natal. Regional philopatry is when wide-ranging individuals return to a natal region to reproduce and give birth, whereas natal philopatry is when females return to their exact birthplace to give birth (Ovenden et al. 2018). Finally, parthenogenesis is defined as reproduction without fertilization, meaning that there is no contribution of the male's sperm (Ovenden et al. 2018). Currently, mitochondrial and nuclear DNA methods are the only type of methodology used for this type of research.

Multiple paternity is accepted as a common reproductive strategy in elasmobranchs, which is seen in both viviparous and ovoviviparous species (B. J. Holmes et al. 2018). Most research has been conducted on coastal and nearshore species. These types of studies use bi-parentally inherited nuclear DNA, such as microsatellites. The tiger shark (*Galeocerdo cuvier*) is large, semi-solitary, and has a long movement range (B. J. Holmes et al. 2018). For more information on this species refer to the appendix. There has been evidence that shows sperm storage for this species; however, it is not known if sperm from different species can be stored. Nine microsatellite loci were examined for four different litters (112 pups) of tiger shark pups (B. J. Holmes et al. 2018). Unfortunately, there was no indication of multiple paternity found in

this study. If multiple paternity does occur, it occurs at very low frequencies, potentially due to the low rate of encounter. There was one pup that indicated the potential for multiple paternity, however due to degraded DNA the results were inconclusive (B. J. Holmes et al. 2018). If tiger sharks are genetically monogamous, then this indicates that these populations are more vulnerable to a loss of genetic diversity than those that exhibit multiple paternity.

Sex-biased dispersal arises when one sex exhibits site fidelity, while the other sex is prone to dispersal (Portnoy et al. 2015). A species might exhibit philopatry for a number of reasons, including: local competition for resources, inbreeding avoidance, and parental investment. Additionally, monogamous species tend to have more territorial males and dispersive females, while polygamous species tend to exhibit female philopatry and male dispersal (Portnoy et al. 2015). In order to determine philopatry, the contrast between biparentally inherited nuclear genome and the maternally inherited mitochondrial genome are studied. Heterogeneity in both markers supports little to no philopatry. Nuclear homogeneity and mtDNA heterogeneity indicated females are mating with males from different nursery areas, with females returning to natal nursery regions for birth (Keeney et al. 2005). In other words, a more pronounced mtDNA structure than nuclear loci indicated a high degree of female philopatry. This information can give us greater insight into how we can better conserve and manage these species.

The blacktip shark (*Carcharhinus limbatus*) in the northwestern Atlantic and Gulf have been found to be highly mobile. However, they are also known to rely on shallow coastal bays and nurseries with the females demonstrating seasonal migrations to these areas (Keeney et al. 2005). For more information on this species refer to the appendix. The specifics of the female's seasonal migrations are poorly understood. Additionally, the lack of geographical barriers suggests that there should be sufficient dispersal throughout the northwestern Atlantic, Gulf, and Caribbean Sea (Keeney et al. 2005). The mtDNA control region and nuclear microsatellite loci of the blacktip shark within the northwestern Atlantic Ocean, Gulf, and Caribbean Sea was compared in order to further understand not only the mating system, but also the stock structure of this species (Keeney et al. 2005). The mtDNA profile was found to show a high level of genetic structure and genetic differentiation in regional comparisons, indicating natal philopatry for this species (Keeney et al. 2005). There were lower levels of genetic differentiation found for males, indicating lower levels of fidelity to breeding sites for males (Keeney et al. 2005).

Additionally, they found genetic differentiation with both markers between northern Yucatan and Belize, indicating that gene flow is limited between these areas (Keeney et al. 2005). Finally, there were no genetic differences detected among Florida nurseries or northwestern Atlantic nurseries with either marker, indicating that there is sufficient movement of both sexes in order to homogenize both markers (Keeney et al. 2005). The findings of this study demonstrate the importance of conserving these important nursery grounds, as well as regional and international management of the genetically distinct populations.

The bonnethead shark (*Sphyrna tiburo*) seasonally migrates to coastal and estuarine waters in the western Atlantic Ocean. For more information on this species refer to the appendix. Unlike other species, the migration of this species appears to be related to food availability and increased access to mates rather than the use of nursery areas (Portnoy et al. 2015). Variations in nuclear SNPs and mitochondrial control regions were compared across the Gulf of Mexico and western Atlantic Ocean to determine the mating system and stock structure for the bonnethead shark (Portnoy et al. 2015). A significant difference in nuclear SNPs were found between the Gulf and the Atlantic, indicating that there is little to no gene flow between the two regions potential due to the Florida Current (Portnoy et al. 2015). Within the Gulf, nuclear SNPs were found to be homogeneous, while mtDNA was found to be heterogeneous, indicating female philopatry and male dispersal (Portnoy et al. 2015). The mtDNA variation pattern indicated an isolation-by-distance motif due to intermediate locations not being significantly different from either of the furthest locations (Portnoy et al. 2015). One major advantage to female philopatry found in this study is that philopatry can facilitate maintenance of locally adaptive variation, with the dispersive sex facilitating movement of potentially adaptive variation among locations and environments (Portnoy et al. 2015).

The bull shark (*Carcharhinus leucas*) is known to rely on estuaries as nursery habitat, as well as travel into river systems (Karl et al. 2011). For more information on this species refer to the appendix. While studies have demonstrated the fidelity of juveniles to specific nursery estuaries, little is known about the migratory patterns of adults. Tracking studies have shown that adult bull sharks have a high vagility, therefore it is believed that there is potential global genetic exchange. The mtDNA control region and nuclear microsatellite loci of bull sharks in the western Atlantic were studied to examine their genetic structure (Karl et al. 2011). The mtDNA has a low diversity for such a widespread species, more similar to reef and nearshore-associated

species (Karl et al. 2011). However, the nuclear microsatellite loci exhibited a very high diversity. The most likely explanation for the differences in diversity between mtDNA and nuclear DNA is female site philopatry, which results in sex-biased gene flow (Karl et al. 2011). Therefore, it is currently believed that female bull sharks exhibit strong natal-site philopatry. These findings are important, because it shows that bull shark nurseries are more genetically isolated than what was previously thought, demonstrating their importance for local, region, and global conservation efforts for this species (Karl et al. 2011).

The occurrence of parthenogenesis has mostly been found in captive animals when they are sexually isolated (Ovenden et al. 2018). There are two main types of parthenogenesis, obligate and facultative (Dudgeon et al. 2017). Obligate parthenogenesis is when all individuals reproduce asexually, however this is currently only observed in reptiles. Facultative parthenogenesis occurs in otherwise sexually producing species. Unfortunately, it is unknown what triggers this mode of reproduction (Ovenden et al. 2018). Parthenogenetic offspring can be identified based on very high genetic homozygosity (Ovenden et al. 2018). One study on captive zebra sharks (*Stegostoma fasciatum*) demonstrated facultative parthenogenesis in two females with different sexual histories (Dudgeon et al. 2017). For more information on this species refer to the appendix. Female one produced female two via sexual reproduction. After skipping only one breeding season, female one began reproducing parthenogenetically, and female two began reproducing parthenogenetically after only one year of maturity (Dudgeon et al. 2017). Due to the rapid transition between reproductive modes, it does not appear to be a rare or sporadic event for this species, but rather in response to some environmental change (Dudgeon et al. 2017). The biggest problem with facultative parthenogenesis is that there is less genetic diversity, and therefore a lower adaptive advantage, as well as increased mutation accumulation. However, since all the offspring produced are female,  it is currently believed that parthenogenesis may play a role as a ‘holding on’ technique in order to maintain their lineage until another mate becomes available (Dudgeon et al. 2017).

*Population Size*

Having an accurate understanding of the population size is crucial, because it can be vital information for the decision making of policy makers. Due to elasmobranchs elusive behavior and wide distribution has made it extremely difficult to create effective policy to protect many

species. Many studies estimate that more species are endangered than are currently listed as such. Molecular biology techniques have the potential to be a very powerful tool for the determination of population size. Molecular and nuclear DNA techniques are currently the best available tool for getting a population estimate. However, eDNA techniques have the potential to become extremely important in the future.

There are two main techniques for population size estimation using nuclear DNA techniques, parameter and individual based. Parameter-based methods relate a genetic characteristic across a sample to the size of the population (Ovenden et al. 2018). Individual-based methods find groups of genetically related individuals and relate them to the number of breeding adults (Ovenden et al. 2018). Both approaches rely on nuclear DNA markers (microsatellite or SNP) and knowledge of the stock structure. Parameter based methods rely upon either estimation of genetic drift or linkage disequilibrium in order to estimate the effective population size (Ovenden et al. 2018). Genetic drift can be estimated in two different ways: 1) a population can be sampled twice separated by at least three generations, or 2) samples that represent cohorts in different age groups taken once. Linkage disequilibrium is the correlation (r) for alleles at all loci pairs. Individual-based methods use what is called a close-kin mark-recapture, which is the recapture of relatives of the original individual (parent-offspring, siblings, or half-siblings) thus repressing the need to capture the same individual multiple times (Ovenden et al. 2018). The method chosen is going to be dependent on the target species, and what circumstances are going to be easiest to sample.

