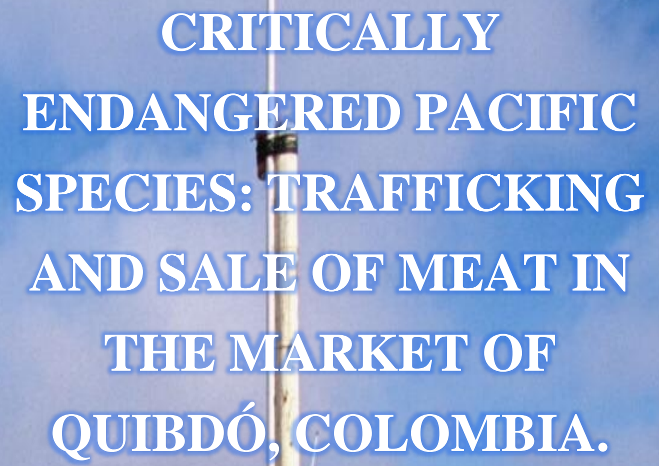

CRITICALLY ENDANGERED PACIFIC SPECIES: TRAFFICKING AND SALE OF MEAT IN THE MARKET OF QUIBDÓ, COLOMBIA.

ECOCEANOS

2021

CRITICALLY ENDANGERED PACIFIC SPECIES: TRAFFICKING AND SALE OF MEAT IN THE MARKET OF QUIBDÓ, COLOMBIA.

SUMMARY

The lack of regulation in the monitoring of shark trade by species is a major challenge that Colombia shares with several countries in the world, mainly due to the difficulties to identify at the species level, using the shark parts traded (dried fins, meat and processed). To address these conservation and management problems, in the present study we applied a multiplex polymerase chain reaction (PCR) assay with species-specific primers based on the ITS2 ribosomal region. In addition, we characterized the shark meat trade in the wholesale market of the city of Quibdó, Colombia, which functions as a collection center for the trade of this product. Results based on the identification of tissues by multiplex PCR reveal that the trade is composed of at least two species. Oceanic whitetip shark (Carcharhinus longimanus) and pelagic thresher shark (Alopias pelagicus). A total of 93.26% (n=138) of the samples analyzed corresponded to species listed in one of the IUCN threat categories. The molecular technique applied in this study has proven to be reliable, fast and useful for the identification of shark species, making it a key tool for strengthening traceability systems and the chain of custody of shark products.

KEYWORDS: CONSERVATION, TRADE, GENETIC IDENTIFICATION, QUIBDÓ, ITS2, PCR MULTIPLEX, SHARKS, IUCN.

INTRODUCTION

Sharks belong to the class Chondrichthyes, which emerged more than 400 million years ago during the Paleozoic era, making them one of the oldest vertebrate groups on the planet (Worm et al. 2012). According to Weigmann (2016), sharks comprise about 500 species belonging to 34 families. Their evident survival on the planet over the years is related to their great evolutionary success that has allowed them to adapt to diverse ecosystems from oceans, seas, estuaries, coral reefs to freshwater systems such as rivers and lakes (Aguilar et al. 2005). Anatomically, they are characterized as jawed aquatic vertebrates with a cartilaginous endoskeleton and denticles throughout their bodies. Teeth are usually not fused to the jaws. Their fertilization is internal by means of the pterygopodia which are the external copulatory appendages of the males. Their fins are supported by unsegmented soft rays (ceratotrichia), they lack a swim bladder, among other characteristics that distinguish them from the rest of the chordates (Nelson 2006); (Castellanos et al. 2013). In terms of their biology, they have a K-type life strategy characterized by slow growth, late sexual maturation, long reproductive cycles and a low fecundity rate (Aguilar et al. 2005).

This strategy is specific to species with low mortality and natural predators (García 2008). However, these characteristics have serious implications for the populations of this taxon, limiting their capacity to withstand overfishing (Moreno et al. 2008). Sharks play an important ecological role in the structure and functioning of marine ecosystems. Some are considered "top predators" because they are at the apex of food webs in such a way that they significantly affect the population size of prey species and thus the composition of the lower levels of food webs (Cortés 1999). Thus, the decline in shark populations could cause a disproportionate increase in mesopredators as they tend to be more generalist species with a higher reproductive rate (Early-Capistrán 2014).

