Forensic species identification of elasmobranch products sold in Costa Rican markets

Jason R. O'Bryhim^{a,b,*}, E.C.M. Parsons^a, Stacey L. Lance^b

^a*Department of Environmental Science & Policy, George Mason University, Fairfax, VA 22030, USA*

^b*Savannah River Ecology Laboratory, University of Georgia, Aiken, SC 29802, USA*

*Author to whom correspondence should be addressed.

Savannah River Ecology Laboratory

Savannah River Site, Building 737-A

Aiken, SC 29802 USA

Tel 803-725-0988

Fax 803-725-3309

email: jobryhim@gmail.com

Abstract

One barrier to establishing catch limits to help protect shark populations is a lack of accurate species-specific extraction rates. This is due to many species looking similar, distinguishing characteristics (fins and head) of sharks commonly being removed, or sharks being grouped together in fisheries data. For this study, we collected elasmobranch (shark and ray) tissue samples from the central markets in San Jose (10 fish vendors or pescadarias) and Heredia (5 pescadarias) from June 2013 to September 2014. We used DNA barcoding techniques to amplify approximately 1050bp of the NADH dehydrogenase subunit 2 (NADH2) gene (n=833). We found that at least nine species of shark (*Alopias pelagicus*, *Carcharhinus falciformis*, *C.*

limbatus, *C. obscurus*, *Mustelus lunulatus*, *Nasolamia velox*, *Rhizoprionodon longurio*, *Sphyrna lewini*, *S. zygaena*) and one ray (*Dasyatis longa*) are being sold in local markets, with *C. falciformis* representing 87.3% of shark samples tested (n=637) and *D. longa* representing 100% of ray samples tested (n=85). Our results suggest that *C. falciformis* continues to be under intense fishing pressure in the waters around Costa Rica despite recent concern over continued population declines. Although the number of Endangered *S. lewini* (4%) being sold in the markets is much less than for *C. falciformis* (87.3%), the numbers are still concerning given their current conservation status.

Keywords: Sharks, Conservation, DNA Barcoding, Species Diversity, Rays

1. Introduction

In the past several decades shark populations have declined due to both expanding directed shark fisheries and increased levels of bycaught sharks (Abercrombie et al., 2005, Dulvy et al., 2014). These declines have resulted in one quarter of shark species being listed as “Vulnerable”, “Endangered”, or “Critically Endangered” by the International Union for Conservation of Nature (IUCN) (Dulvy et al., 2014). Driving these declines is an increased demand for shark products (e.g. fins) resulting in up to 100 million elasmobranchs (sharks and rays) being caught each year (Clarke et al., 2004, Abercrombie et al., 2005, Shivji et al., 2005, Worm et al., 2013, Dulvy et al., 2014, Dent and Clarke, 2015). Market growth in shark products and the increased global fishing pressure experienced by all marine organisms has resulted in reduced catch rates (population declines) for many shark species (Dulvy et al. 2014, Dent and Clarke, 2015). For example, catch rates have declined significantly in the Northwest Atlantic from 1986 to 2003 for hammerhead sharks (*Sphyrna spp.*, by 89%), great white sharks (*Carcharodon carcharias*, by 79%), tiger sharks (*Galeocerdo cuvier*, by 65%), thresher sharks (*Alopias spp.*, by 80%), blue sharks (*Prionace glauca*, by 60%), and mako sharks (*Isurus spp.*, by 70%) (Baum et al., 2003). While blue shark (by >50%) and oceanic whitetip shark (*Carcharhinus longimanus*, by 90%) catch rates in longline fisheries in the Pacific Ocean also declined from 1996 to 2009 (Clarke et al., 2013). In the Mediterranean, smooth hammerhead (*S. zygaena*), blue, mako, porbeagle (*Lamna nasus*), and common thresher shark (*A. vulpinus*) catch rates declined by 96 to 99.99% (Ferretti et al., 2008). In the 1950’s sharks accounted for ~17% of the total catch of longline fisheries in the Gulf of Mexico (Baum and Myers, 2004). However, by the 1990’s they had dropped to only 2% of the total catch (Baum and Myers, 2004). Previous

studies using catch rate data to estimate species abundance have shown that declining catch rates for sharks are representative of population declines (Baum et al., 2003, Baum and Myers, 2004).

In Costa Rica, the impact of the pelagic long-line fishery on shark populations is of particular importance (Dapp et al., 2013). Sharks are rarely the target species in these fisheries (i.e. bycatch), however, pelagic fisheries in Costa Rica have been shown to shift their focus to sharks when their original target species are low in abundance (Swimmer et al., 2010). This has resulted in sharks accounting for up to 15% of all reported landings (Trujillo et al., 2012). Sharks caught in these fisheries also typically do not survive due to either the retention of their bodies for their fins and meat or the high mortality rate associated with the stress of being hooked (Frick et al., 2010, Whoriskey et al., 2011). As a result, catch rates for pelagic sharks in Costa Rica declined by 60% from 1991-2000 (Arauz, 2000, Arauz et al., 2004, Whoriskey et al., 2011). Of particular concern in these fisheries - and others globally - is documenting the catch rates of highly exploited or threatened species like the scalloped hammerhead (*S. lewini*), which is listed as “Endangered” by the IUCN, with population declines between 50-90% depending on ocean basin (Baum et al., 2007). The propensity of individuals of this species to aggregate in predictable locations has made them easier to exploit and increases their vulnerability to fishing pressure (Abercrombie et al., 2005, Baum et al., 2007). Silky sharks (*Carcharhinus falciformis*) are the most commonly caught shark species in Costa Rican long-line fisheries (Dapp et al., 2013). However, due to reductions in catch rates (by 80% in Costa Rica) from both target and non-target fisheries silky sharks have been listed as “Near Threatened” globally and “Vulnerable” in the Eastern Tropical Pacific (Arauz, 2000, Arauz et al., 2004, Dulvy et al., 2008, Whoriskey et al., 2011). Recent observer data also show that the majority of silky sharks caught are below reproductive size and there has been a significant decrease in the reported size of these