Despite the many attempts to estimate the abundance of white sharks (*Carcharodon carcharias*) there is still not an accurate estimate due to the biases of traditional methods. In order to get an accurate population estimate of the white shark in Eastern Australia and New Zealand, juveniles were sampled in order to identify Half-Sibling Pairs (HSPs) (Hillary et al. 2018). The proportion of HSPs to Unrelated Pairs (UPs) can then be used to estimate adult abundance, trend, and survival rate. Therefore, using close-kin mark-recapture methods approximately 280-650 adult white shark individuals were estimated to be located in east Australia and New Zealand (Hillary et al. 2018). For this study, the close-kin mark-recapture method was beneficial, because juveniles are much easier to sample than the adults.

The bull shark (*Carcharhinus leucas*) is a highly migratory species which are heavily harvested both commercially and recreationally in many parts of their range. For more

information on this species refer to the appendix. Therefore, being able to understand and monitor their population size is very important for their conservation. Adult bull sharks were sampled for their nuclear and mitochondrial DNA in the Gulf of Mexico as well as the northwestern Atlantic in order to obtain a population estimation (Karl et al. 2011). A parameter-based model was used in order to determine the effective population size for the Gulf of Mexico and northwestern Atlantic populations separately, as they previously determined there is little to no genetic exchange between the two areas (Karl et al. 2011). The estimated effective population size for the Gulf of Mexico was ~ 150,000, while the northwestern Atlantic was ~ 250,000 (Karl et al. 2011). It is interesting to note, that for both populations, the mitochondrial DNA resulted in a lower estimation than the nuclear DNA. This study was interesting, because it was able to use both nuclear as well as mitochondrial DNA in order to estimate an effective population size, with both methods producing very similar estimates.

eDNA techniques have only recently begun to be used for population size studies. Unfortunately, there are still many issues and unknowns with this methodology that can only be fixed through research. Studies have shown a relationship between the amount of eDNA present, and the abundance of species (Buxton et al. 2017). The amount of eDNA is also dependent on release and degradation rates, which are in turn dependent on biotic and abiotic factors (Le Port et al. 2018). Unfortunately, because these techniques are so new, further studies are needed in order to be able to fully understand these relationships. Another interesting avenue of research is the estimation of a population using metabarcoding techniques. This would be an extremely important tool for the identification of spatial conservation priorities, as it would give us better insight into the habitat use of many species at once (Le Port et al. 2018). More studies are needed in order to better understand the relationship between amplicon abundance and taxon abundance, especially with the presence of primer bias (Le Port et al. 2018). The following studies represent some of the first attempts at trying to use these methods for the quantification of species abundance.

Some studies have shown a potential for peaks in eDNA associated with breeding, resulting in a seasonal shift in eDNA, making it difficult to quantify the population size at any given time (Buxton et al. 2017). One study attempted to quantify this relationship for a semi-aquatic species, the Eastern hellbender (*Cryptobranchus alleganiensis*) (Buxton et al. 2017). The results are interesting, because it indicated that seasonal variations of eDNA concentration are

related to total biomass, rather than abundance/behavior (Buxton et al. 2017). Due to the many factors that can affect the concentration of eDNA that change on a daily to seasonal basis, the authors of this paper suggest that currently these techniques can't be used to map out population trends, but rather give a relative abundance estimate between similar habitats under similar environmental conditions (Buxton et al. 2017). While this study was not directly looking at shark abundance, it does have some interesting results that potentially hold true for sharks and can be tested with further research.

Despite their large size, there is still little known about offshore aggregations of whale sharks (*Rhincodon typus*). In order to estimate the population size of a whale shark aggregation off the coast of Qatar, a polymorphic target region was chosen (DL2) (Sigsgaard et al. 2016). The mutation rate of the DL2 region was estimated to be 0.1% per million years, and was used to estimate the effective female population size (71,600) (Sigsgaard et al. 2016). The effective female population size was scaled to the number of source individuals, and used to estimate a daily number of individuals in the aggregation to be anywhere from 124 – 200. Unfortunately, the estimated mutation rates and number of source individuals are extremely hard to determine accurately (Sigsgaard et al. 2016). Therefore, while the estimates found in this study are not significantly different than those conducted by previous studies, more information and research is needed before these methods can be fully accepted as accurate.

As previously mentioned, quantitative metabarcoding studies are still considered controversial. Because shedding rates differ between communities, species, and even individuals, it makes obtaining an accurate estimation on a community level difficult. One study attempted to demonstrate the differences in shark abundance as well as diversity when comparing more pristine/remote areas, to areas with higher anthropogenic impacts (Bakker et al. 2017). Read abundances were found to be correlated with remoteness in this study (Bakker et al. 2017). While this study was not able to give a population estimate, they were able to demonstrate the potential ability of these metabarcoding techniques to examine more complex, community-level effects. These techniques were further put to the test by using these techniques to examine the entire pelagic marine community in the Caribbean Sea (Bakker et al. 2019). While unable to identify a large portion of the sample to the phylum level, it demonstrates the future possibilities for eDNA metabarcoding techniques.

***Future research***

Molecular biology techniques for shark research are still in their infancy. There is still a lot of research to be done before these studies are more widely used. Therefore, future research in these areas is not only important, but also limitless. eDNA techniques are the newest of all four discussed, and therefore have the greatest potential and need for improvement. However, sequence-based, PCR-based, and mitochondrial and nuclear techniques will also be important for future studies.

eDNA techniques are extremely new in marine research, therefore there is a lot of potential for future research in this area. One major area of future research is the ability to quantitatively estimate shark populations. Research has shown that an increase in the density of target species has resulted in an increase of eDNA concentration, however the concentration is affected by release and degradation rates. The exact nature of these rates is not well understood currently, because there are a lot of factors affecting them. However, future research should help us better understand these rates for given species and ecosystems. eDNA metabarcoding also needs more work in order to quantify multiple species in one sample. Digital droplet PCR is one area of future research that can help make this possible. Digital droplet PCR allows for an accurate estimation of low concentrations of DNA, making it better suited at detecting rare molecules in the sample. Metabarcoding techniques have the potential to give us information about habitat use, home range, and migration pathways. In the future, eDNA techniques could have the ability to not only understand the species diversity of a given area, but also to estimate the number of individuals, without having to do any invasive sampling.

Another potential avenue of research for eDNA is for it to become mobile and autonomous. Currently a big limitation on eDNA techniques is that samples must be shipped to a lab for processes, limiting the range and duration of field work. Recent developments have shown the ability to process these samples in the field using the portable Oxford Nanopore MinION Sequencer, which would allow for rapid detection of species or communities in the field (Truelove, Andruszkiewicz, and Block 2019). Additionally, remote and autonomous techniques could be used for eDNA collection (LePort et al. 2018). Hydroplane drones can collect large amounts of surface water with little effort, greatly increasing sample size. Additionally, ecogenomic sensors would allow for the autonomous collection and analysis of eDNA down to a depth of 4000 m. Currently field portable eDNA metabarcoding techniques are not easy to

implement due to the need for wet-chemistry processing steps. However, advances are being made every day, and hopefully with more research and ingenuity these techniques will hopefully be possible in such a context.

Finally, another exciting area of research using eDNA techniques is the use of alternative methods, samples, or molecules. In this area of research, the possibilities are endless. One study previously mentioned touched upon the idea of using different samples to obtain eDNA, such as the use of iDNA from copepods to describe whale shark population structure (Meekan et al. 2017). This study shows that eDNA can be sampled from a variety of sources, depending on the target species, and the goal of the study. These methods can be valuable when dealing with a very rare or hard to find species. Additionally, RNA has the potential to be used instead of DNA for some studies. RNA is less stable, and therefore degrades faster than DNA. eRNA could therefore provide a more narrow idea of the spatiotemporal location or abundance of a given species (Barnes and Turner 2016). Long-range PCR is a tool that can be used to amplify a longer stretch of DNA than is possible using standard PCR techniques. Therefore, this methodology has the potential to sequence an entire mitogenome in a single amplification. This would allow for greater species-specific identification than is currently achieved with standard PCR. Therefore, eDNA techniques have a long way to go, and are only going to continue to improve and expand with increased research and development.