The National Fisheries Institute is the institution responsible for the Sanitary Control and Regulatory Verification plan for all establishments and entities included in the traceability chain, in order to inspect fishery products throughout the different phases of the chain. These phases are: 1) extraction; 2) wholesale marketing; 3 retail marketing; 4) processing; 5) export; and 6) consumption (Núñez and Wuest 2019). Shark traceability is based on catch and landing records (Sinovas et al. 2015).

From records obtained from traceability chains, it is known that in the Colombian Pacific the viscera of sharks caught are sold in the wholesale market of Quibdó, Colombia, for the production of animal feed supplements. The fins are exported to Asian countries. On the other hand, the meat is marketed nationally, mainly in the interior of the country, where it is sold at prices of other species of higher value, because it is usually sold in fillets, which makes it difficult for consumers to identify correctly.

According to Saubi (2018), species substitution is the most common fraud. There are often irregularities in the chain of custody, i.e. documents related to product traceability. Therefore, lack of knowledge is one of the main obstacles to implementing conservation plans for sharks, as it is difficult to identify at a glance the species being caught when they have undergone mutilation processes (Shivji et al. 2002).

For this reason, the ECOCEANOS CORPORATION has implemented several methodologies for the identification of sharks, the most common being the diagnostic marker based on morphological features. Sharks are easily distinguished from other marine fish due to their body shape and fin structure. Whereas, for taxonomic identification of different shark species, field guides are mainly used to provide dichotomous keys using the most useful diagnostic morphological features such as head, teeth and fins.

This type of identification is difficult when the animal is missing fins and head. For these situations,molecular markers are used, which are polymorphic DNA fragments that allow us to distinguish between different taxonomic groups (Ríos et al. 2009). According to Shivji (2002), the old genetic identification methods developed for sharks were based on molecular markers such as RFLP (Restriction Fragment Length Polymorphisms) which proved to be relatively slow, costly and laborious. Therefore, he proposed the use of other molecular markers such as

internal transcribed spacers (ITS) of ribosomal DNA (rDNA) to examine interspecies (ITS2) and intraspecies (ITS1) variations, having a faster and more economical approach (Zapata et al. 2004).

Eukaryotic rDNA is composed of tandemly repeated clusters of 18S, 5.8S and 28S rRNA genes, which RNA polymerase I transcribes as a precursor molecule. The ribosomal RNA transcription units have exons (coding regions) and introns (non-coding regions) which, through the process of cutting and splicing, are removed for RNA maturation. The coding components of the primary rRNA are the 18S, 5.8S and 28S genes, which are flanked before processing by two ITS sequences determined as ITS1 and ITS2, which are separated by the 5.8S gene (Salgado 2011).

The 5.8S gene is highly conserved and is useful for designing primers, which are oligonucleotide sequences that flank the region of DNA to be amplified.

The ITS2 region, compared to ITS1, has subregions of fairly high conservation, sufficient to be used for phylogenetic analysis at the genus and species level due to its low intraspecific variation, but enough interspecific polymorphism to produce strong diagnostic markers (Shivji et al. 2002).

Lea-Charris et al. (2016) suggest that the relatively short ITS2 region is a good phylogenetic marker at the species and/or genus level. In addition, it is considered a potential region to be used as a genetic barcode because of its characteristics that include the availability of conserved regions to design specific primers and because of its ease of amplification. Amplification of the ITS2 region of rDNA is the most common for molecular identification of sharks, because this region is highly divergent between taxa above the genus level, and therefore species-specific primers are likely to amplify DNA only from related species (Shivji et al. 2002). This is evidenced in the study by Pank et al. (2001) where they indicate that the ITS2 locus in C. plumbeus and C. obscurus is about 1268 bp in size, with a 4% sequence divergence between species.

To perform the analysis more quickly, multiplex PCR (Polymerase Chain Reaction) techniques have been standardized where several shark species can be identified simultaneously (Hidalgo 2013). This type of PCR is a variant of a common PCR which consists of making multiple copies of a DNA target sequence through an amplification process. Whereas in multiplex PCR, more than one pair of primers is added, so that more than one amplification target sequence is used (Méndez-Álvarez and Pérez-Roth 2004).

This multiplex PCR design approach has worked well in different studies based on identification in sharks. Thus, Shivji et al. (2002) used species-specific primers based on ITS2 regions to produce diagnostic amplicons distinguishing between Lamnidae and Carcharhinidae species simultaneously. Using the same molecular technique, Caballero et al. (2012), determined that the success rate in identifying shark samples at the genus and/or species level by applying the multiplex PCR technique was 89% in the Pacific coast of Colombia .