sharks from 2004 to 2010 (Dapp et al., 2013). This could indicate a reduction in the number of adult sharks of this species, which could have significant impacts on its population growth rate and ability to deal with fishing mortality (Dulvy et al., 2008, Dapp et al., 2013).

Like Costa Rica, few nations have developed catch limits or size restrictions for the landing of sharks in their waters and no international or bilateral catch limits exist (Camhi et al., 2008). In many cases there is little interest in managing pelagic sharks because they are mostly caught as bycatch and in most cases the target species of the fishery remains highly productive with more stable populations (Dulvy et al., 2008). Other barriers to establishing catch limits and other protective measures to ensure sustainable extraction rates for sharks include a lack of species-specific data on life history characteristics, extraction rates, and sizes at landing (Bonfil, 2003, Holmes et al., 2009, Trujillo et al., 2012, Spaet et al., 2012). Historically, as elsewhere, landings of sharks would either not be recorded, or recorded data were not defined to species level (Pank et al., 2001, Holmes et al., 2009). The fact that many shark species look similar also made the identification of sharks and recording of species-specific data difficult (Burgess et al., 2005, Holmes et al., 2009). For example, in Costa Rica, silky sharks were commonly mis-identified as blacktip sharks (*Carcharhinus limbatus*) in tuna fisheries (Bonfil et al., 2008, Dulvy et al., 2008). Additionally, the common practice of removing the distinguishing characteristics (fins and head) of sharks yields a relatively un-identifiable carcass (Abercrombie et al., 2005, Shivji et al., 2002). This, and a lack of interest in sharks due to the previously low economic value of their products, resulted in morphologically similar shark species being grouped together in catch records, or landed sharks going unreported altogether (Pank et al., 2001, Bonfil et al., 2008, Dulvy et al., 2008, Holmes et al., 2009). This has made it difficult to monitor fisheries expansion, quantify bycatch mortality, and assess the impact fisheries are having on shark

populations (Abercrombie et al., 2005, Holmes et al., 2009). The absence of accurate catch statistics also hinders the establishment of sustainable management and conservation plans to protect sharks (Shivji et al., 2002).

To help combat the paucity of species-specific fisheries catch data on sharks there is an increasing amount of literature on the identification of sharks and their products (e.g. fins) using various forensic genetic techniques, including DNA barcoding (Abercrombie et al., 2005, Shivji et al., 2005, Ward et al., 2005, Clarke et al., 2006, Ward et al., 2008, Holmes et al., 2009, Barbuto et al., 2010, Liu et al., 2013, Spaet and Berumen, 2015). DNA barcoding uses a short standardized segment of DNA sequence from an unidentified organism and compares it to a reference library (e.g. GenBank, Barcode of Life Database) of sequences of previously identified species to determine the likelihood of that organism being a particular species (Hebert et al., 2003). The ability for DNA barcoding to be an effective tool for identifying species is reliant, however, on the correct taxonomic identifications of the reference sequences entered into the library (Dudgeon et al., 2012). Barcoding allows the identification of individual pieces of sharks (e.g. fins, meat), and helps alleviate the issues of broadly categorized fisheries data (e.g. all species simply labeled shark) for fisheries managers (Tillett et al., 2012).

Our objective, was to use DNA barcoding to conclusively identify the types and quantities of shark species being sold in local markets in Costa Rica's central valley and compare this to current fisheries data. We also looked for changes in species diversity within the markets related to seasonality, to determine if threatened species were more at risk during certain times of the year. These large open-air markets have whole sharks delivered to their vendors from Puntarenas, the main landing dock for pelagic fisheries, several times per week. Therefore, the

sharks being sold in these markets would be representative of the ones being caught by large pelagic fishing vessels (i.e. long-line vessels).