Sequence-based and PCR-based techniques are both very similar in that for the most part they are both currently being used in an academic setting for the improvement of species identification. However, they both have the potential to become very important for the enforcement of legislation at all governance levels. Some areas are currently using these techniques for enforcement, such as the United States, however it must be more globally adopted if it is going to make a difference. Treaties such as CITIES have the potential to be much more effective if these techniques can be used, however many CITIES parties have a lack of funding and expertise, especially in the developing countries (Cardeñosa, Merten, and Hyde 2019). The techniques designed and described by Cardeñosa et al. (2018) represent the fastest and cheapest ($0.94 USD) methodology for the enforcement of CITIES currently available. This technology has the potential to be implemented at many different developing CITIES nations, in order to increase its effectiveness (Cardeñosa, Merten, and Hyde 2019). While these techniques are becoming cheaper, in order to implement these techniques on a large scale, collaborative

initiatives among stakeholders is imperative to ensure enough financial support for these endeavors (Ovenden et al. 2018). Mislabeling has been shown to be a huge problem for many shark products. As databases such as BOLD and BLAST continue to improve and expand, barcoding techniques are going to become extremely important and reliable, making them important tools for determining cases of mislabeling. As mini-barcoding techniques continue to improve, increased management of highly processed samples can occur. Getting shark products out of our cosmetics and pet food is a very important step that mini-barcoding techniques have the potential to help with.

Nuclear and mitochondrial techniques have been shown to be a very powerful tool, yet it tends to be complicated and expensive. Hopefully, with future research these techniques will become more streamlined, and costs will go down, making them more accessible for increased studies. The development and use of SNPs is a growing branch in this branch of research because they have been shown to be very valuable at detecting genetic population structure. Currently, nuclear and mitochondrial techniques have the potential to be used in DNA barcoding studies in order to distinguish between closely related species pairs. Hopefully, as these techniques become more accessible, these SNPs can be used in these situations for an even more reliable species identification (Wong, Shivji, and Hanner 2009). As nuclear and mitochondrial DNA studies continue to grow and expand, hopefully researchers can start investigating how genomes and phenotypes adapt to local variation, in order to better predict the effects of environmental changes. These techniques could also be useful at studying the interactions between traits controlled by genes, and epigenetic changes within and between generations in order to better understand their ability to survive. As more whole genomes become available for shark species, the more we will be able to understand about their evolution and persistence. Nuclear and mitochondrial techniques have the potential to be an extremely powerful tool for shark conversationalists in the near future.

***The future of shark conservation***

After the research is done, how do we apply the findings towards the conservation of the species or ecosystem? The conservation of sharks is a major task that can sometimes feel like an uphill battle for scientists after the important information has been revealed. How can we incorporate some of our genetic findings into the policy making decision? How can we take

different species life-history characteristics into consideration when developing conservation plans? How can we increase public opinion and awareness? Are MPAs effective, and should we be using them? These are just some of the questions that must be considered when discussing shark conservation, and that will be discussed further.

Molecular biology studies have resulted in some very important findings. Arguably, some of the most overlooked findings in terms of conservation policy are those of stock structure studies. Stock structure studies show us where distinct population boundaries lie. Currently, international policies, such as CITIES, are the only major policies in place for a given species, yet they revolve around retention, finning, or trading bans rather than on conserving distinct populations (Domingues, Hilsdorf, and Gadig 2018). Population genetics metrics could greatly help with the assessment of the genetic health of a population. Additionally, genetic health would be a potentially important factor when determining the IUCN status of a given species. High levels of genetic diversity increase an individual's fitness, as well as the population's resilience (Domingues, Hilsdorf, and Gadig 2018). Considering the general lack of historic population sizes, as well as current population sizes for a given species, these genetic metrics could be extremely valuable if introduced into conservation practices.

Sharks have a wide range of life-history strategies that vary greatly. Sharks range in size from about 20 cm to about 12 meters long. Sharks also have a wide range of other characteristics including mating strategies, range, and diet. Therefore, different species are going to require different levels of protection. Some species have been shown to bounce back to fishing pressure better than other dues to mating strategies. Studies have shown that larger species are more likely to be vulnerable to fisheries before maturation and have lower rebound potentials than smaller species (Frisk, Miller, and Fogarty 2001). Therefore, greater care should be taken to make size-based fisheries limits for species who have either late maturation, or a large size at maturation. While larger species should, and typically are of greater focus for conservation efforts, the life history strategies are very important to consider when creating a management plan. If the species tends to take a long time to reach maturation, has few pups at a time, or has long periods of time between mating events, then they will require greater protections. Additionally, if the species has a very specific nursery area, especially one that is in a nearshore area, then this area is going to be of greater conservation concern. Unfortunately, the wide variety of life-history characteristics are not known or considered by policy makers. Many species are capable of supporting a

significant level of fishing, if it could be based off of scientific-based limits (Dulvy et al. 2017). When dealing with policy makers, it is important to convey the importance of these life-history strategies to them in order to have sufficient protection in place for the survival of the species.

As a conversationalist, the public is incredibly important to consider. In the world of policy making, the public is extremely important. Without public support, laws will either not pass, or not be supported and enforced. Additionally, community concern is a huge tool for conversationalists, because with enough of it, the public can influence their policy makers into doing something about the problem. The question is, how do we get the public concerned about sharks, when many of them gained all of their knowledge about sharks from sensationalized movies that paint sharks as vicious man-eating monsters? Studies have shown that the more knowledge an individual has about a species, or any environmental issues, the more positive the attitude towards policy, even if they do not have a particularly good perception of sharks (O'Bryhim and Parsons 2015). Additionally, it has been shown that most people who have knowledge about sharks gained it from watching "Shark Week", or other shark documentaries (O'Bryhim and Parsons 2015). Therefore, programs such as "Shark Week" have the potential to be very important tools for changing public opinion on a broad scale. However, one big problem with "Shark Week", is that not all of the programming is factual, and many of it is highly sensationalized. Therefore, it is important as scientists to not only get involved in our local community and talk about sharks, but it is also important to consider how the media can be used for the positive portal of sharks in order to increase public knowledge and support for conservation initiatives.

Another important tool to consider as a shark conversationalist, is ecotourism. Shark ecotourism is growing in popularity, especially as knowledge about sharks continues to grow and misconceptions fade. Shark diving is one of the most important forms of shark ecotourism, and it currently generates $314 million USD annually (Haas, Fedler, and Brooks 2017). Ecotourism can be an incredibly powerful tool at creating public concern and support. By showing people how beautiful and relatively harmless a species is in their natural habitat, while learning more about them and their conservation concerns can help generate a lot of public support. Additionally, ecotourism can be a great way to get support from local communities to enact important conservation legislation. The Bahamas is a very important example of how important ecotourism can become to a country's economy. In the early 1990s, the Bahamas placed a ban on

longline fishing, followed by the establishment of a shark sanctuary in 2011 (Haas, Fedler, and Brooks 2017). The result is that they have a healthy and vibrant shark community, as well as generating approximately $78 million annually to the GDP (Haas, Fedler, and Brooks 2017). The shark ban has therefore been successful at bringing tourist dollars to the islands, as well as generating jobs for would be shark fishermen. Therefore, ecotourism could be an attractive solution for some countries as an alternative, in cases where intense shark management is required.

Marine Protected Areas are important area-based conservation approaches, yet they have some major considerations and flaws that must be considered before being put into place. Unfortunately, many MPAs were not established based on scientific evidence, but rather from a political standpoint to fill quotas. Therefore, it is really important that future MPAs are designed with science-based evidence in mind. As with any conservation initiative, the most important thing to consider when designing an effective MPA is the life-history strategies of the given species. For example, many reef sharks show high site fidelity and residency, meaning that a smaller MPA would be sufficient. However, any type of migratory or dispersal actions must be accounted for when designing an MPA (Osgood and Baum 2015). The same holds true for highly mobile species. While MPA's that span the species' entire global range is not practical, it is important to look at key areas for the species, such as mating or nursery areas, as well as what time of year they occupy these areas (Lascelles et al. 2014). MPAs have the potential to be powerful tools at not only conserving important species but conserving entire ecosystems. That is why it is important to design MPAs from a multi-species and ecosystem-based approach (Osgood and Baum 2015). Unfortunately, many MPAs are criticized for being too small in relation to range, as well as lacking appropriate management plans. MPAs can legally only be placed within an EEZ, meaning that there is a lot of area outside of any jurisdiction that lacks any protection (Lascelles et al. 2014). Some smaller, island countries have created what are called Large Marine Protected Areas (LMPA), which span their entire economic zone (Mizrahi et al. 2019). They don't currently span a large percentage of the world's oceans ( > 3%), but they have the potential to increase yields and profits, reduce income inequality between fishing nations, and allow some mobile shark species a potential for recovery (Letessier, Bouchet, and Meeuwig 2015). Unfortunately, if they are insufficiently enforced, they can lead to a false sense of protection and stop the development of other conservation measures that would be more

beneficial (Mizrahi et al. 2019). Additionally, if all shark fishing activities stop without proper stakeholder engagement, there will be people who suddenly have no way to provide for their families, making the LMPA more likely to fail (Mizrahi et al. 2019). Therefore, these LMPAs can be powerful tools, if implemented in the correct places with local support. One of the most important tools for future MPA development is the use of dynamic ocean management, or 'moving MPAs' (Letessier, Bouchet, and Meeuwig 2015). Dynamic ocean management is the idea that management responds to spatial and temporal changes in the marine environment (Lascelles et al. 2014). MPAs represent a very important area of conservation, however care must be taken in order to ensure that these MPAs are as effective as they can be.