These previous studies demonstrate that the molecular technique of multiplex PCR with species-specific primers is a fast and efficient method (Nachtigall et al. 2017). Since, this technique requires only PCR without additional enzymatic processing or sequencing of the amplified products to obtain a species diagnosis, unlike RFLP or phylogenetic analysis (Pinhal et al. 2012).

For this taxon, the current regulatory environment is insufficient to safeguard this fishery resource and strengthened conservation strategies are required. Therefore, this study highlights the importance of molecular methods in the identification of shark species and the need to implement them at strategic control points.

The purpose of this study is to identify the shark species present in the Quibdó wholesale market by means of multiplex PCR. Specifically, to establish the percentage of species found in the market through the use of species-specific primers. In addition, to characterize the commercialization of shark meat in the market.

METHODOLOGY

Study area.

Shark samples were collected at the Quibdó Wholesale Market, located on Avenida Río Atrato, Colombia (see Figure 1). The Wholesale Market has an area of 1916 square meters with a capacity for 77 commercial premises. It has a direct radius of influence of 3 km (Quirola 2015). Being one of the largest seafood markets in the city, there is intense commercial activity that activates the economy generating sources of employment for the local population (Emberá Indigenous).

Figure 1. Wholesale market of the city of Quibdó, Colombia.

Collection of fish muscle tissue samples.

Before starting sample collection, the Framework Contract for Access to Genetic Resources DNB-CM-2021-045 issued by the Ministry of the Environment was obtained.

Each tissue sample was placed in Ziploc bags labeled with a code indicating the initial of the common name of the species and the date of collection. The fish samples were transported in a cooler to the Laboratory of Phenotypic and Genotypic Variability of the Faculty of Biological Sciences of the National University of Colombia where they were stored at -20°C (Pank et al. 2001).

DNA extraction from fish tissue samples.

For the extraction of genomic DNA from shark tissue, a team of 2 biologists from the University of Quibdó and 2 volunteers from the ECOCEANOS Corporation used the GeneJET Genomic DNA Purification Kit (Thermo Scientific). Each tissue was cut into small 25 mg pieces and then disintegrated with a mortar containing 180 µL of digestion solution. The product obtained was placed in a 1.5 ml Eppendorf tube, and 20 µL of proteinase K solution was added and mixed well by pipetting. The sample was incubated at 56 °C for approximately 3 hours until the tissue was completely lysed and no particles remained. Then, it was removed from the water bath and 20 µL of RNase A solution was added, mixed by pipetting and incubated for 10 minutes at room temperature. After the set time elapsed, 200 µL of lysis solution was added and mixed well by stirring manually for 15 seconds until a homogeneous mixture was obtained. Then, 400 µL of 50% ethanol was added and again mixed by pipetting. The lysate obtained was transferred to a GeneJET genomic DNA purification column and centrifuged for 1 min at 6000 rpm. The collection tube was discarded and the GeneJET genomic DNA purification column was placed in a new 2 ml collection tube to add 500 µl of wash buffer I (with ethanol added). It was centrifuged for 1 min at 8000 rpm and the flow-through was discarded to again place the purification column in the collection tube. Next, 500 µL of wash buffer II (with ethanol added) was added to the GeneJET Genomic DNA purification column and centrifuged for 3 minutes at 12000 rpm. The collection tube was discarded, while the GeneJET genomic DNA purification column was transferred to a 1.5 ml Eppendorf tube. 100 µL of elution buffer was added to the center of the membrane of the GeneJET genomic DNA purification column and allowed to incubate for 2 min at room temperature. Then, it was centrifuged for 1 minute at 8000 rpm. Finally, the GeneJET purification column was discarded and the purified DNA was stored at -20 °C (Thermo Fisher Scientific 2016).

Identification of shark species by multiplex PCR.

For the multiplex PCR, two universal primers and ten species-specific primers were used. The triplex PCR

consisted of placing the universal shark primers (FISH5.8SF and FISH28SR) together with each of the species-specific primers in the same reaction.

Thermal cycling conditions and reagent concentration were performed based on Pinhal (2012). Each 50 µl of PCR reaction contained 10-25 ng/µL of DNA, 12.5 pmol of each primer, 1X PCR Buffer, 2.0 mM MgCl2-, 200 µM of dNTPs and 1 unit of Taq DNA Polymerase. Multiplex PCR was amplified following the following conditions: an initial denaturation at 94°C for 15 minutes, followed by 35 cycles of 94°C for 1 minute, 65°C for 1 minute and 72°C for 2 minutes, with a final extension of 5 minutes at 72°C. At the end of cycling, samples were kept at 4°C or -20°C until analyzed by 1.2% agarose gel electrophoresis.