2. Methods

2.1 Sample Collection

We collected a total of 833 tissue samples between June 2013 to September 2014 from the central markets in San Jose (n =10 “pescadarias” or fish vendors) and Heredia (n =5 pescadarias), Costa Rica. We sampled all pescadarias selling shark products during 28 (San Jose) and 22 (Heredia) separate sampling trips (days). We made six sampling trips in the fall (September – November), eight in winter (December – February), eight in spring (March – May), and six in summer = June – August). Sporadically, we noticed products being sold that were labeled as “Raya” (Ray) and we collected tissue samples from these products for analysis as well (included in the 833 total samples). Shark meat being sold at pescadarias from these locations is generally sold as either a fillet or a “chuleta” (a cross section of the shark including a single vertebra), while ray meat is generally sold as fillets. For each sampling trip (day) we collected a single sample of each of the available cuts of shark or ray products from each pescadaria. This was done to reduce the possibility of sampling the same individual more than once. In some instances, we collected tissue samples from whole sharks that had yet to be processed into smaller cuts. We used an 8mm disposable biopsy punch to take samples from the different cuts of shark or ray meat. We then stored the shark and ray tissues in a RNA preservation buffer (0.018 M Sodium Citrate, 0.014 M EDTA, 3.78 M Ammonium Sulfate in DEPC-treated water) and kept them at -4° C.

2.2 DNA Barcoding

We extracted total genomic DNA from the tissue samples using the DNeasy Tissue Kit (Qiagen, Valencia, CA), following protocols recommended by the manufacturer. We then amplified an approximately 1050bp region of the NADH dehydrogenase subunit 2 (NADH2) gene for species identification using the ASNM and ILEM primer combination described in Naylor *et al.* (2012). We conducted the polymerase chain reaction (PCR) amplifications within a total volume of 25µl containing 10mM Tris pH 8.4, 50mM KCL, 0.2 mM each dNTP, 1.5mM MgCl₂, 0.4µM each primer, 1 U Amplitaq Gold Polymerase® (Life Technologies), and 4 µl of template DNA. The PCR thermal cycling profile we used consisted of: 5 min at 94°C; 35 cycles of 30 s at 94°C, 30 s at 54°C, and 1 min at 72°C; followed by 10 min at 72°C. Amplifications were performed using GeneAmp 9700 thermal cyclers (Applied Biosystems). To check PCR products for quality and relative concentration we electrophoresed them on a 1% TBE agarose gel containing GelRed and visualized them on an AlphaImager® (Alpha Innotech). We purified PCR products by combining 10 µL of PCR product with 2 units of Exonuclease I (New England BioLabs) and 10 units of shrimp alkaline phosphatase I (New England BioLabs) in a total volume of 15 µL and incubating at 37°C for 20 min followed by 80°C for 15 min. We conducted cycle sequencing reactions on the cleaned products using one eighth reactions of BigDye® Terminator Cycling Sequencing Ready Reaction Mix v3.1 (Applied Biosystems). After purifying the sequencing reactions with sephadex we ran them on an ABI 3130xl (Applied Biosystems) capillary sequencer.

2.3 Data Analysis

Forward and reverse sequences were aligned using Sequencher v5.2.4 to generate a full-length consensus sequence for each sample. We aligned all consensus sequences using the MUSCLE tool and trimmed them to ~1000bp. In some cases, the forward sequencing reaction

failed and we used only the reverse sequence for species identification. The reverse reaction consistently yielded 700bp. To verify using only this sequence, for 100 samples that produced both a forward and reverse sequence we compared species ID results using the full 1000bp or the reduced 700bp. In no cases did the species ID differ when using the shorter sequence. We used MEGA v6.06 to create two neighbor joining (NJ) trees, one for the 1000bp consensus sequences and one for the 700bp reverse sequences, in order to cluster identical sequences onto a single node. We then performed species identifications for each cluster (node) using a representative sequence entered into GenBank's BLAST program with a 98% match criteria necessary for accurate species identification. To determine shark species diversity within seasons, pescadarias (fish vendor), and markets (San Jose and Heredia) we calculated the Fisher's α -value (Fisher et al., 1943). Then to compare the variation in shark species diversity among seasons, pescadarias (fish vendors) sampled, and the San Jose and Heredia markets we used an analysis of similarity (ANOSIM). In cases where shark species diversity was significantly different we used *non-metric multidimensional scaling* (NMDS) to visualize which species grouped significantly more with particular seasons, pescadarias, or markets. The NMDS collapses multi-dimensional information (e.g. multiple locations) into two or three, so that they can be visualized and interpreted. Due to ray products not being sold at every location sampled and only one species being represented in all the samples, they were not included in further analyses.

3. Results

3.1 Species Identification

Of the 833 samples tested, 722 successfully amplified (401 full-length consensus sequences, 321 reverse only sequences) and resulted in positive species identifications (637 shark and 85 ray). Overall, nine species of shark and one ray were represented in the samples

(Table 1). Silky sharks accounted for the vast majority (87.3%) of shark samples tested. Two species of hammerhead shark (scalloped and smooth) were also found in the markets making up 4% and 2% of the samples, respectively. The pelagic thresher (*A. pelagicus*) (3.3%) and blacktip shark (1.9%) were the only other two shark species to account for more than 1% of the samples tested. The longtail stingray (*Dasyatis longa*) accounted for 100% of all ray samples tested.

3.2 Seasonal Variation

Shark species diversity varied significantly among seasons (Table 2) ($R = 0.2246$, $p = 0.001$). However, based on the low R -value obtained by the ANOSIM analysis very little of the variation in species diversity is explained by season. Both the Fisher's α -values and species richness are similar for each season (Table 2). Silky sharks, which account for the majority of the samples, and scalloped hammerheads, were present in all seasons sampled. The smooth hammerhead and the pelagic thresher were both found in the three of the four seasons. The remaining species account for very few of the samples.