One of the biggest disadvantages for the current use of molecular biology techniques in conservation practices, is a general lack of cross-disciplinary communication. Conferences concerning genetics and fisheries managers rarely overlap. Therefore, there is little crossover of information. By increasing knowledge and accessibility to these types of studies beyond just geneticists would greatly increase the likelihood that these methods can be seen as useful and practical to policy makers. Unfortunately, there are no simple solutions. Overall, management programs cannot be perfect, but a mixture of strategies should be used that consider the species-specific characteristics as well as the associated fisheries in order to create a sustainable balance.

### *Conclusion*

Sharks are a very diverse group of species, which are important members of their community. Many species are apex predators, meaning that their removal has important top-down effects. They are highly threatened, not only from overfishing, but also bycatch, pollution, and habitat degradation. Sharks are targeted for most of their parts, including their skin, teeth and jaws, meat, and most importantly their fins. Current conservation measures are lacking for most species, and those that are in place tend to be lacking in management and enforcement. Molecular biology tools have the potential to be incredibly important tools in aiding in the conservation of sharks.

The four main molecular biology categories of techniques discussed are: mitochondrial and nuclear DNA, environmental DNA, specific sequence-based techniques, and general PCR techniques. All of these techniques have unique advantages and disadvantages. In general, the techniques used are going to depend greatly on the type of study. For example, eDNA techniques

are useful at studying elusive or rare species in their environment without having to actually sample any individuals. PCR based techniques are most useful when a inexpensive and easy method of identifying a specimen to the species level for a few targeted species is required. Sequence-based techniques are most useful when it is necessary to identify a specimen to the species level, with the use of a database including many species. Mitochondrial and nuclear techniques have a wide range of uses and can produce some very powerful results. However, the methodology is highly variable, complicated, and expensive. Therefore, all of the techniques described are important techniques for the conservation of shark species.

Molecular biology techniques have been used to study many things, but the vast majority are studies concerning identifying a species. There are many species that are important for one reason or another and are of increased concern to identify at a market. Some studies have used these techniques to describe the species composition found at different fin trading markets, in order to identify species of importance in the fin trade. Additionally, highly processed samples, such as pet food, are difficult, but important to study in terms of identifying if there are any shark products included. Species identification however is not the only type of study that can be conducted using molecular biology tools. Things such as species composition, stock structure, mating systems, and population size have all been studied using molecular biology techniques. More and more research is starting to emerge in these areas of increased importance.

Future studies will continue to improve these techniques' ability to study sharks and better understand their biology. The biggest hurdle for turning this type of research into important conservation measures, is a lack of communication between geneticists and fisheries managers and policymakers. These tools have the potential to not only help the management and enforcement of current policies, but also to provide useful information for future policies as well. That is why it is important for any scientists interested in working towards better shark conservation initiatives to understand the value of these techniques.

## *Appendix*

Further Information on Discussed Species in Alphabetical Order

*Alopias pelagicus*

The common name for *Alopias pelagicus* is the pelagic thresher shark. This is a primarily oceanic species that occasionally moves inshore with a circumglobal distribution (Froese and Pauly 2019). They have a depth range from 0-300m, but they are usually found anywhere from 0-152m deep. It ranges from 260 - 292 cm long. They use their long tail to stun prey for feeding and are ovoviviparous (Froese and Pauly 2019).

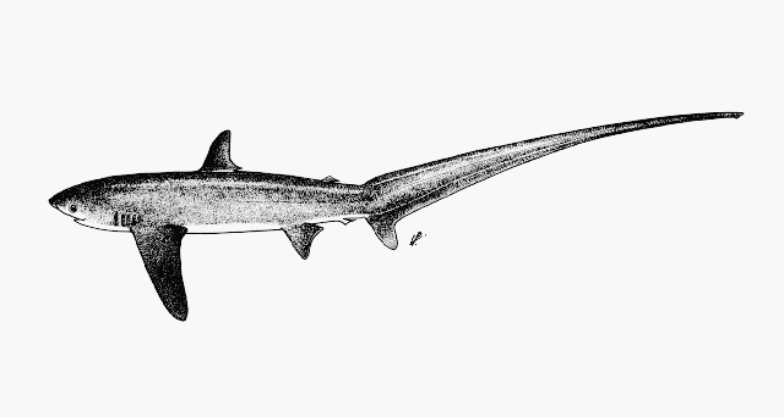

Figure 9: *Alopias pelagicus* obtained from (Froese and Pauly 2019)

*Alopias superciliosus*

The common name for *Alopias superciliosus* is the bigeye thresher shark. It has a circumglobal distribution. It can be found in tropical and temperate seas in coastal waters and over the continental shelf (Froese and Pauly 2019). It has a depth range from 1-500m. It ranges from 154- 341 cm. The bigeye thresher is ovoviviparous and uses its long tail to stun its prey (Froese and Pauly 2019).

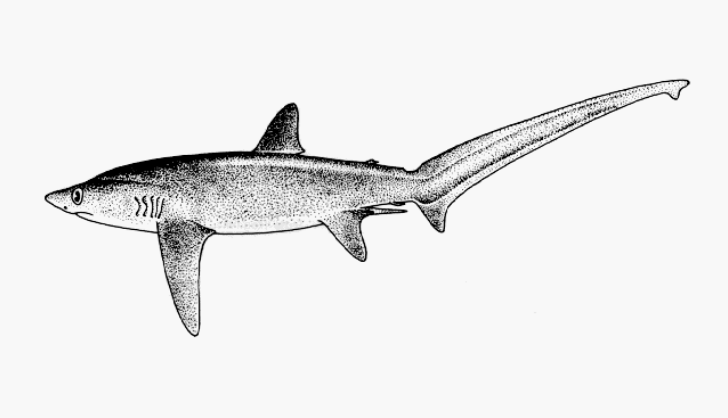

Figure 10: Depiction of *Alopias superciliosus* obtained from (Froese and Pauly 2019)

*Alopias vulpinus*

The common name for *Alopias vulpinus* is the thresher shark. It has a cosmopolitan distribution in temperate and tropical waters (Froese and Pauly 2019). It is an oceanic and pelagic species, but it is most commonly found near land. It has a depth range of 0-650m but is commonly found at 0-200m (Froese and Pauly 2019). It ranges from 226 - 400 cm long. It is ovoviviparous, and it uses its long tail to stun prey (Froese and Pauly 2019).

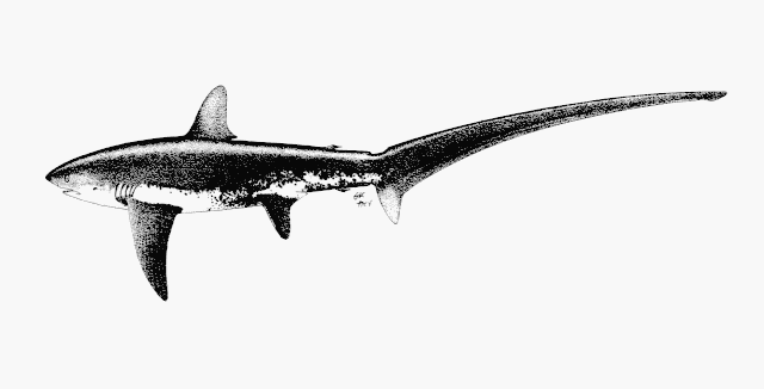

Figure 11: Depiction of *Alopias vulpinus* obtained from (Froese and Pauly 2019)

*Carcharhinus altimus*

The common name for *Carcharhinus altimus* is the bignose shark. They have a circumglobal distribution in tropical and warm seas (Froese and Pauly 2019). It is rare in shallow waters and is more commonly found near the bottom around shelf breaks and drop-offs. They have a depth range of 12-810 m but are more commonly found around 80-220 m (Froese and

Pauly 2019). They range from 205 - 282 cm long. They are viviparous and feed on bony fish, other sharks, stingrays, and cuttlefish.

Figure 12: Female *Carcharhinus altimus* obtained from (Froese and Pauly 2019)

*Carcharhinus amblyrhynchoides*

The common name for *Carcharhinus amblyrhynchoides* is the graceful shark. Little is known about this species. It is found in the Indo-Pacific, mainly in inshore and coastal areas (Froese and Pauly 2019). The upper levels of its depth range are unknown, but it does not go deeper than 50 m. It has an unknown length range, with the max length recorded to be 167 cm female (Froese and Pauly 2019). It feeds mainly on crustaceans and cephalopods and is viviparous.