To optimize the process, quadruplex PCR was applied for the two species belonging to the family Carcharhinidae, using the two universal shark primers and the species-specific primers for Prionace glauca (Linnaeus, 1758) and Carcharhinus falciformis (Müller and Henle, 1839). Thermal cycling conditions and reagent concentrations were the same as those applied to triplex PCR (Pank et al. 2001).

A pentaplex PCR assay was also performed on the families Carcharhinidae, Alopiidae and Sphyrnidae. In the first family, the three species-specific primers, Carcharhinus longimanus and Carcharhinus galapagensis, were combined in a single reaction with the two universal primers. Likewise, for the family Alopiidae, the two universal primers were combined with the three species-specific primers: Alopias pelagicus (Nakamura, 1935), Alopias superciliosus (Guérin, 1830) and Alopias vulpinus (Bonnaterre, 1788). Similarly, for the family Sphyrnidae, the three species-specific primers Sphyrna zygaena (Linnaeus, 1758), Sphyrna lewini (Griffith & Smith, 1834) and S.mokarran (Rüppel, 1837) were placed in a single reaction with the two universal primers. The thermal cycling conditions and reagent concentrations were the same as those applied to triplex PCR (Pank et al. 2001).

Agarose gel electrophoresis.

To visualize the amplification products in each PCR reaction, 1.2% agarose gel electrophoresis was performed. PCR products before loading on the gel were prepared by adding 2 µl of loading buffer and 8µL of the PCR reaction. The 100 bp ladder was used and electrophoresis was performed at 110 V for 2 hours (Hidalgo 2013).

Market observation and sample information.

Shark meat trade dynamics were investigated through market observations and informal conversations with

fishery inspectors and shark traders. Questions were asked to learn about the origin of the samples, their economic value and others concerning this trade (Jabado et al. 2015); (Núñez and Wuest 2019).

Statistical analysis.

Descriptive statistical methods were applied in this study. Frequency measures were used to determine the proportion of shark species present at the time of the population assessment, using prevalence analysis, the formula for which is:

$$P = \frac{A}{A + B}$$

Where, A is the total number of individuals of a certain species and A+B is total population sampled at the time the study was conducted (Fajardo-Gutiérrez 2017).

RESULTS

Extraction and determination of DNA quality of samples.

A total of 150 shark tissue samples were collected at the Quibdó wholesale market. For DNA extraction, the protocol proposed in the manual of the GeneJET Genomic DNA Purification Kit (Thermo Scientific) was followed with an incubation time adjustment at 56°C for 3 hours. As for the quality of the extracted DNA, the samples presented a well-defined band near the well in which the DNA mixture was placed, evidencing its good quality.

Species identification by multiplex PCR.

When triplex PCR was performed on all shark samples, the species-specific primers demonstrated complete species specificity. Tissues identified as Alopias pelagicus had a band size of 1200bp for the ITS2 positive control and a band size of 385 bp corresponding to the species-specific forward primer and universal reverse primer. The triplex PCR assay was also used to identify Carcharhinus longimanus, the samples identified as that species amplified in two bands; one in the positive control amplicon (1470bp) and the other in the species-specific amplicon (1085bp).

From this preliminary study Carcharhinus longimanus (oceanic whitetip shark) is the most commercialized shark species in the city of Quibdó; the second most commercialized species is Alopias pelagicus (pelagic thresher shark) present in 25.67% of the total samples.

Based on the global assessments of the IUCN Red List, the two species identified in this study belong to the group of <threatened species>. Of which, the species Carcharhinus longimanus (Carcharhinus longimanus) is classified in the <CR> category, while Alopias pelagicus (The pelagic thresher shark) is listed as <EN>. 100% of the species in this study are included in CITES Appendix II.

DISCUSSION

Species identification by multiplex PCR.