3.3 Variation among pescadarias and markets

Overall there was no difference in shark species diversity between the two markets ($R=0.1199$, $p=0.053$, Table 1), but there was a difference among pescadarias ($R=0.1141$, $p=0.002$, Table 3). Examining the species diversity index scores (Fisher's α -values) and the species richness for each market clearly shows that there is no difference between the San Jose and the Heredia markets (Table 1), but that there are differences among the individual pescadarias for a variety of species, both within and between markets (Table 3). Interestingly, within the San Jose market only the pescadaria "Caracol" had silky shark abundance below 90%, with 41% of samples collected from that location identified as either scalloped or smooth hammerhead sharks.

4. Discussion

It is clear that Costa Rica has an active market for elasmobranch products that encompasses a variety of different species. Interestingly, the number of species of sharks ($n = 9$) and rays ($n = 1$) identified in this study was below observer data from landings of Costa Rican longline vessels (12-18 species of shark, 2-6 species of ray) (Whoriskey et al., 2011, Dapp et al., 2013). This is likely the result of certain species having little market value or being caught in too low abundance to bother shipping to markets.

4.1 Variation across seasons, markets and pescadarias

Overall, we found there to be significant variation in shark species diversity across seasons and pescadarias, but not markets. However, none of these variables individually explained a large proportion of the variance. Most of our results are driven by the dominance of silky sharks across seasons (88-91%), markets (~78-91%), and pescadarias (45-100%). The wider range of variation in proportion of silky sharks across the pescadarias provides is of interest. Silky sharks made up 100% of the samples for seven pescadarias and $\geq 90\%$ for five others. Most of these were found in the San Jose market. In general, the diversity of products was higher in the Heredia market where four of five pescadarias sold multiple shark species; while only four of the 10 in San Jose did. It is not known whether the dominance of silky sharks in the markets reflects catch rates and/or preference from the vendors for the species. During sampling it was observed that silky shark meat had a very clean white coloring, like other highly prized teleost fish in the market, compared to samples of other species of shark (pelagic thresher, hammerhead), which either had a more red or brown/black coloring. Perhaps the coloring of the meat has led to silky sharks being more desirable to customers allowing them to garner a higher price. This was also true for ray meat, which was found to be tougher than shark and also has a

darker coloring than silky shark meat. In some cases, samples were actually listed as pelagic
thresher or silky shark in the market, and in those cases we noted the price was higher for silky
shark meat. However, discussions with pescadaria owners indicated that most had no idea what
type of shark species they were purchasing, or that there were even different types coming to the
market. Potentially they are purchasing sharks simply based on the way the meat looks and
therefore selecting the more valued silky shark exclusively.

Of the eight pescadarias that sold multiple shark species products, half were located in
the Heredia market. This could be the result of the Heredia market being smaller than the San
Jose market causing pescadaria owners in Heredia to purchase cheaper species. However, based
on conversations with pescadaria owners, seafood products coming from the Pacific coast, first
arrive at the San Jose market and then travel to the Heredia market. Therefore, Heredia market
pescadaria owners may have a greater variety of species because they are left with shark species
that the pescadaria owners in San Jose do not want.

4.2 Species specific findings

Though we identified nine species of sharks, a vast majority (87.3%) of the products
being sold in Costa Rican markets came from silky sharks. This finding further corroborates
previous studies based on observer data that silky sharks are the most commonly caught shark
species in Costa Rican long-line fisheries (Whoriskey et al., 2011, Dapp et al., 2013). Previous,
observer programs on long-line vessels found silky sharks accounted for 60-70% of recorded
shark landings (number of individuals) from 1991-2000, and 58.2% in 2003 (Arauz et al., 2004).
Based on our results it appears that silky sharks are still the most exploited pelagic elasmobranch
species in Costa Rican waters. Similar results have been reported from a pelagic longline survey
in the neighboring countries of Panama, El Salvador, and Guatemala, where they found silky

sharks to account for 79.80%, 63.3% and 44.29% of the shark catch, respectively (Porrás, 1996).

The pelagic thresher shark species is currently listed as “Vulnerable” by the IUCN, with the population in the Eastern Tropical Pacific estimated to have declined by up to 83% (Ward and Myers, 2005). This species also has a low annual rate of population increase (2-4%), resulting in it being at particular risk from continued fisheries exploitation (Baum et al., 2003). In this study, whereas the pelagic thresher shark only accounted for 3.3% of all shark species sampled, it did make up a relatively large portion (10.9%) of shark meat being sold at the Heredia market. The pelagic thresher is one of the top five elasmobranch species landed in pelagic longline fisheries (Whoriskey et al., 2011, Dapp et al., 2013), thus we were surprised not to find it in the much larger San Jose central market.

