# 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

## ARTICLE INFO

## 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 1050 bp 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.

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## 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 96–99.99% (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 to 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 and 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, 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, 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, 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, 2008; Clarke et al., 2006; 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 vertebrae), 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 8 mm 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 $-4\,^{\circ}C$.

### 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 1050 bp 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 25 µL containing 10 mM Tris pH 8.4, 50 mM KCL, 0.2 mM each dNTP, 1.5 mM $MgCl_2$, 0.4 µM each primer, 1 U Amplitaq Gold Polymerase® (Life Technologies), and 4 µL of template