This study is the first evaluation of shark species composition in the main fishing market of Quibdó, Colombia. The methodology applied in this study allows the identification of samples only if the specific primers for the target species are available. The success rate in the identification of samples to the species level was 98.67% using the multiplex PCR technique. While 1.33% involved two samples that belong to the family Carcharhinidae but their species is unknown. Both samples amplified in the positive control band for that family (1470bp) (Magnussen et al. 2007). The possible species are: Carcharhinus altimus (Springer, 1950), Carcharhinus leucas (Muller & Henle, 1839), Carcharhinus limbatus (Müller & Henle, 1839), Carcharhinus longimanus (Poey, 1861), Carcharhinus porosus (Ranzani, 1839) and Galeocerdo cuvier (Péron and Lesueur, 1822). They are present in continental waters of Colombia, and have also been reported as common species in fishing landings (Aguilar et al. 2005); (Fowler et al. 2005); (Martínez-Ortíz et al. 2007); (Herrera 2021). Several studies have noted low yield coamplification of the positive control amplicon

when sharks; P. glauca (blue) and C. falciformis (silky) were the target species (Pank et al. 2001); (Shivji et al. 2002); (Chapman et al. 2003); (Abercrombie 2004); (Hidalgo 2013). However, in this study this phenomenon was only observed when I. oxyrinchus (mako) was the target species. Shivji et al. (2002) suggest that this is a putative "primer proximity" competitive interaction, i.e., as the mating site of the first species-specific primer is relatively close to the mating site of the first 5.8SF positive control, some kind of primer competition is created.

It is worth mentioning that the inconsistency of the universal shark amplicon is not a sign of identification failure since the universal shark primer is included in the multiplex PCR only in order to avoid false negative results. That is, if you know that the samples are exclusively shark, there is the option of not using the positive control primer (Shivji et al. 2002).

The use of species-specific primers based on previously designed ITS2 regions of ribosomal DNA has been very useful in determining shark species. Likewise, the standardized combinations of primers proposed by Hidalgo (2013) reflect reliable diagnoses.

The use of multiplex PCR with efficient results requires planning strategies, multiple attempts and optimization of the reaction conditions in order to avoid the appearance of problems that could compromise the efficiency of the method, since it is very susceptible to execution errors and contaminants (Bolivar et al. 2014). Likewise, there are other molecular techniques such as multiplex real-time PCR, which is a variant of the technique described in this study, with the characteristic that it eliminates the need to perform electrophoresis, thus reducing the time to diagnose the species (Cardeñosa et al. 2018).

These methods are increasingly being used to strengthen traceability systems and chain of custody for shark products (Shivji et al. 2002); (Chapman et al. 2003). These molecular techniques together with identification guides, are tools that together provide sufficient information to justify withholding shipments on the illegal presence of CITES-listed species and thus prevent illegal catches from having a market (Cardeñosa et al. 2018). In fact, these new molecular research techniques have proven to be efficient in different countries such as Hong Kong and Peru, which is evidence that scientific techniques are tools for the management of fishery resources and what is needed is political will to implement shark conservation measures (Pardo 2020).

Unfortunately, Colombia does not consider these molecular tools in its National Action Plan for the conservation and management of the shark resource 2020-2024, which aspires that the use of observer reports, inspectors and marketing guides are sufficient tools to control the landing of fish, as well as the internal and external marketing of shark and its derivatives, when evidently this is not the case.

Market observation and sample information.

The most significant species in landings according to traders are Carcharhinus longimanus and Alopias pelagicus. This is possibly due to the fact that Alopias pelagicus has a seasonal tendency of decreasing landings between March and August in the Colombian Pacific, dates that coincide with the sampling phase of this study. The shark meat trade in the city of Quibdó is considered to be booming. This is reflected in the fact that there are several stalls in the city's market dedicated to the sale of this fishery product, which satisfy local and national demand. Therefore, it is evident that this is not incidental fishing when the economy of this trade is based on the increase in demand.

The World Wildlife Foundation (WWF 2021), estimates that more than 200 countries are importing

and exporting shark and ray meat for a global trade valued at $2.6 billion between 2012 and 2019.

Shark meat trade is related to the high demand for fins as the main product of international interest. Consequently, the value of the fins is higher than the meat. In fact, the latter, being considered of poor quality, tends to be traded at a low price compared to other bony fishes. According to Oceana (2007) and Clarke et al. (2013), the low quality of the meat is due to its strong and unpleasant ammonia odor resulting from the degradation of urea in the tissue. In addition, its consumption is not recommended due to the presence of methylmercury at levels that exceed the permissible limits for humans (De Pinho et al. 2002); (Storelli et al. 2003). In spite of this, the consumption of this product is not only limited to Colombia, but several countries in the world such as Sri Lanka, Oman, Mexico and African countries depend on shark meat as a source of protein.