Two hammerhead species, the scalloped and smooth hammerhead, were both found within the market samples. Although accounting for relatively low abundance of shark samples, the current conservation status, “Endangered” and “Vulnerable” respectively, makes any documentation of landings of these species important. Previous studies by non-governmental organizations using observer-based programs on longline fishing vessels found these two species to account for lower quantities (<1% for both species) of the total catch compared to our results (Whoriskey et al., 2011, Dapp et al., 2013). When comparing overall elasmobranch species diversity between the Costa Rican markets and the observer program data, we see that fewer elasmobranch species are actually sold in markets than are landed by the fishing vessels. This could be due to lower market values for many of the other elasmobranch species landed, resulting in these species being discarded. One pescadaria, “Caracol”, in the San Jose market accounted for the majority of hammerhead samples identified, and the reason behind this is unclear. However, determining if this pescadaria receives its elasmobranch products from

different sources, compared to the others, could help in the understanding of how these species products are utilized in Costa Rica.

The longtail stingray was the only ray species recorded. Each of the samples that we identified as longtail stingrays were also labeled as stingray (raya) in the markets. In previous studies, the pelagic stingray (*Pteroplatytrygon violacea*) accounted for the first or second highest abundance of ray landings in longline fisheries (Whoriskey et al., 2011, Dapp et al., 2013). In those studies, all landed stingrays were observed to be discarded, which may explain why we did not identify any pelagic stingrays in the market samples. It is uncertain, however, why longtail stingrays would be retained while other stingrays are discarded. Through the fall of 2013 and into the winter, spring and summer of 2014 more pescadarias began selling ray meat. More data would be needed to determine if this result is due to natural fluctuations in abundance or whether it indicates a growing market for this product in Costa Rica. The longtail stingray is currently listed as “Data Deficient” by the IUCN with an unknown population status. Therefore, careful monitoring of the exploitation of this species in Costa Rica could prove important in determining current abundance and for the sustainability of the population in the region.

5. Conservation implications

This study represents the first species identification of elasmobranch products in Costa Rican markets using DNA barcoding. As mentioned above, our findings have conservation implications for silky sharks and scalloped hammerheads. Ward and Myers (2005) estimated that the silky shark populations have declined in abundance and biomass by ~90%, prompting their IUCN redlist status (Dulvy et al., 2008). At the 2015 annual meeting of the IATTC, new regulations were established to protect silky sharks by requiring them to be released when landed as bycatch in pelagic fisheries (IATTC, 2015, Project Aware, 2015). The Costa Rican

Government refused to support these regulations making the current status of silky shark populations, along with the continued high fishing pressure they are experiencing, all the more concerning for the fate of this species in the eastern tropical Pacific.

The Endangered scalloped hammerhead was also found in the Costa Rican markets, and due to their current conservation status, any landings of scalloped hammerhead sharks in pelagic waters (likely mature adults) could have negative consequences for their populations. Landings of this species should continue to be monitored for any further reductions in catch rates, which could indicate further population declines and need for stronger protection. Due to the discrepancies between the elasmobranch species diversity reported in this study compared to previous observer-based studies, the DNA barcoding of markets likely only explains part of the story for elasmobranch species facing fishing pressure in Costa Rica. However, the species in the market are likely those facing the greatest fishing pressure currently and are therefore at the greatest risk of overexploitation.

Due to markets not encompassing all species landed by pelagic vessels, the inability of observer programs to monitor a majority of the pelagic vessels potentially landing sharks, and the misidentification by observers of species with similar characteristics, we recommend the use of DNA barcoding at landing docks. This will provide a more accurate representation of all species of elasmobranch currently under fishing pressure in Costa Rica and determine whether certain species become targeted more often. This will require significant support from the Costa Rican government as gaining access to pelagic vessels, particular foreign flagged ones, can be difficult and dangerous. Nevertheless, Costa Rican pelagic fisheries and open-air markets sell elasmobranch species that are currently overexploited and threatened with extinction, so the issue is urgent.

6. Acknowledgements

We would like to thank everyone in Costa Rica who helped make this research possible including: Dr. Ted Bradley, Randall Arauz, Maike Heidemeyer, Andy Bystrom, Taylor Clarke, Dr. Ingo Wehrtmann, PRETOMA, the University of Costa Rica, and the vendors who were willing to participate in this research. We also would like to thank the Rufford Foundation for being the main funding agency of this research as well as the Savannah River Ecology Laboratory, George Mason University, and the Explorers Club Washington Group. This research was also partially supported by the U. S. Department of Energy under Award Numbers DE-FC09--07SR22506 to the University of Georgia Research Foundation.

7. References

- Abercrombie, D., Clarke, S., & Shivji, M. (2005) Global-scale genetic identification of hammerhead sharks: Application to assessment of the international fin trade and law enforcement. *Conservation Genetics* 6, 775–788.
- Alpizar, M. A. Q. (2006) Participation and fisheries management in Costa Rica: From theory to practice. *Marine Policy* 30, 641– 650.
- Arauz, R. M. (2000) Impact of high seas longline fishery operations on shark and sea turtle populations in the Economic Exclusive Zone of Costa Rica. Sea Turtle Restoration Project. < <http://www.pretoma.org/downloads/pdf/longline2000.pdf> > [Accessed 20 June 2015]
- Arauz, R. M., Cohen, Y., Ballesterio, J., Bolaños, A., & Pérez, M. (2004) Decline of shark populations in the Exclusive Economic Zone of Costa Rica. Proceedings of the International Symposium on Quantitative Ecosystem Indicators for Fisheries Management. UNESCO, FAO. Paris, France. March 2004.
- Barbuto, M., Galimberti, A., Ferri, E., Labra, M., Malandra, R., Galli, P., & Casiraghi, M. (2010) DNA barcoding reveals fraudulent substitutions in shark seafood products: the Italian case of “palombo” (*Mustelus spp.*). *Food Research International* 43, 376–381.
- Baum, J. K., Myers, R. A., Kehler, D. G., Worm, B., Harley, S. J. & Doherty, P. A. (2003) Collapse and conservation of shark populations in the Northwest Atlantic. *Science* 299, 389-392.
- Baum, J. K., & Myers, R. A. (2004) Shifting baselines and the decline of pelagic sharks in the