Figure 13: Image of *Carcharhinus amblyrhynchoides* obtained from (Froese and Pauly 2019)

*Carcharhinus amboinesis*

The common name for *Carcharhinus amboinesis* is the pigeye shark. It is commonly found in marine, brackish water, and is typically associated with reefs (Froese and Pauly 2019). It is usually found more inshore, or in shallow bays and estuaries. It is mostly demersal but can be found throughout the water column (Froese and Pauly 2019). It is found anywhere from 0 - 150 m. It ranges from 198 - 223 cm long, and is viviparous (Froese and Pauly 2019).

Figure 14: Male *Carcharhinus amboinesis* obtained from (Froese and Pauly 2019)

*Carcharhinus brevipinna*

The common name for *Carcharhinus brevipinna* is the spinner shark. This species is marine and reef-associated (Froese and Pauly 2019). It can be found anywhere from inshore to offshore. It is found anywhere from 0 - 100m deep and is usually 170 - 266 cm long (Froese and Pauly 2019). It makes vertical spinning leaps out of the water through a school of fish in order to capture food. This species is viviparous and is a schooling species (Froese and Pauly 2019).

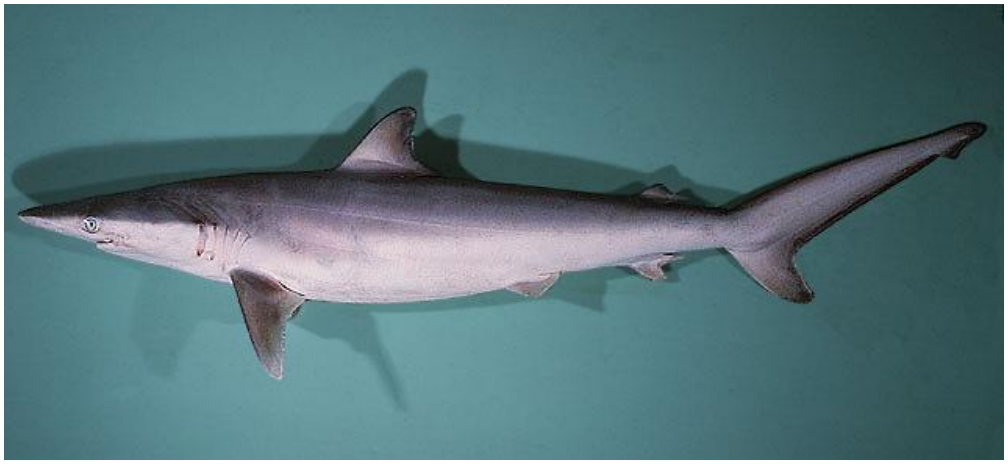

Figure 15: Female *Carcharhinus brevipinna* obtained from (Froese and Pauly 2019)

*Carcharhinus falciformis*

The common name for *Carcharhinus falciformis* is the silky shark. It has a circumtropical distribution and is found anywhere from the continental shelf, to the open sea, and even occasionally inshore (Froese and Pauly 2019). This species is most commonly found in deep-water reefs. It has a depth range of 0 - 4000m but is most commonly found between 0 -500 m deep (Froese and Pauly 2019). It usually ranges from 202 - 260 m long. This species is quick-moving, aggressive, and solitary (Froese and Pauly 2019). It is viviparous.

Figure 16: *Carcharhinus falciformis* obtained from (Froese and Pauly 2019)

*Carcharhinus galapagensis*

The common name for *Carcharhinus galapagensis* is the galapagos shark. It has a circumtropical distribution for a preference for waters around islands (Froese and Pauly 2019). It is common, but habitat-limited, preferring clear water with coral and rocky bottoms. It has a depth range of 1 - 286 m but is usually found between 30 - 180 m (Froese and Pauly 2019). It ranges between 215 - 245 cm long. While it is a coastal species, it is capable of crossing large distances of open ocean between islands (Froese and Pauly 2019). It mainly feeds on bottom fishes, squid, and octopi, and is viviparous.

Figure 17: Male *Carcharhinus galapagensis* obtained from (Froese and Pauly 2019)

*Carcharhinus limbatus*

The common name for *Carcharhinus limbatus* is the blacktip shark. This species is an inshore and offshore shark that is commonly found in brackish waters (Froese and Pauly 2019). It is often found off river mouths and estuaries, muddy bays, mangrove swamps, lagoons, and coral reef drop-offs. It is amphidromous, meaning it migrates from fresh to salt water at least once in its life (Froese and Pauly 2019). It has a depth range of 0 - 100m, but is usually found between 0 - 30 m. It ranges from 120 - 194 cm long and is viviparous (Froese and Pauly 2019).

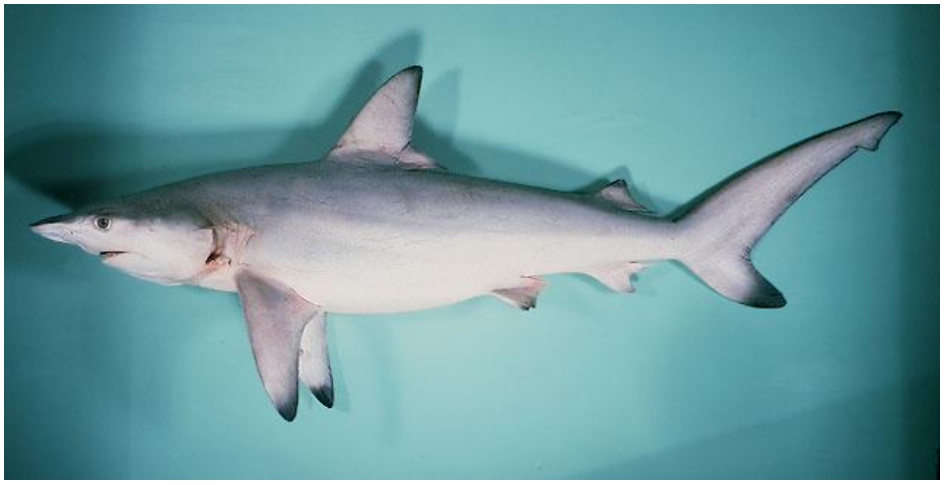

Figure 18: Female *Carcharhinus limbatus* obtained from (Froese and Pauly 2019)

*Carcharhinus leiodon*

The common name for *Carcharhinus leiodon* is the smooth tooth blacktip shark. Very little is known about this species. It is only known to be found in the Gulf of Aden (Froese and Pauly 2019). It is a demersal species. The length range of this species is unknown, but the maximum recorded length is a 75 cm male (Froese and Pauly 2019). This species is believed to be viviparous. There is no verified image available of this species.

*Carcharhinus leucas*

The common name for *Carcharhinus leucas* is the bull shark. It can be found in marine, freshwater, or brackish waters (Froese and Pauly 2019). It has a cosmopolitan distribution in tropical and subtropical waters. It inhabits shallow waters, such as bays, estuaries, rivers, and lakes (Froese and Pauly 2019). It is capable of covering great distances moving between fresh and brackish waters whenever. It has a depth range of 1 - 152 m but can usually be found between 1 - 30 m (Froese and Pauly 2019). It is usually between 180 - 230 cm long and is viviparous.

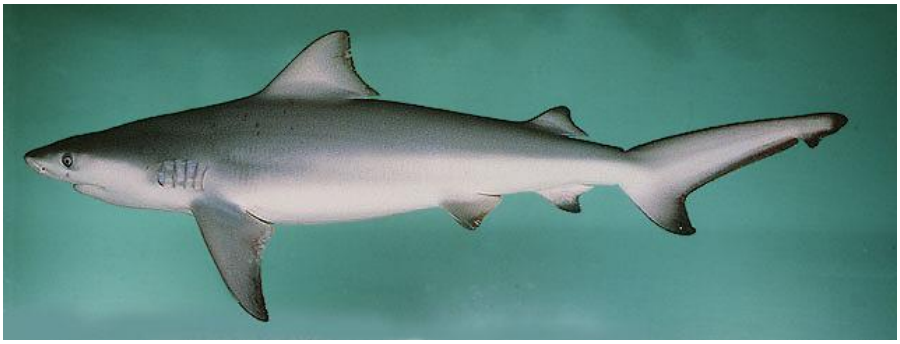

Figure 19: *Carcharhinus leucas* obtained from (Froese and Pauly 2019)

*Carcharhinus longimanus*

The common name of *Carcharhinus longimanus* is the oceanic whitetip shark. It has a circumglobal distribution in warm to tropical waters (Froese and Pauly 2019). It is a highly mobile, oceanic deep-water species which occasionally comes close to shore. It has a depth range of 0 - 230 m, and is usually found between 0 - 152 m (Froese and Pauly 2019). It ranges between 180 - 200 cm long. It is frequently accompanied by *Remora, Coryphaena*, pilot fishes, and tortoises (Froese and Pauly 2019). It is a viviparous species with segregation based on size and sex in some areas.