Likewise, the growth of the phenomenon known as "mislabeling" has been evidenced, which consists of replacing a species of high economic value with one of lower value (Saubi 2018). The study conducted by Mateo (2014) in the Colombian Pacific markets identifies that 26.67% of fish fillets collected under the name of corvina, billfish, wreckfish, tuna, etc. are actually shark and of these, 40% are endangered species. All this is related to irregularities in the traceability of these fishery products, which are major obstacles to implementing conservation plans for sharks, especially for protected species.

CONCLUSIONS

Of the 150 samples identified by multiplex PCR, 125 were determined to correspond to: Carcharhinus longimanus. This demonstrates that the methodology was effective in determining whether the sample was acquired from the target species or not. 100% of the individuals identified in this study are in one of the IUCN threat categories. This demonstrates the unsustainable management of the market and highlights the need to implement new tools focused on a more transparent traceability system for threatened shark species. In the present study, we present a rapid, reliable, relatively inexpensive molecular tool that allows the identification of shark species from any part of the body. Multiplex PCR is a simplified methodology and its implementation at strategic control points will provide reliable data on shark catch and trade.

CITES

1. Megalofonou P, Damalas D, Deflorio M and De Metrio G. 2009. Modeling the environmental, spatial, temporal and operational effects of bycatches of trawl bycatch in the Mediterranean longline fishery. Journal of Applied Ichthyology25: 47-55.

2. Méndez-Álvarez S and Pérez-Roth E. 2004. Muliplex PCR in clinical microbiology. Elsevier 22(3): 183-192.

3. Menéndez E. 2008. Marketing channels of shark bycatch products and their impact on the economy of the artisanal fishing sector of Mantadurante canton during 2007.

4. Moreno F, Acevedo K and Paramo J. 2008. Contributions to the biology of sharks and deemersal rays in the northern Caribbean region of Colombia. Acta biológica colombiana13(3): 121-130.

5. Myers R, Baum J, Shepherd T, Powers S and Peterson C. 2008. Cascading effects of the loss

of apex predatory sharks from a coastal ocean. Science 315: 1846-1850.

6. Nachtigall P, Rodrigues-Filho L, Sodré D, Vallinoto M and Pinhal D. 2017. A multiplex PCR approach for molecµLar identification and conservation of the critically endangered daggernose shark. Endangered Species Research 32: 169-175.
7. National Geographic. 2018. Mako shark, the lightning of the sea. (Available at:https://www.ngenespanol.com/naturaleza/revista-impresa-agosto-2017-tiburonmako-informacion/. Accessed on: 05 December 2021).
8. Nelson J. 2006. Fishes of the world. New Jersey: John Wiley and Sons, Inc. Cited in: Del MoralFlores L, Morrone J, Alcocer J and Pérez-Ponce de León G. 2016. Diversity and biogeographic affinities of the sharks, rays and chimaeras (Chondrichthyes: Elasmobranchii, Holocephali) of Mexico. Journal of Tropical Biology 64(4): 1469-1486.
9. Núñez J and Wuest R. 2019. The shark fishery value chain in Peru. 100pp.
10. Oceana. 2007. End of the line: global threats to sharks. (Available at: https://oceana.org/reports/end-line-global-threats-sharks. Accessed on: 14 October 2021).
11. Pank M, Stanhope M, Natanson L, Kohler N and Shivji M. 2001. Rapid and simultaneous identification of body parts of morphologically similar sharks Carcharhinus obscurus and Carcharhinus plumbeus (Carcharhinidae) by multiplex PCR.
12. Marine Biotechnology 3(3): 231-240.
13. Wirsing, A.J., Heithaus, M.R. and Dill, L.M. 2007. Living on the edge: dugongs prefer to forage in microhabitats that allow escape from rather than avoidance of predators.
14. Ramírez, R. del R. and E. Medina. 1998. Diagnosis of the Shark Resource in the Republic of Panama. 28 page
15. Robles, Y. and L.A. Mountains. 2011. Diagnosis for fishing exploitation and socioeconomic analysis of mangroves and related ecosystems in the district of David. Technical Report presented to the MarViva Foundation, National Authority for the Environment and International Conservation. 190 pages
16. Rodríguez-Arriatti, Y.N. 2010. National Action Plan for the Conservation and Management of Shark Fisheries. Aquatic Resources Authority of Panama (ARAP), 001 Fishing Technical Document. Panama City, Panama, 48 pp.
17. Vega, A.J., Y. Robles, and A. Del Cid. 2011. Impact of artisanal fishing on sharks and rays in Coiba National Park and its area ofinfluence, Gulf of Chiriquí. In: Fisheries biological studies in the Gulf of Chiriquí, Pacific of Panama (A.J. Vega, ed.).