- Gulf of Mexico. *Ecology Letters* 7,135–145.
- Baum, J., Clarke, S., Domingo, A., Ducrocq, M., Lamónaca, A. F., Gaibor, N., Graham, R., Jorgensen, S., Kotas, J. E., Medina, E., Martinez-Ortiz, J., Monzini Taccone di Sitizano, J., Morales, M. R., Navarro, S. S., Pérez, J. C., Ruiz, C., Smith, W., Valenti, S. V., & Vooren, C. M. (2007) *Sphyrna lewini*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.2. <www.iucnredlist.org> [Accessed 21 June 2015]
- Bonfil, R. (2003) Consultancy on elasmobranch identification and stock assessment in the Red Sea and Gulf of Aden. In: Final Report presented to the Regional Organization for the Conservation of the Environment of the Red Sea and Gulf of Aden, Jeddah.
- Bonfil, R. (2008) The Biology and Ecology of the Silky Shark, *Carcharhinus falciformis*. In *Sharks of the Open Ocean: Biology, Fisheries, Conservation*, 1st edn. (Camhi, M. D., Pikitch, E. K. & Babcock, E. A., eds), pp. 114-127. Oxford, UK: Blackwell Publishing.
- Burgess, G. H., Beerkircher, L. R., Cailliet, G. M., Carlson, J. K., Cortes, E., Goldman, K. J., Grubbs, R. D., Musick, J. A., Musyl, M. K., & Simpfendorfer, C. A. (2005) Is the collapse of shark populations in the Northwest Atlantic Ocean and Gulf of Mexico real? *Fisheries* 30, 19 – 26.
- Camhi, M. D. (2008) Conservation Status of Pelagic Elasmobranchs. In *Sharks of the Open Ocean: Biology, Fisheries, Conservation*, 1st edn. (Camhi, M. D., Pikitch, E. K. & Babcock, E. A., eds), pp. 397-417. Oxford, UK: Blackwell Publishing.
- Clarke, S. C., McAllister, M. K., & Michielsens, G.J. (2004) Estimates of Shark Species Composition and Numbers Associated with the Shark Fin Trade Based on Hong Kong Auction Data. *Journal of Northwest Atlantic Fishery Science* 35, 453 – 465.
- Clarke, S. C., Magnussen, J. E., Abercrombie, D. L., McAllister, M. K., & Shivji, M. S. (2006) Identification of shark species composition and proportion in the Hong Kong shark fin market based on molecular genetics and trade records. *Conservation Biology* 20, 201–211.
- Clarke, S. C., Harley, S. J., Hoyle, S. D., & Rice, J. S. (2013) Population trends in Pacific oceanic sharks and the utility of regulations on shark finning. *Conservation Biology* 27, 197 – 209.
- Dapp, D., Arauz, R., Spotila, J. R., & O'Connor, M. P. (2013) Impact of Costa Rican longline fishery on its bycatch of sharks, stingrays, bony fish and olive ridley turtles (*Lepidochelys olivacea*). *Journal of Experimental Marine Biology and Ecology* 228, 228 – 239.
- Dent, F., & Clarke, S. (2015) State of the global market for shark products. *FAO Fisheries and Aquaculture Technical Paper* 590.