Figure 20: *Carcharhinus longimanus* obtained from (Froese and Pauly 2019)

*Carcharhinus obscurus*

The common name for *Carcharhinus obscurus* is the dusky shark. This species is highly migratory, and can be found in both marine and brackish waters (Froese and Pauly 2019). It is found in coastal and offshore waters, but not oceanic. It has a depth range of 0 - 400 m and is

usually found between 200 - 400 m (Froese and Pauly 2019). It ranges from 220 - 300 cm long. This species shows seasonal migration over parts of its range and is viviparous.

Figure 21: *Carcharhinus obscurus* obtained from (Froese and Pauly 2019)

*Carcharhinus plumbeus*

The common name for *Carcharhinus plumbeus* is the sandbar shark. This species is found in marine and brackish waters (Froese and Pauly 2019). It is found inshore and offshore, and is common at bays, river mouths, and in harbors. It avoids sandy beaches, the surf zone, coral reefs and rough bottom, and surface waters (Froese and Pauly 2019). It has a depth range of 0 - 500 m, and is commonly found at 20 - 65m. It is usually 126 - 183 cm long and is viviparous (Froese and Pauly 2019). It does show sexual dimorphism and is known to make seasonal migrations in some parts of its range.

Figure 22: *Carcharhinus plumbeus* obtained from (Froese and Pauly 2019)

*Carcharhinus sorrah*

The common name for *Carcharhinus sorrah* is the spot-tail shark. This species is found on the continental and insular shelves, primarily near reefs (Froese and Pauly 2019). It is found in both marine and brackish water. It has a depth range of 0 - 140 m, and is mainly found between 1- 73 m (Froese and Pauly 2019). The length range of this species is unknown, but the maximum recorded length is a 160 cm male. It lives near the seabed during the day, and near the surface at night (Froese and Pauly 2019). It usually has short movements (50 km), but they can be more than 1,000 km. It is viviparous, and mainly feeds on teleosts.

Figure 23: *Carcharhinus sorrah* obtained from (Froese and Pauly 2019)

*Carcharhinus tilstoni*

The common name of *Carcharhinus tilstoni* is the Australian blacktip shark. This species is only found at the continental shelf in Australia (Froese and Pauly 2019). It is found on the continental shelf from close inshore to ~ 150 m deep (0 - 150 m depth range). The size range is not known, but the longest individual is a 200 cm male (Froese and Pauly 2019). It is usually found near the sea bed during the day and at the surface at night. It usually feeds on teleosts, is viviparous, and forms large aggregations (Froese and Pauly 2019).

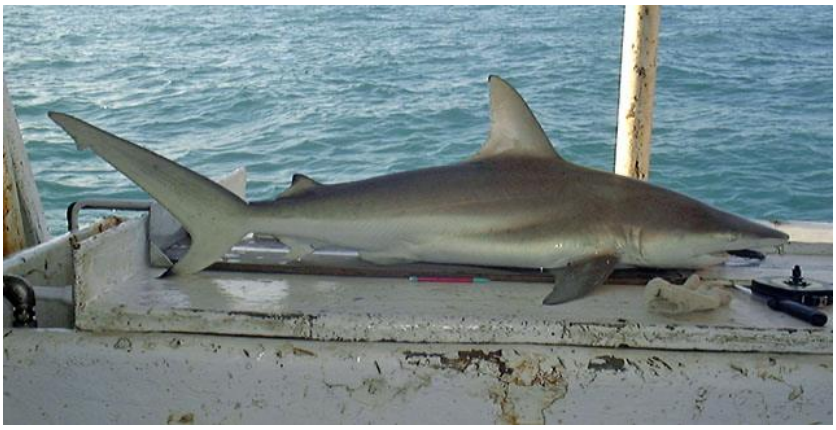

Figure 24: *Carcharhinus tilstoni* obtained from (Froese and Pauly 2019)

*Carcharias taurus*

The common name of *Carcharias taurus* is the sand tiger shark or the grey nurse shark. This species has a circumtropical distribution (Froese and Pauly 2019). This species is littoral and is often found on or near the bottom. It has a depth range of 1 - 191 m and is usually found between 15 - 25 m (Froese and Pauly 2019). It ranges between 220 - 230 cm long. It is the only known shark to gulp and store air in its stomach in order to maintain neutral buoyancy (Froese and Pauly 2019). It is found both individually, as well as in schools. It is ovoviviparous and is known to have seasonal migration in parts of its range (Froese and Pauly 2019).

Figure 25: *Carcharias taurus* obtained from (Froese and Pauly 2019)

*Carcharodon carcharias*

The common name of *Carcharodon carcharias* is the great white shark, or the white shark. This species is cosmopolitan to temperature waters in both the northern and southern

hemisphere, but not the tropics (Froese and Pauly 2019). It is primarily found close inshore to the surf line, and can penetrate shallow bays. They have a depth range of 0 - 1200 m but are usually found between 0 - 250 m (Froese and Pauly 2019). It has a length ranging from 450 - 500 cm. It is pelagic, and capable of migration across oceanic regions. They are usually solitary, or in pairs, but can be found in feeding aggregations (Froese and Pauly 2019). They are ovoviviparous, and feed on a wide variety of prey.


Figure 26: Male C*archarodon carcharias* obtained from (Froese and Pauly 2019)

*Cetorhinus maximus*

The common name for *Cetorhinus maximus* is the basking shark. This species has a cosmopolitan distribution in cold to warm temperate water and is rarely found in equatorial waters (Froese and Pauly 2019). They have a depth range of 0 - 2000 m. They are between 500 - 1000 cm long, making them the second largest shark (Froese and Pauly 2019). This species is highly migratory. They are found singularly, in pairs, in groups of three or more, or even in huge schools (Froese and Pauly 2019). They prefer waters with a temperature range between 8 - 16°C. They feed on zooplankton via filter feeding, so they make extensive horizontal and vertical movements along the continental shelf and shelf edge to obtain food (Froese and Pauly 2019). They undergo long transoceanic migrations, covering over 9,000 km. They are ovoviviparous.


Figure 27: *Cetorhinus maximus* obtained from (Froese and Pauly 2019)

*Galeocerdo cuvier*

The common name of *Galeocerdo cuvier* is the tiger shark. It has a circumglobal distribution in tropical and temperate seas (Froese and Pauly 2019). This species is highly migratory and is bottom associated. It is frequently found in river estuaries, wharves and jetties, and in coral atolls and lagoons (Froese and Pauly 2019). They have a depth range of 0 - 800 m, and are commonly found between 0 - 140 m. They range in length from 210 - 350 cm. They are nocturnal feeders and are ovoviviparous (Froese and Pauly 2019).

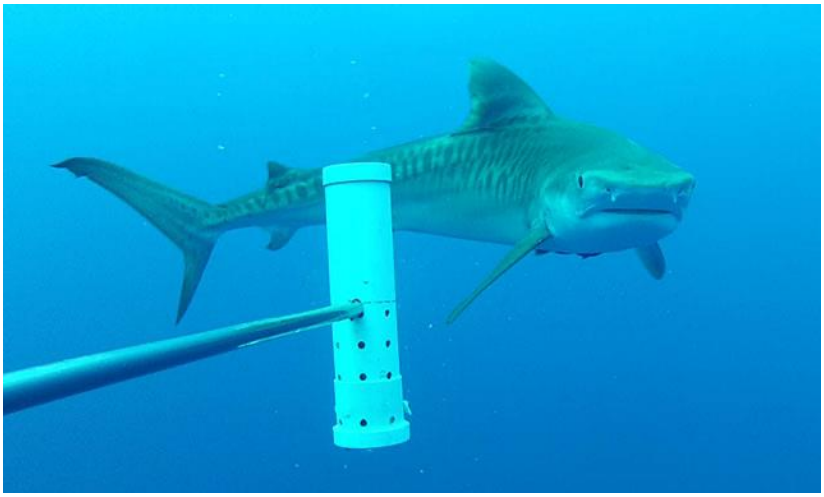

Figure 28: *Galeocerdo cuvier* obtained from (Froese and Pauly 2019)

*Isogomhodon oxyrhynchus*

The common name for *Isogomhodon oxyrhynchus* is the daggernose shark. This is a demersal species that is found in marine and brackish waters (Froese and Pauly 2019). They are only found in the western Atlantic, specifically, Trinidad, Guyana, Suriname, French Guiana, and Brazil. This is an inshore species that is found over rocky bottoms (Froese and Pauly 2019). They occupy an unknown depth range, but they have not been seen below 15 m. They also have an unknown length range, but the largest recorded individual was a 160 cm male (Froese and Pauly 2019). They feed on small school fish and are viviparous.