- Dudgeon, C. L., Blower, D. C., Broderick, D., Giles, J. L., Holmes, B. J., Kashiwagi, T., Krück, N. C., Morgan, J. A. T., Tillett, B. J., & Ovenden, J. R. (2012) A review of the application of molecular genetics for fisheries management and conservation of sharks and rays. *Journal of Fish Biology* 80, 1789 – 1843.
- Dulvy, N. K., Fowler, S. L., Musick, J. A., Cavanagh, R. D., Kyne, P. M., Harrison, L. R., Carlson, J. K., Davidson, L. N. K., Fordham, S. V., Francis, M. P., Pollock, C. M., Simpfendorfer, C. A., Burgess, G. H., Carpenter, K. E., Compagno, L. J. V., Ebert, D. A., Gibson, C., Heupel, M. R., Livingstone, S. R., Sanciangco, J. C., Stevens, J. D., Valenti, S. & White, W. T. (2014) Extinction risk and conservation of the world's sharks and rays. *eLife* 3, e00590.doi:10.7554/eLife.00590.
- Dulvy, N. K., Baum, J. K., Clarke, S., Compagno, L. J. V., Cortés, E., Domingo, A., Fordham, S., Fowler, S., Francis, M. P., Gibson, C., Martínez, J., Musick, J. A., Soldo, A., Stevens, J. D., & Valenti, S. (2008) You can swim but you can't hide: the global status and conservation of oceanic pelagic sharks and rays. *Aquatic Conservation* 18, 459 – 482.
- Ferretti, F., Myers, R. A., Serena, F., & Lotze, H. K. (2008) Loss of large predatory sharks from the Mediterranean Sea. *Conservation Biology* 22(4), 952–964.
- Fisher, R.A., Corbet, A.S., & Williams, C.B. (1943) The relation between the number of species and the number of individuals in a random sample of animal population. *Journal of Animal Ecology* 12, 42 – 58.
- Frick, L. H., Reina, R. D., & Walker, T. I. (2010) Stress related physiological changes and post release survival of Port Jackson sharks (*Heterodontus portusjacksoni*) and gummy sharks (*Mustelus antarcticus*) following gill-net and longline capture in captivity. *Journal of Experimental Marine Biology and Ecology* 385, 29–37.
- Hebert, P. D. N., Cywinska, A., Ball, S. L., & DeWaard, J. R. (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society B* 270, 313–321.
- Holmes, B. H., Steinke, D., & Ward, R. D. (2009) Identification of shark and ray fins using DNA barcoding. *Fisheries Research* 95 (2–3), 280–288.
- IATTC (Inter-American Tropical Tuna Commission) (2015) Recommendations by the staff for conservation measures in the eastern Pacific Ocean. Document IATTC 89-04d. 89th Meeting Guayaquil, Ecuador.
- Liu, S. V., Chan, C. C., Lin, O., Hu, C., & Chen, C. A. (2013) DNA barcoding of shark meats identify species composition and CITES-listed species from the markets in Taiwan. *PloS One* 8, e79373.
- Naylor, G. J. P., Caira, J. N., Jensen, K., Rosana, K. A. M., White, W. T., & Last, P. R. (2012) A DNA sequence-based approach to the identification of shark and ray species and its

- implications for global elasmobranch diversity and parasitology. *Bulletin of the American Museum of Natural History* 367.
- Pank, M., Stanhope, M., Natanson, L., Kohler, N., & Shivji, M. (2001) Rapid and simultaneous identification of body parts from the morphologically similar sharks *Carcharhinus obscurus* and *Carcharhinus plumbeus* (Carcharhinidae) using multiplex PCR. *Marine Biotechnology* 3, 231 – 240.
- Porras, O. (1996) Campañas de pesca comercial simulada realizadas con palangre en la Zona Económica Exclusiva del litoral Pacífico de Panamá, El Salvador, y Guatemala. Programa Regional de Apoyo al Desarrollo de la Pesca en el Istmo Centroamericano. Unión Europea-OLDEPESCA.
- Project Aware. (2015) IATTC Takes Steps to safeguard rays, but leaves finning ban weak, and sharks under-protected. <<http://www.projectaware.org/sites/default/files/imce/IATTC%202015%20press%20release.pdf>> [Accessed 18 June 2015]
- Shivji, M., Clarke, S., Pank, M., Natanson, L., Kohler, N., & Stanhope, M. (2002) Genetic identification of pelagic shark body parts for conservation and trade monitoring. *Conservation Biology* 16, 1036 –1047.
- Shivji, M. S., Chapman, D. D., Pikitch, E. K., & Raymond, P. W. (2005) Genetic profiling reveals illegal international trade in fins of the great white shark, *Carcharodon carcharias*. *Conservation Genetics* 6, 1035 –1039.
- Spaet, J. L. Y., Thorrold, S. R., & Berumen, M. L. (2012) A review of elasmobranch research in the Red Sea. *Journal of Fish Biology* 80(5), 952 – 965.
- Spaet, J. L. Y., & Berumen, M. L. (2015) Fish market surveys indicate unsustainable elasmobranch fisheries in the Saudi Arabian Red Sea. *Fisheries Research* 161, 356 – 364.
- Swimmer, Y., Suter, J., Arauz, R., Bigelow, K., López, A., Zanela, I., Bolaños, A., Ballesterio, J., Suárez, R., Wang, J., & Boggs, C. (2010) Sustainable fishing gear: the case of modified circle hooks in a Costa Rican longline fishery. *Marine Biology* 158, 757–767.
- Tillett, B. J., Field, I. C., Bradshaw, C. J. A., Johnson, G., Buckworth, R. C., Meekan, M. G., & Ovenden, J. R. (2012) Accuracy of species identification by fisheries observers in a north Australian shark fishery. *Fisheries Research* 127-128, 109 – 115.
- Trujillo, P., Cisneros-Montemayor, A. M., Harper, S., & Zeller, D. (2012) Reconstruction of Costa Rica's marine fisheries catches, 1950 – 2008. *The University of British Columbia Fisheries Centre Working Paper* 2012-03.
- Ward, P., & Myers, R. A. (2005) Shifts in open ocean fish communities coinciding with the commencement of commercial fishing. *Ecology* 86(4), 835 – 847.

- 512 Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R. & Hebert, P. D. N. (2005) DNA barcoding
513 Australia's fish species. *Philosophical Transactions of the Royal Society B* 360, 1847 –
514 1857.
- 515
- 516 Ward, R. D., Holmes, B. H., White, W. T. & Last, P. R. (2008) DNA barcoding Australasian
517 chondrichthyans: results and potential uses in conservation. *Marine and Freshwater*
518 *Research* 59, 57–71.
- 519
- 520 Whoriskey, S., Arauz, R., & Baum, J. K. (2011) Potential impacts of emerging mahi-mahi
521 fisheries on sea turtle and elasmobranch bycatch species. *Biological Conservation* 144,
522 1841–1849.
- 523
- 524 Worm, B., Davis, B., Kettner, L., Ward-Paige, C. A., Chapman, D. D., Heithaus, M.R., Kessel,
525 S., & Gruber, S. H. (2013) Global catches, exploitation rates, and rebuilding options for
526 sharks. *Marine Policy* 40, 194 – 204.
- 527

Table 1. Percentage (%) of each shark species identified from all samples and from the two markets (San Jose and Heredia), the bottom rows represent the Fisher's α and Species Richness values.

Species	Total	Markets	
		San Jose	Heredia
	(n=637)	(n=454)	(n=183)
<i>Sharks</i>			
Pelagic thresher shark (<i>Alopias pelagicus</i>)	3.3	0.2	10.9
Silky shark (<i>Carcharhinus falciformis</i>)	87.3	90.8	78.7
Dusky Shark (<i>Carcharhinus obscurus</i>)	0.5	0.7	-
Sicklefin smooth-hound (<i>Mustelus lunulatus</i>)	0.5	0.4	0.6
Blacktip shark (<i>Carcharhinus limbatus</i>)	1.9	0.2	6
Whitenose shark (<i>Nasolamia velox</i>)	0.3	-	1
Pacific sharpnose shark (<i>Rhizoprionodon longurio</i>)	0.2	0.2	-
Scalloped hammerhead shark (<i>Sphyrna lewini</i>)	4	5.5	0.6
Smooth hammerhead shark (<i>Sphyrna zygaena</i>)	2	2	2.2
<i>Fisher's α</i>		1.38	1.44
<i>Species Richness</i>		8	7

Table 2. Percentage (%) of each shark species identified from samples collected each season, the bottom rows contain the Fisher's α and Species Richness values for each season.

Species	Fall (n=140)	Winter (n=111)	Spring (n=230)	Summer (n=156)
Pelagic thresher shark (<i>Alopias pelagicus</i>)	4	-	3	5
Silky shark (<i>Carcharhinus falciformis</i>)	81	91	89	88
Dusky Shark (<i>Carcharhinus obscurus</i>)	-	-	-	2
Sicklefin smooth-hound (<i>Mustelus lunulatus</i>)	1	-	1	-
Blacktip shark (<i>Carcharhinus limbatus</i>)	5	1	1	1
Whitenose shark (<i>Nasolamia velox</i>)	-	-	<1	1
Pacific sharpnose shark (<i>Rhizoprionodon longurio</i>)	-	-	<1	-
Scalloped hammerhead shark (<i>Sphyrna lewini</i>)	4	6	5	1
Smooth hammerhead shark (<i>Sphyrna zygaena</i>)	5	2	-	3
Fisher's α	1.27	1.03	1.36	1.29
Species Richness	6	5	7	6

Table 3. Percentage (%) of each shark species identified from samples collected at individual pescadarias, the last columns contain the Fisher's α and Species Richness values.

Species	Smooth hammerhead (<i>Sphyrna zygaena</i>)	Scalloped hammerhead (<i>Sphyrna lewini</i>)	Pacific sharpnose (<i>Rhizoprionodon longurio</i>)	Whitenose (<i>Nasolamia velox</i>)	Blacktip (<i>Carcharhinus limbatus</i>)	Sicklefin smooth-hound (<i>Mustelus lunulatus</i>)	Dusky (<i>Carcharhinus obscurus</i>)	Silky (<i>Carcharhinus falciformis</i>)	Pelagic thresher (<i>Alopias pelagicus</i>)	Fisher's α	Species Richness
Pescadarias in San Jose											
Bagre (n=49)	-	-	-	-	-	-	-	100	-	0.18	1
Caracol (n=79)	11	30	-	-	-	-	4	54	-	0.88	4
Corvina (n=48)	-	-	2	-	-	-	-	98	-	0.42	2
Despensia (n=40)	-	-	-	-	-	-	-	100	-	0.18	1
Dolfin (n=44)	-	-	-	-	-	-	-	100	-	0.18	1
Dorado (n=51)	-	-	-	-	-	-	-	100	-	0.18	1
Galapagos (n=42)	-	2	-	-	2	5	-	91	-	1.07	4
Malecon (n=17)	-	-	-	-	-	-	-	100	-	0.23	1
Marisco (n=42)	-	-	-	-	-	-	-	100	-	0.18	1
Rey (n=42)	-	-	-	-	-	-	-	98	2	0.44	2
Pescadarias in Heredia											
Dos Mares (n=39)	10	-	-	3	3	3	-	82	-	1.49	5
Marina (n=40)	-	-	-	-	-	-	-	100	-	0.18	1
Nino (n=10)	-	10	-	-	-	-	-	90	-	0.75	2
Pulpo (n=51)	-	-	-	-	16	-	-	45	39	0.70	3
Unica (n=43)	-	-	-	2	5	-	-	93	-	0.73	3