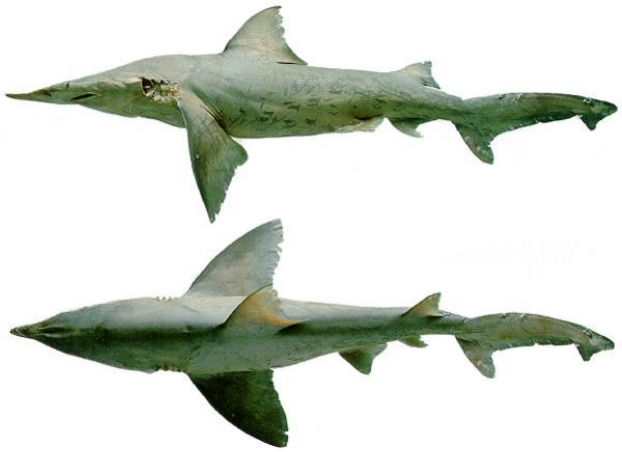

Figure 29: Female *Isogomhodon oxyrhynchus* obtained from (Froese and Pauly 2019)

*Isurus oxyrinchus*

The common name for *Isurus oxyrinchus* is the shortfin mako. This species has a cosmopolitan distribution in temperate and tropical seas (Froese and Pauly 2019). They are oceanic, but sometimes found inshore. They have a depth range of 0 - 750 m, and are usually found between 100 - 150 m (Froese and Pauly 2019). They range from 275 - 285 cm long. They are ovoviviparous, and have shown to have a sexually segregated population structure (Froese and Pauly 2019). They are considered the fastest of the sharks and can leap out of the water when hooked.


Figure 30: Male *Isurus oxyrinchus* obtained from (Froese and Pauly 2019)

*Lamna nasus*

The common name of *Lamna nasus* is the porbeagle shark. This species has a circumglobal distribution, with centers of distribution in the North Atlantic and temperate waters of the southern hemisphere, they are not found in equatorial seas (Froese and Pauly 2019). This species is migratory, moving along the continental shelves, moving to more inshore surface waters in the summer, and offshore in deeper water in the winter. They are one of the most cold-tolerant sharks, with extremes of (- 1°C) and 23°C (Froese and Pauly 2019). Additionally, they are known for being able to temporarily tolerate salinities as low as 10 in order to chase prey. They are found as individuals, as well as in school (Froese and Pauly 2019). They are ovoviviparous, with the females growing larger than the males.

Figure 31: *Lamna nasus* obtained from (Froese and Pauly 2019)

*Mobula tarapacana*

The common name of *Mobula tarapacana* is the Chilean devil ray. This species has a circumtropical distribution. It is mostly oceanic, but it is found in coastal waters. This species has a depth range of 0 - 1896 m, but is usually found between 0 - 20 m. The size range is unknown, but the largest recorded individual is a 328 cm male. This species is mainly solitary, but it sometimes does form groups. It is ovoviviparous.

Figure 32: *Mobula tarapacana* obtained from (Froese and Pauly 2019)

*Mustelus mustelus*

The common name for *Mustelus mustelus* is the smooth-hound shark. This species is demersal and is only found in the Eastern Atlantic (Froese and Pauly 2019). It is found on the continental shelves and uppermost slopes, mainly near the bottom. This species has a depth range of 5 - 624 m and is usually found between 5 - 50 m (Froese and Pauly 2019). The length range of this species is unknown, but the largest recorded individual is a 200 cm male. It feeds mainly on crustaceans, but it also eats cephalopods and bony fish (Froese and Pauly 2019). This species is viviparous.

Figure 33: Female *Mustelus mustelus* obtained from (Froese and Pauly 2019)

*Mustelus palumbes*

The common name for *Mustelus palumbes* is the whitespotted smooth-hound. This demersal species is only found in the Southeastern Atlantic, specifically South Africa (Froese and Pauly 2019). This species is found on the continental shelf and upper slope from the intertidal region to deeper waters. This species has a depth range of 0 - 443 m (Froese and Pauly 2019). The length range of this species is unknown, but the longest individual recorded is a 120 cm male. They feed on crustaceans, octopi, bony fish, and fish offal (Froese and Pauly 2019).This species is believed to be ovoviviparous.

Figure 34: *Mustelus palumbes* obtained from (Froese and Pauly 2019)

*Prionace glauca*

The common name for *Prionace glauca* is the blue shark. This species is found in marine and brackish water, and it has a circumglobal distribution in temperate and tropical waters

(Froese and Pauly 2019). They may travel large distances. They are usually found at least 150 m deep, with a depth range of 1- 1000 m (Froese and Pauly 2019). They range from 170 - 221 cm long. They have a wide-ranging diet, feeding on fish, small sharks, squids, pelagic red crabs, cetacean carrion, and occasionally birds and garbage (Froese and Pauly 2019). This species is viviparous and has sexual dimorphism.

Figure 35: *Prionace glauca* obtained from (Froese and Pauly 2019)

*Pseudobatos horkelii*

The common name of *Pseudobatos horkelii* is the Brazilian guitarfish. This species is demersal, and found in the Western South Atlantic, from Rio de Janeiro to Argentina (Froese and Pauly 2019). This species is found from the coastline to the continental edge. They have a depth range of 1 - 150 m (Froese and Pauly 2019). The length range of this species is unknown, but the longest recorded individual was 138 cm male. They feed on crustaceans, cephalopods, polychaetes, and small fish (Froese and Pauly 2019). This species is ovoviviparous.

Figure 36: Juvenile *Pseudobatos horkelii* obtained from (Froese and Pauly 2019)

*Rhincodon typus*

The common name for *Rhincodon typus* is the whale shark. This species has a circumglobal distribution, in all of the tropical and warm temperate seas except for the Mediterranean (Froese and Pauly 2019). They are mainly seen offshore, but do come close inshore, occasionally entering lagoons or coral atolls. This species has a depth range of 0 - 1928m and is usually found between 0 - 100 m (Froese and Pauly 2019). The whale shark is the largest shark species, with a length range of 440 - 560 cm. The largest recorded whale shark was a 2,000 cm female (Froese and Pauly 2019). They are found as individuals, or in large aggregations of over 100 individuals. This species is highly migratory, and is known to migrate between ocean basins, returning to the same sites annually. They feed on planktonic and nektonic prey (Froese and Pauly 2019). When actively feeding the sharks turn their heads from side to side with part of their head lifted out of the water. The mouth opens and closes 7 - 28 times per minute, which are synchronized with the opening and closing of the gill slits (Froese and Pauly 2019). This species is ovoviviparous, with litter sizes over 300 pups.

Figure 37: *Rhincodon typus* obtained from (Froese and Pauly 2019)

*Rhizoprionodon acutus*

The common name for *Rhizoprionodon acutus* is the milk shark. This species is found in marine, freshwater, and brackish water (Froese and Pauly 2019). It is found on the continental shelves, often on sandy beaches and rarely in estuaries. This species is found anywhere from 1 - 200 m deep (Froese and Pauly 2019). It ranges in size from 70 - 80 cm. It feeds mainly on small

pelagic and benthic bony fishes, cephalopods, and other invertebrates (Froese and Pauly 2019). This species is viviparous, and is believed to be amphidromous.

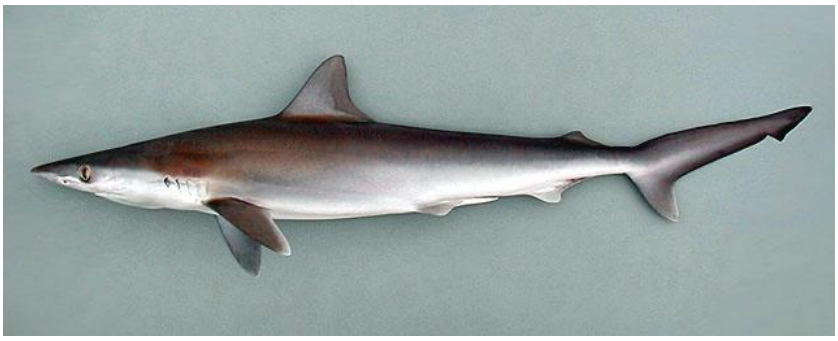

Figure 38: Male *Rhizoprionodon acutus* obtained from (Froese and Pauly 2019)

*Rhynchobatus australiae*

The common name for *Rhynchobatus australiae* is the bottlenose wedgefish. This species is demersal, and only found in the Indo-West Pacific (Froese and Pauly 2019). This species inhabits inshore waters on the continental shelves. The depth range for this species is 0 - 60 m (Froese and Pauly 2019). The size range of this species is unknown, the longest recorded individual is a 300 cm female. They feed on bottom crustaceans, mollusks, and bottom-dwelling fish (Froese and Pauly 2019). This species is ovoviviparous.

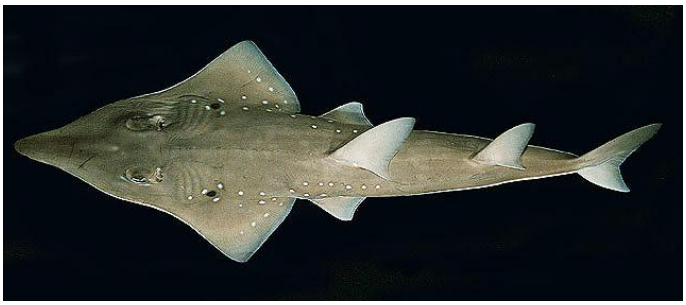

Figure 39: *Rhynchobatus australiae* obtained from (Froese and Pauly 2019)

*Rhynchobatus djiddensis*

The common name for *Rhynchobatus djiddensis* is the giant guitarfish. This species is found in marine and brackish waters in the Western Indian Ocean only (Froese and Pauly 2019). The depth range for this species is 1 - 75 m. The length range of this species is unknown, but the largest recorded individual is a 310 cm male (Froese and Pauly 2019). This species occurs

inshore and in shallow estuaries. It feeds on crabs, lobsters, bivalves, small fish, and squids (Froese and Pauly 2019). This species is ovoviviparous.

Figure 40: *Rhynchobatus djiddensis* obtained from (Froese and Pauly 2019)

*Sphyrna lewini*

The common name for *Sphyrna lewini* is the scalloped hammerhead. This species is found in marine and brackish waters and has a circumglobal distribution in coastal warm temperature and tropical seas (Froese and Pauly 2019). This species occurs over continental and insular shelves and adjacent deep water, and also enters more inshore waters entering enclosed bays and estuaries. This species has a depth range of 0 - 1000 m but is usually found between 0 - 25 m (Froese and Pauly 2019). This species ranges from 140 - 273 cm long. Large schools of small migrating individuals move poleward in the summer in certain areas, as well as having permanent resident populations (Froese and Pauly 2019). This species is viviparous.

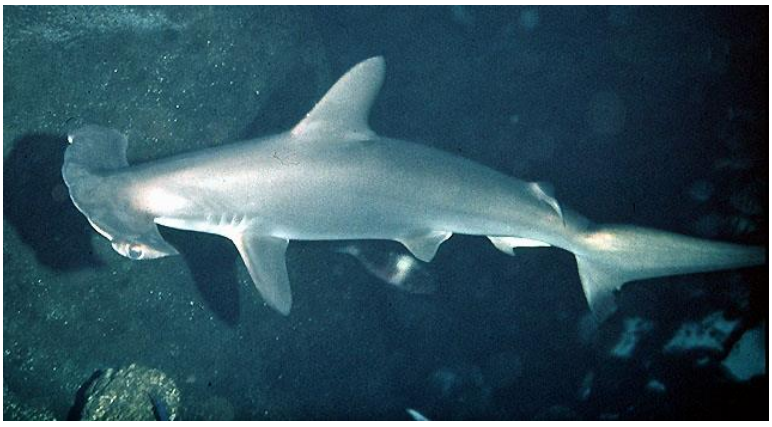

Figure 41: *Sphyrna lewini* obtained from (Froese and Pauly 2019)

*Sphyrna mokarran*

The common name for *Sphyrna mokarran* is the great hammerhead. This species is found in marine and brackish waters and has a circumglobal distribution in coastal warm temperature and tropical seas (Froese and Pauly 2019). This species is found close inshore as well as offshore, over the continental shelves, island terraces, and in passes and lagoons. They are often associated with the bottom and reefs (Froese and Pauly 2019). This species has a depth range of 1 - 300 m, but is usually found between 1 - 100 m. This species has a length ranging from 210 - 300 cm. They prefer to feed on stingrays and other batoids, groupers and sea catfish, but they prey on others as well (Froese and Pauly 2019). They are a viviparous species.

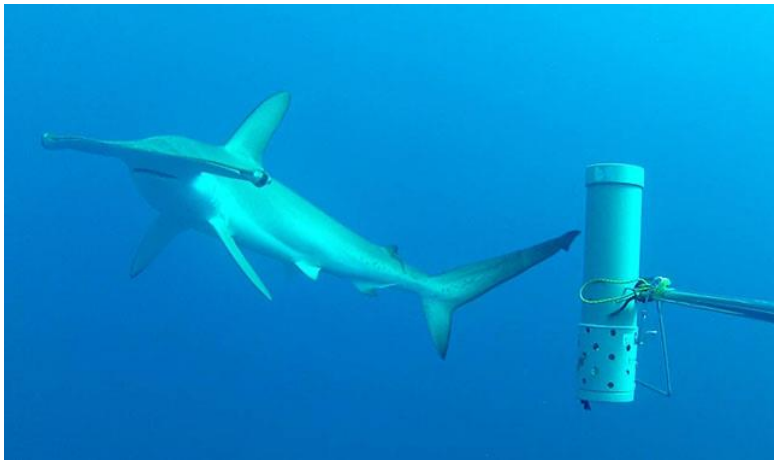

Figure 42: *Sphyrna mokarran* obtained from (Froese and Pauly 2019)

*Sphyrna tiburo*

The common name for *Sphyrna tiburo* is the bonnethead shark. This species is found in marine and brackish water in the Western Atlantic and Eastern Pacific (Froese and Pauly 2019). This species often occurs in shallow water, including estuaries, shallow bays, and over coral reefs. This species has a depth range from 10 - 80 m but is mainly found between 10 - 25 m (Froese and Pauly 2019). They have a length ranging from 80 -90 cm. They spend the night on shallow grass flats searching for invertebrate prey and move into deeper waters during the day. They mainly feed on crustaceans, but they also eat bivalves, octopi, and small fish (Froese and Pauly 2019). This species is viviparous and exhibits sexual segregation.

Figure 43: Male *Sphyrna tiburo* obtained from (Froese and Pauly 2019)

*Sphyrna zygaena*

The common name for *Sphyrna zygaena* is the smooth hammerhead. This species is found in marine and brackish water (Froese and Pauly 2019). This species is widespread in temperate and tropical seas both inshore and offshore. They are often associated with the bottom. This species has a depth range of 0 - 200 m but is usually found between 0 - 20 m (Froese and Pauly 2019). The length range of this species is unknown, but the largest individual is a 500 cm male. They migrate northward in the summer (Froese and Pauly 2019). The young often form large aggregations of hundreds of individuals. They are viviparous (Froese and Pauly 2019).

Figure 44: *Sphyrna zygaena* obtained from (Froese and Pauly 2019)

*Stegostoma fasciatum*

The common name for *Stegostoma fasciatum* is the zebra shark. This species is found in marine and brackish water and is found in the Indo-West Pacific (Froese and Pauly 2019). This species is found inshore on sand, rubble, or coral bottoms. This species has a depth range of 0 - 90 m but is usually found between 5 - 30 m (Froese and Pauly 2019). The length range of this species is unknown, but the largest recorded individual is 354 cm long. They are recorded to have entered freshwater and are believed to be amphidromous (Froese and Pauly 2019). They are also believed to be nocturnal, feeding on mollusks and small bony fish. They are slow swimming, but able to swim through narrow cracks and crevices (Froese and Pauly 2019). This is an oviparous species.

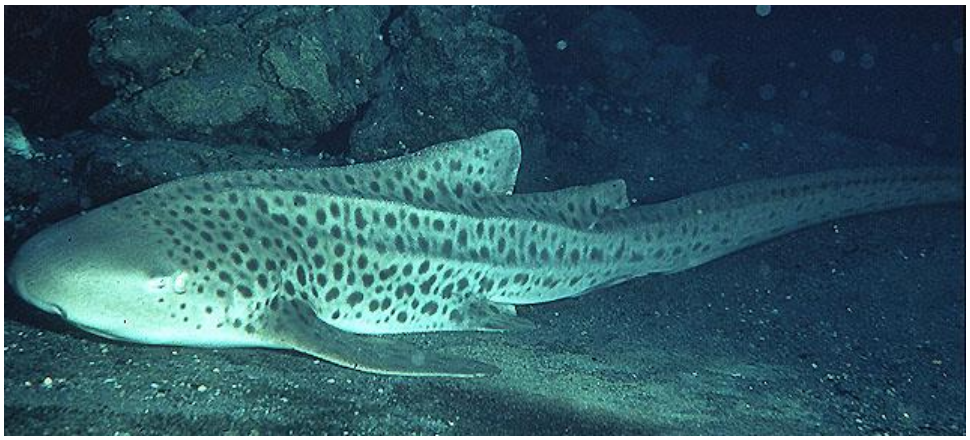

Figure 45: *Stegostoma fasciatum* obtained from (Froese and Pauly 2019)

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